Northwest Pear Research Review

Red Lion, Yakima

17-19 Feb 2014

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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
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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.

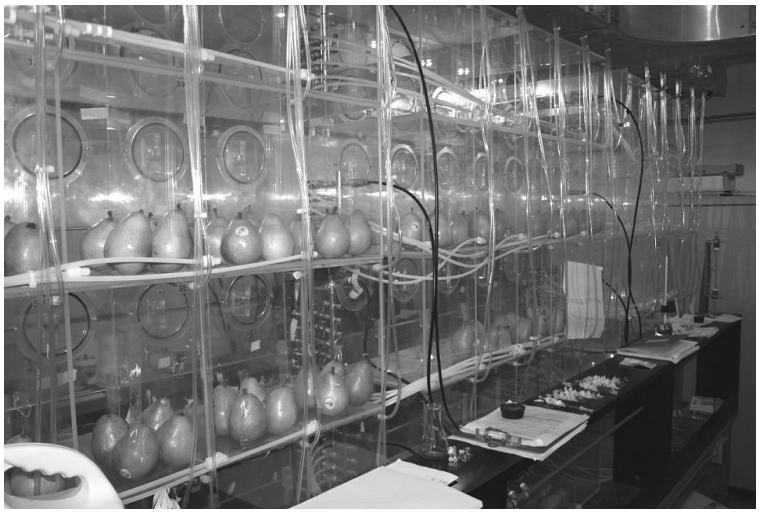

Figure 1. Anjou fruit in 6.0 liter chambers in existing flow-through respiration system.

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.

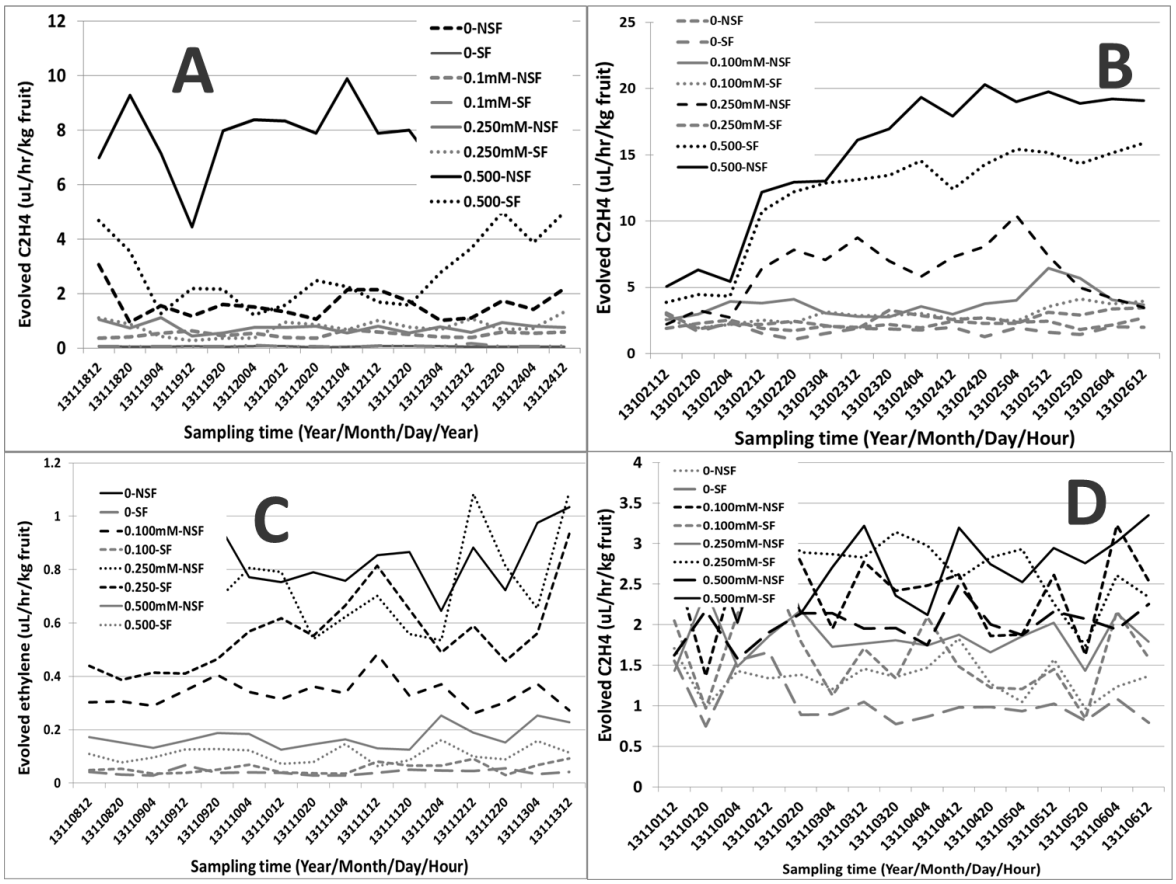

Figure 2. Evolved ethylene (A) from 12-hour RC2-exposed Bartlett, (B) evolved CO2 from 12-hour exposed Bartlett, (C) evolved ethylene from 12-hour exposed Anjou, and (D) and 12-hour exposed CO2. Ripening acceleration is dramatically lower in all experiments relative to longer drench periods.

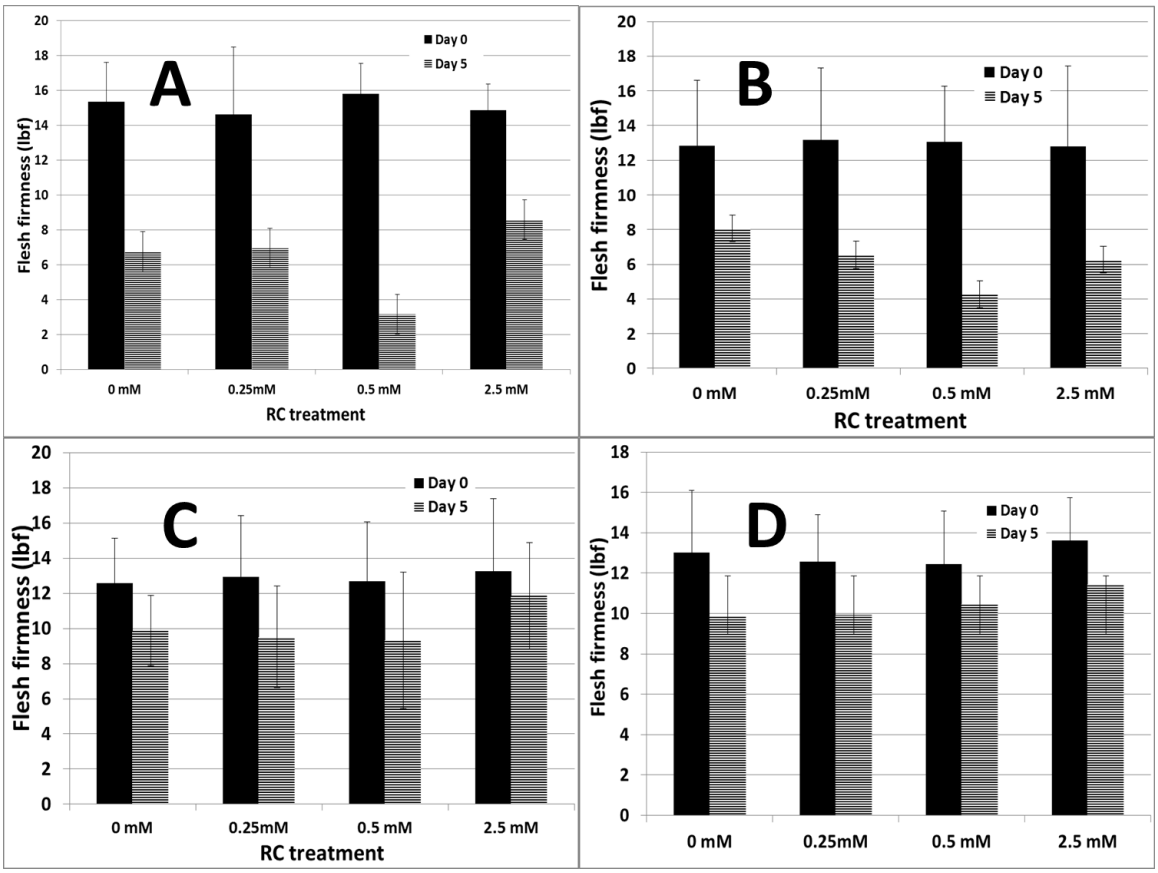

Figure 3. Flesh firmness reduction in SmartFresh-treated, RC2 exposed Anjou fruit from the 2013 season. (A) and (B) represent firmness reduction in Bartlett and Anjou (respectively) after 24 hour RC2 exposure. (C) and (D) represent firmness reduction in Bartlett and Anjou (respectively) for 12 hour RC2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

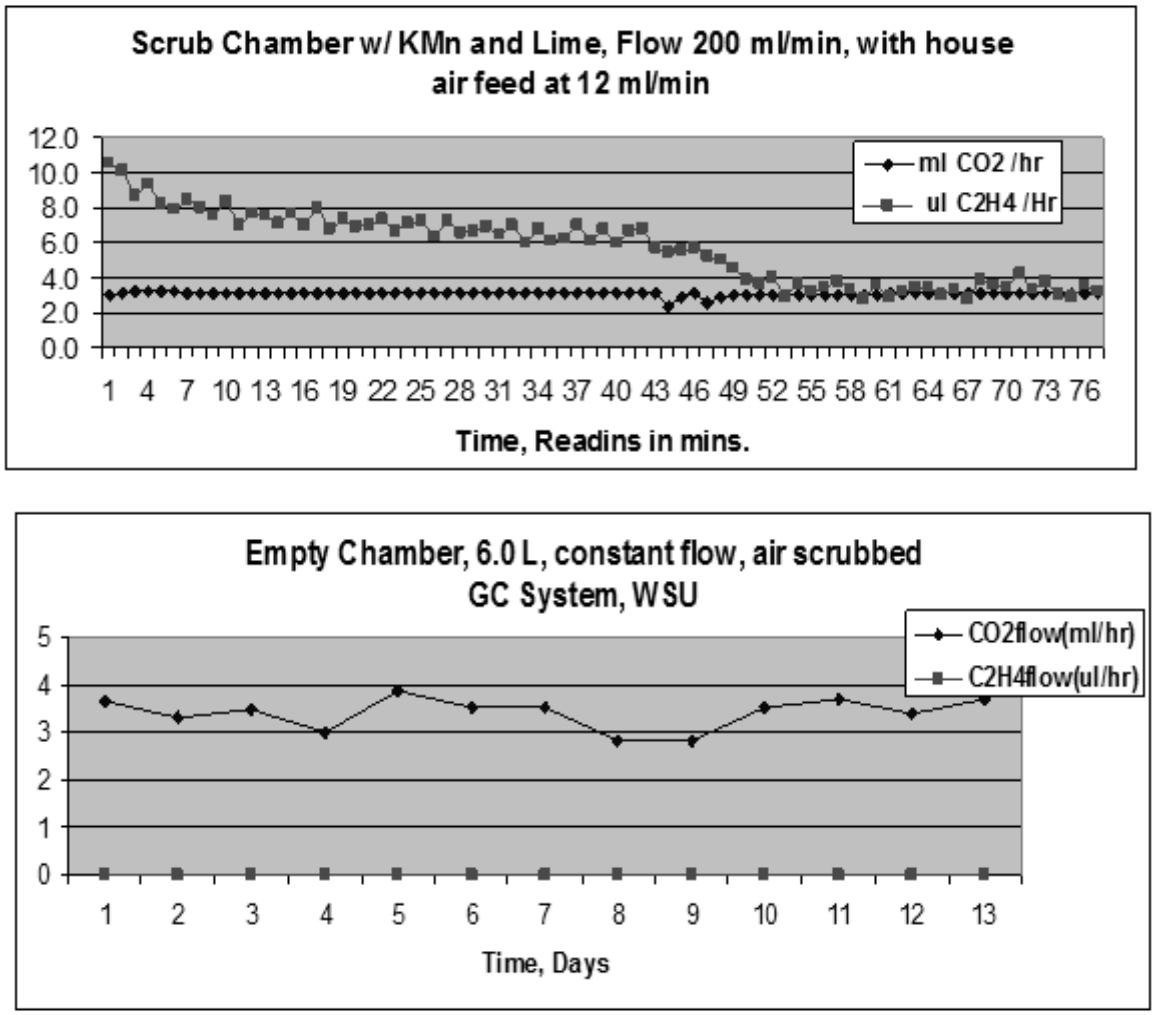

Figure 4. Blanking calibration in GC based system (top) and CID-900 instrument (bottom) with 400 mL/min dynamic flow through sample chamber. Note continuously lower ethylene values than GC-based technology.

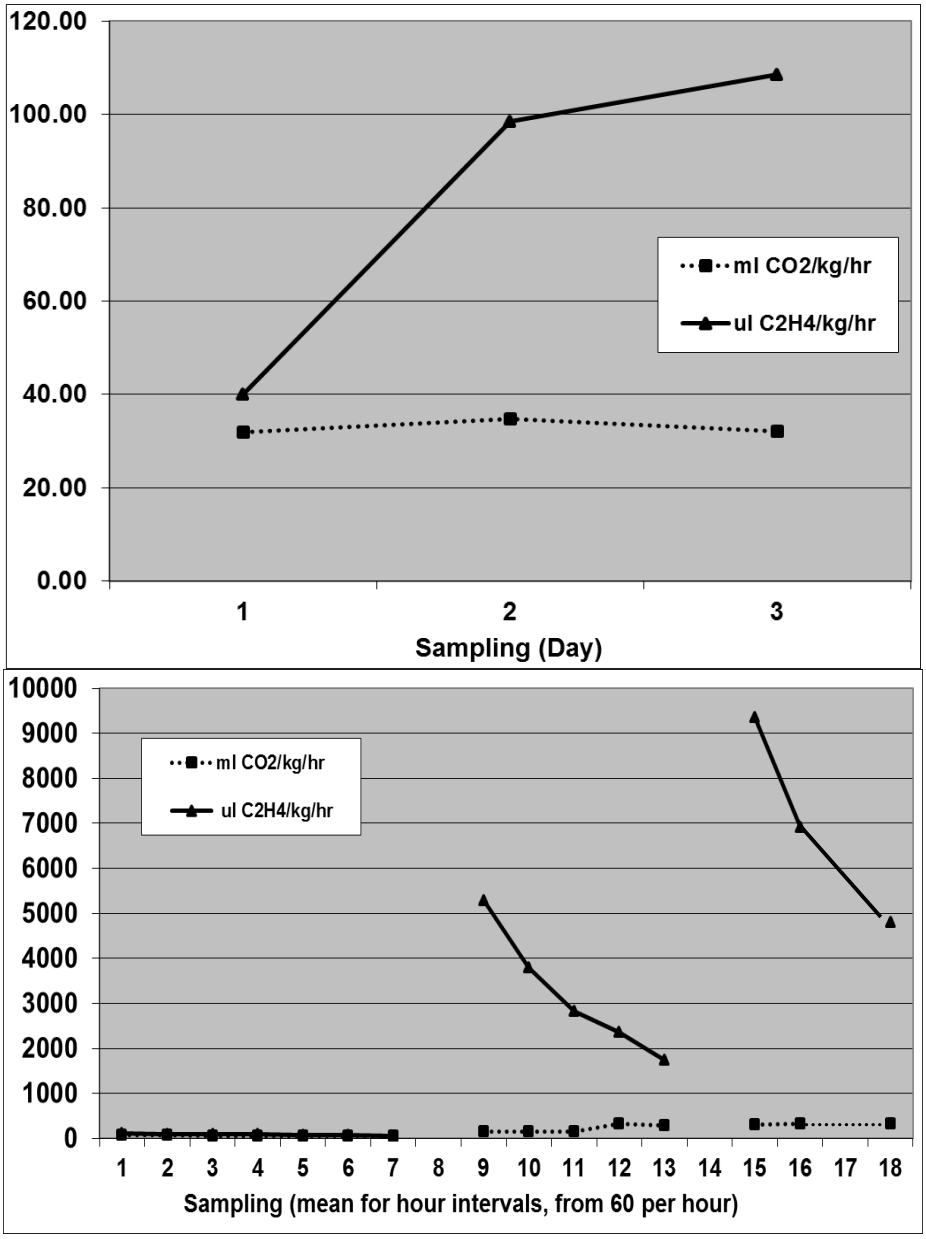

Figure 5: Comparison of existing gas-chromatography based respiration monitoring (Top) and CID-900 real-time ethylene and carbon dioxide monitoring instrument (Bottom). A dynamic flow was established in the headspace of the chamber measured by the CID-900 instrument between sampling events 7 and 9 at 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

1. Bai, J. et al., 2006. J. Hort. Sci. Biotech. 81:959-964.
2. Chen and Spotts. 2005. Int. J. Fruit Sci. 5(3):3-17.
3. Rudell et al., 2005. Inhibition of ethylene action alters apple and pear fruit responses to CA environments with elevated carbon dioxide oxygen and/or temperature. Controlled Atmosphere Conference Proceedings.
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5. Argenta et al., 2003. J. Agric. Food Chemistry. 51:3858-3864.
6. Bai and Chen. 2005. Acta Hort (ISHS) 671:325-331.
7. Villalobos-Acuña et al., 2011. Postharvest Biol. and Technol. 59:1-9.

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.

FINAL PROJECT REPORT

Project Title: Ripening capacity and decay control in winter pears

PI: David Sugar
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Email: david.sugar@oregonstate.edu
Cooperators: E.J. Mitcham, A. Dhingra

Other funding sources: None

Total Project Funding:

Item	**2012**	**2013**
Salaries	19,853	19,853
Benefits	13,102	13,102
Wages		
Benefits		
Equipment		
Supplies	2,000	2,000
Travel		
Miscellaneous		
Total	34,955	34,955

OBJECTIVES

1. Determine appropriate durations of ethylene conditioning, 50 °F conditioning, and ethylene followed by 50 °F conditioning of Anjou pears after 1, 3, 5 months of storage at 30-31 °F, and of Comice pears after 2, 4, and 8 weeks of storage at 30-31 °F. Evaluate the treatment effects on fruit ripening ability, shipping firmness, and eating quality.

2. Evaluate new treatment options for postharvest decay control, with emphasis on preparing fruit for long-term field-run storage through combinations of orchard treatments, in-orchard bin drenches, and packinghouse line-sprays.

SIGNIFICANT FINDINGS:

Objective 1:

1. Ethylene conditioning for 72 hours, 50 °F temperature conditioning for 10 days, or 24-48 hours in ethylene followed by 5 days at 50 °F can induce earlier and more complete ripening capacity and enhance eating quality in Anjou and Comice pears than conventional cold temperature conditioning.
2. The benefits of ethylene and temperature conditioning Anjou and Comice pears were still significant after 1-3 months (Anjou) and 2-4 weeks (Comice) at 31 °F.
3. Conditioning after 5 months (Anjou) and 6 weeks (Comice) at 31 °F was of little or no benefit to ripening.
4. Shipping firmness may be compromised by excess conditioning. Charts relating specific conditioning regimes to shipping firmness were developed for three harvest dates.

Objective 2:

1. Summer calcium and 1-week-pre-harvest fungicide sprays were highly beneficial in reducing postharvest decay.
2. Pre-harvest treatment with Fungiphite (potassium phosphite) significantly reduced postharvest decay, though to a lesser extent than Pristine fungicide.
3. Summer calcium and pre-harvest Pristine and Fungiphite mitigated a delay in postharvest line-spray treatment with Penbotec.
4. Thermofog treatment with pyrimethanil or fludioxonil reduced postharvest decay, especially gray mold and side rot.

RESULTS AND DISCUSSION:

Objective 1:

Successful ethylene conditioning of Anjou and Comice pears harvested at the onset of maturity required 72 hours in ethylene at ~68 °F to induce the capacity to ripen fully within 7 days at room temperature. When using temperature alone to condition pears, 50 °F was a more efficient temperature than either warmer or colder temperatures. Ethylene and 50 °F conditioning can be combined using shorter durations of each. The benefits of these treatments are to facilitate earlier marketing of pears with the capacity to ripen, and to enhance the eating quality of the pears when ripe. Conditioning regimes for Anjou and Comice pears that include time at 50 °F can result in enhanced fruit aroma and an improved eating experience.

Ethylene and 50 °F conditioning applied to Anjou and Comice pears were still beneficial in inducing full ripening within 7 days at room temperature after the fruit had been stored for 1-3

months (Anjou) and 2-4 weeks (Comice) at 31 °F (Figs. 1-6). After 5 months (Anjou) and 6 weeks (Comice) at 31 °F, subsequent conditioning was of little or no benefit to ripening. Harvest date influences the amount of conditioning needed to induce full ripening capacity.

Although ethylene and 50 °F conditioning enhance ripening capacity, excess exposure to either ethylene or 50 °F can result in fruit with compromised shipping firmness. In Figs. 1-6 below, two thresholds of shipping firmness are indicated: 10 lbf and 8 lbf. Assuming that these reflect a typical range of acceptable firmness values for domestic shipping, there are conditioning regimes that result in adequate shipping firmness and other conditioning regimes that result in shipping firmness values too soft to safely ship. For example, in Comice harvested at the onset of maturity and stored 2 weeks before conditioning, pears conditioned for 5 days at 50 °F without ethylene retained shipping firmness > 10 lbf, and after 10 days at 50 °F had shipping firmness ~8 lbf. With later harvest dates, both the duration of conditioning necessary to achieve full ripeness and the shipping firmness decreased.

It is notable that in Anjou pears, even after 1 month at 31 °F prior to conditioning, full ripeness within 7 days at room temperature was not achieved by ethylene treatments shorter than 72 hours. Without ethylene, in Anjou pears stored 1 month at 31 °F prior to conditioning, 10 days at 50 °F induced ripening capacity, and a shipping firmness > 10 lbf was retained in fruit from all three harvest dates tested (Fig. 4).

Objective 2:

Summer calcium chloride treatments and 1-week-pre-harvest fungicide sprays have consistently reduced postharvest decay in Bosc pears (Figs. 7 & 8). This approach has been most effective against side rot (*Cladosporium*, *Alternaria*, and *Phialophora*) and blue mold (*Penicillium*), while of little benefit against gray mold (*Botrytis*). Only Pristine was used as a pre-harvest fungicide in 2012 and 2013 trials, although in previous results Topsin-M has also been effective. Pre-harvest treatments reduced overall decay and mitigated the effect of delays in application of postharvest line-sprays with Penbotec.

In both 2012 and 2013, a putative resistance-stimulant, Fungiphite (potassium phosphite), reduced postharvest decay following treatments applied 1 week before harvest (Figs. 7 & 8). The level of decay control was not as great as that provided by Pristine, but decay reduction was significant.

Thermofog treatments with pyrimethanil (Fig. 9) and fludioxonil (Fig. 10) in CA rooms reduced decay in Bosc pears in 2012 and 2013, respectively. In 2013, decay appeared to be further reduced by SmartFresh application.

Fig. 1. **Comice** pears stored **2 weeks** at 31 °F before conditioning. Left column indicates degree of ripeness achieved in 7 days following ethylene + 50 °F conditioning. Horizontal line at 4 lbf indicates onset of ripeness with buttery-juicy texture. Right column indicates shipping firmness immediately following conditioning treatments. Horizontal lines at 10 and 8 lbf indicate estimated thresholds for safe shipping. Rows differ in harvest date: 0, 7, or 14 days after entering the maturity range.

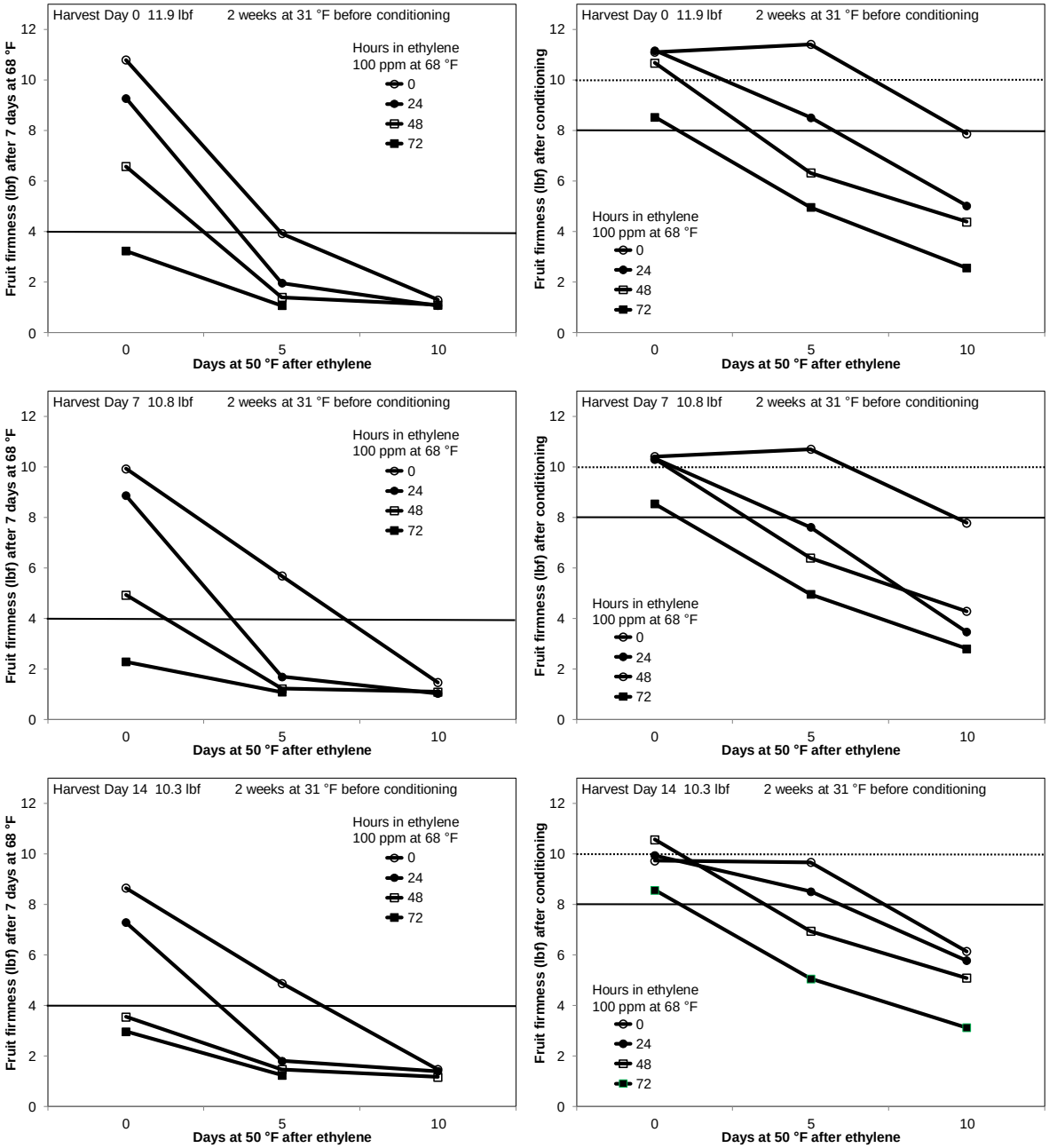


Fig. 2. **Comice** pears stored **4 weeks** at 31 °F before conditioning. Left column indicates degree of ripeness achieved in 7 days following ethylene + 50 °F conditioning. Horizontal line at 4 lbf indicates onset of ripeness with buttery-juicy texture. Right column indicates shipping firmness immediately following conditioning treatments. Horizontal lines at 10 and 8 lbf indicate estimated thresholds for safe shipping. Rows differ in harvest date: 0, 7, or 14 days after entering the maturity range.

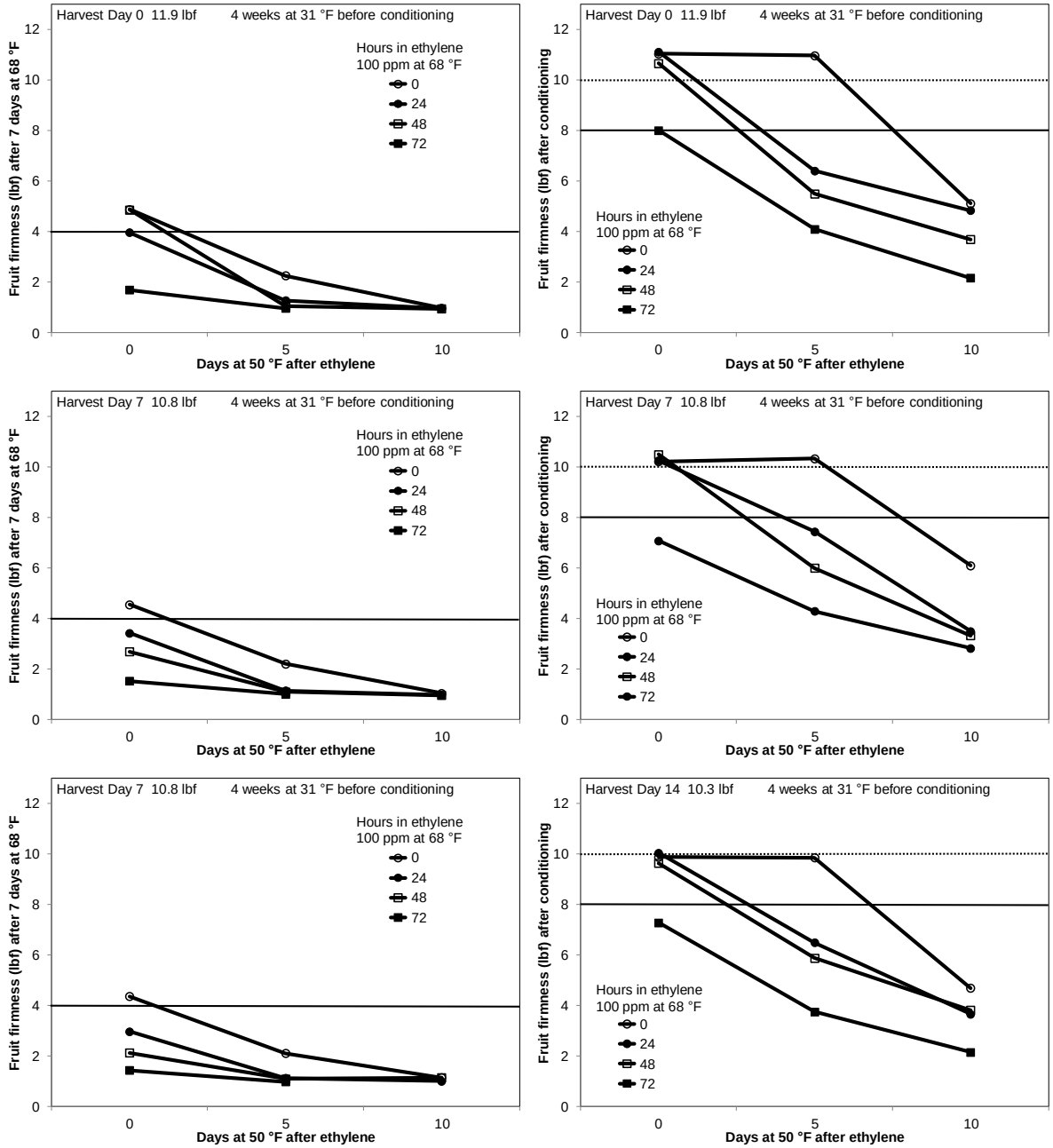


Fig. 3. **Comice** pears stored **6 weeks** at 31 °F before conditioning. Left column indicates degree of ripeness achieved in 7 days following ethylene + 50 °F conditioning. Horizontal line at 4 lbf indicates onset of ripeness with buttery-juicy texture. Right column indicates shipping firmness immediately following conditioning treatments. Horizontal lines at 10 and 8 lbf indicate estimated thresholds for safe shipping. Rows differ in harvest date: 0, 7, or 14 days after entering the maturity range.

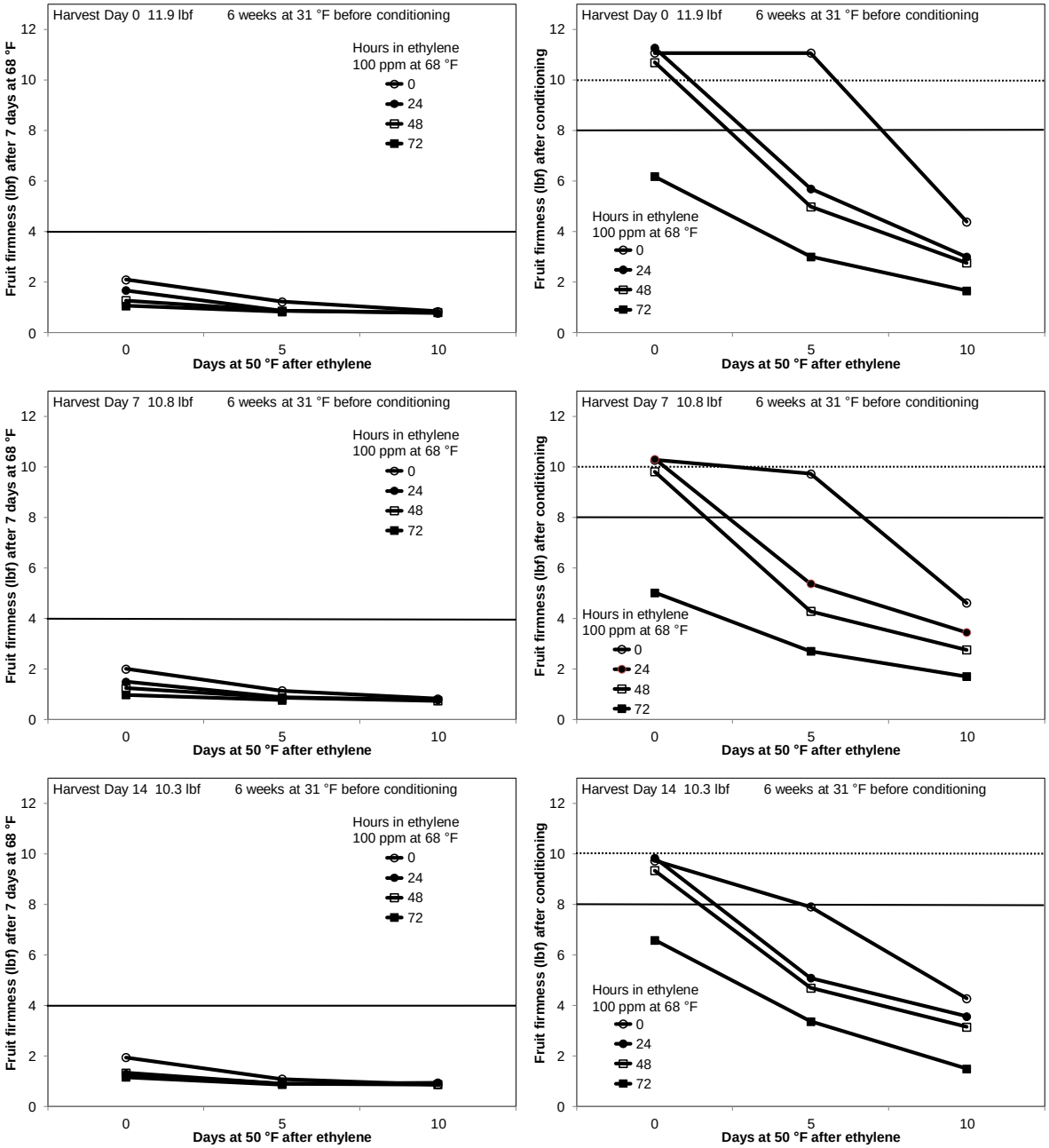


Fig. 4. **Anjou** pears stored **1 month** at 31 °F before conditioning. Left column indicates degree of ripeness achieved in 7 days following ethylene + 50 °F conditioning. Horizontal line at 4 lbf indicates onset of ripeness with buttery-juicy texture. Right column indicates shipping firmness immediately following conditioning treatments. Horizontal lines at 10 and 8 lbf indicate estimated thresholds for safe shipping. Rows differ in harvest date: 0, 7, or 14 days after entering the maturity range.

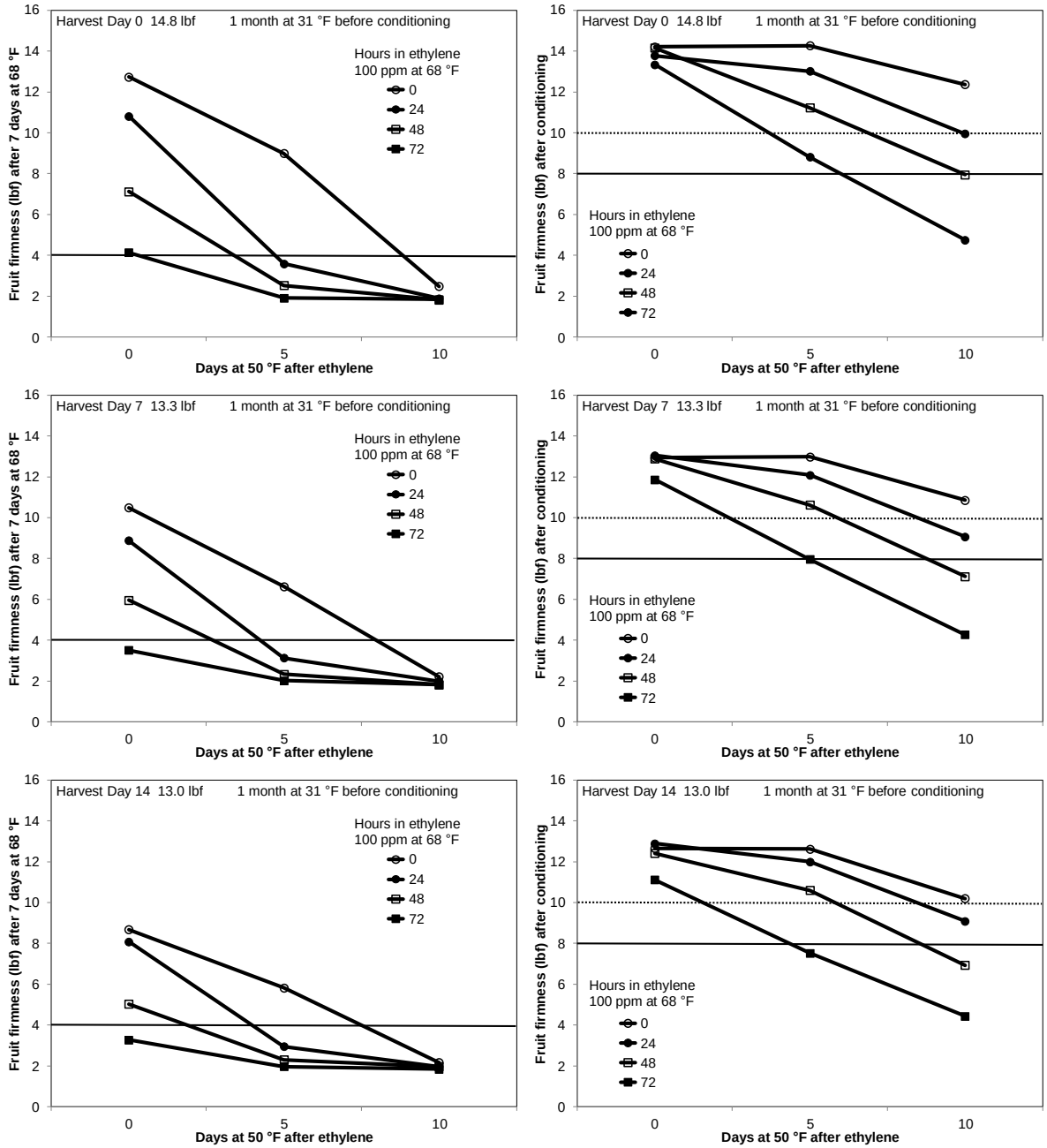


Fig. 5. **Anjou** pears stored **3 months** at 31 °F before conditioning. Left column indicates degree of ripeness achieved in 7 days following ethylene + 50 °F conditioning. Horizontal line at 4 lbf indicates onset of ripeness with buttery-juicy texture. Right column indicates shipping firmness immediately following conditioning treatments. Horizontal lines at 10 and 8 lbf indicate estimated thresholds for safe shipping. Rows differ in harvest date: 0, 7, or 14 days after entering the maturity range.

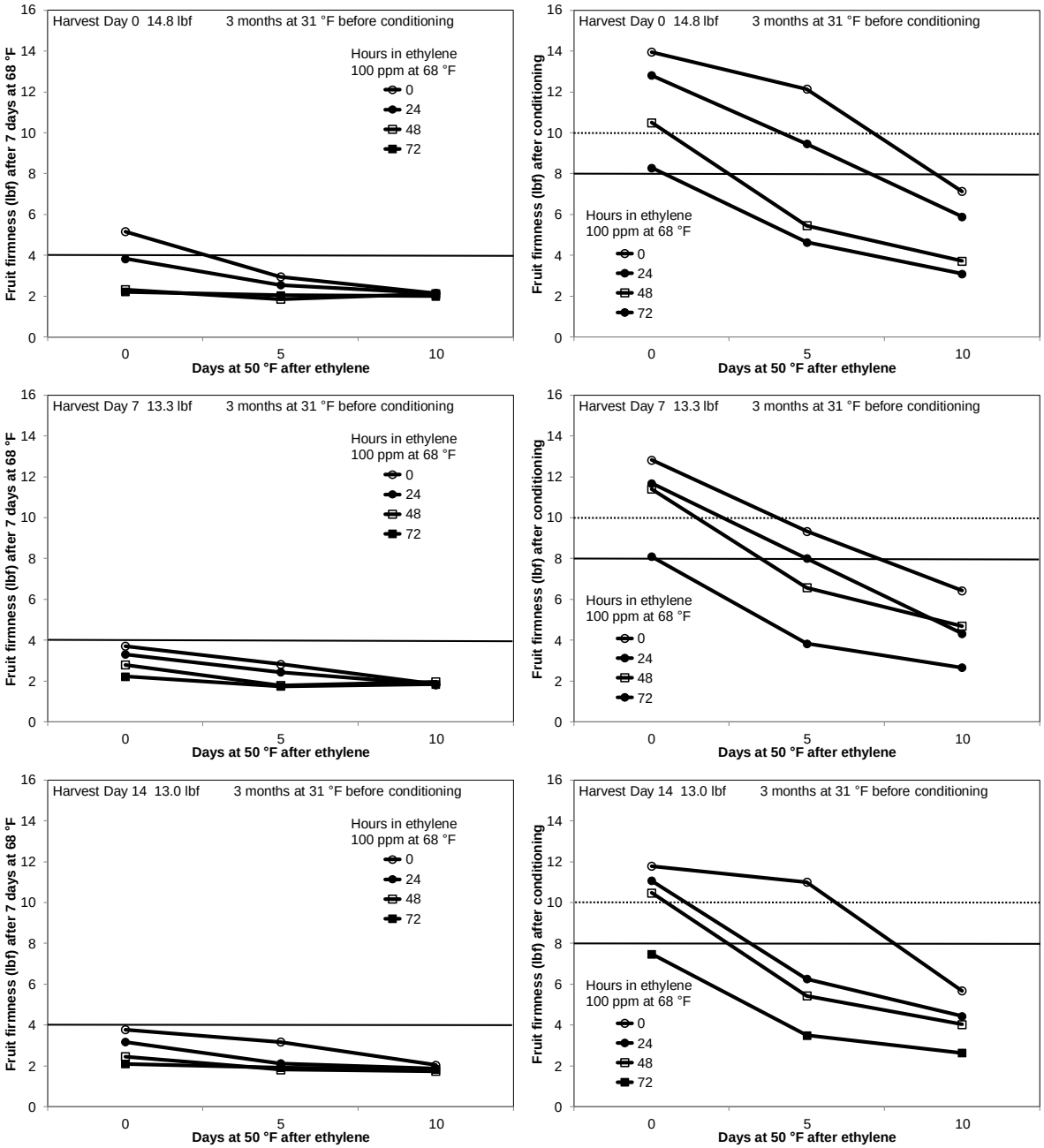


Fig. 6. **Anjou** pears stored **5 months** at 31 °F before conditioning. Left column indicates degree of ripeness achieved in 7 days following ethylene + 50 °F conditioning. Horizontal line at 4 lbf indicates onset of ripeness with buttery-juicy texture. Right column indicates shipping firmness immediately following conditioning treatments. Horizontal lines at 10 and 8 lbf indicate estimated thresholds for safe shipping. Rows differ in harvest date: 0, 7, or 14 days after entering the maturity range.

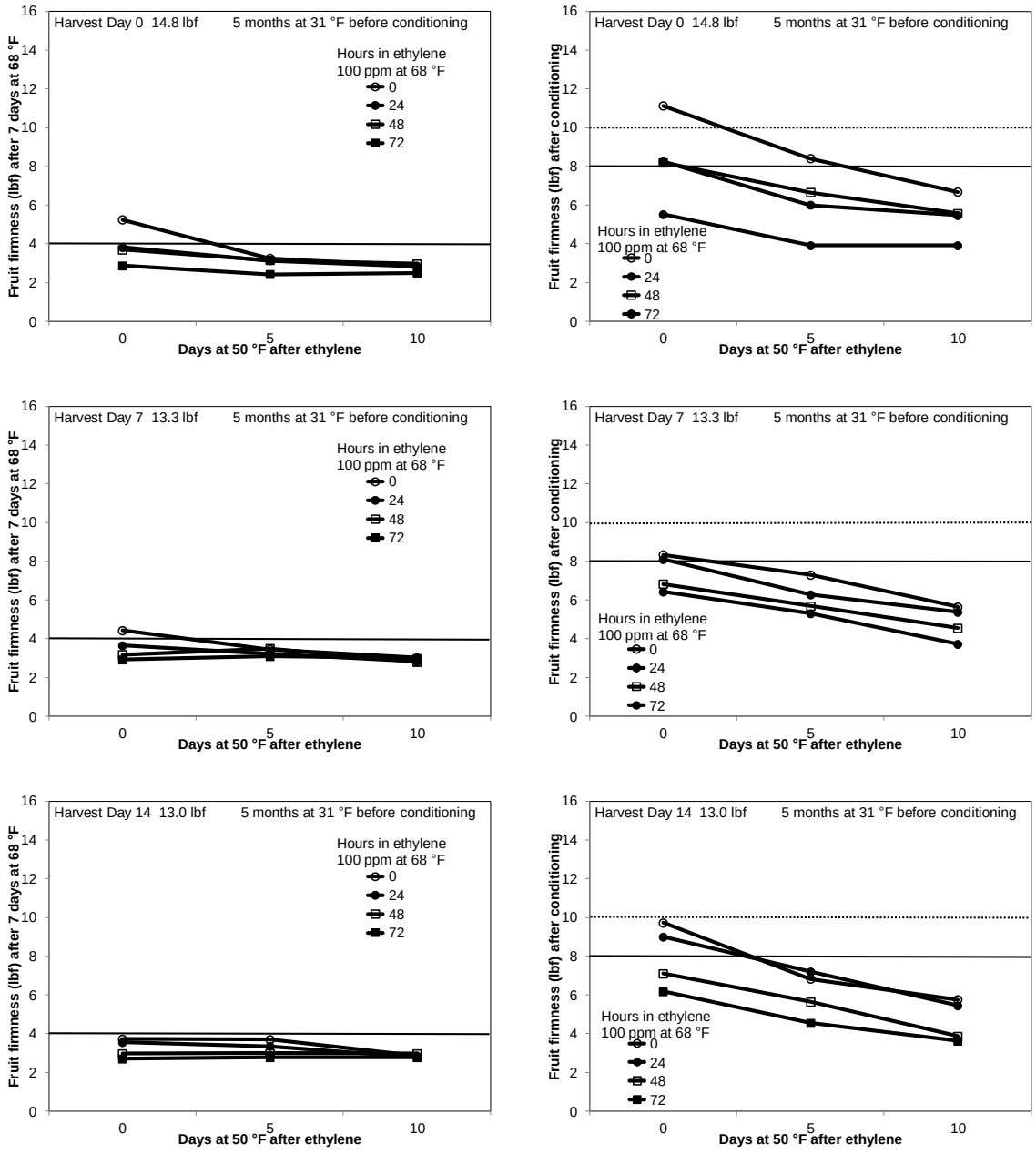


Fig. 7. Effect of orchard treatments with **Fungiphite, Pristine, or Pristine + Fungiphite** on incidence of decay at wounds in Bosc pears when postharvest line-spray application of pyrimethanil (Penbotec) was delayed for up to 9 weeks after harvest and wounding. Combined data 2012 and 2013.

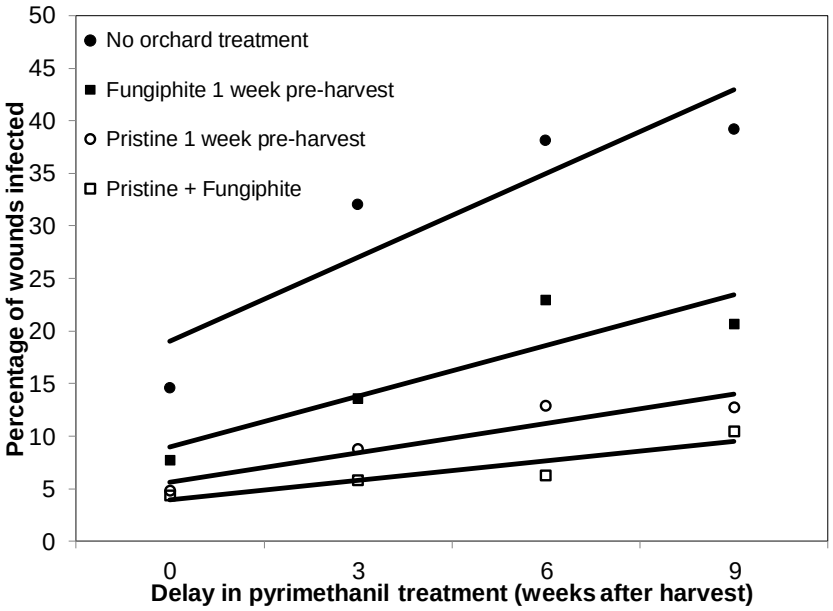


Fig. 8. Effect of summer orchard treatments with **calcium chloride** followed by pre-harvest **Fungiphite, Pristine, or Pristine + Fungiphite** on incidence of decay at wounds in Bosc pears when postharvest line-spray application of pyrimethanil (Penbotec) was delayed for up to 9 weeks after harvest and wounding. Combined data 2012 and 2013.

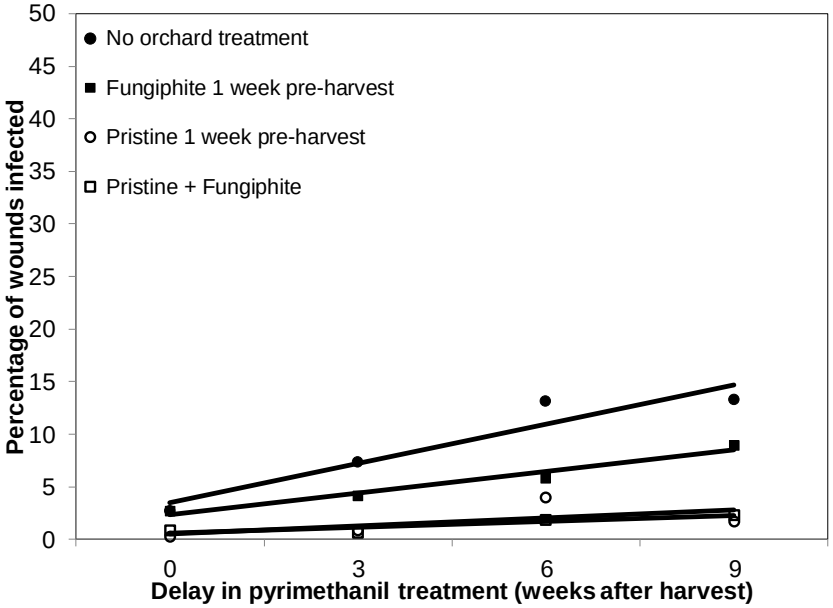


Fig. 9. Effects of **thermofog** treatment with **pyrimethanil** followed by CA storage on gray mold (*Botrtyis cinerea*) decay in artificially inoculated Bosc pears, with and without additional **SmartFresh** treatment. 2012-2013 data.

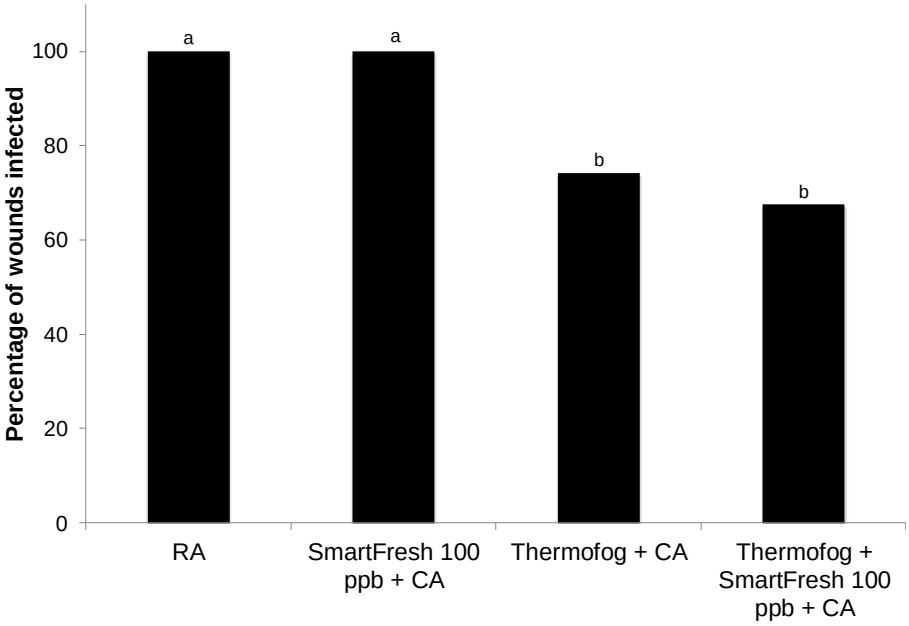
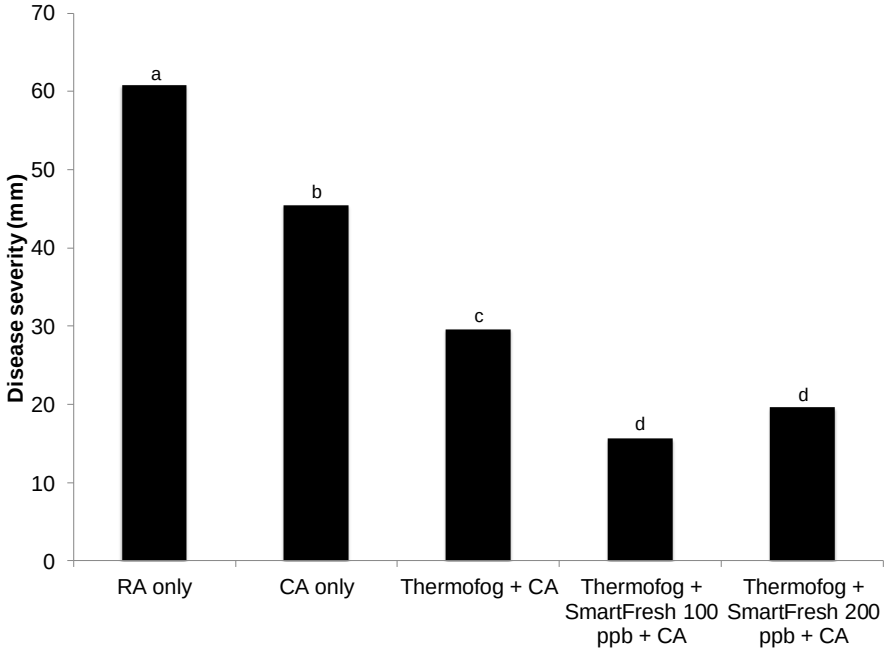


Fig. 10. Effects of **thermofog** treatment with **fludioxonil** followed by CA storage on gray mold (*Botrtyis cinerea*) decay in artificially inoculated Bosc pears, with and without additional **SmartFresh** treatment. 2013-2014 data.



EXECUTIVE SUMMARY:

Successful conditioning of Anjou and Comice pears harvested at the onset of maturity to induce full ripening capacity was accomplished by either 72 hours in ethylene, 10 days at 50 °F, or 24-48 hours in ethylene plus 5 days at 50 °F. Charts relating specific ethylene or 50 °F conditioning regimes to ripening and shipping firmness were developed. Postharvest decay in Bosc pears was significantly reduced by summer calcium chloride sprays, Pristine fungicide applied 1 week pre-harvest, and Fungiphite putative resistance stimulant applied 1 week pre-harvest. The most effective decay control was achieved by combinations of these treatments. Thermofog application of pyrimethanil or fludioxonil followed by CA storage and SmartFresh treatments reduced gray mold decay and side rot in Bosc pears.

I wish to express my gratitude to the Research Subcommittee, the Fresh and Processed Pear Committees, the previous Winter Pear Control Committee, the Washington Tree Fruit Research Commission, and Pear Bureau Northwest for many years of support for my research. I have very much enjoyed being part of the pear community during my tenure at Oregon State University, and wish the pear industry continued success and advancement.

FINAL PROJECT REPORT

Project Title: Genetic analysis of advanced pear rootstock

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Cooperators: Richard Bell, USDA; Stefano Musacchi, Italy (WSU); Feli Fernandez, UK; Joan Bonny, IRTA Spain and Francois Laurens, INRA France

Total Project Funding: **Year 1:** 49,362

Other funding sources – none

Budget 1
Organization Name: WSU
Telephone: 509 335 4564

Contract Administrator: Carrie Johnston
Email address: carriej@wsu.edu

Item	2013
Salaries	
Benefits	
Wages[a]	36,510
Benefits	2,352
Equipment	
Supplies[b]	10,000
Travel	1,500
Miscellaneous	
Plot Fees	
Total	49,362

Footnotes:

a. Wages to partially support the time of a graduate student and an undergraduate student on the project
b. DNA quantification, quality analysis, PCR, comprehensive genetic analysis and subsequent family structure analysis - ~250 samples

OBJECTIVES

The objective of this 1 year project was to determine the genetic relationships amongst the advanced a nd elite pear rootstock germplasm available outside of the USA which we plan to import over the next few years to jump start pear rootstock breeding.

Even before the plant material is imported via Prosser or USDA Beltsville, there is a need to understa nd the genetic diversity of the material. This will enable the prioritization of the individuals to be imp orted and the order in which they should be imported. The aim is to import representatives of most di verse selections to have the largest degree of genetic diversity.

An additional bonus of the genetic analysis prior to the introduction of the germplasm through the CP C is that there will be a baseline fingerprint already available that can then be utilized to confirm true ness-to-type at a later date. This is critical as exemplified with the recent genetic analysis of OHxF87 where it has been found that Bartlett and not Farmingdale is the male parent. There are several other e xamples of mix ups recently with the Geneva rootstocks. Therefore, utilization of a systematic approa ch was proposed to be employed in this project.

Objective 1: Genetic diversity analysis of advanced rootstock selections obtained from collaborating pear breeding programs. (Year 1)

Specific Goals:

a. Perform DNA marker analyses for ~250 individuals, score markers using a binary code (0, 1)
b. Convert information into a unique genetic identifier and a barcode
c. Perform population structure analysis to identify genetic relationships among individuals
d. Submit the data and results to GDR-Database for Rosaceae

SIGNIFICANT FINDINGS

- Population structure analysis identified 3 main populations amongst the DNA samples representing 29 advanced rootstocks obtained from University of Bologna.
- Grouping of individuals does not correspond to the indicated parental descent implying potential mixing of inventory.
- The DNA marker analysis method produces substantial information for a robust population analysis.

RESULTS AND DISCUSSION

In order to begin DNA marker analyses, a primer screen was performed to identify primer combinatio ns that would amplify the greatest number of polymorphic loci that would be most informative about population structure since there are no previous reports of application of Targeted Region Amplified Polymorphism (TRAP) markers in pear. A total of 48 primer combinations were tested with 39 prime r combinations, each amplifying between ~20 to ~75 loci from a single genotype were identified thro ugh this primer screen.

Perform marker analyses for ~250 individuals:
Primers were selected based upon the number of loci amplified in the primer screen. Three primer pa irs (six total combinations) were used for PCR. DNA for 34 pear samples was received from Stefano

Musacchi at University of Bologna. PCR was performed in triplicate for each of these samples. PCR products were subsequently visualized on a polyacrylamide gel. Gel images were scored for each individual using a binary system of 0 or 1, indicating absence or presence of a marker, respectively. Results were recorded in an Excel spreadsheet for analysis.

Conversion of information into a unique genetic identifier and barcode:
The custom software seeDNA© developed by the Dhingra lab was used to simultaneously generate a unique genetic identity (GID) code, a scannable barcode, and two dimensional gel image for each individual. Each two dimensional gel image and barcode contains all of the scoring information obtained from the PCR. Each color in the 2D gel represents a different primer combination (these are indicated by arrows on Figure 1A). The lengths of the horizontal lines correspond to the molecular weight of that particular marker. This information is unique to the individual from which the DNA was taken. With that in mind, the images shown in Figure 1B can be used as ID tags in nurseries or by growers in the field. Scannable barcodes and QR codes allow for easy access of information regarding the individual. In addition to functioning as an ID tag to aid in inventory management, the information on this tag can be used to reveal potentially mistaken identity based on mislabeling. To accomplish this, DNA from the unknown sample can be analyzed and compared to the marker scoring information from a sample.

seeDNA© was also used to compare similarity between individuals. Figure 1A shows samples that are 83% identical to the pear sample number 26 (1B), an open pollinated cross. Sample 26 is shown on the far left while the two most similar relatives are depicted in the middle and right of 1A. Note that although there are a significant amount of similarities between the three gel images there are several markers that differ between these individuals. Information such as this may be helpful in identifying potential parental redundancy between these individuals. The results from seeDNA© are in agreement with those from both STRUCTURE and NTSys.

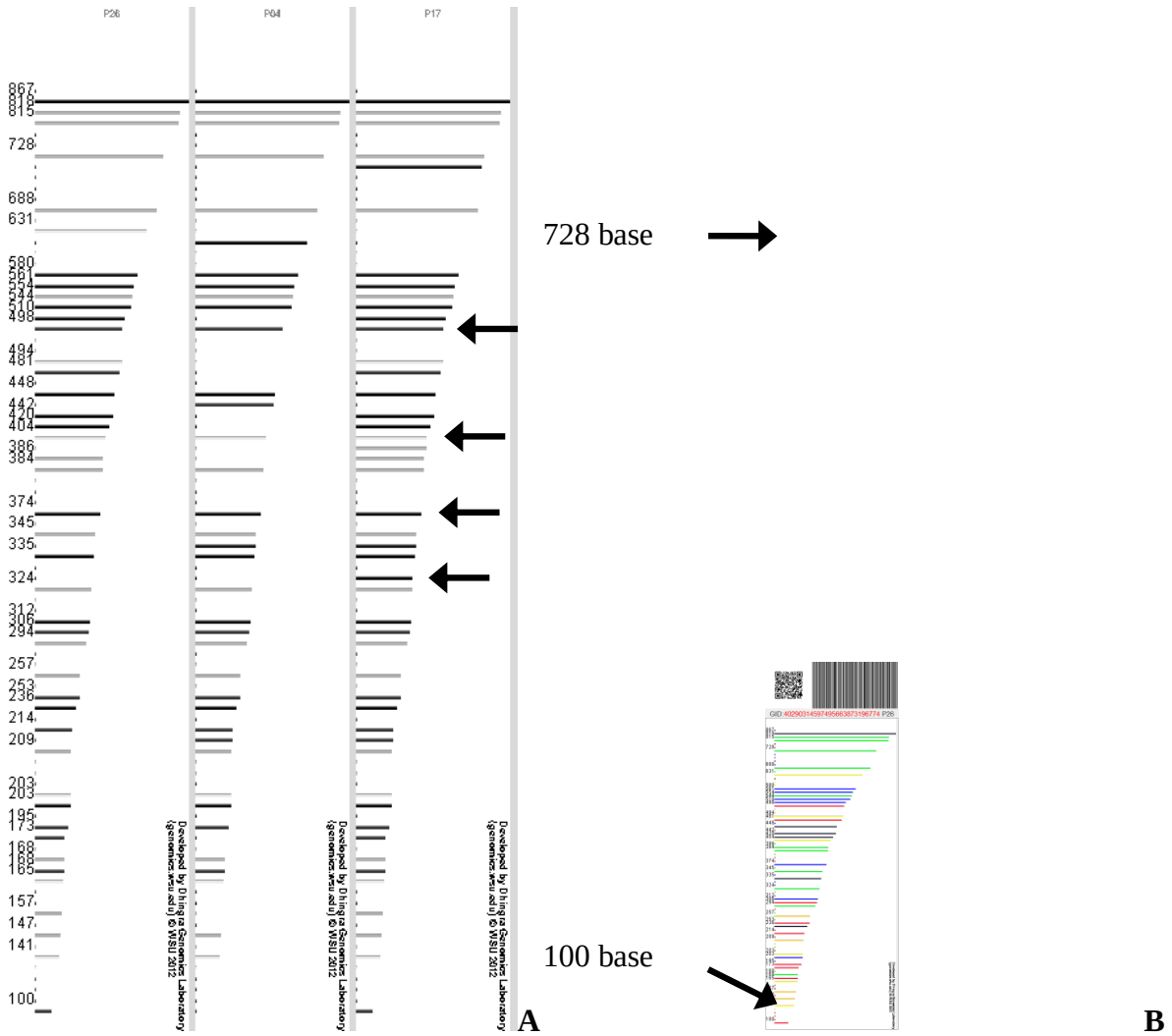
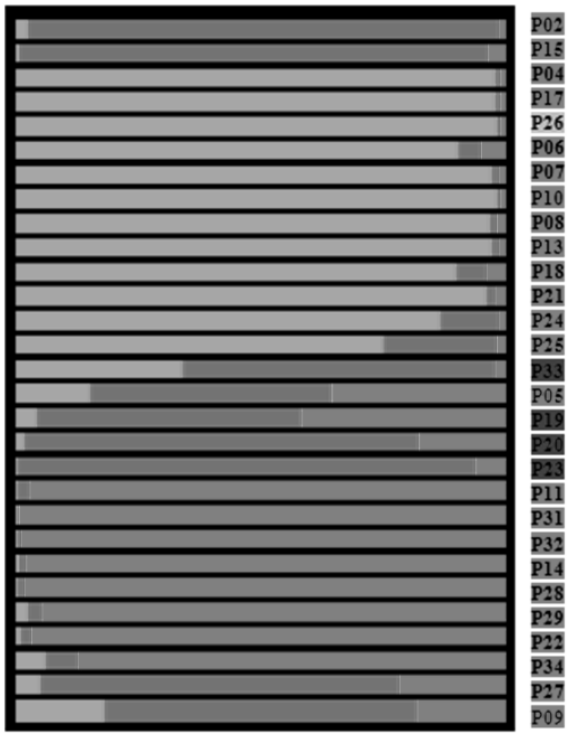


Figure 1: seeDNA© generated output. Comparison of individuals 83% similar to sample number 26 (A) and identification tag containing genetic identification (GID) code, sample name, two dimensiona l gel image, and scannable barcode (B).

Population structure analysis:
The polymorphic loci information was used for population structure analysis. TRAP primers produce d 86 polymorphic markers among the 29 of the 34 pear samples. We were unable to obtain sufficient amplification of markers for 5 samples; DNA for these samples has been requested to complete the an alysis. The program STRUCTURE was used to assign individuals into populations based on probabili ty and the presence or absence of a marker, i.e. genotype data. Results were also analyzed by the prog ram NTSys which creates a phylogenetic tree using statistical algorithms and a specified similarity co efficient.

The genetic relationships among these individuals were assessed using STRUCTURE and NTSys. ST RUCTURE output is shown (Figure 3) as horizontal bars, each bar representing a single individual. Within each horizontal bar are colored portions, each color is one of the populations determined by th e program. The proportion of a color with a horizontal bar indicated the proportion of genes from that individual that belong to that particular population. For example samples 11, 31, 32, 14, 28, 29, 22, 3 4, and 27 have a large proportion of genes that belong to the yellow population. When additional infor

mation is factored in to these results, we see that these individuals are all crosses from the same two p arents Abate Fetel x sel. 79504074. Other information regarding desirable traits, location, and growth habits can be factored in to identify even more correlations between these individuals and populations . Relationships between these individuals become more identifiable when this output is paired with th e phylogenetic tree produced by NTSys. Figures 3 and 4 can be laid side by side to show the populati ons (or families) and the admixture within each of the individuals in those families.



Cross Information by Color
US309 x NIJISSEIKI
Passa Crassana x Decana
Abate x California
Abate x sel. 79504074
Abate x M.R. Bartlett
Abate x Rosired
Decana x M.R. Bartlett
Open pollination
Butirra Hardy x Kaiser

Figure 3: Output from the program STRUCTURE in dicating the proportion of an individual (a row) that belongs to a particular population (a color: blue, orange, or yellow) as determined by the program. Sa mple numbers are listed to the right of each row and are color coordinated according to the parental cr oss.

NTSys was useful in identifying relationships among the pear individuals analyzed. This phylogenetic tree can be used to discern similarity between individuals. Returning to the sample number 26 exampl e, you can see that the two most related samples to 26 are numbers 4 and 17. While these individuals may have desired traits on their own, it would be at the discretion of the breeder whether it would be beneficial to make a cross between such genetically similar individuals. Among the individuals analy zed thus far are several progeny of Passa Crassana (Passe Crassane in French). Passa Crassana is an i deal parent as it has compact habits and is frost resistant (USDA-ARS-NGRP). The Asian pear Nijiss eiki, another suitable parent, produces a medium size tree and is only moderately susceptible to fire bl ight.

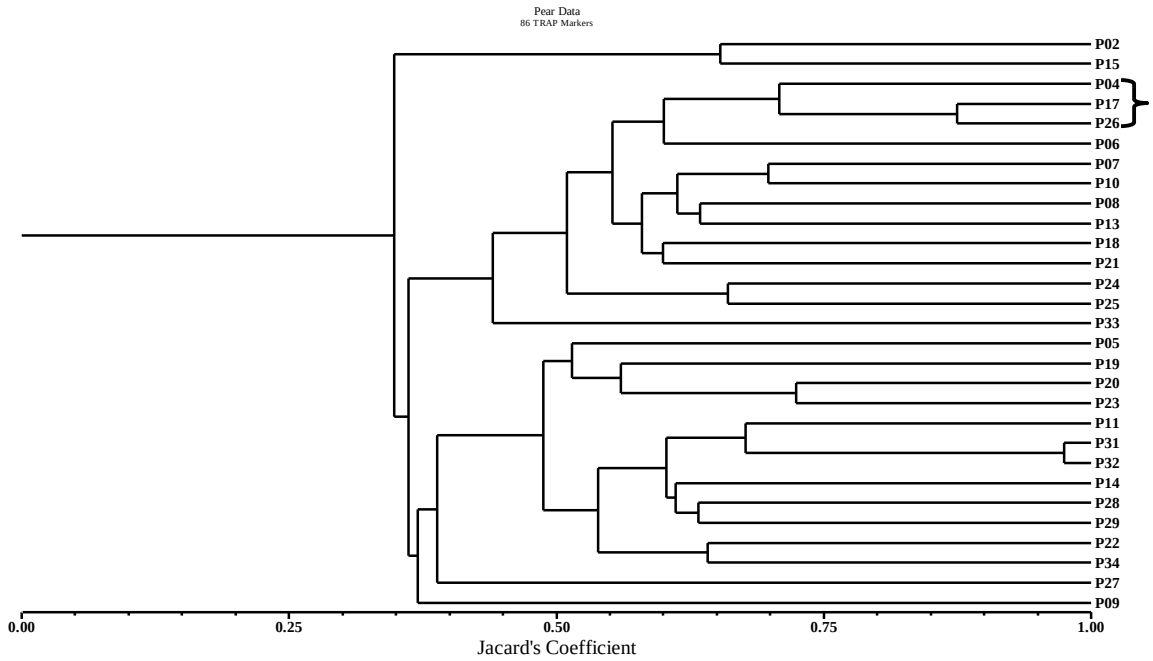


Figure 4: Phylogenetic tree created using the program NTSys. Samples are in the same vertical order as the STRUCTURE output so as to facilitate side by side comparison.

We have determined that these methods are successful in identifying genetic relationships among diff erent species of *Pyrus*. Progress on this project has shown that there is a significant amount of genetic diversity available in advanced and elite pear rootstock material from Italy. DNA from additional sam ples will be received in Feb-March 2014. With the addition of the material, we will have a broad unde rstanding of the genetic relationships among these advanced selections. This knowledge will assist br eeders in making more informed crosses to create dwarfing and disease resistant rootstocks.

The information generated through this project will be submitted to GDR. This will serve as a catalog of genetic information about the imported individuals. As more genomic information becomes availab le for pears, we can begin to link marker information to traits of interest. We have established a valua ble tool for pear rootstock and variety breeding.

Once all the samples are analyzed in 2014 an update shall be provided to WTFRC.

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USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (G RIN)*. [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=%20PI+131662 (23 January 2014)

USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (G RIN)*. [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1180597 (23 January 2014)

Executive Summary

Significant progress
Utilizing gene-based markers, we have cataloged the elite rootstock selections from University of Bologna into 3 major sub-populations. This will guide what rootstocks need to be imported and in what order. The information has been provided to Dr. Stefano Musacchi who shall now combine this information with agronomic traits and make a decision on the import process.

Summary of findings
Targeted Region Amplified Polymorphism type of markers generated suitable amount of polymorphic loci to resolve population structures. It was expected that the siblings derived from listed parents shall group together. However, based on information provided by Dr. Musacchi, the parents of the populations were not controlled so outcrossing is possible. This analysis has resulted in the proper identification of the parents as well.

Future directions
Successful resolution of population structure with the markers used for 29 samples will be utilized for the remaining samples. This information will be utilized in the future selection of parental material for pear rootstock breeding.

FINAL REPORT

Project Title: Developing rooting strategies for clonal pear rootstocks

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Cooperators: Todd Einhorn, Oregon State University, Yongjian Chang, North American Plants,

Other funding sources

Agency Name: California Pear Advisory Board
Amount awarded: $38000

Total Project Funding:

Budget History:

Item	Year 1:
Salaries	22000
Benefits	12000
Wages	2000
Benefits	
Equipment	
Supplies	2000
Travel	
Plot Fees	
Miscellaneous	
Total	38000

JUSTIFICATION:

The development and use of clonal pear rootstocks was long restricted by the lack of effective and rapid propagation systems. Clonal rootstocks were difficult to propagate both traditionally and *in vitro*. Many promising rootstocks were abandoned because of difficulty with traditional propagation or poor growth *in vitro* (Proebsting, WTFRC reports 2003-7). Development of new pear media makes it possible to produce all types of pears *in vitro* (Reed et al., 2012; Reed et al., 2013). A wide range of both scion and rootstock selections now have excellent growth and multiplication with several mineral nutrient improvements. The types and combinations of mineral nutrients in growth media greatly affected the growth and development of the 20 cultivars tested, and new formulations provide good growth for this diverse group (Fig. 1). Quince selections also propagate well on these media.

Development of effective rooting protocols will allow for *in vitro* or direct rooting of shoots so plantlets can be acclimated, grown up in nurseries and made available to growers. Earlier studies of pear rooting showed that there is great variation in root production *in vitro*; some treatments were highly effective (60-80%), while some pears had low rooting rates or did not root on any of the treatments (Reed, 1995; Yeo and Reed, 1995). These studies were done with pears grown on Murashige and Skoog medium (MS) (Murashige and Skoog, 1962); rooting as well as growth may be improved on medium with a better nutrient formulation. Rooting of propagules is a limiting factor in commercial production of many new rootstock selections.

An improved multiplication medium for propagating clonal rootstocks, PRS medium, is now available, but the final step is to determine effective rooting protocols. We have done some preliminary experiments with 'Horner 51', 'OH×F87' and 'OH×F333' that indicate some genotype related effects of the propagation and rooting media as well as the hormone used for rooting. Once these protocols are developed, the techniques can be used by commercial micropropagation companies to produce a steady supply for the industry. Testing these rooting techniques in a commercial setting will verify the efficacy for transfer to the micropropagation industry. Transferring these outcomes to commercial tissue culture labs, will improve production efficiency and shorten the production period for new pear rootstocks. The best rooting protocols developed in this study could be used to screen additional selections without additional large-scale testing. If the callus induction test proves to be predictive of rooting ability, it could be used to prescreen rooting hormone for each selection.

This is the final portion of research needed to make pear micropropagation viable for rapidly producing clonal rootstock selections for nurseries and growers. All of these techniques will be freely available (no patent or licensing required). These objectives strongly support the pear industry priority of improved propagation techniques for clonal rootstocks.

OBJECTIVES:

1) Determine effect of rooting hormone types and concentrations on callus formation.
2) Compare PRS and MS medium formulations for efficiency of root production.
3) Test rooting protocols on rootstock selections for *in vitro* rooting.
4) Test direct rooting to soilless medium in a commercial setting.
5) Transfer this information to the micropropagation industry for use.

Results

1. Rooting test with 5 pear genotypes with NAA and IBA in DMSO (dimethyl sulfoxide). Five genotypes (9 shoots/ box), 'OH×F69', 'OH×F87', 'OH×F513', 'Horner 51' and 'Pyro 2-33' planted on PRS medium with no growth regulators. **Treatments:** shoots were dipped for 5 seconds in one of the plant growth regulator (PGR) solutions dissolved in DMSO at (0, 1, 5, 10 and 15 mM): **IBA** (indole-3-butyric acid: MW 203.24 g mol^{-1}) and **NAA** (1-naphthaleneacetic acid: MW 186.21 g mol^{-1}).
Results: Controls (no PGR) did not root, NAA was more effective than IBA (Table 1).

Table 1. Percent rooting of five pear rootstocks after dipping in 1-15 mM NAA or IBA dissolved in DMSO. Controls without treatment did not root. Data taken at 4 weeks.

Genotype	NAA (% rooted)				IBA (% rooted)			
	1 mM	5 mM	10 mM	15 mM	1 mM	5 mM	10 mM	15 mM
Horner 51	11.1	66.7	55.6	44.4	33.3	22.2	33.3	44.4
OH×F 69	33.3	66.7	55.6	77.8	11.1	66.7	77.8	33.3
OH×F 87	0.0	50.0	100.0	88.9	0.0	44.4	55.6	88.9
OH×F 513	44.4	75.0	100.0	88.9	11.1	55.6	55.6	66.7
Pyro 2-33	0.0	100.0	88.9	100.0	33.3	66.7	44.4	66.7

1. The second test with higher concentrations of PGR was run with the same genotypes as above and replicated. Five genotypes (9 shoots/ box) × 2 replications × 10 treatments. PRS media with no BA: 80 boxes. IBA and NAA at 0, 5, 10, 15 and 20 mM were tested.

Results: Controls (no PGR) did not root. NAA was more effective than IBA (Table 2).

Table 2. Percent rooting of five pear rootstocks after dipping in 5-20 mM NAA or IBA dissolved in DMSO. Controls without treatment did not root. Data taken at 4 weeks.

Genotype	**NAA** (% rooted)				**IBA** (% rooted)			
	5 mM	10 mM	15 mM	20 mM	5 mM	10 mM	15 mM	20 mM
Horner 51	55	33	72	72	72	77	83	83
OH×F 69	100	100	100	83	83	77	66	94
OH×F 87	94	88	83	100	83	77	72	77
OH×F 513	100	100	94	100	77	100	72	72
Pyro 2-33	88	100	72	77	66	28	61	67

3. Test with polyethylene glycol 400 (PEG 400). Five genotypes planted on PRS medium with no growth regulators with three PGR solutions (NAA 10 mM, IBA 20 mM, and a combination of 5 mM NAA and 10 mM IBA) in 40% PEG 400 in deionized water. Treatments: Dipping for 2 seconds in one of 3 PGR concentrations.

Results: Controls (no PGR) did not root. Shoot quality was better than those rooted with DMSO and most maintained green leaves (Table 3, Figure 1).

Table 3. Percent *in vitro* rooting of five pear rootstocks after dipping in NAA, IBA, or the two combined and dissolved in PEG 400. Controls did not root. Data was taken at 3 weeks.

Genotype	NAA 10 mM	IBA 20 mM	NAA+IBA
Fox 11	100	100	100
Horner 10	78	89	100
OH×F 69	100	100	100
OH×F 87	55	88	73
OH×F 97	100	100	100

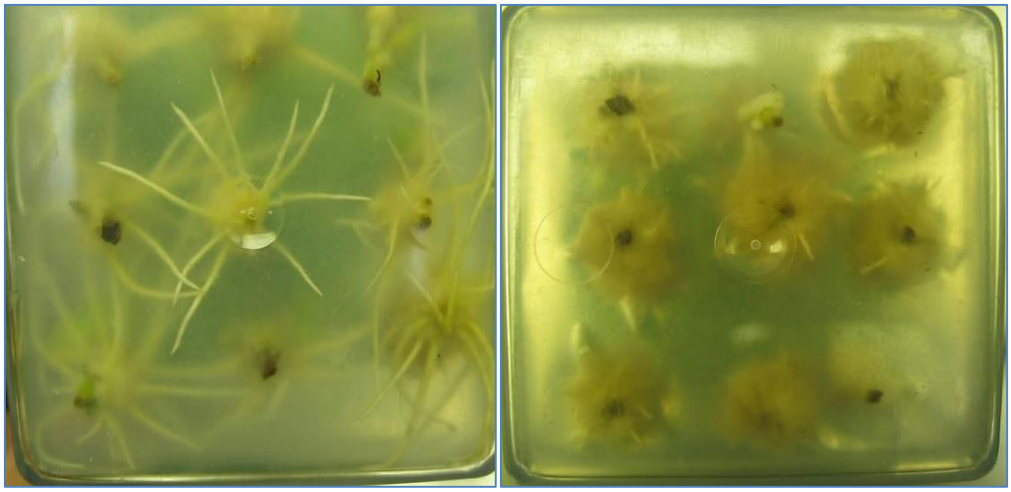


Fig. 1. *In vitro* rooted 'OH×F97' shoots at 3 weeks after treatment with PEG and PGRs. Left: NAA 10 mM, Right: 5 mM NAA + 5 mM IBA.

4. *Ex vitro* direct rooting. Four genotypes ('Horner 4', 'OH×F69', 'OH×F87', and 'OH×F97') were used for *ex vitro* rooting with four replications per treatment. Shoots were cultured on two growth media (MS and PRS) for 4 weeks and transferred to fresh medium for two more times for stabilization (total 12 weeks subculture period). Those shoots grown on the two propagation media were dipped in two rooting hormones at the PGR levels determined in the previous tests (NAA 15 mM and IBA 5 mM combined with NAA 5 mM) dissolved in 40% PEG 400 with DI water, and directly planted in a soilless mix (NA Plants proprietary mix). Shoots were placed under mist in a greenhouse at NA Plants Inc. Rooting was evaluated 4 weeks after planting. Most of the 'Horner 4' treatment could not be evaluated.

Control shoots grown on MS produced an occasional root in some of the genotypes. Control shoots grown on PRS of 'OH×F87' and 'OH×F97' produced some roots and the plants were healthier than those on MS.

Shoots treated with NAA or a mix of NAA and IBA: rooting was variable by treatment and genotype (Fig. 2). 'OH×F97' rooted on all treatments, but the PRS with NAA and IBA had the most roots and

plantlets with the best appearance. ‘OH×F69’ rooted best with the PRS NAA+IBA mix. ‘OH×F87’ had good rooting on the NAA+IBA mix for both growth media but the PRS grown plants had a better appearance and longer roots. ‘Horner 4’ rooted very well on the PRS NAA+ IBA mix (the treatments could not be evaluated) (Fig. 3).

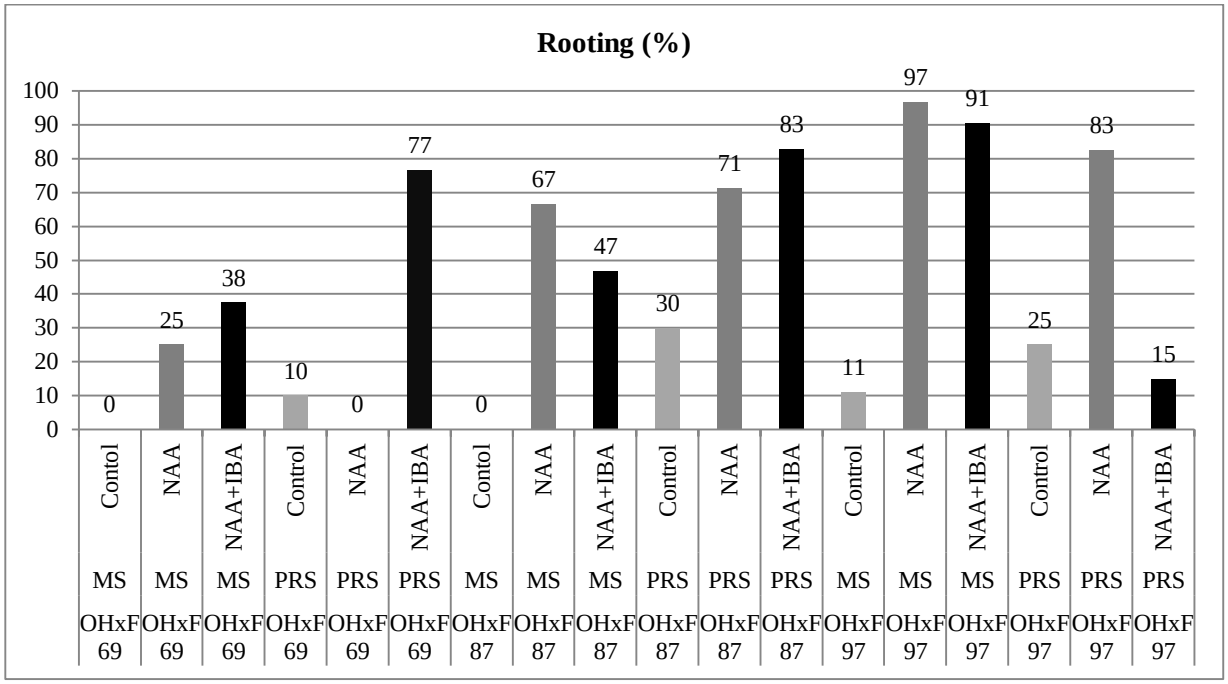
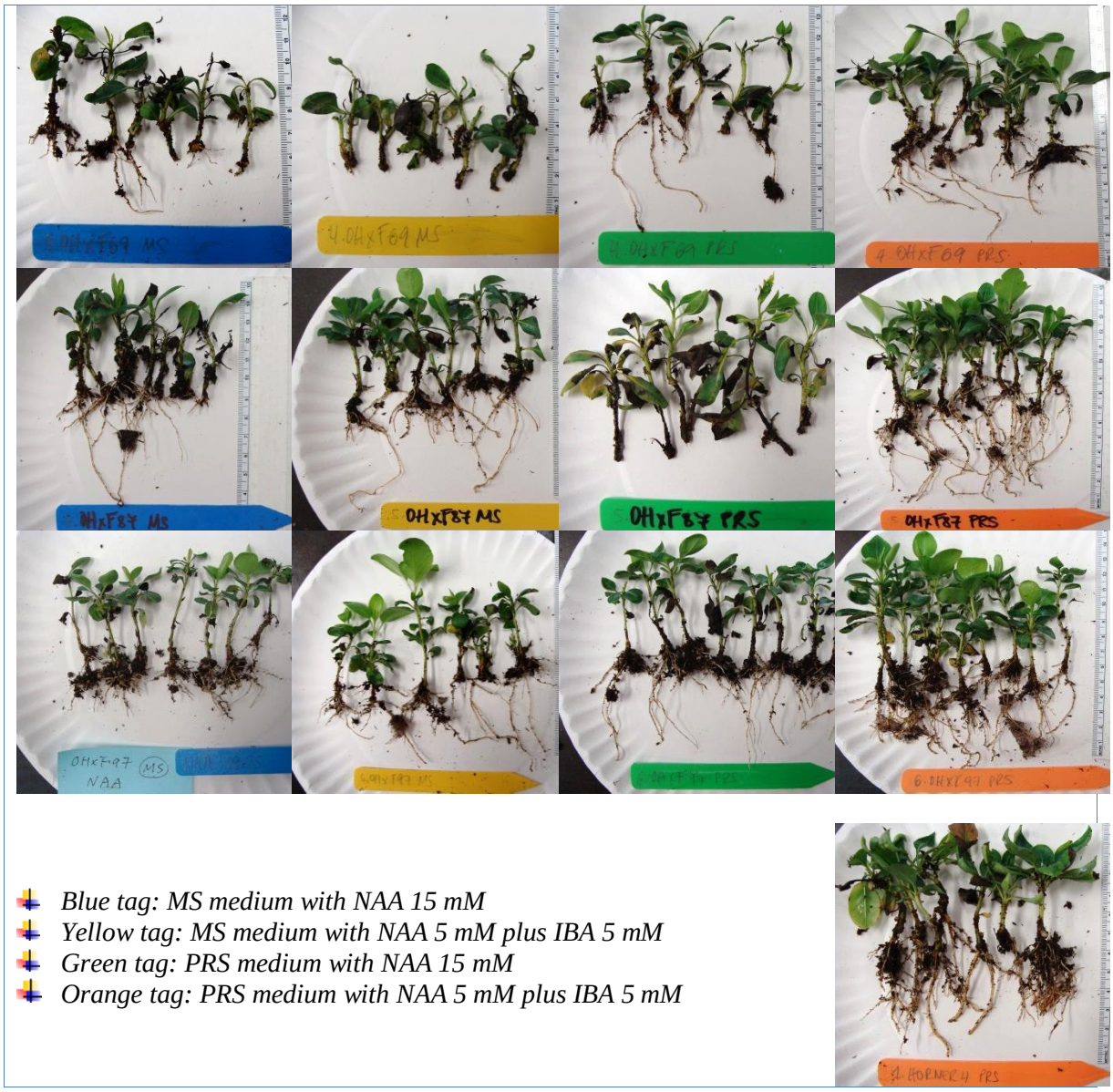


Fig. 2. Rooting of shoots grown on either MS or PRS medium and rooted *ex vitro* with two PGR treatments



- *Blue tag: MS medium with NAA 15 mM*
- *Yellow tag: MS medium with NAA 5 mM plus IBA 5 mM*
- *Green tag: PRS medium with NAA 15 mM*
- *Orange tag: PRS medium with NAA 5 mM plus IBA 5 mM*

Fig. 3. Pictures of *ex vitro* rooted plantlets from the NAA and NAA+IBA treatments on shoots grown on either MS or PRS medium before rooting.

Conclusions:

1. Callus was not always indicative of rooting success
2. *In vitro* rooting was best with NAA for most genotypes when DMSO was used as a solvent.
3. PEG 400 was a better solvent for rooting than DMSO; producing better rooting and healthier plantlets. NAA and IBA combined provided a high rate of rooting for most genotypes.
4. Shoots grown and rooted on the new PRS medium with combined 5 mM IBA and 5 mM NAA produced healthy plantlets with good root systems and a high rooting percentage.

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Reed, B.M., S. Wada, J. DeNoma, and R.P. Niedz. 2013. Improving *in vitro* mineral nutrition for diverse pear germplasm. In Vitro Cell. Dev. Biol. - Plant. 49: 343-355.

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Executive summary:

The development and use of clonal pear rootstocks was long restricted by the lack of effective and rapid propagation systems. Clonal rootstocks were difficult to propagate both traditionally and *in vitro*. Many promising rootstocks were abandoned because of difficulty with traditional or *in vitro* propagation. Development of new pear media now makes it possible to produce all types of pears *in vitro*. In addition an improved multiplication medium for propagating clonal rootstocks, Improved Pear Rootstock medium (PRS), is now available. A wide range of both scion and rootstock selections now have excellent growth and multiplication with several mineral nutrient improvements through our series of studies and these new media. The types and combinations of mineral nutrients in the growth media greatly affected the growth and development of the 20 pear cultivars tested, and these new formulations now provide good growth for this diverse group.

Rooting is a limiting factor in commercial production of many new root stock selections. Development of effective rooting protocols will allow for *in vitro* or direct *ex vitro* rooting of shoots so plantlets can be acclimated, grown up in nurseries and made available to growers. *In vitro* as well as *ex vitro* testing of efficient rooting techniques in a commercial setting can verify the efficacy for micropropagation industry. The current study determined effective rooting protocols for the rootstock cultivars. Promising rootstocks were grown on the new PRS medium and compared for rooting with rootstocks grown on the standard MS medium. We determined that NAA or NAA combined with IBA were the best plant growth regulators to use for pear rootstocks. We compared root production of shoots propagated on either PRS or MS medium, revealing that PRS medium produced superior plantlets and better rooting for the genotypes tested compared to MS medium. We determined that polyethylene glycol was an efficient solvent for applying the rooting chemicals. In direct rooting tests a combination of NAA and IBA produced excellent rooting on plantlets grown on PRS medium and rooting in a soilless substrate in a commercial setting. Transferring these outcomes to commercial tissue culture labs, will improve production efficiency and shorten the production period for new pear rootstocks. The best rooting protocols developed in this study could be used to screen additional selections without additional large-scale testing. As a whole, these studies provide information that makes pear micropropagation a good option for rapidly producing clonal rootstock selections for nurseries and growers. All of these techniques are freely available through series of publications.

- Reed, B.M., Wada, S., DeNoma, J., and Niedz, R.P., 2013. Improving *in vitro* mineral nutrition for diverse pear germplasm. *In Vitro Cell. Dev. Biol. - Plant* 49:343-355.
- Wada, S., Niedz, R.P., DeNoma, J., and Reed, B.M., 2013. Mesos components ($CaCl_2$, $MgSO_4$, KH_2PO_4) are critical for improving pear micropropagation. *In Vitro Cell. Dev. Biol. - Plant* 49:356-365.
- Reed, B.M., Wada, S., DeNoma, J., and Niedz, R.P., 2013. Mineral nutrition influences physiological responses of pear *in vitro*. *In Vitro Cellular & Developmental Biology-Plant*: 1-11.
- Wada, S., Maki, S., Niedz, R.P., and Reed, B.M., 2014. *In vitro* response and ionic mineral analysis of genetically diverse pear species to increased mesos components ($CaCl_2$, $MgSO_4$, KH_2PO_4) in the growth medium. *Scientia Hortic. In review.*
- Wada, S., Niedz, R.P., and Reed, B.M. *In vitro* nitrogen requirements vary with pear species *In Vitro Cell. Dev. Biol. - Plant. In preparation.*
- Wada, S. and Reed, B.M. *In vitro* mineral nutrition and rooting protocols for dwarfing pear rootstock selections. *In preparation.*

FINAL PROJECT REPORT

Project Title: Accelerating pear breeding progress with early-flowering plants

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Address 2: Appalachian Fruit Research Station
City/State/Zip: Kearneysville, WV 25430

Cooperators: None

Total Project Request: **Year 1:** $14,308

Other funding sources: None

Budget 1
Organization Name: USDA, ARS **Contract Administrator: Ingrid Charlton**
Telephone: 215-233-6554 **Email address: Ingrid.charlton@ars.usda.gov**

Item	**2013**
Salaries	
Benefits	
Wages	$10,490
Benefits	$ 840
Equipment	
Supplies	$ 2978
Travel	
Miscellaneous	
Plot Fees	
Total	$14,308

OBJECTIVES

The objectives were to transform 'Bartlett' pear scion cultivar and 'Old Home' x Farmingdale' pear rootstocks with *PtFT1* and *BpMADS4* genes. Following selection and verification of transgenic clones, tree architecture and flowering were to be evaluated in the greenhouse. Flowering clones were to be artificially pollinated with cross-compatible parents and fruit and seed development, objective measures of fruit quality (size, % soluble solids, titratable acidity), and seed yield would be evaluated. Finally, one clone of 'Bartlett' and one clone of OHF97 would be selected for future studies of horticultural practices to minimize the generation cycle and maximize fruit/seed production.

SIGNIFICANT FINDINGS

Several experiments were conducted at two-week intervals to genetically transform 'Bartlett', OHF97,OHF87 and 'Conference' with the *PtFT1* gene. All leaf explants of the uninoculated controls produced callus, with no necrosis (leaf death), but the percentage of regeneration was low (9%). Callus was produced on non-necrotic leaf explants for 'Bartlett' (92%), 'Conference' (91%), OHF97 (65%) and OHF87 (35%). No new adventitious shoots, however, were produced which survived selection on the kanamycin antibiotic-containing tissue culture medium. Changes in the protocol have been instituted to increase the efficiency of plantlet regeneration and recovery of transgenic plantlets.

RESULTS AND DISCUSSION

Stock shoot tip cultures were multiplied to provide sufficient leaves for transformation/plantlet regeneration experiments. Several experiments were conducted at two-week intervals to genetically transform 'Bartlett', OHF97,OHF87 and 'Conference' with the *PtFT1* gene. All leaf explants of the uninoculated controls produced callus, with no necrosis (leaf death), but the percentage of regeneration was low (9%). Callus was produced on non-necrotic leaf explants for 'Bartlett' (92%), 'Conference' (91%), OHF97 (65%) and OHF87 (35%). No new adventitious shoots, however, were produced which survived selection on the kanamycin antibiotic-containing tissue culture medium. Changes in the protocol have been instituted to increase the efficiency of plantlet regeneration and recovery of transgenic plantlets. An experiment has commenced to transform these cultivars with the *BpMADS4* gene. Additional experiments are underway to enhance our regeneration/transformation success. [Changes in the standard protocol/medium composition were made by my technician without my knowledge, which may have led to reduced plantlet regeneration/transformation.]

Work on this project was delayed because the funding was not available until August 20, 2013, and hiring the Biological Aide candidate required another 3 weeks.

EXECUTIVE SUMMARY

In order to respond efficiently to present and future breeding priorities and challenges, accelerated strategies are needed to more rapidly transfer important genes from less than optimum germplasm resources into commercially feasible genetic backgrounds. Development of this technology would be particulary important to deal with potential challenges imposed by climatic factors and water availability. Genetic transformation with genes which control flowering, such as *PtFT1*from poplar and *BpMADS4* from birch offer one technology to reduce the non-flowering period to one year, substantially reducing the generation cycle. This technology has been successfully applied to apple using *BpMADS4* and is being developed for plum and pear with *PtFT1* at our research station. This project seeks to develop the genetically transformed early-flowering plants and greenhouse production system to allow rapid cycling breeding for pear which will enhance incorporation of valuable traits from unadapted pears into commercial varieties.

Summary of findings

Several experiments were conducted at two-week intervals to genetically transform 'Bartlett', OHF97, OHF87 and 'Conference' with the *PtFT1* gene. All leaf explants of the uninoculated controls produced callus, with no necrosis (leaf death), but the percentage of regeneration was low (9%). Callus was produced on non-necrotic leaf explants for 'Bartlett' (92%), 'Conference' (91%), OHF97 (65%) and OHF87 (35%). No new adventitious shoots, however, were produced which survived selection on the kanamycin antibiotic-containing tissue culture medium. Changes in the protocol have been instituted to increase the efficiency of plantlet regeneration and recovery of transgenic plantlets.

Future directions

Experiments will be conducted to increase the efficiency of the tissue culture-based plantlet regeneration and transformation system. Work planned for the coming year includes continued transformation of 'Bartlett' and 'Old Home' x 'Farmingdale' 97 pear rootstock with *PtFT1* and *BpMADS4*. 'Conference', a pear cultivar with a high adventitioius regeneration capacity, will also be transformed. Transgenic clones generated with be evaluated in the greenhouse for tree architecture and flowering. Flowering clones will be pollinated with cross-compatible parents and fruit development, objective measures of fruit quality (size, % soluble solids, titratable acidity), and seed yield will be assessed. Single clones of 'Bartlett' or 'Conference' and one clone of OHF97 will be selected for future studies of horticultural practices to minimize the generation cycle and maximize fruit/seed production.

FINAL PROJECT REPORT

Project Title: Inheritance of chilling-dependent pear fruit ripening

PI: Richard L. Bell
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City/State/Zip: Kearneysville, WV 25430

Cooperators: None

Other funding sources: None

Total Project Funding: $10,938

Budget History:

Item	2013
Salaries	
Benefits	
Wages	$8,202
Benefits	$ 656
Equipment	
Supplies	$2,080
Travel	
Plot Fees	
Miscellaneous	
Total	**$10,938**

ORIGINAL OBJECTIVES

1. Determine the segregation of an absolute and quantitative requirements for fruit chilling in the two crosses by evaluating fruit ripening after 7 days at 20°C following 0, 30, and 60 days at -1°C.
2. Determine the genetic composition of the parents and selected seedlings with no, moderate, and high chilling requirements for allelic variants of ACS genes, and determine the relationship between genotype and chilling-requirement.

SIGNIFICANT FINDINGS

- Seedlings segregated for chilling-independence (n=19) and chilling-dependence (n=13) for fruit ripening. This ratio, tested by chi-square analysis, is consistent with genetic control by a single gene or genetic locus.
- There were no genetic differences among the parents and the seedlings for the ACS1 and ACS2 using an agarose gel detection system and the ACS1 and ACS2 allele-specific primers reported by El-Sharkawy et al. (2004). These results were duplicated in the laboratory of Nahla Bassil at the USDA, ARS, National Clonal Germplasm Repository in Corvallis, Oregon. Dr. Bassil also tried genotyping an additional 7 cultivars and breeding selections, including 'Passa Crassane' and 'Old Home', which El-Sharkawy reported were chilling-dependent and chilling-independent, and which he reported to differ in their ACS2 genotypes. She did not find any differences. These results are also consistent with results obtained by Dr. Amit Dhingra of Washington State University. However, using a capillary electrophoresis system, Dr. Bassil detected a small fragment (81 base pairs) of ACS1b which may be associated with chilling-dependence.

RESULTS AND DISCUSSION

In 2013, the presence or absence of a chilling requirement for pear fruit ripening was determined for 32 seedlings of the cross 'Beurre d'Anjou' (high chilling requirement) and US76128-009 (low chilling requirement). Seedlings segregated for chilling-independence (n=19) and chilling-dependence (n=13) (Table 1). This ratio is statistically consistent with a 1:1 segregation indicating single gene control. With data from 2011, a total of 45 seedlings have been evaluated. Among those seedlings which exhibited chilling-dependence, five required 4 weeks of chilling, three required 8 weeks of chilling, and five did not ripen after 8 weeks of chilling, and apparently require more than 8 weeks. The chilling requirement of an additional five could not be accurately determined due to insufficient fruit for all treatments. The genetic control of quantitative difference in chilling requirement could not be determined from this type of analysis, but is probably due to different genes than that controlling the chilling dependence versus independence.

No differences in ACS1 and ACS2 genotypes were detected using an agarose gel detection system and the ACS1 a/b and ACS2 a/b allele-specific primers reported by El-Sharkawy et al (2004) (Table 2). We concluded that new primers designed from the ACS1 and ACS2 gene and/or promoter sequences are needed to genotype pears for these genes involved in ethylene biosynthesis and to answer the question of whether they control fruit chilling response in a qualitative or quantitative manner. Similar results were obtained in the laboratory of Dr. Nahla Bassil, who collaborated on the study. The DACS1a primer amplified a larger fragment than reported by El-Sharkawy et al. (2004). The primers for DACS1b, DACCS2a, and DACS2b amplified products similar to those reported by El-Sharkawy, but no polymorphisms were observed in 'Old Home' and 'Passa Crassane', the two

pear cultivars used in his study, nor in seven other pear cultivars which varied in date of maturity, and presumably in chilling requirement (Table 3). However, polymorphic products of low molecular weight were found with the DACS1b (73 bp, 75 bp, 79 bp and 81 bp) and DACS2b (109 bp and 115 bp) primers were found (Table 4). The 81 bp DACS1b fragment was associated with those cultivars and selections which had a chilling requirement. The significance of this fragment must be verified with additional cultivars, selections and segregating seedling populations of known chilling requirement.

REFERENCES

El-Sharkawy, I., B. Jones, L. Gentzbittel, J.-M. Lelièvre, J. C. Pech and A. Latché. 2004. Differential regulation of ACC synthase genes in cold-dependent and –independent ripening in pear fruit. Plant Cell and Environment 27:1197-1210.

Table 1. Segregation of chilling-independence versus chilling-dependence[1].

	Chilling-Dependent		
Chilling-Independent	4 weeks	8 weeks	8 weeks+
19	5	2	6
Total 19		13	

[1] Chi-square analysis detected no deviation from the hypothesized 1:1 ratio (Pr> ChiSq = 0.37).

Table 2. Amplified fragment sizes (in number of DNA base pairs) for 'Old Home' and 'Passe Crassane' (Experiment 1)

	ACS gene and allele			
Cultivar	ACS1a	ACS1b	ACS2a	ACS2b
Old Home	~900	405	266	437
Passe Crassane	~900	405	266	437

Table 3. Amplified fragment sizes (in number of DNA base pairs) for eight pear cultivars (Experiment 2).

			DACS1		DACS2	
CPYR Number	and Cultivar	Maturity	Expected	Observed	Expected	Observed
1057	Angelica di Saonara	Early	785/405	405	437	437/266
1165	Buerre Bosc	Late	785	405	266	437/266
148	Abate Fetel	Intermediate	785/405	405	437/266	437/266
77	Doyenne du Comice	Late	785	405	266	437/266
448	Pautalia	Early	785/405	405	437	437/266
431	Old Home	Intermediate	785/405	405	437/266	437
441	Passe Crassane	Late	785	405	266	437

Table 4. Amplified fragments sizes based on capillary electrophoresis using M13-tagged primers.

		DACS1b		DACS2a		DACS2b	
CPYR Number and Cultivar		Expected	Observed	Expected	Observed	Expected	Observed
441	Passe Crassane	421	73, 81	266	317	489	109
63	Anjou	421	79, 81	266	317	489	109
431	Old Home	421	79, 81	266	317	489	109, 115
	US78307-045	421	79, 81	266	317	489	109
	US84907-166	421	75, 79	266	317	489	109
	US76128-009	421	75, 79, 81	266	317	489	109
	Sunrise	421	79	266	317	489	109
	NJ B9 R1 T117	421	75, 79	266	317	489	109

EXECUTIVE SUMMARY

Winter pears, such as 'Beurré d'Anjou', 'Buerré Bosc' and Doyenné du Comice', require a period of cold storage and/ or ethylene exposure to initiate normal ripening. The inability to ripen, while perhaps contributing to long storage life, poses challenges to the pear industry in their ability to deliver a ready-to –eat product to market. The development of new cultivars with which are more amendable to post-harvest manipulation requires knowledge of the inheritance of the post-harvest chilling requirement. In addition, knowledge of the underlying genomic basis for the trait and the genetic composition of important cultivars and breeding parents will improve the ability to breeding new cultivars with desirable post-harvest ripening. Preliminary data for a seedling population derived from hybridizing 'd' Anjou' (chilling –dependent with long requirement) and US76128-009, a chilling-dependent selection with a short requirement) suggested that chilling-dependence is a dominant trait, and that the amount of chilling is variable, likely determined by an unknown number of genes. The purpose of this study was to determine the inheritance of the absolute requirement for chilling as well as to characterize the nature of the quantitative variation in chilling requirement.

Summary of findings

Approximately half of the 45 seedlings exhibited chilling independence for fruit ripening, while the others exhibited chilling dependence, with the amount of chilling required varied from 4 to 8 weeks or more. This finding is consistent with genetic control by a single gene. It had been hypothesized that the genes ACS1 and ACS2 controlled chilling-dependence. Using gene primers published by El-Sharkawy, we found no relationship of these genes with chilling dependence/independence. We were unable to duplicate El-Sharkawy's results with the cultivar he used in his study, 'Old Home' and 'Passe Crassane'. We hypothesized that different primers designed from the gene sequences would be necessary to further investigate the relationship. Subsequent communication from other researchers confirmed that these genes do not control chilling dependence/independence. We did find a possible relationship of a small DNA fragment (81 base pairs) with chilling dependence.

Future directions

Evaluation of additional seedlings would enhance the robustness of the analysis of the genetic control of chilling dependence, especially the quantitative variability in the amount of chilling needed to initiate normal ripening. We plan to do that in the growing season. In addition, new seedlings of the cross between 'Anjou' and 'Harrow Delight', which has not chilling requirement, will be generated by hybridization. We plan to determine the relationship between genotype, global gene expression patterns, and chilling-requirement using advanced genomic approaches, Pnome and RNA-Seq. We will validate the association of the 81 bp fragment with chilling independence in additional pear cultivars and seedlings. The overall goal will be to develop new primers or markers designed based on the gene sequences derived from the European pear genome sequence.

FINAL PROJECT REPORT **YEAR**: 2 of 2

Project Title: Fire blight epidemiology and improved post-infection control

PI: Ken Johnson
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Cooperators: Rachel Elkins (UC Cooperative Extension - Lake County, CA)
Steve Castagnoli (OSU Extension - Hood River County, OR)
Tim Smith, (WSU Extension - Chelan County, WA)
David Sugar (OSU SOARC – Medford, OR)

Total Project Request: **Year 1:** $15,667 **Year 2:** $16,137

Other funding sources

Agency: California Pear Advisory Board $46K (Elkins $25K, Johnson $12K, others $10K)
Syngenta Crop Protection (Johnson $15K)

Budget History:

Item	**2011-12**	**2012-13**	
Salaries Faculty Res. Assist.	7,337	7557	
Benefits OPE 56%	4,109	4232	
Wages undergrads	1,200	1236	
Benefits OPE 8%	96	99	
Equipment	0	0	
Supplies	1,925	1983	
Travel	500	515	
Miscellaneous		0	
Plot Fees	500	515	
Total	**$15,667**	**$16,137**	

Footnotes: Annually**:** FRA 2 mo + fringe, 130 hr undergrad labor, 2K M&S, 1K local travel & plot fee, 3% inflation

OBJECTIVES:

1a) Evaluate paints of an inducer of systemic acquired resistance as an aid to cutting of fire blight in diseased pear trees

1b) Evaluate an inducer of systemic acquired resistance in combination with antibiotics for protection of pear trees from fire blight

2a) Evaluate the effect of a delayed dormant, copper sanitation treatment on detectability of the fire blight pathogen in samples of flowers collect at various stages of pear bloom

2b) Evaluate new LAMP technologies that will facilitate widespread adoption of molecular scouting within regional fruit production districts.

SIGNIFICANT FINDINGS

- For a 3nd season, a paint of acibenzolar-S-methyl (Actigard) used in combination with cutting of blight reduced the severity of 're-ignited' fire blight cankers in pear.
- In pear, the addition of Actigard to antibiotic treatments enhanced fire blight control over antibiotics alone in both experimental and commercial orchards.
- For a 6th season, molecular scouting during the bloom period detected and characterized the build-up of fire blight pathogen populations in pear flowers with the pathogen detection being greatest near petal fall.
- For a 3rd season, the LAMP molecular scouting protocol demonstrated that a delayed-dormant timing of a copper bactericide suppressed positive pathogen detection in flower samples.
- The protocol and technologies used for molecular detection of the fire blight pathogen were refined and adapted for use with LAMP machines, which allow for the assay to be performed in the orchard.

RESULTS

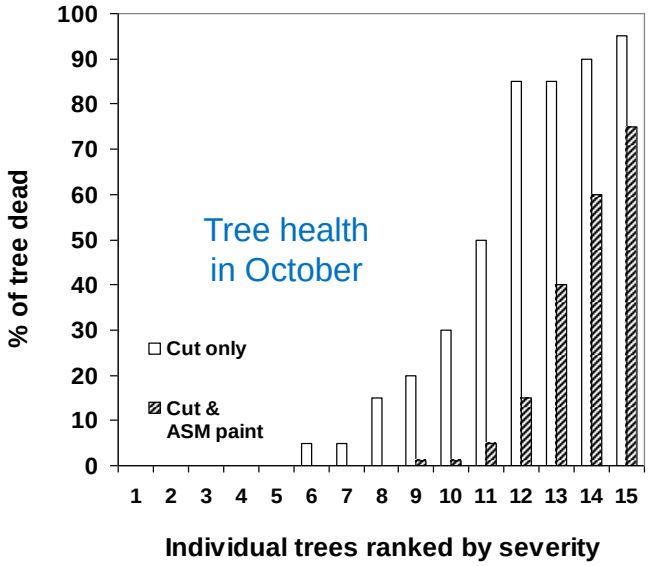
Obj. 1a) Evaluate paints of an inducer of systemic acquired resistance as an aid to cutting of fire blight in diseased pear trees

Experiments were conducted in 2012 and 2013 to evaluate Actigard as an aid to cutting of blight in pear trees.

In 2012, in a 4-yr-old Bosc pear block, 1 to 5 fire blight strikes developed on each tree as a result of the pathogen inoculation at full bloom. Individual trees were then grouped into experimental blocks based on number of strikes per tree. Blight was cut on 6 June and if cuts re-ignited, repeated on 27 June. Cuts were made at 6-8" below the proximal margin of the canker. Immediately after each cutting, an Actigard paints (30 g/L [1 oz./qt.] in 2% Pentrabark) was applied to 30-40 cm (12-16") of symptomless branch below the cut. Over the summer, cankers re-

ignited in about half of the trees. Canker size and effect on tree health were evaluated in early October. Compared to cut only, the Actigard paint treatment significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers (Fig.1).

Fig. 1. Effect of an Actigard paint on re-ignited fire blight cankers in 4-yr-old 'Bosc' pear. Trees were inoculated on 27 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 6 and 27 June. Immediately after cutting, an Actigard paint (Actigard 30g/L in 2% Pentrabark) was applied to the 30-40 cm (12-16") of symptomless branch below the cut. Canker severity (weight) and % of tree dead from fire blight were assessed on October 4. Bars represent individual trees ranked in order of disease severity (% tree dead).



In 2013, a 13-year-old Bartlett pear block was inoculated with E. amylovora as part of trial to evaluate treatments to prevent floral infection. After strikes were counted, we pulled the treatment flags and re-grouped the individual trees into experiment blocks based on number of strikes per tree; 14 paired replications of cut only and cut plus Actigard paint were organized in a randomized block design with an average of 9.5 strikes per tree. Blight was cut on 5 June; cuts were made at 6-8" below the proximal margin of the canker. Immediately after cutting, the Actigard treatment received a paint of this material (30 g/L) on the 30-40 cm (12-16") of symptomless branch below each cut. Over the summer, cankers re-ignited at about ¼ of the cuts; the number and sizes of these cankers were evaluated in late September. Compared to cut only, the Actigard paint treatment significantly reduced ($P \leq 0.05$) number and severity (weight) of the re-ignited fire blight cankers (Fig.2 **next page**).

Discussion of Actigard paints. Like apple, fire blight susceptible pear cultivars respond to treatments of the SAR inducer, acibenzolar-*S* methyl (Actigard), resulting in slowed canker expansion in diseased trees. In earlier greenhouse research, the effect of Actigard on suppression of fire blight was most dramatic when drenches were applied to potted trees, but in the field, Actigard drenches have not provided a significant response. Consequently, our experiments with SAR induction as an aid to the restoration of tree health has shifted to Actigard paint treatments applied to the symptomless branches after cutting.

Further rationale for the shift to paints was observed in 2011 greenhouse-grown apple (see January 2012 apple crop protection final report). Trunk paints of Actigard showed levels of disease resistance gene induction (termed 'PR-gene') that were on par with the levels of PR-gene induction achieved by pot drench. The measurement of PR-gene induction provides a marker on whether or not a SAR inducer is providing consistent induction of host defense responses (i.e., an enhanced ability to fend off pathogens). In contrast to pot drenches and trunk paints, foliar sprays have been less consistent in PR-gene induction.

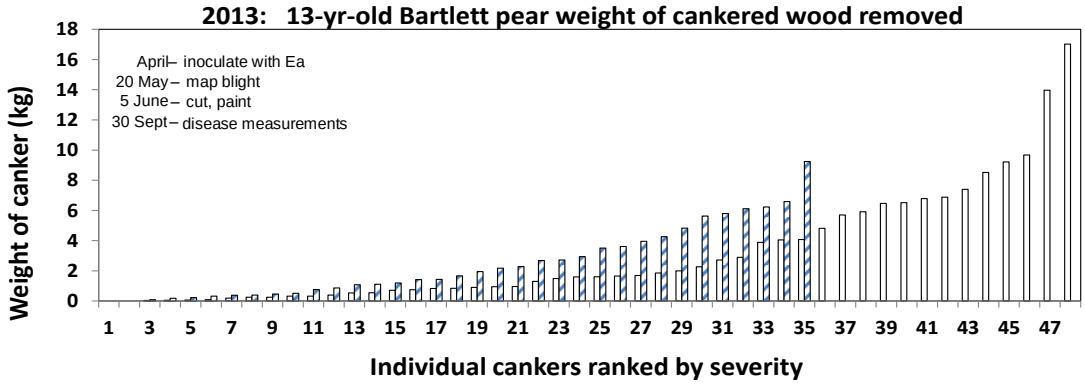


Fig. 1. Effect of branch paints the SAR-inducer, Actigard, on re-ignited fire blight cankers in 13-yr-old 'Bartlett' pear. Trees were inoculated with the fire blight pathogen on 9 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 5 June. Immediately after cutting, Actigard was applied by paint (Actigard 30g/L in 2% Pentrabark) to the 30-40 cm (12-16") of symptomless branch below the cut. Weight of cankered branches removed was assessed on September 30. Data are the ranked severity (weight) of individual cankers 'Actigard-painted' trees (hatched bar) compared to individual 'cut only' trees (open bar).

For the body of data collected from both pear and apple (see January 2012 apple and pear final reports and January 2014 apple crop protection final report), Actigard treatments applied by paint have been most suppressive when the pathogen was present but the amount of active disease in the tree was small. For example, in the greenhouse, paint or spray treatments made at the time of inoculation (pathogen present, small amount of disease) were more effective than treatments made one month prior (no pathogen) or one month after inoculation (increased amount of disease). In the field, Actigard paints applied to a symptomless branch below a surgical cut to remove a canker have provided a stronger response than trunk paints applied to trees where cankers were left to run.

Actigard could prove practical as aid to cutting blight in pear trees, either reducing severity of re-ignited cankers or reducing the incidence of re-ignition. A paint application of Actigard has been incorporated into the proposed pome fruit label, which is expected to receive an EPA registration in 2015. Nonetheless, although we have achieved positive results with the chosen rate and timing of Actigard paints, further exploration of both of these variables could improve our understanding on of this technology.

Obj. 1b) Evaluate an inducer of systemic acquired resistance in combination with antibiotics for protection of pear trees from fire blight

In 2013, Actigard (acetyl-S-methyl) was evaluated in plot trials and in commercial orchards for enhanced suppression of floral infection by the fire blight pathogen. In an experimental pear orchard, relative to the water-treated control, oxytetracycline alone significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree. The addition of Actigard treatments in combination with oxytetracycline improved the control of fire blight compared to the antibiotics alone (Fig. 3). This result is consistent with apple trials (not shown) conducted 2011-2013 (see 2013 apple report).

Under the 2013 Actigard experimental use permit, 4 ha-sections (one pull tank) in commercial pear orchards located in northern California, southern and northern Oregon and north central Washington were treated at full bloom and petal fall with Actigard at 2 oz./A. During the bloom period, the orchardists also applied an antibiotic program to the entire orchard. In May, the Actigard treated area and an adjoining no-Actigard area were assessed for fire blight by counting strikes or by counting trees with blight (Medford only). Overall, the Actigard plus grower program had 37% fewer strikes than the adjoining area that received only the grower program (Fig. 4)

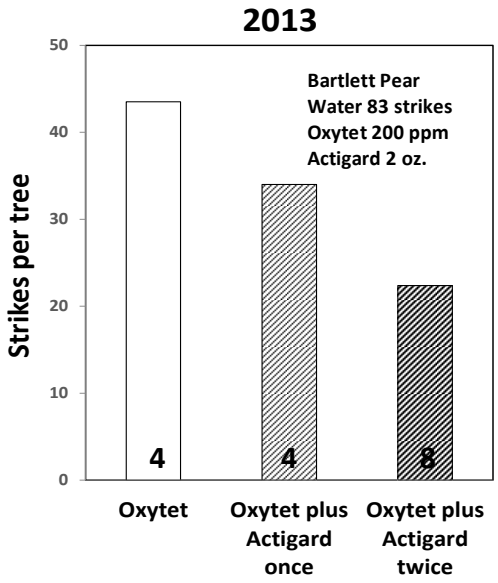


Fig. 3. Fire blight strikes per tree as affected by treatment with oxytetracycline once or in a program with 1 or 2 additional Actigard treatments. Trial conducted in an experimental Bartlett pear orchard near Corvallis, OR. The antibiotic was applied at full bloom; Actigard treatments varied from 30% bloom to petal fall. Numbers within bars are the number of replicate trees averaged for each mean

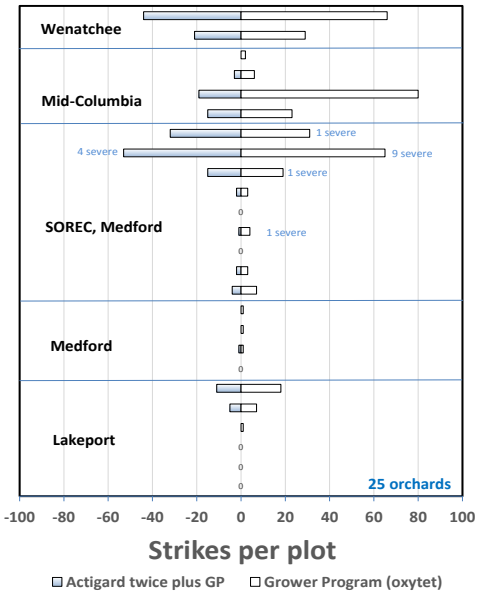


Fig. 4. Fire blight strikes per tree as affected by grower's antibiotic program alone or grower's program plus two additional Actigard treatments. Trials were conducted in commercial pear orchards at the locations shown. Actigard treatments were applied at full bloom and petal fall.

Discussion. We have made significant progress in understanding effective rates of Actigard for the various methods of application. Induction of systemic acquired resistance appears to have its greatest protective effect when blight symptoms are minimal (just prior to or near time of infection, or after cutting). Actigard shows value as program partner with antibiotics during bloom, and we speculate it may be effective as long residual protectant for rattail, shoot infection or trauma blight phases of fire blight. In 2013, we also conducted a shoot blight trial on potted Concorde pear trees (data not shown). The potted trees were prayed with Actigard (2 oz./100 gal.) or untreated and then inoculated with high pathogen does (10^8 CFU/ml); under this extreme pressure, Actigard reduced shoot infection by 30%.

Obj. 2a. Evaluate the effect of a delayed dormant, copper sanitation treatment on detectability of the fire blight pathogen in samples of flowers collect at various stages of pear bloom

Treatment of pear trees with a copper-based bactericide at the 'green tip' phenological stage prior to flowering has been recommended historically to suppress primary inoculum of *Erwinia amylovora* associated with overwintering fire blight cankers. In the western United States, however, this practice became little used as treatment with effective antibiotic materials became common. We re-evaluated this practice by utilizing a primary bloom scouting protocol in conjunction with a loop-mediated isothermal DNA amplification assay (LAMP) for specific detection of *E. amylovora* in samples of flowers.

From 2010 to 2013, 4 ha-sections in commercial pear orchards located in northern California were split into two plots with the orchardist applying horticultural oil only to one plot and horticultural oil plus a fixed copper bactericide (6 lbs/A) to the other plot 2-4 weeks before first bloom. During the bloom period, the orchardists also applied their usual antibiotic program to the entire orchard.

Flowers from each 2 ha-plot were sampled at mid-bloom, at full bloom, and once or twice at petal fall. On each sampling date, three samples of 100-flower clusters (~ 600 flowers) were made in each 2-ha plot following a standard W-shaped scouting pattern with the LAMP protocol performed on the floral wash of each sample.

Overall, *E. amylovora* was detected rarely at mid-bloom (5% of samples) but detected commonly at petal fall (37% of samples) (Fig. 5). In 3 of 4 seasons, positive pathogen detection in flower samples increased more rapidly as a function of bloom stage in oil only plots compared to oil plus copper-treated plots. Among orchards, development of fire blight was sporadic but was decreased significantly ($P \leq 0.05$) by the copper treatment in two of 4 seasons. Among seasons, differences in detection of epiphytic *E. amylovora* by LAMP-based scouting indicated that orchardists could potentially benefit from 'point-of-care' LAMP testing in individual orchards each season.

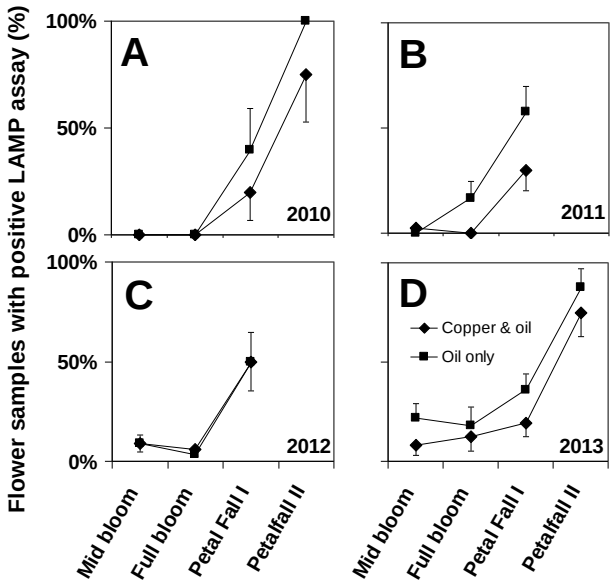
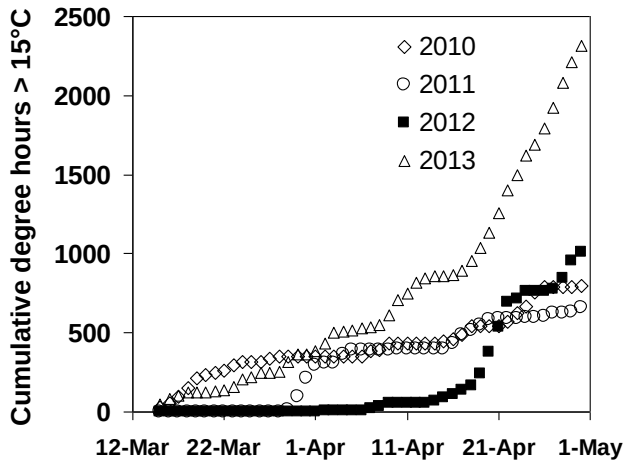


Fig. 5. Effect of delayed dormant timings of copper plus oil (♦) and oil only (■) on the detection of *Erwinia amylovora* in 100-flower cluster samples from commercial pear orchards in Yuba, Sacramento and Lake Counties in California. Detection of the fire blight pathogen was based on a loop mediated isothermal DNA amplification (LAMP) assay performed on washes of sampled flowers, which was confirmed by dilution plating of the same wash onto a selective culture medium. Each point is the mean of 29 to 41 100-flower cluster samples.

2012 was the season we failed to see an effect of delayed dormant copper on positive pathogen detection in pear flower samples. The spring of 2012 had a very cool beginning, which

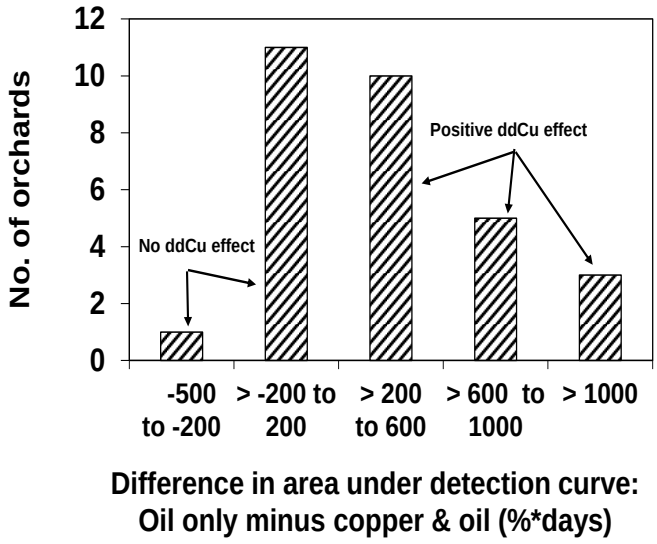
compared to the other seasons resulted in a longer period (5-6 weeks) between the copper treatment and flowering (Fig. 6). Due to this longer period, we speculate that the copper residue had weathered (declined) to non-bactericidal levels at the beginning of the bloom period.

Fig. 6. Cumulative degree hours > 15°C for Lakeport, CA from March 15 to April 30 for the years 2010 to 2013.



For the seasons where we observed an effect of delayed-dormant copper on positive pathogen detection in pear flower samples (2010, 2011, and 2013), we evaluated the data from individual orchards first by calculating the 'area under the detection curve' (AUDC) for each treatment, and then secondly subtracting the area obtained for the oil plus copper treatment from the oil only treatment (Fig. 7). The units on AUDC are '% · days', and thus an AUDC value of e.g., 600, can be interpreted as an average of a 60% difference in Cu treated compared to untreated for a period of 10 days (60% · 10 days = 600). Overall, the magnitude of the difference between AUDC for oil plus copper subtracted from the oil only was > 200 (a positive delayed-dormant copper effect) for 2/3 of the orchards, and > 600 for 8 of the 30 orchards. Thus, delay dormant copper is an effective treatment when inoculum of the pathogen is expected to arise from within the orchard block.

Fig. 7. Histogram depicting the magnitude of the difference in area under the detection curve (AUDC) for *Erwinia amylovora* in 100-flower cluster samples collected from 2 ha sections of commercial pear orchards treated at a delayed dormant (green tip) timing with horticultural oil only or with oil plus copper. The orchards were located in Lake and Yuba Counties of California with 30 paired 2-ha sections treated and monitored during the years for 2010, 2011 and 2013. Each AUDC was comprised of three 100-flower cluster samples collected at each of several bloom stages: mid-bloom, full bloom, petal fall I, and petal fall II.



The overall performance of the LAMP assay over the four seasons of the California study in shown in Table 1. LAMP assay results were confirmed by plating the flower wash on culture media

semi-selective for the fire blight pathogen. Of 705 LAMP assays, there were 156 'true' positives for *E. amylovora* and zero 'false' negatives based on comparison to media isolation. There were also 16 positive LAMP assays that were not confirmed by media isolation. For this situation, the result could either be a false positive for LAMP or a false negative for media isolation. After working with LAMP and for 6 six years and media isolation for many more, we are more inclined to conclude the 16 non-corresponding data points are false negative for media isolation due to competing bacteria overgrowing the smaller *E. amylovora* colonies on the culture media; this concern is especially problematic when is a small number of pathogen cells in the sample.

Table 1. Comparison of the LAMP assay and dilution plating methods of detection of *Erwinia amylovora* washes of 100-flower cluster samples collected within commercial pear orchards in Lake and Yuba Counties, CA during the years of 2010 to 2013.

Per 100-cluster sample: (n = 705)	**Results of media isolation for *E. amylovora***	
Result of LAMP assay for *E. amylovora*	negative	positive
negative	533	0
positive	16	156

Discussion of LAMP surveys. LAMP has proven to be an outstanding research tool, and has provided valuable information for understanding the impact of fire blight management treatments such as delay-dormant copper sanitation. Nonetheless, whether LAMP-based scouting will have a place in commercial IPM programs will not be known until the assay can be performed in the orchard (see sub-objective 2B). Degree-hour models, e.g. Cougarblight, Zoller 'California', and Maryblyt have evolved to be accurate in assessing conditions for inoculum presence and build-up. The results of the LAMP surveys, however, provide of a direct assessment the prevalence of pathogen inoculum, and can guide research on effective timings and materials for disease control. One result of LAMP-based scouting has been the observation that the detectability of the fire blight pathogen continues to increase in the late stages of bloom; this suggests that fire blight control programs could be enhanced by additional copper-based sanitation during bloom or by extending protective treatments into the period near and after petal fall.

2b) Evaluate new LAMP technologies that will facilitate widespread adoption of molecular scouting within regional fruit production districts

The rationale for this sub-objective is to improve and simplify the LAMP technology on which molecular scouting is based so that other individuals can perform the assay at the 'point-of-care', i.e., the orchard. Currently, for each flower-wash sample, our lab-based protocol requires i) concentration of the sample wash, ii) DNA extraction from concentrated wash, and iii) LAMP assay on extracted DNA. To perform the assay in the field, we need to eliminate DNA extraction and ideally, concentration of the sample (or simplify it). In the last few years, technology developed by Optigene, Ltd. (Horsham, West Surrey, England) has shortened the LAMP reaction time from 45 to 20 minutes, and their 'mastermix' has combined multiple reagents into a single reagent (liquid) that is added to the LAMP reaction tube. Optigene (and others) also have developed 'LAMP machines' (Optigene's machine is called 'Genie II') that run multiple assays at one time and potentially eliminate the need for DNA extraction.

Step 1: Adaption of LAMP to fluorescence-based machines. Figure 8 shows the adaption of our *E. amylovora* LAMP protocol to new Optigene technologies. To achieve these results, we designed and evaluated new primers (named 'Amy13'primers) targeted to chromosomal DNA of the fire blight pathogen. We also developed a highly-specific, fluorescent 'assimilation probe' that creates a machine-readable signal when DNA of the target (fire blight pathogen DNA) is amplified. The assimilation probe technology was first evaluated in flower samples collected in 2012, and used exclusively for the California flower samples analyzed in 2013 (see Obj. 2a above).

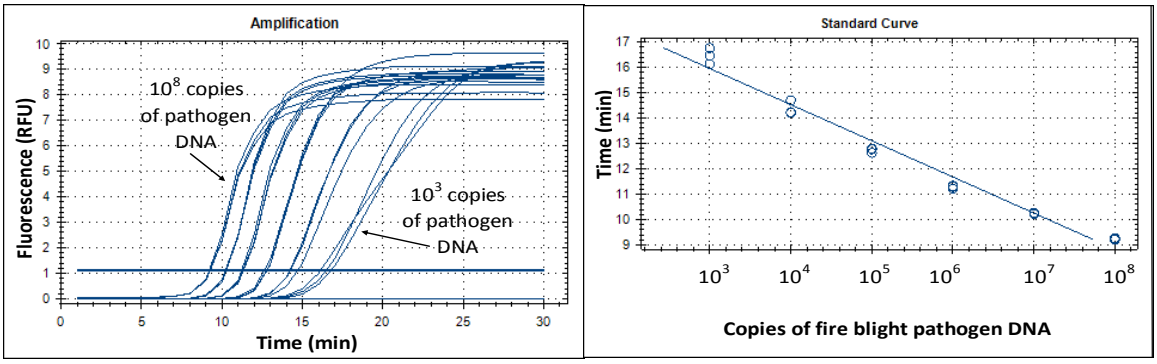


Fig. 8. Fluorescence-based loop-mediated isothermal amplification (LAMP 'ISO-001 polymerase) assay performed on DNA extracted from *Erwinia amylovora* cells grown in pure culture. Graph on left is cumulative fluorescence units emitted over time as influenced by a 1:10 dilution series of *E. amylovora* DNA (clustered groups of lines represent individual dilutions). Graph on right is time to achieve a 'positive' result as a function of amount of *E. amylovora* DNA.

Step 2: Evaluation of alternative polymerase, 'TIN', for amplification of DNA extracted from pathogen cells grown in per culture. LAMP reactions require an enzyme (a polymerase) that makes the chains of DNA after the pathogen-specific keys (primers) have located pathogen DNA in the sample. Elimination of the DNA extraction step requires the use of a heat tolerant polymerase (named 'TIN') that can withstand a 10 minute pre-heating of the sample to 95°C (203°F) to lyse cells prior to the LAMP reaction (which is then run at 65°C for 50-60 minutes). With extracted pathogen DNA, we first evaluated the TIN polymerase without pre-heating at 95°C. Compared to polymerase ISO-001, LAMP reactions with TIN required a few additional minutes (compare times Fig. 9 to Fig. 8) but sensitivity was not significantly altered by the switch to the heat-tolerant TIN-polymerase.

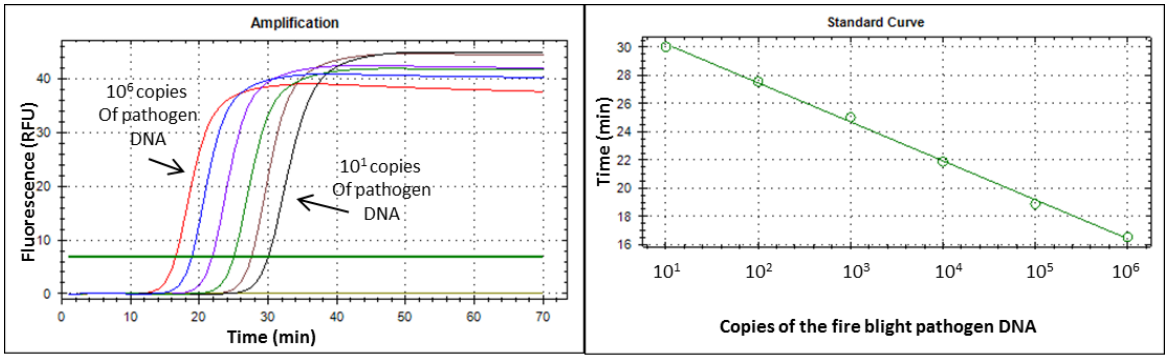


Fig. 9. TIN polymerase, fluorescence-based loop-mediated isothermal amplification (LAMP) assay performed on DNA extracted from *Erwinia amylovora* cells grown in pure culture. Graph on left is cumulative fluorescence units emitted over time as influenced by a 1:10 dilution series of *E. amylovora* DNA. Graph on right is time to achieve a 'positive' result as a function amount of *E. amylovora* DNA.

Step 3. Evaluation of alternative polymerase, 'TIN', for amplification of DNA in whole cells of the pathogen grown in per culture. The purpose of the additional 95°C treatment is to break open (lyse) pathogen cells and release the DNA. By lysing the cells in the preheating step (10 min), the LAMP reaction can be done in a single tube that is placed into the machine only once. Some organisms will lyse at 95°C without additional amendments to the sample; others require the addition of a lysing agent (e.g., NaOH:SDS or InstaGene matrix) to aid the DNA release. Initially, for whole cells of *E. amylovora*, reduced sensitivity of DNA amplification was observed in the 'Genie II' LAMP reactions when the cells were first pre-heated to treatment for 10 minutes. The addition of either NaOH:SDS or Instagene matrix to the LAMP reaction tubes resulted in positive amplification of cells down to 10^0 CFU per ml (i.e., assay sensitivity restored) (Fig. 10).

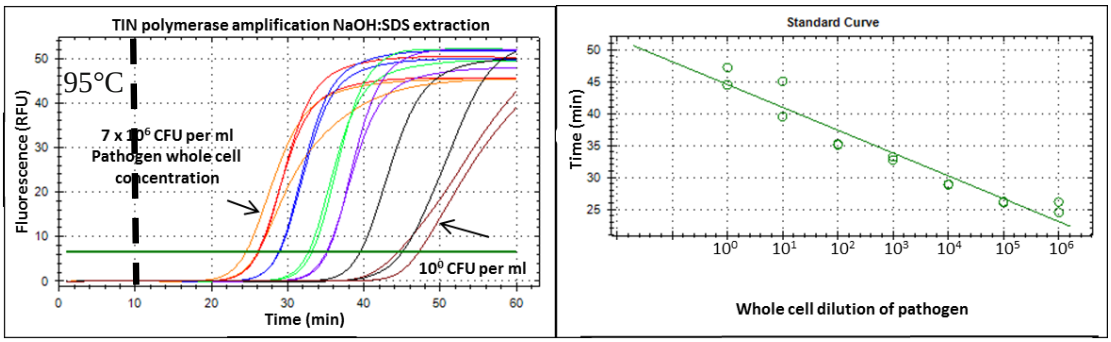


Fig. 10. TIN polymerase fluorescence-based loop-mediated isothermal amplification (LAMP) assay performed on whole cells of *Erwinia amylovora* grown in pure culture. Graph on left is cumulative fluorescence units emitted over time as influenced by a 1:10 dilution series of *E. amylovora* whole cells. NaOH:SDS was added to the reaction tube to aid in DNA release during the heating of cells to 95°C. Graph on right is time to achieve a 'positive' result as a function amount of *E. amylovora* whole cell concentration (CFU/ml).

Step 4. Evaluation of polymerase, TIN', for amplification of whole pathogen cells of the pathogen in a floral wash. Whole cells of *E. amylovora* were serially diluted and mixed with a wash of field-sampled flowers void of pathogen DNA. After adding NaOH:SDS and preheating the sample at 95°C, positive amplification of pathogen cells was observed down to 10^2 CFU per ml (*data not shown*). As expected, however, the time to obtain a positive amplification was increased by 10-15 minutes compared to cells suspended in water. This increase in reaction time reduced sensitivity is due to the presence (competition/interference) of DNA and other biochemicals from other organisms in the sample.

Step 5. Evaluation polymerase, 'TIN', for amplification of E. amylovora in naturally infested, non-concentrated wash samples collected in spring 2013. A subset of 30 floral washes from the California study (above) were saved (frozen). Previously, with the lab-based LAMP protocol and media isolation, 24 of these samples were found to be positive for *E. amylovora* DNA and 6 were negative. These saved floral washes were 'non-concentrated', meaning we omitted the step of our lab protocol where 30 ml of floral wash is embedded onto a filter membrane and then resuspended in 1 ml of liquid. When samples were incubated at 95°C for 10 minutes in either NaOH:SDS or InstaGene matrix, amplification occurred in 92 and 100% of positive samples, respectively. No amplification was observed in the 6 negative samples. Amplification required up to 70 minutes, suggesting that the omitted concentration step has a significant impact on time to get a positive signal.

Discussion of new LAMP technologies. New LAMP technologies have greatly simplified the process of performing a LAMP assay for specific detection of *E. amylovora*. Moreover, compared to the original technology based on the visualization of turbidity (precipitate formation), the new florescence-based technology is better suited to quantification of the *amount* of target DNA present in a sample (Figs. 8-10). Compared to LAMP assays performed on lab-grown pure cultures, samples of flowers from orchards show more variability in the estimated amount of pathogen, but it should be possible to distinguish between those samples with greater amounts of pathogen (fast time to reaction) vs. those with small amounts of pathogen (long time to reaction). We have also streamlined the handling and preparation of flower samples for LAMP, such that multiple samples can processed within a shorter period of time.

We have not purchased a LAMP machine because the companies have overpromised the availability of models designed for filed use (e.g., Optigene initiated a re-design of model Genie III before it was ever marketed). The Genie II machine used for this research was on loan, and it is not designed for field-use. Optigene's website is indicating the field-designed, Genie III LAMP machine, will be marketed sometime in 2014 (http://www.optigene.co.uk/instruments/instrument-genie-iii/). We also were working with another company's (diagenetix.com/product-and-technology/smart-dart-platform) inexpensive field-based LAMP machine. In our evaluation of their prototype, we concluded that they have several technical issues to overcome before the machine can be used reliably as an aid to decision making.

All-in-all, we are close to an ability to run LAMP-based scouting in the orchard, but this ability will require a reliable LAMP machine (Optigene's Genie III fulfills this requirement but cost will be a consideration). Once a field-based machine is in hand, there are still a few technical issues to iron out in how samples are processed, but these issues are minor.

EXECUTIVE SUMMARY

Project Title: **Fire blight management in organic and conventional apple**
Investigator: Ken Johnson, Oregon State University

SIGNIFICANT FINDINGS:

Systemic acquired resistance:

- For a 3nd season, a paint of acibenzolar-S-methyl (Actigard) used in combination with cutting of blight reduced the severity of 're-ignited' fire blight cankers in pear.
- In pear, the addition of Actigard to antibiotic treatments significantly enhanced fire blight control in both experimental and commercial orchards.

Industry implications: Actigard, with its unique mode-of-action (induction of host defense genes), shows value as program partner with antibiotics for fire blight prevention during bloom. Even with very good products for prevention of fire blight, the disease still occurs and its clean-up can be difficult, especially in young orchards. We continue to achieve promising results with Actigard as an aid to clean-up of blight in pear trees.

LAMP-based molecular scouting:

- For a 6th season, molecular scouting during the bloom period detected and characterized the build-up of fire blight pathogen populations in pear flowers with pathogen detection being greatest near petal fall.
- For a 3rd season, molecular scouting for the fire blight pathogen in commercial orchards demonstrated that a delayed-dormant timing of a copper bactericide suppressed positive pathogen detection in flower samples.
- The protocol and technologies used for molecular detection of the fire blight pathogen were refined and adapted for use with LAMP machines, which will allow for the assay to be performed in the orchard.

Industry implications: Having an ability to quickly detect the fire blight pathogen in flowers has to potential to improve the timing of treatments for fire blight control and to lessen the impact of this disease. For example, in a 4-yr study that utilized molecular scouting in commercial pear orchards, we demonstrated that a copper bactericide applied at the 'green tip' phenological stage delayed the build-up the fire blight pathogen in flowers during the bloom period. The technology that we used to detect the pathogen, loop-mediated isothermal amplification of DNA, is very new and rapidly evolving. We are keeping pace with this evolution with a goal of developing molecular scouting for wide-spread use in orchard IPM programs.

FINAL PROJECT REPORT: PR-12-101

Project Title: Efforts to disrupt winterform re-entry using repellents or attractants

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State/Zip: WA 98951

Cooperators: ISCA Technologies, Riverside, California
Jocelyn Millar, University of California, Riverside

Other funding sources: None

Total Project Funding: $38,000

Budget History:

Item	Year 1: 2012	Year 2: 2013
Salaries	$14,615	$14,615
Benefits	$ 4,385	$ 4,385
Total	$19,000	$19,000

OBJECTIVES

Develop approaches to disrupt colonization of orchards by post-diapause winterforms (*repellents*) and to delay mating of colonists following arrival (*pheromone*).

- Determine whether SPLAT products developed by ISCA and shown to repel other psyllids can be used to disrupt re-entry by returning winterform psylla.
- Determine whether saturation of atmosphere with 13-MeC27 pheromone slows mating by returning colonists.

SIGNIFICANT FINDINGS

Repellents

- SPLAT-DMDS (disulfide compound) failed to repel winterform pear psylla in a series of small and large cage studies
- SPLAT-DMDS failed to affect psyllid colonization of pear trees and egglaying in a large field trial
- New SPLAT product (proprietary; developed from volatiles extracted from a plant essential oil) shown to repel both winterform and summerform psylla in olfactometer trials. However, product was not effective under field conditions.
- Pear ester shown to repel both winterform and summerform pear psylla in olfactometer trials. Product was not effective under field conditions.

Pheromone (13-MeC27)

- The GC-MS trace identifying the 13-MeC27 compound was found to include two peaks hidden by the 13-MeC27 peak.
    - Those peaks were identified, and the compounds were synthesized (Jocelyn Millar, UCR). Olfactometer trials showed that one of the compounds (11-MeC27) is attractive to male psylla.
- Small cage studies suggested that saturation of cages with 13-MeC27 slowed rate that females were mated, suggesting the compound interfered with mate location.
- Efforts were initiated to develop a sprayable formulation of the pheromone compatible with grower practices. The low volatility of the compound prompted concerns about use of septa with which to saturate an orchard for disruption trials (i.e., need for excessive number of point sources).
    - The compound is fully soluble in oil, and was found to retain its activity when mixed in 1% horticultural oil (+ water): olfactometer trials, arrestment trials, and field trials confirmed activity.

METHODS

Objectives were addressed using a series of cage studies, olfactometer assays, and field tests. Additional detail provided below in **Results & Discussion**.

RESULTS & DISCUSSION

1. SPLAT-DMDS: effects on colonization (cage study). Pear seedlings (1.5 foot tall) were placed at opposite ends of a screened cage (6 ft long x 2 ft x 2 ft). The repellent was placed at one end of the cage, at the base of seedlings. Winterform psylla (100 mixed sex) were released in the center of the cage, and location was determined 24 hours later. ***Results.*** A significant preference was noted for one end of the cage, irrespective of treatment, due to a slight light gradient (Figure 1, top two bars).

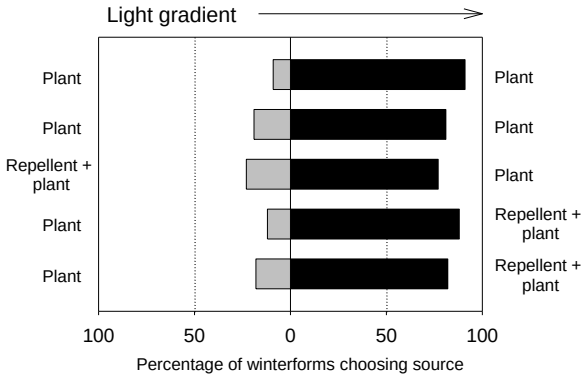

Figure 1. Large cage study with SPLAT-DMDS

Having the repellent at that end of the cage did not overcome that light effect (Figure 1, bottom two bars). This study provided no evidence that the compound is repellent to psylla. No phytotoxicity was seen associated with the compound.

2. SPLAT-DMDS: effects on departure (cage study). Winterform psylla were allowed to settle on pear seedlings at one end of the cage. A second set of seedlings were placed at the opposite end of the cage, and the repellent was applied to the bottom of the original set of plants, just below the feeding psylla. All plants were examined for psylla at 2 hours and at 24 hours. Control trials (no repellent) were run in parallel. ***Results.*** There was no evidence that the repellent prompted movement off of the treated plants (Figure 2). Psylla were observed feeding within several inches of the compound.

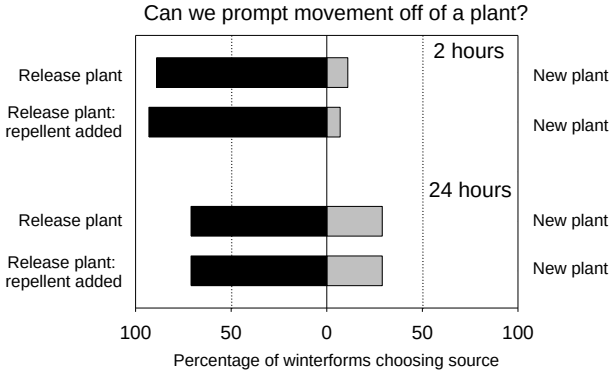

Figure 2. Cage study to examine whether SPLAT-DMDS prompts movement off of plants.

3. SPLAT-DMDS: field trial. A field trial with the SPLAT-DMDS formulation was done in a small (48 tree; 4 rows x 12 trees) orchard located at the Moxee farm to determine whether the compound interfered with winterform colonization of trees during the re-entry period (March/April). Three trees

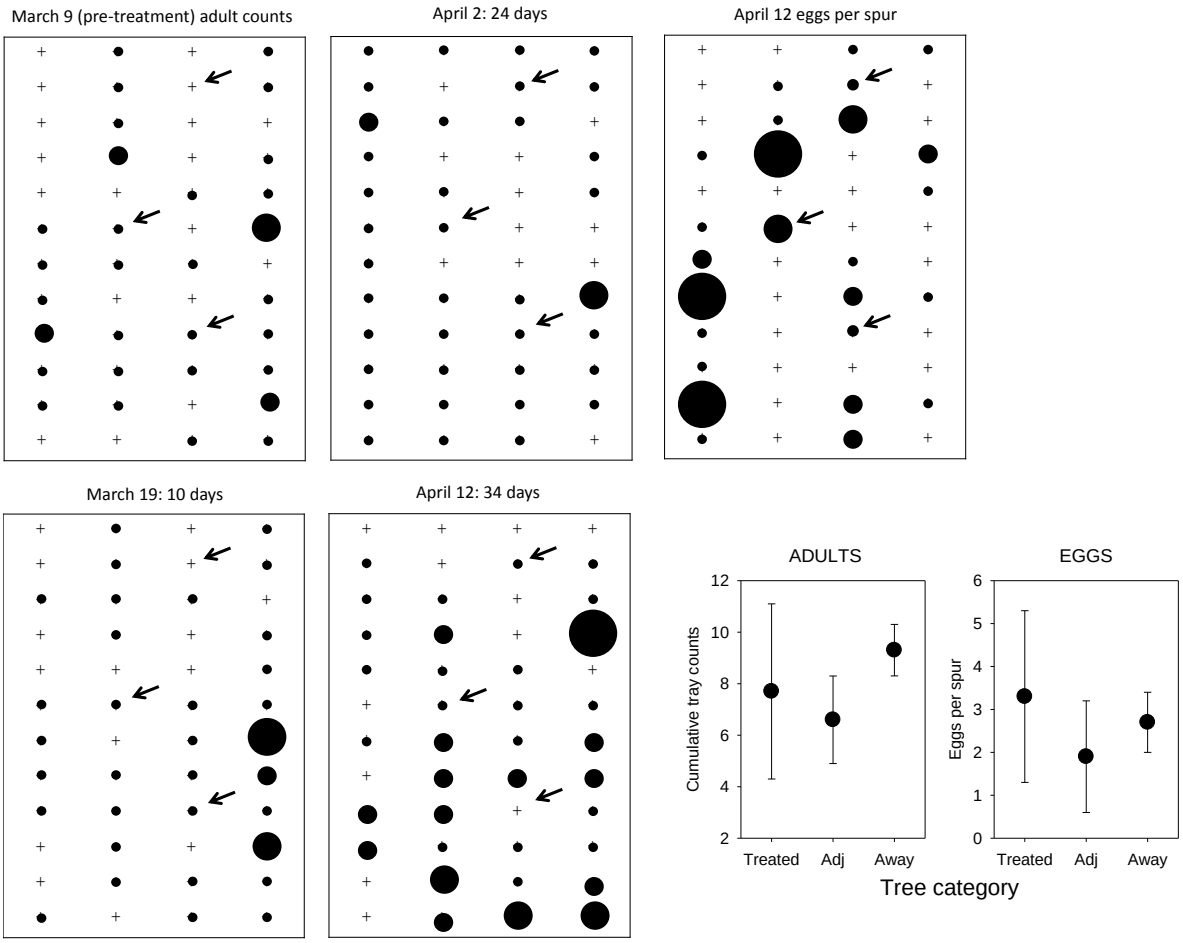

Figure 3. Field trial (48-tree orchard). Arrows: location of SPLAT-DMDS. Size of circle proportional to numbers. Four left-most panels: adults. Graphs in bottom right show count data summarized by whether tree was treated, adjacent to a treated tree, or away from treated tree. + indicates no psylla.

in the center two rows were chosen to receive the compound (Fig. 3 arrows). The compound was applied where lower-most limb attached to the trunk. All 48 trees were then sampled at intervals to determine adult numbers and egg numbers. ***Results.*** There was no evidence that the repellent slowed colonization of the three treatment trees (Figure 3; trees marked with arrows) or neighboring trees, nor did it affect egglaying (size of the circle in each figure is proportional to numbers). The graph at the bottom right compares cumulative numbers of adults and eggs on the treatment trees, the trees immediately adjacent to the treatment trees, and trees away from the treatment trees. There was no evidence that the SPLAT product affected distribution of adults or eggs.

4. SPLAT-New product: olfactometer trials. Olfactometer trials were done with a new SPLAT product (proprietary) shown to repel potato psyllid. The compound was extracted from volatiles emitted by a plant essential oil. The olfactometer trial compared pear leaves vs pear leaves + SPLAT product. ***Results.*** The product was shown to repel both winterform and summerform psylla (Figure 4). A field trial in 2013 was conducted of identical design to that used for the DMDS trial in 2012. Weekly samples were again taken but failed to show any repellency of the product (weekly data not shown; summary data shown in Figure 5).

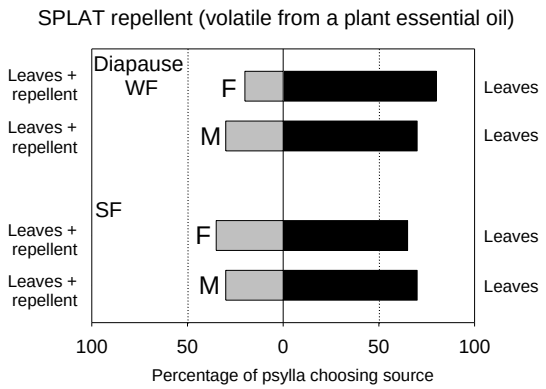

Figure 4. Olfactometer test of new SPLAT psyllid repellent (proprietary).

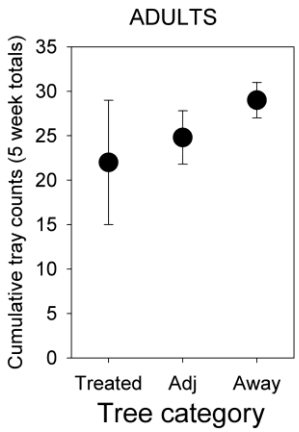

Figure 5. Tray counts on trees treated with SPLAT product, trees adjacent to treated trees, and trees distant from treated trees.

5. Pear ester: olfactometer trials. Olfactometer trials were done with pear ester in efforts to find a new attractant for pear psylla. ***Results.*** Pear ester was actually repellent to both summerform and winterform psylla in olfactometer trials (Figure 6), rather than attractive; this was possibly a concentration effect. A field trial failed to demonstrate activity in the field (data not shown).

6. Pheromone, new compound: olfactometer trials. Two compounds "hidden" in GC-MS profiles by the 13-MeC27 peak were discovered by J. Millar, identified (9-MeC27, 11-MeC27), and then synthesized. Synthetic formulations were evaluated for attractiveness to summerform males. ***Results.*** The 11-MeC27 product was attractive to male summerforms (Figure 7).

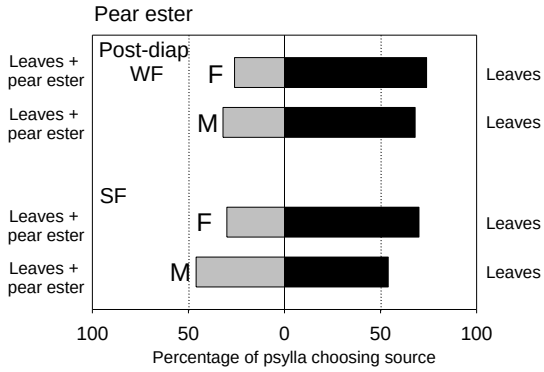

Figure 6. Repellency of pear ester to winterform and summerform psylla in olfactometer trials.

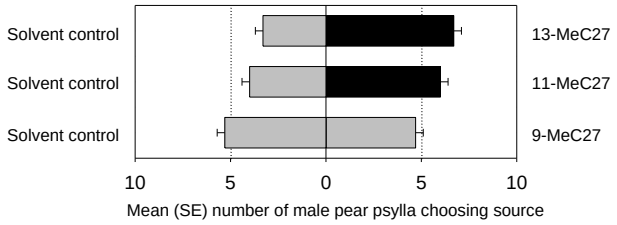

Figure 7. Olfactometer trial showing attractiveness of 11-MeC27 to male summerforms.

7. Pheromone, effects on mate-finding of male winterforms. Small cage studies were conducted to determine whether saturation of a cage with 13-MeC27 interferes with how rapidly males find and mate previously unmated female winterforms. Unmated psyllids were collected from a pear orchard in late winter before mating had begun in the field. Cages (with and without pheromone) received a mix of pear seedlings and pear cuttings. I added 50 females to each cage, and allowed them 24 hours to settle. After 24 hours, 50 males were added to each cage. Females were removed at 24 hours and 48 hours following addition of males, and dissected to determine spermatophore numbers (an index of mating frequency). ***Results.*** Saturation of cages with 13-MeC27 led to a statistical drop in number of spermatophores passed (compare black bars [=disruption cages] with gray bars [=control cages] in Figure 8). Results are consistent with the hypothesis that saturation of small cages with 13-MeC27 slowed rates at which males were able to locate females for mating.

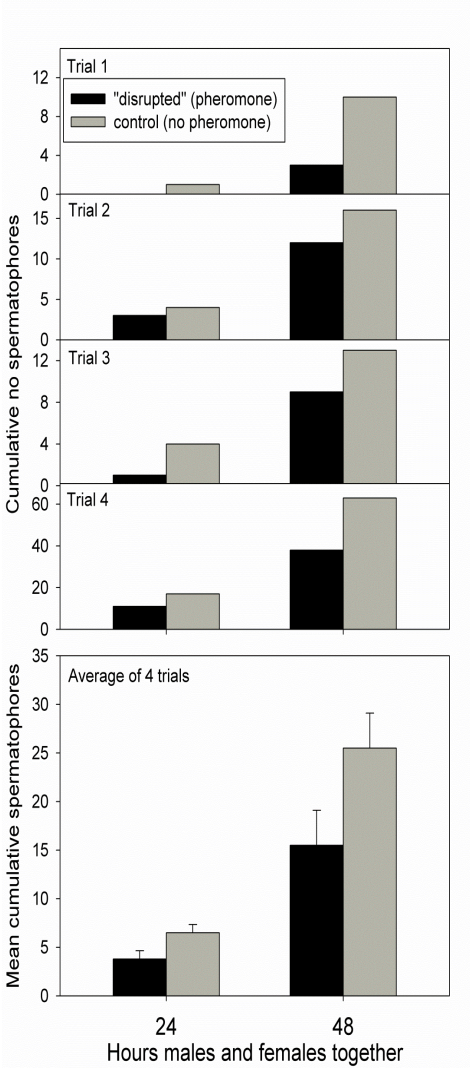

Figure 8. . Spermatophores per 25 females in "disruption" cages and pheromone-free cages**.**

8. Oil as carrier of 13-MeC27. Due to low volatility of 13-MeC27, an individual septa would presumably provide a signal over only a limited area. Saturation of an orchard with the active volatile would thus require many point sources. Pear growers apply oil (1-2% in water) in late winter to control mites and other soft-bodied arthropods. Because 13-MeC27 is a hydrocarbon, it is fully soluble in oil. I examined in a series of assays whether 13-MeC27 in oil was biologically active. ***(A). Olfactometer trial***. I examined response of male winterform psyllids to oil-treated shoots vs oil+pheromone-treated shoots. Shoots were treated with 1% oil or 1% oil+pheromone, with rates chosen to provide a signal of about 50 female equivalents on the oil+pheromone side of the olfactometer. Two trials, each with 100 males (10 runs x 10 males per run) were conducted. ***Results***. In both trials, a statistically significant number of males chose the pheromone side of the olfactometer (Figure 9). ***(B). Physical contact assay.*** I next examined whether treatment of shoots with oil+pheromone led to increased time males spend in physical contact with the shoot. Shoots treated with 1% oil or 1% oil+pheromone (concentration of pheromone as in the olfactometer trial) were paired in small circular arenas (15 cm in diameter). Shoots were placed with cut ends in water, 5 cm apart. Five winterform males were added to each chamber, and location of each male was recorded at 10 minute intervals over a 2 hour assay period. The assay included 20 replications of 5 males each. ***Results.*** Mean cumulative number of male psyllids in contact with the pheromone-treated shoot was significantly larger than number of males in contact with the oil-only shoot (Figure 10).

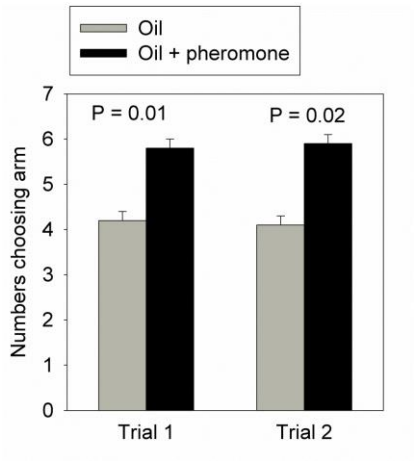

Figure 9. Results of olfactometer trial with oil as carrier.

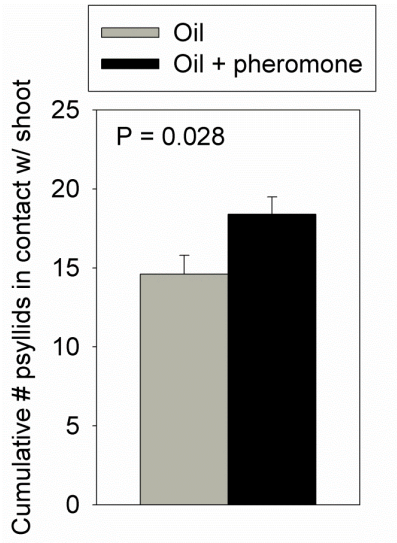

Figure 10. Arrestant properties of pheromone with oil as carrier.

Figure 11. Mesh cylinder (with tangle-foot) encircling treated shoot.

(C). Field trials with oil+pheromone formulation. Finally, field trials were done twice late in the winterform generation (April 2013). Paired shoots were sprayed with 10 ml of 1% oil in water or 1% oil+pheromone (n=10 pairs). Volume of pheromone per shoot was enough to provide a signal equivalent to about 50 females. Shoots were each about 18 inches in length. Each shoot was then encircled with a mesh cylinder that had been coated with tangletrap (Figure 11). Traps were left in the field for 3 days. ***Results.*** In the early-April trial, I found a substantial and highly significant increase in numbers of male psyllids on pheromone-treated shoots compared to oil-only shoots (by almost 20 psylla per trap; Figure 12: top panel). Females showed no preferences. Psyllid numbers declined substantially by the second trial in mid-April (Figure 12: bottom panel), as this was extremely late in the generation, and males were only marginally more abundant in the pheromone treatment.

Conclusions from all assays with oil as carrier. Assays showed that 13-MeC27 retained its biological activity when dispensed in 1% horticultural oil, suggesting that it may be possible to develop a sprayable formulation of the attractant.

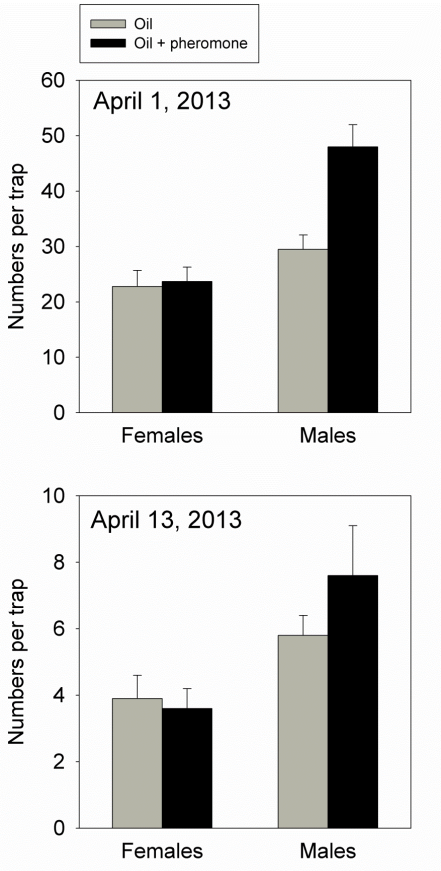

Figure 12. Winterform attraction in field to pheromone-treated shoots using oil as carrier.

EXECUTIVE SUMMARY

Management of pear psylla requires some level of control of the post-wintering winterform generation. Pear psylla at that time of year may be vulnerable to certain control approaches, due to characteristics of the psyllid's life history. These traits include (a) the requirement, following wintering, to rapidly locate a host for feeding (to replenish reserves expended in winter) and egglaying; and, (b) the life-history fact that pear psylla females overwinter in an unmated condition. (a) Strategies that could be used to slow or delay colonization of pear orchards through the use of repellents would lead to lowered densities of egglayers, added stress on psyllids to replenish expended reserves, and reduced fitness of egglayers. (b) Strategies that could be used to delay mating would lead to delays in egglaying combined with the production of early-season infertile eggs.

Repellents. Two compounds known to be repellent to other psyllid species were formulated into SPLAT release media by ISCA. Neither product exhibited field activity against returning winterforms, despite evidence for repellence in olfactometer trials.

Pheromone disruption. Small cage trials suggested that saturation of cages with the 13-MeC27 sex attractant led to lowered rates of mating. However, the very low volatility of the pheromone would make it difficult to obtain a similar level of saturation under orchard conditions in the absence of logistically infeasible numbers of point sources. Discussions with J. Millar (UCR) led to the suggestion that the attractant could be applied as part of the grower's typical dormant and delayed dormant oil sprays, given that the attractant (a hydrocarbon) would be completely soluble in horticultural oil. Laboratory and field trials suggested that the compound retains its biological activity in 1% oil (+ water).

Future directions. *Repellents*. I will be examining (with ISCA) a sprayable formulation of a potato psyllid repellent, rather than as a SPLAT-dispensed formulation. *Pheromone*. A field trial will be done Feb.-April 2014 to examine whether the sprayable formulation of 13-MeC27 affects mating rates of winterform psylla under orchard conditions.

FINAL PROJECT REPORT

Project Title: Suppression of pear psylla using elicitors of host-defenses

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Cooperators: David R. Horton, USDA-ARS, 5230 Konnowac Pass Road, Wapato, WA

Other funding sources: None

Total Project Request: $16,170

Budget History

Item	**2013**
Salaries	$12,000
Benefits	$870
Wages	
Benefits	
Equipment	
Supplies	$2000[2]
Travel	$300[3]
Miscellaneous	
Plot Fees	$1000
Total	$16,170

OBJECTIVES

Summary statement: The overall goal of this one-year study was to determine whether applications of commercial elicitors of host-plant defenses (Actigard, Employ, and ODC) reduce pear psylla performance and whether future experiments on induced defenses against pear psylla are warranted.

Objective 1: Evaluate the effects of foliar applications of three commercial defense elicitors, Actigard, Employ, and ODC, on survival and development rates of pear psylla nymphs on two pear cultivars, Bartlett and D'Anjou.

Objective 2: Evaluate the effects of defense elicitors on oviposition by pear psylla adults using choice and no-choice assays.

Objective 3: Determine whether observed effects of defense elicitors on pear psylla performance are caused by systemic induced defenses in pear or by direct contact with chemical elicitors.

Objective 4: Evaluate the effects of defense elicitors on nymph feeding behavior and honeydew production.

SIGNIFICANT FINDINGS

Objective 1: Foliar applications of the defense elicitors, Employ, Actigard, and ODC, each reduced the population growth of pear psylla on both Bartlett and D'Anjou pear.

Objective 2: Ovipositing females tended to settle and oviposit on untreated trees rather than on trees treated with Employ, Actigard, or ODC in choice assays. In no-choice assays, females oviposited fewer eggs on trees treated with Employ than on untreated trees or trees treated with ODC.

Objective 3: The effects of Employ, Actigard, and ODC on pear psylla were due to systemic plant defenses in treated trees.

Objective 4: Defense responses activated by the tested defense elicitors did not alter nymph feeding behavior.

RESULTS AND DISCUSSION

Defense elicitors are chemicals that induce broad-spectrum resistance against pathogens and insects. Several commercial defense elicitors including Employ, Actigard, and ODC are used to control plant pathogens on certain crops. Although these products are marketed for control of pathogens, each of these elicitors also activates plant defenses that reduce the population growth of aphids, which have similar feeding behaviors and strategies as pear psylla. It is not known whether defense elicitors activate defenses against pear psylla.

Several independent studies were conducted to assess the effects of defense elicitors on pear psylla preference and performance. In our first set of experiments, 10 field-collected adults were confined to an actively growing shoot of each treated tree, and the numbers of nymphs and adults were counted after 30 days. Trees were treated with Employ, Actigard, or ODC 24 hours before the insects were released into cages; control trees were left untreated. This study was conducted three times (trials) with different cohorts of insects. Insect releases occurred on 7-June (trial 1), 10-July (trial 2), and 13-August (trial 3). The numbers of nymphs on plants in trial 1 were nearly 10× greater compared with those in trial 2, and nearly 40 × greater compared with the number of nymphs

observed in trial 3 (Figure 1). Despite the differences in nymph populations observed on plants in the three trials, the results of each trial were generally consistent. All three defense elicitors reduced the numbers of nymphs present on the trees in trials 1 and 2 (Table 1; Figures 1A & B), but did not reduce the numbers of living adults (Tables 1 and 2). In trial 3, numbers of nymphs (Table 1; Figure 3A) and adults (Tables 1 and 2) were significantly lower on trees treated with Actigard than on trees treated with other elicitors. Trees treated with ODC supported fewer nymphs than trees treated with Employ, and numbers of nymphs on control plants were intermediate to those on trees treated with Employ and ODC (Figure 3A). The observed differences in nymph populations among trials may have been due to seasonal changes in psylla oviposition and development rates, or seasonal changes in pear physiology which could influence psylla performance and the strength of induced defenses activated by the elicitors.

In a separate study, the effects of defense elicitors on oviposition preference were examined by placing a tree from each treatment into each of 5 cages and releasing 10 reproductively mature females in the center of the cage. The number of adults and eggs on each tree were counted after 3 days. Results indicated that when given a choice, adults settled and oviposited more frequently on control trees compared with trees treated with Employ, Acitgard, or ODC (Figure 2A). These results indicate that induced defenses activated by foliar applications of Employ, Actigard, and ODC deter oviposition by pear psylla.

To examine the systemic effects of defense elicitors on adult oviposition and nymph development, a single leaf on each tree was protected from foliar applications using a plastic sleeve while all other leaves were treated with Employ, Actigard, ODC, or control. A single reproductively mature female was confined to the untreated leaf of each tree for 3 days before counting the number of eggs. The plants were returned to the greenhouse after removing the adults, and the numbers and mean life-stage of the surviving offspring were observed after 30 days. Under these no-choice conditions, females laid fewer eggs on trees treated with Employ compared with trees treated with control or ODC (F=5.1; d.f.=3, 26; P<0.001; Figure 2B). Fewer surviving offspring were observed on trees treated with Employ, Actigard, and ODC compared with control (F=4.1; d.f.=3, 16; P=0.025; Figure 2C), but the weighted mean life-stage of surviving offspring did not differ between treatments (F=0.06; d.f.=3, 10; P=0.98; Figure 2D). These results indicate that Employ, Actigard, and ODC each induce systemic defenses in pear that reduce oviposition and survival rates by pear psylla, but do not influence development rates of nymphs. In preliminary experiments, we treated nymphs with each elicitor and maintained the insects on artificial diet for 48 hours. Results of these preliminary experiments did provide support that elicitors have direct effects on psylla, and support our findings that treatment of trees with elicitors activates systemic plant defenses that reduce nymph survival.

Acquired defenses reportedly reduce feeding rates of aphids. We measured the amount of honeydew produced by pear psylla nymphs that were confined to leaves of pear trees treated with Employ, Actigard, ODC, or control to test whether acquired defenses also reduce feeding rates of pear psylla. Results of our experiments did not provide evidence that feeding rates were altered by acquired defenses in pear (F=0.53; d.f.=3, 12; P=0.67).

Our study is the first to investigate the effects of induced defenses on pear psylla, and indicate that three commercially available defense elicitors reduce oviposition and nymph survival by pear psylla on Bartlett and D'Anjou pear. These findings are consistent with previous reports that defense elicitors reduce performance and population growth of aphids, which are phloem-feeders with similar feeding behaviors as psylla. Results of our study suggest that elicitors of induced defenses could contribute to the integrated pest management of pear psylla, but further research is required to gain a better understanding of the mechanisms of induced defenses against pear psylla, and the efficacy of defense elicitors against psylla on an ecological scale.

Table 1. Statistical analyses of the numbers of surviving nymphs and adults on Bartlett and D'Anjou trees 30 days after releasing 10 adults on each tree.

	Trial 1	Trial 2	Trial 3
Total number of nymphs			
Cultivar	F=21.2; d.f.=1, 42; P<0.001	F=112.7; d.f.=1, 40; P<0.001	-
Treatment	F=561.4; d.f.=3, 42; P<0.001	F=305.1; d.f.=3, 40; P<0.001	F=22.5; d.f.=3, 14; P<0.001
Cultivar ×Treatment	F=74.2; d.f.=3, 42; P<0.001	F=92.5; d.f.=3, 40; P<0.001	
Total number of adults			
Cultivar	F=0.03; d.f.=1, 42; P=0.853	F=0.01; d.f.=1, 40; P=0.905	-
Treatment	F=0.97; d.f.=3, 42; P=0.416	F=0.17; d.f.=3, 40; P=0.918	F=3.96; d.f.=3, 14; P=0.031
Cultivar ×Treatment	F=0.09; d.f.=3, 42; P=0.966	F=0.35; d.f.=3, 40; P=0.788	

Table 2. Mean (± S.E.) number of surviving adults on Bartlett and D'Anjou trees 30 days after releasing 10 adults on each tree.

	Trial 1	Trial 2	Trial 3
Bartlett			
Control	6.9 ± 1.02	5.4 ± 0.92	2.0 ± 0.69 ab
Employ	8.0 ± 1.09	5.8 ± 0.98	2.0 ± 0.57 ab
Actigard	7.0 ± 1.02	6.2 ± 1.00	0.55 ± 0.83 b
ODC	6.9 ± 1.01	6.0 ± 0.98	3.7 ± 0.83 a
D'Anjou			
Control	6.7 ± 1.02	6.0 ± 1.06	-
Employ	8.4 ± 1.12	6.5 ± 1.03	-
Actigard	6.4 ± 0.98	5.4 ± 0.98	-
ODC	6.9 ± 1.01	5.4 ± 0.93	-

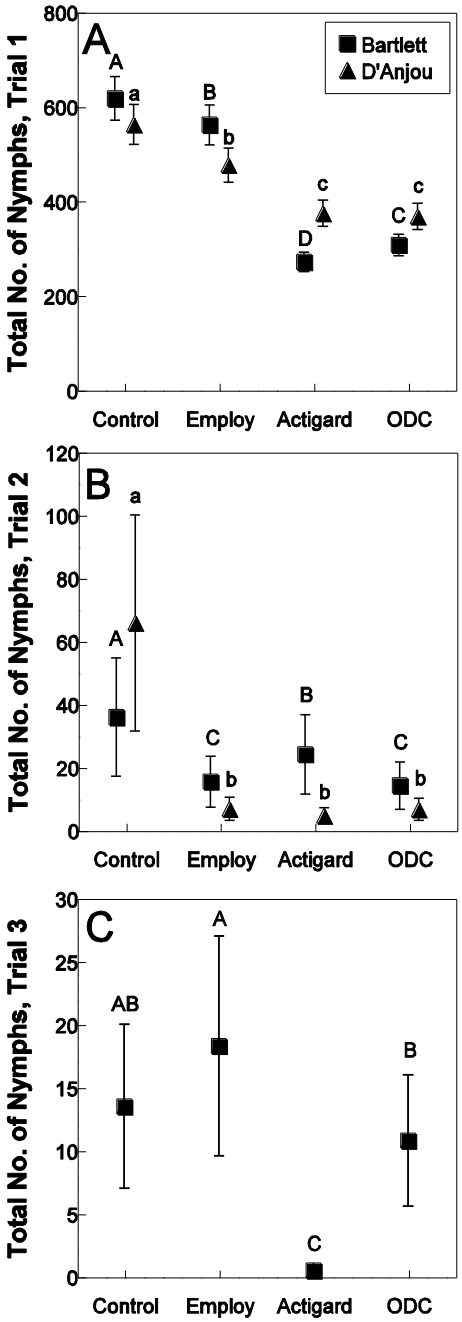

Figure 1. Total number of nymphs present on trees thirty days after releasing adults on trees treated with Control, Employ, Actigard, or ODC. Capital letters and lower-case letters indicate significant differences among treatments on Bartlett and D'Anjou trees, respectively.

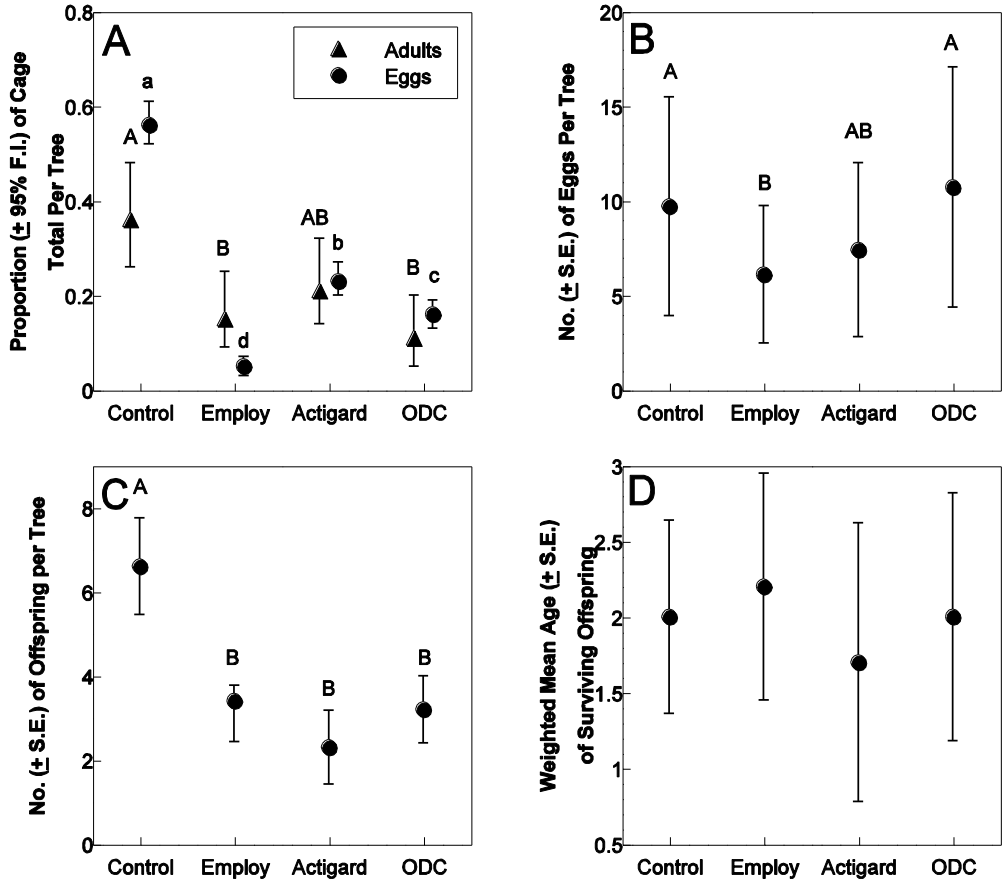

Figure 2. Proportion of the total number of eggs in each cage oviposited on trees treated with different treatments in choice oviposition assays (A), total number of eggs oviposited on trees in no-choice assays (B), mean number of surviving offspring on trees 30 days after treatment applications (C), and the weighted mean age of offspring after 30 days (D). In Figure A, different capital letters denote significant differences among adults whereas different lower-case letter denote significant differences among eggs.

EXECUTIVE SUMMARY

The objective of this study was to examine the effects of commercially available elicitors of host-plant defenses on pear psylla preference and performance. This proof-of-concept study was conducted to determine whether larger field studies on induced defenses against pear psylla are warranted.

Summary of Findings

Results of our study provide evidence that the defense elicitors Employ, Actigard, and ODC each activate systemic defenses in pear that reduce oviposition preference and nymph survival by pear psylla.

Future Directions

Further research is required to gain a better understanding of the mechanisms of induced defenses against pear psylla. It is not clear how defenses that are typically associated with protection from pathogens are effective against phloem-feeding insects such as pear psylla, but it seems possible that induced plant defenses indirectly reduce psylla survival by reducing populations of the insect's obligate bacterial endosymbiont, *Carsonella ruddi* . Further research is also needed to test the efficacy of defense elicitors against psylla on an ecological scale. Within-plant variability of induced defenses on larger trees may lessen the direct impact of these defenses on populations of pear psylla. However, induced plants may attract natural enemies that provide an additional level of control beyond direct defenses. It is also important to assess the effects of nutrient availability on induced defenses. Results of previous studies suggest that increasing potassium or magnesium availability may increase the strength of induced defenses and the efficacy of the defense elicitors. Knowledge of the mechanisms in which these defenses reduce pear psylla and of the ecological-level effects of induced defenses is required in order to fully implement defense elicitors into integrated pest management programs for this insect.

FINAL PROECT REPORT

Project Title: Conservation biological control of pear psylla in PNW pears

PI:	Peter W. Shearer, Ph.D.	**Co-PI (2):**	Kaushalya G. Amarasekare, Ph.D.
Organization:	OSU Mid-Columbia Ag. Res. & Ext. Ctr.	**Organization**:	OSU Mid-Columbia Ag. Res. & Ext. Ctr.
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Cooperators:

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Steve Castagnoli, OSU MCAREC, Hood River, OR
David Horton, USDA-ARS, Wapato, WA
Tom Unruh, USDA-ARS, Wapato, WA
Gene Miliczky, USDA-ARS, Wapato, WA

Total Project Request: **Year 1**: $71,571

Other funding sources: None

Budget 1

Organization Name: OSU MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2013
Salaries	$35,253
Benefits	$19,741
Wages	$11,520
Benefits	$957
Equipment	$0
Supplies	$2,500
Travel	$1,600
Miscellaneous	
Plot Fees	
Total	$71,571

Footnotes:

Postdoctoral Researcher: 9 months year 1.
OPE Postdoctoral Scholar: 56%
Hourly help: 1 person, 6 month, $12/hour,
OPE Hourly help: 8.31%
Supplies: Traps, pheromone lures, natural enemy attractants, sampling supplies,
Travel: to field plots

Project Title: Conservation Biological Control of Pear Psylla in PNW Pears

Objectives:

1. Determine why large-scale, area-wide mating disruption for codling moth in pear results in reductions in pear psylla population levels and insecticide sprays to manage it.

2. Develop actionable deliverables for use by pear industry, university Extension and researchers.

Significant Findings:

- Large-scale, area-wide mating disruption for codling moth in pear orchards and overall reduction of pesticide sprays for codling moth and pear psylla control can result in stabilized pest and natural enemy populations in Mid-Columbia pear orchards. This is the third study we have observed this in.

- Established natural enemy populations can be difficult to disrupt with pesticides sprays.

- Spiders were the most dominant predators observed during this study.

- Tree banding provided a better assessment of abundance of spiders, lacewings and earwigs than beating tray samples.

- Low populations of pear psylla were observed throughout the study period, despite the use or lack of insecticide use after the petal fall abamectin sprays. This supports previous observations regarding the benefits of codling moth mating disruption and reduced pesticide applications for pear psylla management.

Objective 1: Determine why large-scale, area-wide mating disruption for codling moth in pear results in reductions in pear psylla population levels and insecticide sprays to manage it

This study was carried out in a pear orchard in White Salmon, WA. The orchard was under a long-term area-wide codling moth mating disruption program with only rare applications of psyllicides applied after the petal fall abamectin applications. Four large green Bartlett blocks were selected from different locations within the orchard and each block was sub-divided into two sections for each treatments, i.e. non-disrupted and disrupted. Insecticides were not applied to the non-disrupted plots during the summer cover season (Table 1). A mixture of Altacor, Delegate and oil (Omni Supreme Spray) at high label rate was applied to the other plots on 25 May and 12 June, 2013 to disrupt natural enemies. We then assessed effects of disruption on pear psylla and natural enemies.

Table 1. Insecticide applications for test sites.

Application Dates	Product	Active ingredient	Rate/ acre	Disrupt	Non-disrupt
24-Feb-13	Omni Oil	Mineral Oil (98%)	3-4 gal	X	X
8-Mar-13	Omni Oil	Mineral Oil (98%)	2 gal	X	X
	Esteem 0.35 WP	Pyriproxyfen (35%)	5 oz		
1-Apr-13	Nexter	Pyridaben (75%)	8 oz	X	X
	Penncozeb 75 DF	Ethylenebisdithiocarbamate (58.1%)	8 lb		
21-Apr-13	Penncozeb 75 DF	Mancozeb (75%)	8 lb	X	X
	Surround WP	Kaolin (95%)	50 lb		
10-May-13	Zoro	Abamectin (1.9%)	20 fl oz		
	Omni Oil	Mineral Oil (98%)			
25-May-13	Delegate	Spinetoram (25%)	7 oz	X	
	Altacor	Chlorantraniliprole (35%)	4 oz		
	Omni Oil	Mineral Oil (98%)	2 qt		
12-Jun-13	Delegate	Spinetoram (25%)	7 oz	X	
	Altacor	Chlorantraniliprole (35%)	4 oz		
	Omni Oil	Mineral Oil (98%)	2 qt		
27-Jul-13	Omni Oil	Mineral Oil (98%)	2 qt	X	X
	CYD-X HP	Codling Moth Granulosis virus (0.06%)	1.5 oz		

The blocks were sampled weekly for important natural enemies using beating trays, assessing spur or shoot leaf samples, trapping of natural enemies using Herbivore Induced Plant Volatiles (HIPV) and bi-weekly banding of trees with strips of corrugated cardboard. Pear psylla eggs and nymphs were monitored weekly using spur leaves (early season) (5 spur leaves from 10 trees/block, 200 leaves/treatment/week) or shoot leaves (later in the season) (3 leaves/shoot, 1 shoot/tree, 10 shoots per block, 120 leaves/treatment/week). Weekly beating tray samples were used to monitor pear psylla adults, spiders, Coccinellids, *Trechnites*, immature natural enemies such as lacewing and Coccinellid larvae, *Deraeocoris brevis*, *Orius*, and *Campylomma verbasci* nymphs. Three types HIPV lures were used to monitor green lacewing *Chrysopa nigricornis* (Squalene), other green lacewing species (e.g. *Chrysoperla* sp.) (AMP [Acetic Acid, Methyl salicylate and Phenylacetaldehyde]), syrphids (Phenylacetaldehyde and Geraniol) in addition to using traps for *Campylomma* spp. (*Campylomma* pheromone). A trap for each lure was placed in each of the four blocks per treatment. Tree banding was conducted using a passive trap that consists of individual 1.5" wide cardboard bands placed on trunks of 20 trees per replicate (n=80 bands per treatment). These bands were used to assess the abundance of lacewing larvae, spiders and earwigs and were placed in the field twice a month and left in the field for approximately14 days. Treatment differences were analyzed with a ProcMixed model (SAS) and all significant different differences are noted on graphs using an asterisk (* = Significance at $P \leq 0.05$ (LS Means) (Proc Mixed, SAS).

Spiders collected from the beating tray samples and tree banding were stored in isopropyl alcohol and sent to USDA-ARS Wapato, WA for identifications. The results of spider identification are not yet available. All other natural enemies mentioned above and collected were identified and results are presented below.

RESULTS AND DISCUSSION:

Very few adult pear psylla were detected in beating tray samples in both non-disrupted and disrupted plots throughout the study period (Fig 1). Low numbers of psylla eggs and nymphs from spur leaves and shoot leaves were detected in the two treatments (Fig 2). Pear psylla egg, nymph and adult counts were well below economic threshold levels throughout the season.

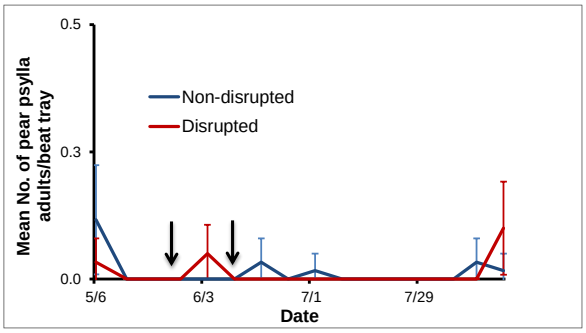

Figure 1: Mean number of pear psylla adults collected from beating trays. Arrows indicate application timings of Delegate and Altacor.

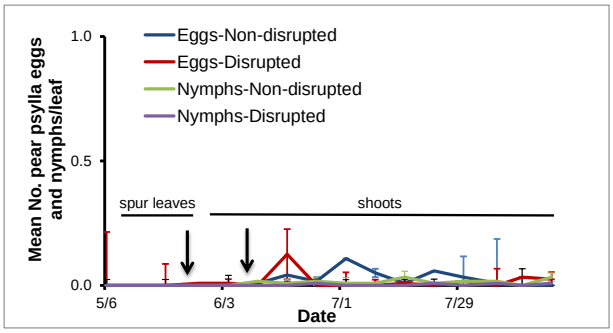

Figure 2: Mean number of pear psylla eggs and nymphs collected from spur leaves and shoot samples. Arrows indicate application timings of Delegate and Altacor.

Earwigs are important biological indicators when evaluating effects of pesticides in agricultural cropping systems. A large number of earwigs were collected from tree banding although only a negligible number were collected from beating trays (Fig 3). There was a decline in number of earwigs collected after the second spray in mid-June, but this was apparent in both treatments.

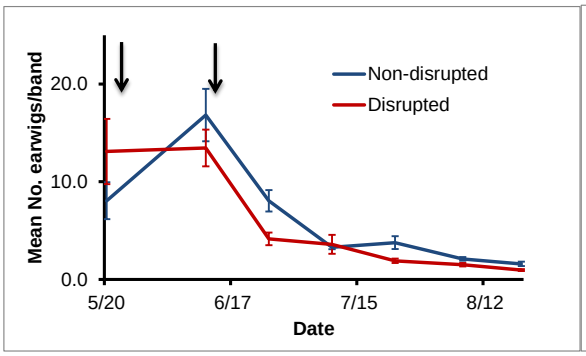

Figure 3: Mean number of earwigs collected from tree banding. Arrows indicate application timings of Delegate and Altacor.

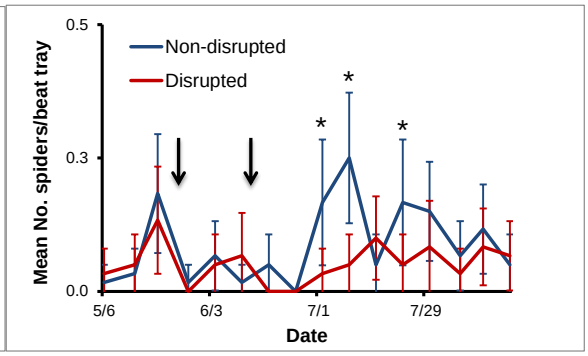

Figure 4: Mean number of spiders collected from beating trays. . Arrows indicate application timings of Delegate and Altacor.

Spiders were the dominant natural enemy collected from beating trays and tree banding (Fig 4 & 5). We observed higher levels of spiders on beat trays in the non-disrupted block on 3 sample dates (Fig. 4). More spiders were collected from tree banding than from beating tray samples. The spider counts from tree banding demonstrate the continuous abundance of spider populations in both non-disrupted and disrupted plots. Although low in numbers, continuously high spider populations were observed from beating tray samples in non-disrupted plots.

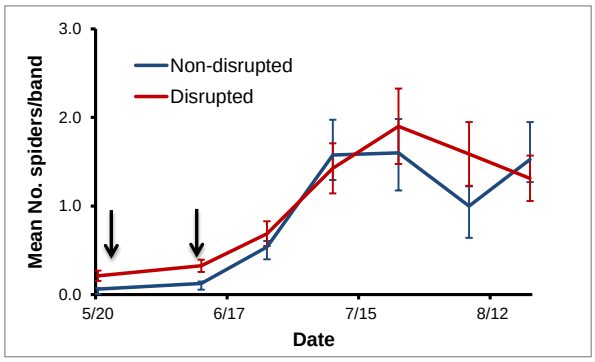

Figure 5: Mean number of spiders collected from tree banding. Arrows indicate application timings of Delegate and Altacor.

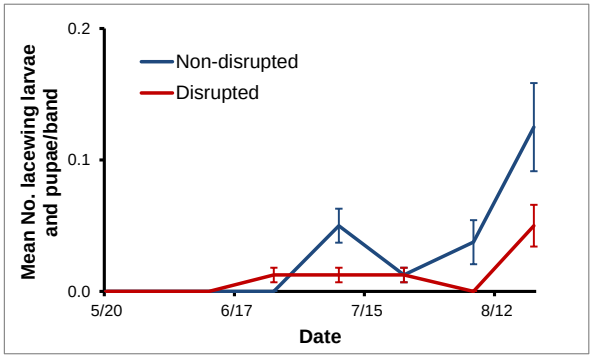

Figure 6: Mean number of lacewing larvae and pupae collected from tree banding. Arrows indicate application timings of Delegate and Altacor.

The abundance of lacewing larvae and pupae that were collected from cardboard bands attached to tree trunks tree banding were low in both non-disrupted and disrupted plots (Fig 6). The number of the green lacewing *C. nigricornis* were caught in weekly on HIPV traps was low (Fig 7). We were able to capture more *C. nigricornis* during the months of July and August than in May and June in both disrupted and non-disrupted plots. There was no difference in number of other green lacewing adults (*Chrysoperla* sp.) collected from HIPV traps placed in both non-disrupted and disrupted plots (Fig 8). Although low in numbers, *Chrysoperla* populations were continuously present in both non-disrupted and disrupted plots throughout the study period.

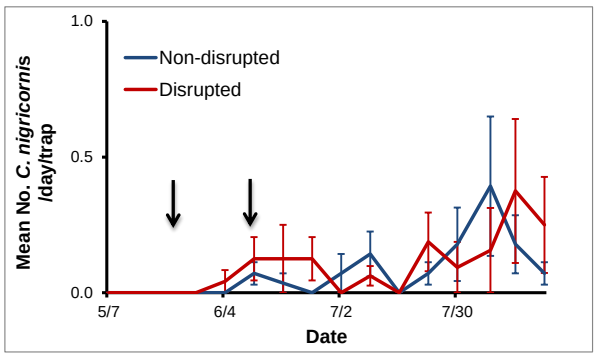

Figure 7: Mean number of *C. nigricornis* collected from HIPV traps. Arrows indicate application timings of Delegate and Altacor.

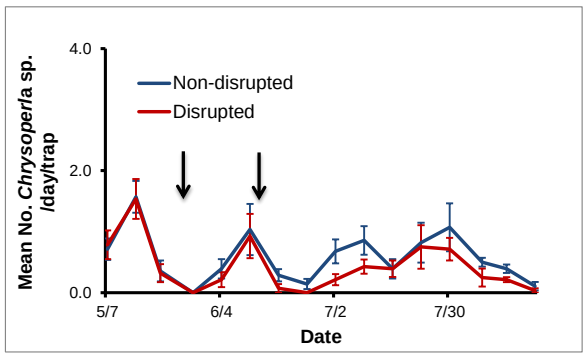

Figure 8: Mean number of *Chrysoperla* spp. collected from HIPV traps. . Arrows indicate application timings of Delegate and Altacor.

We observed a low number of adult Coccinellids from beating trays samples (Fig. 9). There were statistically more Coccinellids observed in the non-disrupted block on three sample dates. The number of Coccinellids collected increased in July after the two sprays in May and June and then gradually declined in August. Only a few Coccinellid pupae were collected from tree banding throughout the study period (Fig. 10).

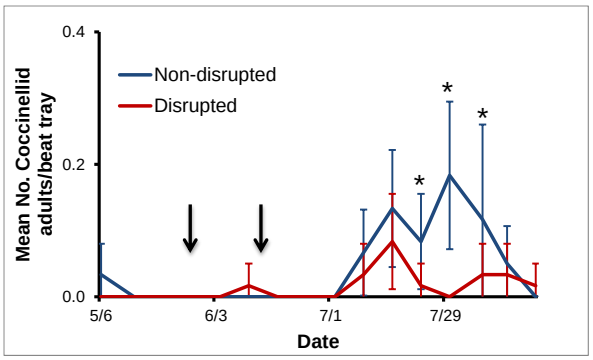

Figure 9: Mean number of *Coccinellid* adults collected from beating trays. Arrows indicate application timings of Delegate and Altacor.

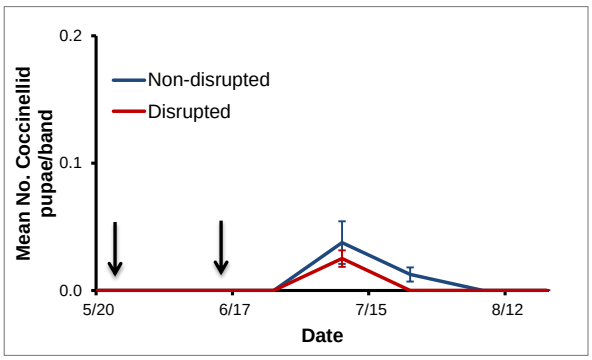

Figure 10: Mean number of *Coccinellid* pupae *collected* from tree banding. Arrows indicate application timings of Delegate and Altacor.

Adult *Campylomma* verbasi started appearing in pheromone traps in early July and then the numbers rapidly increased on traps in both non-disrupted and disrupted plots (Fig 11). The traps placed in the non-disrupted plots had more *Campylomma* adults than in the traps placed in the disrupted plots suyring the last two sample dates (Fig. 11). A low number of syrphids were caught from weekly HIPV trappings (Fig 12). Syrphids were mainly caught during the months of July and August rather than in May and June in both disrupted and non-disrupted plots.

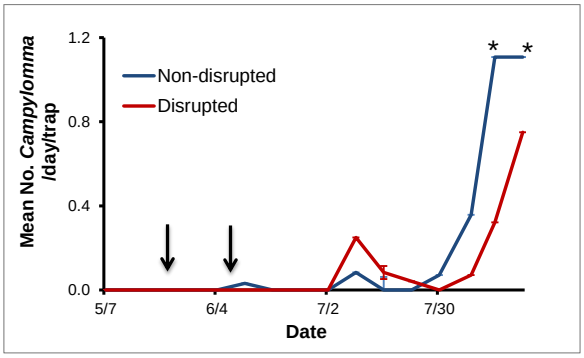

Figure 11: Mean number of *Campylomma* spp. collected from HIPV traps. Arrows indicate application timings of Delegate and Altacor.

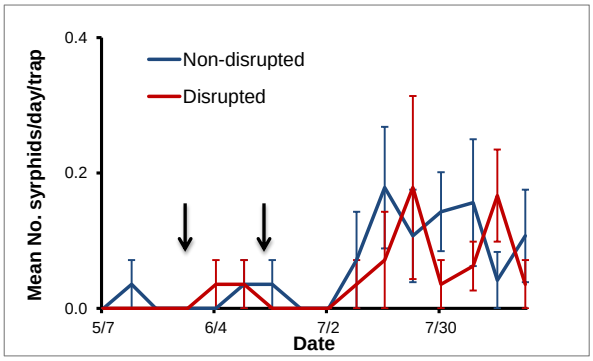

Figure 12: Mean number of syrphids collected from HIPV traps. Arrows indicate application timings of Delegate and Altacor.

Overall, spiders were the main natural enemy in this orchard. We assume they are helping keep pear psylla populations below treatment threshold levels. Buildup of spider populations may have occurred due to the long term mating disruption of codling moth and subsequent reduction of pesticide use for codling moth and pear psylla control.

Objective 2. Develop actionable deliverables for use by pear industry, university Extension and researchers.

- Recent industry experience in Mid-Columbia pear orchards suggests that biological control of summer pear psylla is possible during the summer cover season. Results from study indicate the need for rigorous evaluation of the potential for biological control to regulate pear psylla in contemporary pear pest management programs that utilize codling moth mating disruption. However, the overall lack of differences in natural enemy abundance in the disrupted and

non-disrupted blocks indicated something else might be occurring. Another theory that should be investigated is the impact or lack thereof of pesticide-induced hormolygisis, where pest reproductive output is stimulated by low dose of insecticides. These were main components that were removed from the original proposal.

- Information generated from this project will be made available to PNW growers and PCAs through ongoing outreach activities such as regional industry meetings, industry publications, and WSU DAS and Enhanced BC websites.
- Looking to the future, this project will provide a new start for collaborative PNW research on pear pest management including OSU, WSU, and ARS researchers.

Executive Summary

Project Title: Conservation biological control of pear psylla in PNW pears

Results of this study supports previous observations that long-term mating disruption for codling moth in pears and subsequent reduction in pesticide applications for codling moth control benefits pear psylla management and biological control. Reduction in pesticide use can protect beneficial organisms that are important for pear psylla biological control resulting less fewer insecticide applications for this pest, specifically during summer months. Overall reduction in the use of pesticides in an orchard provides many benefits including lower pest management costs.

The pear orchard we used for this study was under a long-term codling moth mating disruption program. One major result is that the grower uses fewer insecticide and miticide applications, protects natural enemies and minimize pear psylla and spider mite outbreaks. The uniqueness about this particular orchard is that these circumstances have helped to build up large populations of spiders throughout the orchard. Spiders were the most dominant natural enemy detected in this particular orchard irrespective of the method of monitoring used. We detected negligible abundance of pear psylla throughout the study.

This study shows that spiders can be a dominant yet often overlooked biological control component in pear orchards and possibly an important regulator of pear psylla populations. This information that demonstrates long-term mating disruption for codling moth can lead to reduction in pesticide use, improve natural enemy abundance and reduction in secondary pest outbreaks will benefit pest managers in their IPM programs. This study suggests that biological control of summer pear psylla is possible in Mid-Columbia pear orchards during summer months.

CONTINUNING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project number:

Project Title: Controlling postharvest disorders of pears during storage and export

PI:	Yan Wang	**Co-PI (2):**	
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Address 2:		**Address 2**:	
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Cooperators: Todd Einhorn, David Sugar, Xingbin Xie, Wade Root (Duckwall-Pooley Fruit)

Total Project Request: **Year 1:** $25,090 **Year 2: $25,751** **Year 3:** $26,431

Other funding sources
Agency Name: Syngenta Corp.
Amt. awarded: $6,000
Notes: Support for postharvest fungicide evaluations with Syngenta products.

Budget
Organization Name: Agricultural Research Foundation **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2013	2014	2015
Salaries	13,088[1]	13,481	13,885
Benefits	1,250[2]	1,300	1,352
Wages	6,715[3]	6,917	7,124
Benefits	537[4]	553	570
Equipment			
Supplies	3,000[5]	3,000	3,000
Travel	500[6]	500	500
Miscellaneous			
Total	25,090	**25,751**	26,431

Footnotes:
[1]Postdoctoral Research Associate (Dr. Xingbin Xie): 1/3 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 500hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 8% of the wage.
[5]Supplies: maintaining cold rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

OBJECTIVES

1. Controlling senescent disorders (yellowing, senescent scald, internal breakdown) of summer pears by 1-MCP, ReTain™, and MAP.

1. Ensure a consistent 1-MCP efficacy on maintaining storage and export quality of 'Bartlett'.
2. Determine the effect of 1-MCP and ReTain™ on storage quality and ripening capacity of 'Starkrimson'.
3. Optimize MAP conditions for storage and export of 'Bartlett' and 'Starkrimson'.

2. Reducing scuffing of pears ('Comice', 'Bartlett', 'Anjou').

1. Develop cultivar-specific wax coating application protocol to reduce friction forces on epidermal cells to reduce scuffing by lubricating fruit surface.
2. Reduce enzymatic discoloration by antioxidant-ethoxyquin.
3. Determine if 1-MCP treated 'Bartlett' and 'Anjou' are more resistant to scuffing.

3. Controlling storage decay of pears by introducing new postharvest fungicides.

1. Evaluate the efficacy of a premix formulation of Difenoconazole and Fludioxonil, compared with Scholar (fludioxonil) and Penbotec (pyrimethanil) on gray and blue molds of pears.

In the Pear Research Priority Summary developed by PNW Pear Research Committee in 2013, postharvest disorders, decay, and 1-MCP use are listed as important Current and Chronic issues. The present project complemented with the continuing project "Delivering 1-MCP treated pears with predictable ripening capacity" addresses the research needs to extend packing season, maintain quality, reduce postharvest losses, and facilitate international marketing of PNW pears.

SIGNIFICANT FINDINGS: year-1

Objective 1. Controlling senescent disorders of summer pears.

1. 1-MCP efficacy on inhibiting senescence of 'Bartlett' is extremely inconsistent at commercial application. To ensure 1-MCP efficacy on maintaining color and FF of 'Bartlett' following storage + export (temperature fluctuations):
    a. Fruit internal ethylene concentration (IEC) should be < 0.2-0.3 ppm at the time of 1-MCP treatment.
        i. Fruit should be harvested at 19-18lb, especially for fruit harvested from higher production elevations.
        ii. Treat fruit within 12 days after harvest.
        iii. Eliminate field heat quickly after harvest and store fruit at 30°F during the delay.
    b. Eliminate exogenous ethylene in the treating room (if > 300ppb) by venting before treatment.

2. 'Starkrimson' produces a higher amount of ethylene and has a higher respiration rate and therefore a shorter storage life compared to other PNW pear cultivars. Both 1-MCP treatment and a pre-harvest ReTain™ spray delayed ripening and reduced senescent disorders of 'Starkrimson' following storage.
    a. Postharvest treatment of 1-MCP at 300ppb.
    b. Spray of ReTain™ at 60-120ppm 1 week before harvest.

3. The right MAP liner (i.e., ~12% O_2 + 5-6% CO_2 at 30°F) with the right packing procedures (Packing at fruit pulp temperature of 40°F then forced-air cooling to 30°F within 4-5d) could

extend storage life and avoid over-ripening of 'Bartlett' at export transit temperatures between 30-40°F in the earlier packing season and 30-36°F in the late packing season.

'Starkrimson' is very susceptible to high CO_2/O_2 injury, therefore, MAP does not work for 'Starkrimson'.

Objective 2. Reducing scuffing of pears

1. Pear industry applies wax coatings (i.e., carnauba) at relatively low solid (i.e., 5-6%) to avoid anaerobic injury on pears. We found out that some cultivars (i.e., 'Comice') are more tolerant to high CO_2/O_2 ratio than others.
2. Compared to the current industry standard (5-6%), wax coating at solid of 7-8% reduced scuffing without negative effect on quality of 'Comice'.
3. Ethoxyquin at 1000ppm mixed in wax coating slowed down chlorophyll degradation and reduced scuffing expression of 'Comice'.
4. 1-MCP reduced scuffing of 'Anjou' and 'Bartlett' by maintaining high FF to resist mechanical damaging and maintaining chlorophyll to mask scuffing expression.

Objective 3. Controlling storage decay of pears by introducing new postharvest fungicides.

1. The pre-mixed formulation of Difenoconazole and Fludioxonil (Syngenta product) applied as drenching at 16 oz. per 100 gallons controlled blue and gray mold decays at levels equivalent to Penbotech and Scholar alone. The different modes of action between Difenoconazole and Fludioxonil in the pre-mix may retard resistance development in the pathogens.

METHODS

Objective 1. Controlling senescent disorders of summer pears.

1) 'Bartlett'. Study the effects of harvest maturity, delayed treatment, production lot, and exogenous ethylene on efficacy of 1-MCP:

1. Harvest maturity: early commercial harvest (18-19lb); late commercial harvest (17-18lb); and over-mature (16-17lb).
2. Delayed treatment (1 and 12 days) and temperature during the period of delayed treatment: 12 day-delay at 32°F, 37°F, and 41°F.
3. Production lots (elevations), 500ft and 2000ft.
4. Exogenous ethylene presenting in the treating rooms: 0, 300ppb and 1ppm.

A $40m^3$ cold room at 32°F will be used to treat fruit with 1-MCP at the commercial rate of 300ppb for 24h. Treated fruit will be stored at 30°F. Fruit color, I_{AD}, IEC, FF, senescent scald, internal breakdown, and flavor will be evaluated before and during storage for 4 months.

After storing for 3 and 4 months at 30°F, subsets of the fruit treated with 1-MCP will be transferred to a cold room at 40°F for 3 weeks to simulate export transit conditions. Fruit color, I_{AD}, FF, IEC, disorders and flavor will be evaluated before and after the simulated export

2) 'Starkrimson'. Study effects of postharvest 1-MCP treatment and pre-harvest ReTain™ spray on storage quality and ripening capacity of 'Starkrimson'.

1. Postharvest treatment with 1-MCP at 300 ppb as described above.
2. Pre-harvest sprays with ReTain™ at 30, 60, and 120 ppm 1 week before commercial harvest.

Fruit quality and ripening capacity will be determined after 1, 2, 3, and 4 months of cold storage at 30°F.

3) MAP. Continue the MAP-pear project funded by CGFG to further study:

1. Evaluate the commercial MAP liners: LifeSpan®, Extend®, Breatheway®, and PrimPro®. Standard macro-perforated PE liner as control.
2. Effect of additional temperature scenarios simulated of sea transportation (32°F, 40°F, 50°F) on CO_2/O_2 injury.
3. Effect of Ethylene Absorbance within MAP on fruit quality at simulated transit temperatures. Supposing that ethylene accumulated in the packaging environment will accelerate over-ripening of pear fruit at elevated transportation temperatures.

Objective 2. Reducing scuffing of pears.
Fruit respiration and ripening physiology influenced by variety, temperature, O_2 and CO_2 will be studied on the major PNW cultivars: 'Starkrimson', 'Bartlett', 'Bosc', 'Comice', and 'd'Anjou'. Anaerobic thresholds of each cultivar based on carnauba wax solids (0, 5%, 10%, 15%, and 20%) will be determined.

1. Increase wax solid to reduce scuffing for cultivars which are resistant to anaerobic injury. Trials will be run in Duckwall-Pooley Fruit Co.
2. Determine if antioxidant-ethoxyquin mixing in wax coating can reduce scuffing expression. Trials will be run in Duckwall-pooley Fruit Co.
3. 1-MCP treated 'Bartlett' and 'Anjou' stored for 3 and 5 months, respectively, will be run on a packingline (MCAREC) to determine scuffing resistance.

Objective 3. Controlling storage decay of pears by introducing new postharvest fungicides.
A pre-mix formulation of Fludioxonil with Difenoconazole, Difenoconazole alone, Scholar, and Penbotec will be obtained from Syngenta. Artificially inoculated 'Bosc' pear fruits with spore solutions of *Botrytis cinerea* and *Penecillium expansum* will be drenched with the fungicides at label recommended rates. Decay incidence, decay severity, and sporulation will be evaluated after 3-5 months of storage at 30°F.

RESULTS AND DISCUSSION

OBJECTIVE 1 (CONTROLLING SENESCENT DISORDERS OF SUMMER PEARS)

1. Ensure a consistent 1-MCP efficacy on 'Bartlett'. 1-MCP efficacy on inhibiting senescence of 'Bartlett' has been reported being extremely inconsistent from year to year and from lot to lot.

1.1. Effect of 'Bartlett' harvest maturity on 1-MCP efficacy.
There were 3 harvest maturities: H1=19lbf (IEC=0ppm); H2=17.2lbf (IEC=0.2ppm); H3=16.5lbf (IEC=1.0ppm). 1-MCP treatment maintained fruit peel chlorophyll and FF for H1 and H2 fruit without senescent disorders (yellowing, senescent scald and internal breakdown) for 4-5 months of cold storage. However, 1-MCP treated F3 fruit lost chlorophyll and FF significantly after 2 months and developed senescent disorders after 4 months of cold storage at 30°F (Fig. 1).

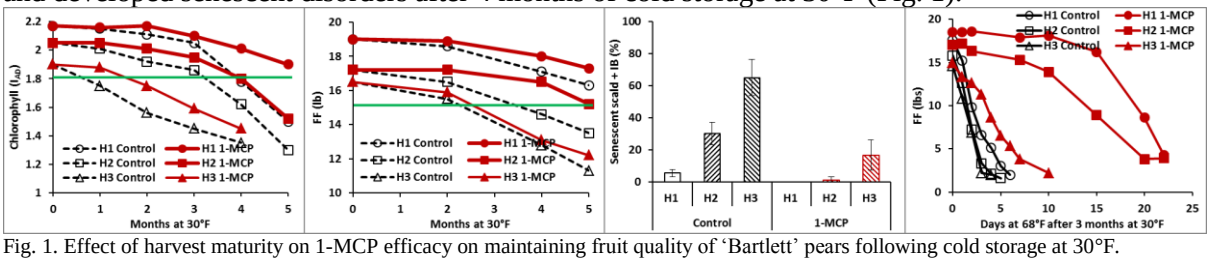

Fig. 1. Effect of harvest maturity on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' pears following cold storage at 30°F.

After 3 months of cold storage, fruit were transferred to a cold room at 41°F for 3 weeks to simulate export transit conditions. 1-MCP treatment maintained higher peel chlorophyll content and FF for only H1 fruit after the simulated export transit. Both H2 and H3 fruit treated with 1-MCP lost chlorophyll and FF significantly and developed senescent scald and IB (Fig. 2).

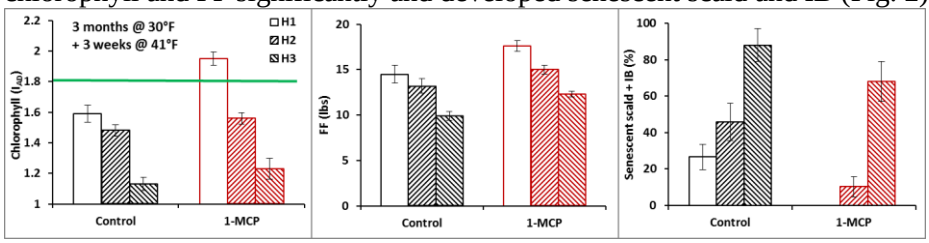

Fig. 2. Effect of harvest maturity on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' pears following 3 months of cold storage at 30 °F plus 3 weeks at 41 °F.

1.2. Effect of delayed treatment after harvest on 1-MCP efficacy.

It may take 10d or longer time to fill a storage room. 'Bartlett' fruit were harvested at 19-17lbs (IEC<0.2ppm). Fruit were stored at cold rooms at 30, 37, and 41°F for 12d until 1-MCP treatment. IEC did not increase at 30°F, but increased at 37 and 41 °F during the 12d delay. A delayed treatment of fruit that have been stored at 30 °F for 12d did not reduce 1-MCP efficacy on maintaining fruit peel chlorophyll and FF of 'Bartlett' pears after 4 months of cold storage, compared to treating fruit immediately after harvest. However, storing fruit at 37 and 41 °F for 12d after harvest reduced 1-MCP efficacy on maintaining quality of 'Bartlett' after 4 months of cold storage (Fig. 3).

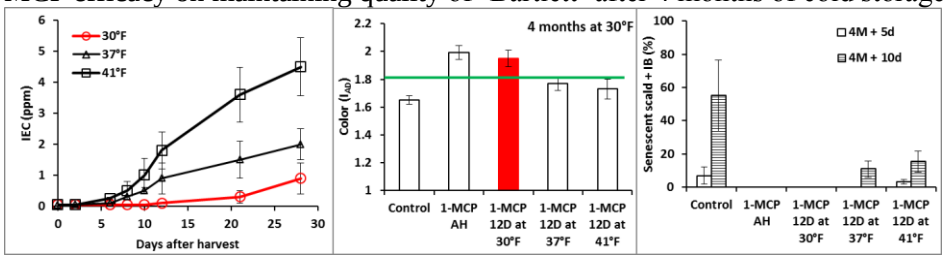

Fig. 3. Effect of treatment delays on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' after 4 months of cold storage at 30 °F.

1.3. Effect of production elevation on 1-MCP efficacy.

'Bartlett' fruit were harvested at 3 maturities based on FF from two elevations: 500ft and 2000ft. at the same FF, fruit from the higher elevation (2000ft) had higher IEC, especially for H2 (17lbf) and H3 (16.5lbs). H2 and H3 from elevation of 2000ft reduced 1-MCP efficacy on maintaining color and FF after 4 months of cold storage, compared to H1.

1.4. Effect of exogenous ethylene in treating room on 1-MCP efficacy.

Exogenous ethylene at 300ppb in treating room reduced 1-MCP efficacy on maintaining chlorophyll and FF and senescent scald-free after 4 months of cold storage (Fig. 4).

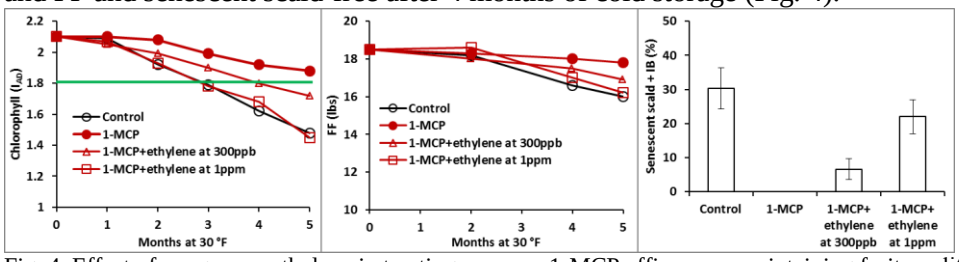

Fig. 4. Effect of exogenous ethylene in treating room on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' following 4 months of cold storage at 30°F.

2. Determine the effects of 1-MCP and ReTain^{TM} on storage quality of 'Starkrimson'.

2.1. 1-MCP

1-MCP retarded ethylene production rate and reduced respiration rate during 3 months of cold storage at 30°F. 1-MCP treatment did not affect FF, SSC, and TA, but significantly reduced watery flesh and core breakdown after 3 months of cold storage. 1-MCP treated 'Starkrimson' ripened to below 4 lbf after 2 weeks at 68°F following 2 and 3 months of cold storage (Fig. 5).

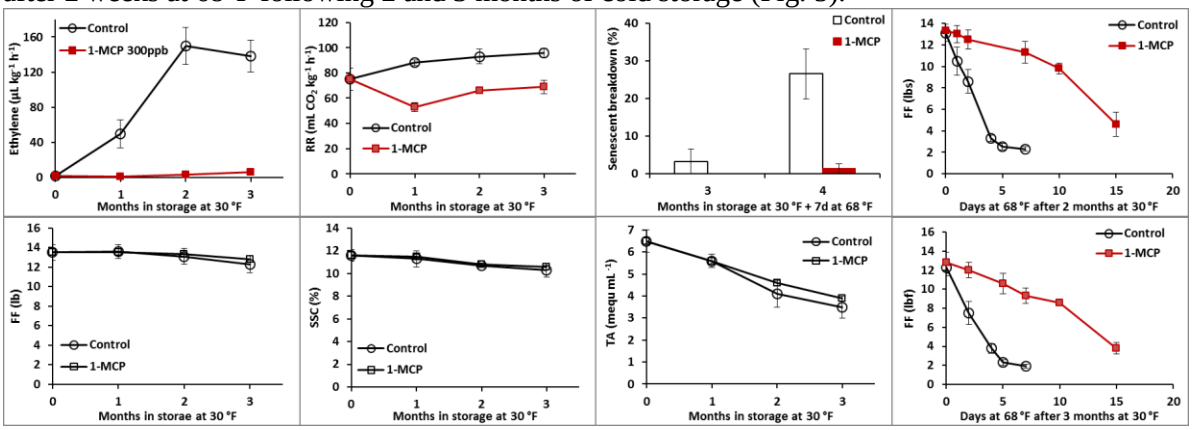

Fig. 5. Effect of 1-MCP on storage quality and ripening capacity of 'Starkrimson' pears stored for 3 months at 30°F.

2.2. Pre-harvest spray of ReTain^{TM} (reporting the result next year)

3. Optimize MAP conditions for storage and export of 'Bartlett' and 'Starkrimson'.

3.1. 'Bartlett'.

MAP1&2 with high O_2 and high CO_2 (i.e., 5-12% O_2 + 5-6% CO_2) may replace CA to extend 'Bartlett' storage life with green color and high FF without internal browning (IB) for up to 4 months at 30°F. MAP3 with low O_2 and high CO_2 (i.e., 2% O_2 + 6% CO_2) resulted in fruit with IB after 3 months of storage at 30°F. MAP4 with high O_2 and low CO_2 (i.e., 18% O_2 + 2% CO_2) had minimal effect on delaying fruit yellowing and softening in cold storage (Fig. 6). Fruit packed in MAP1&2 had a higher TA and could ripen to 4lbs within 5d at 68°F (data not shown).

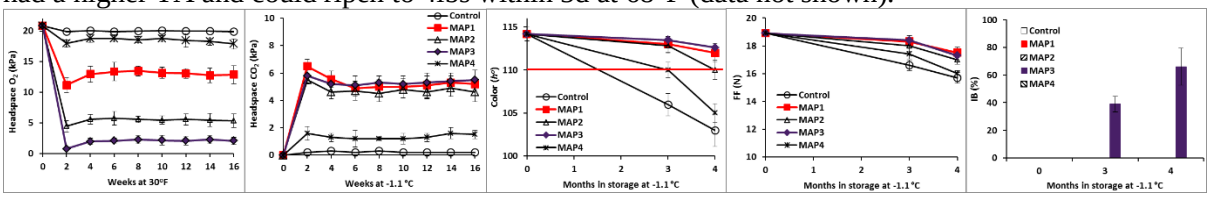

Fig. 6. Effect of atmospheres in MAP on storage quality of 'Bartlett' pears during 4 months of cold storage at 30°F.

Ethylene absorbent sachets effectively absorbed ethylene in MAP liners, however, it did not affect fruit quality following 4 months of cold storage. Therefore, ethylene accumulated in MAP did not affect storage quality of MAP packed 'Bartlett' pears during 4 months of cold storage at 30°F.

Elevated temperatures (simulating the situation of export transportation) from 36 to 50°F did not change CO_2 much but reduced O_2 concentration (from 10% to 1%) in MAP significantly. Storage durations (1 and 3 months) did not affect O_2 and CO_2 in MAP at elevated temperatures. Elevated temperatures higher than 45°F (O_2<2% + CO_2>8%) induced IB. Late season fruit showed higher incidence and severity of IB. there were two types of IB: Wet Brown Flesh and classic Pithy Brown Core. Elevated temperatures reduced FF of 'Bartlett' in MAP. To maintain FF higher than 15lbs (the critical value for resistance to scuffing) at export arrival, transit temperature should be less than 40

and 36°F in earlier and late packing seasons, respectively. Elevated temperatures did not affect fruit color of ‘Bartlett’ packed in MAP (Fig. 7).

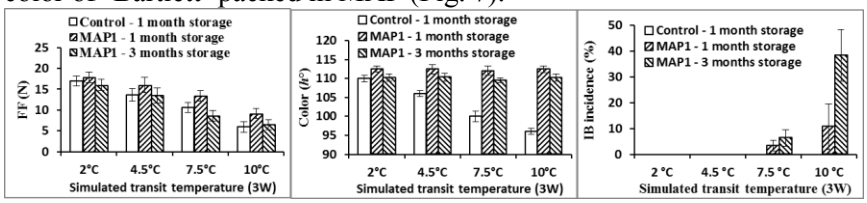

Fig. 7. Effect of elevated temperatures on fruit quality of MAP packed ‘Bartlett’.

3.2. ‘Starkrimson’

‘Starkrimson’ developed IB after 2 months of storage in MAP with atmospheres of either of 9.5% O_2 + 6.0% CO_2 or 18.0% O_2 + 2.5% CO_2. ‘Starkrimson’ was highly susceptible to CO_2 injury, possibly related to its high respiration rate and internal CO_2 concentration.

OBJECTIVE 2 (REDUCING SCUFFING)

2.1. Respiration physiology of pear cultivars. Pears are more sensitive to high CO_2injury. Therefore, waxes (e.g., carnauba) for pears are lower in solids (5-6%) than those for apples (18-22%). However, low solid waxes are less effective in minimizing scuffing. Fruit were waxed after harvest using a commercial carnauba wax coating at solids of 0, 5, 10, 15, and 20%. After satisfying chill requirement plus 7d at 68°F, ‘Starkrimson’ and ‘Bosc’ developed anaerobic metabolism at wax solid of 20%; ‘Starkrimson’, ‘Bosc’, ‘Bartlett’, and ‘d’Anjou’ developed IB or abnormal ripening at wax solids higher than 10%. Injury was not found in ‘Comice’ at any of the wax solid treatments (Fig. 8).

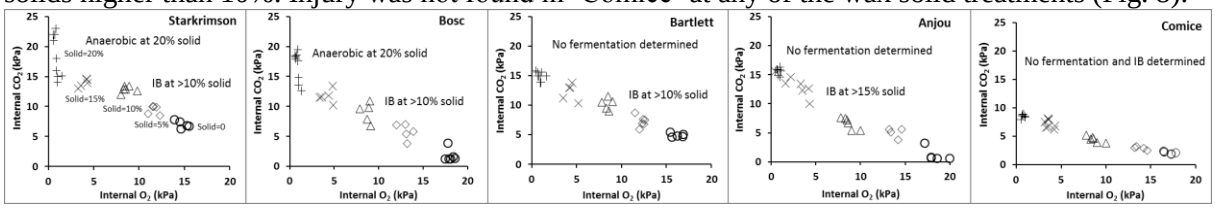

Fig. 8. Internal O_2 and CO_2 concentrations affected by wax solids of a commercial carnauba wax coating after fruit ripening of 5 European pear cultivars.

2.2. Reduce scuffing by increasing wax solids and adding ethoxyquin. Compared to the commercial wax solids of 5-6%, wax solids at 7-8% plus ethoxyquin at 1000ppm reduced scuffing without negative effect on fruit quality of ‘Comice’ pear. Carnauba wax coating at higher solids may reduce abrasion force on fruit peel during online processing and ethoxyquin may reduce the enzymatic reaction and therefore expression of the discoloration.

OBJECTIVE 3 (CONTROLLING STORAGE DECAY)

The pre-mix of Fludioxonil with Difenoconazole, applied as drenching within 18h after inoculation, was very efficient and comparable with Penbotec and Scholar on controlling both blue and gray molds of pears during cold storage (Fig. 9). The rate at 16 oz was more efficient than 11.4 oz on gray mold. Both rates were equal and efficient on blue mold.

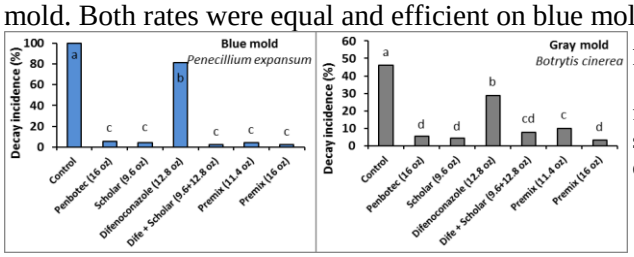

Fig. 9. Blue mold and gray mold decays in inoculated ‘Bosc’ pears treated by experimental and standard fungicides and evaluated after 3 and 5 months of cold storage at 30°F. Rates are in fluid ounces per 100 gallons of water.

CONTINUNING PROJECT REPORT **YEAR: 2** of 3
WTFRC Project number:

Project Title: Deliver 1-MCP treated d'Anjou pears w/predictable ripening capacity

PI: Yan Wang
Organization: OSU MCAREC
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Address: 3005 Experiment Station
City/State/Zip: Hood River/OR/97031

Cooperators: Xingbin Xie, David Sugar, Paul Chen, Nate Reed (AgroFresh Inc.)

Total Project Request: **Year 1:** $25,613 **Year 2:** $25,777 **Year 3: $26,461**

Other funding sources
none

Budget 1
Organization Name: Agricultural Research Foundation **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Salaries			
Benefits			
Wages	15,000[1]	15,450	15,914
Benefits	7,113[2]	7,327	7,547
Equipment			
Supplies	3,000[3]	2,500	2,500
Travel	500[4]	500	500
Miscellaneous			
Total	25,613	25,777	**26,461**

Footnotes:
[1]Wages: 500hr each for 2 part-time employees at $10/hr and $20/h, respectively. 3% increase is factored into Year 2 and 3.
[2]OPE: $10/hr Temp employee calculated at 8.47% +2.43/mo., $20/hr Unclassified Employee calculated at 28.57%+$1230.51 per month. Both have a 3% increase per year.
[3]Supplies: maintaining cold rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.
[4]Travel: field trips to packinghouses and orchards.

OBJECTIVES

The goal of this project is to develop commercial protocols for controlling postharvest disorders of pears through postharvest application of 1-MCP at commercially manageable dosage (100-300ppb) while allowing ripening to outstanding eating quality. The key objectives are to:

1. Determine the effects of storage temperatures (30°F, 34°F, 36°F) on storability, superficial scald and ripening capacity of 1-MCP treated d'Anjou pear after storage.
2. Evaluate if delayed treatments with 1-MCP can maintain fruit ripening capacity of d'Anjou pear without losing superficial scald control after storage.
3. Study the effect of simultaneous treatment with 1-MCP and ethylene on storability, superficial scald and ripening capacity of 1-MCP treated d'Anjou pear after storage.
4. Study the effects of post-storage conditionings at temperature of 50°F or ethylene of 100ppm on superficial scald and ripening capacity of 1-MCP treated d'Anjou pears.
5. Evaluate the effects of harvest maturity, production elevation (500 vs. 2000ft), and production year on storability, superficial scald control, and ripening capacity of 1-MCP treated d'Anjou pear after storage.

Goals, activities, and anticipated accomplishments for the next year:

1. Study the effect of simultaneous treatment with 1-MCP + ethylene at 300, 600, and 1000ppb on ripening capacity, scald control, and storability of Anjou.
2. Optimize the post-storage ethylene conditioning protocol for "positive temperature storage" and "simultaneous treatment with 1-MCP + ethylene".
3. Study and optimize combinations between "positive temperature storage", "1-MCP + ethylene simultaneous treatment", and "post-storage ethylene conditioning" to extend storage life, control scald, and maintain ripening capacity of Anjou fruit.

SIGNIFICANT FINDINGS (year 1-2)

Ripening capacity referred in this report was defined as the capability of 1-MCP treated Anjou fruit to soften below 4-6 lbf within 7d at 68°F following cold storage.

1. Storing at 34°F, 1-MCP treated Anjou restored ripening capacity with minimal scald severity and incidence after 7d at 68°F following 5-8 or 6-8 months of cold storage in 2011 and 2012, respectively. Control fruit fulfilled the chilling requirement and obtained ripening capacity following 2 or 3 months of cold storage in 2011 and 2012, respectively.

2. Simultaneous treatment with 1-MCP at 150ppb + ethylene at 300ppb allowed the 1-MCP treated Anjou fruit producing ethylene and developing ripening capacity with minimal scald within 7d at 68°F following 5-8 or 6-8 months of cold storage in 2011 and 2012, respectively.

3. Post-storage ethylene conditioning (100ppm for 72h at 68°F) could ripen the 1-MCP treated Anjou within 7d at 68°F following 7-8 or 8 months of cold storage at 30°F, 4-5 or 4-6 months of cold storage at 34°F in 2011 and 2012, respectively

4. Ethylene is the most efficient compound for ripening 1-MCP treated Anjou. All other ripening compounds including Abscisic acid (ABA), Jasmonic acid, methyl jasmonate, salicylic acid … may not have commercial value on ripening 1-MCP treated Anjou.

5. Fruit from high elevation (i.e., 2000ft) are less responsive to 1-MCP than fruit from low elevation (i.e., 500ft). That means 1-MCP treated Anjou fruit from high elevations are relatively easier to be ripened than that from lower elevations following cold storage.

6. Delaying the treatment with 1-MCP after harvest may not be a practically useful protocol for ripening 1-MCP treated Anjou pears. Fruit treated with 1-MCP 1-22d after harvest did not develop ripening capacity within 7d at 68°F following 4-8 months of cold storage. Fruit treated with 1-MCP 23-29d after harvest developed unacceptable scald within 7d at 68°F following 4-8 months of cold storage.

7. Regarding ripening capacity, there were no differences in responsiveness to 1-MCP between H1 = 15lbf (early commercial harvest maturity) and H2 = 13lbf (late commercial harvest maturity). Fruit harvest at H3 = 12.5lbf developed ripening capacity better than H1 and H2 in 2011, but not in 2012.

METHODS

Defect-free fruit were packed into 20kg wooden boxes with perforated polyethylene liners. Packed fruit were immediately transported to MCAREC and stored at 30°F. 1-MCP (SmartFresh: AgroFresh, Spring House, PA, USA) treatment at 150ppb was carried out according to procedures provided by the manufacture in an air-tight 40M^3 room at 32°F for 24h on the second day after harvest. An electronic fan was used to circulate the air in the treating room.

1. Storage temperature

After ventilation, 1-MCP treated fruit were transferred to storage rooms at 30°F, 32°F, and 36°F. Control fruit were included in each storage temperature. After each month of cold storage, fruit IEC (internal ethylene concentration), storability (fruit firmness [FF], skin color), and ripening-related gene expression were evaluated after 1d at 68°F, and fruit ripening capacity (FF, buttery-juicy texture, SSC [soluble solid content], TA [titratable acidity]) and superficial scald were evaluated after 7 and 15d at 68°F.

2. Delayed treatment

Fruit were exposed to 1-MCP at 150ppb at 2, 3, 4-weeks-delay-after-harvest in an air-tight 40M^3 room at 32°F for 24h. After ventilation, treated fruit were transferred to a storage room at 30°F. Fruit evaluations were the same as described in 1. IEC, FAR [α-farnesene], and CTs [conjugated trienes] of control fruit were measured every week until 12 weeks at 30°F.

3. Exposure fruit with 1-MCP and ethylene simultaneously

Immediately after exposure of fruit to 1-MCP at 150ppb, a calculated amount of ethylene (300, 600, 1000ppb) was injected into the air-tight 40M^3 room at 32°F. Fruit were treated with 1-MCP and ethylene simultaneously for 24h. After ventilation, the treated fruit were transferred to a storage room at 30°F. Fruit evaluations were the same with described in 1.

4. Ethylene and temperature conditioning after storage

After each months of storage, 1-MCP treated fruit were moved to an air-tight ethylene ripening room with ethylene concentration at 100ppm at 68°F for 48h, or transferred to an ethylene-free room at 50°F for 15d. Then, fruit were transferred to 32°F for 2 weeks. Fruit evaluations were the same as described in 1.

5. Ripening compounds

Dipping 1-MCP treated fruit in solutions of abscisic acid (ABA), jasmonic acid (JC), methyl jasmonic (JA), salicylic acid (SA), RC-2…… at recommended concentrations for 1-24h following cold storage.

6. Harvest maturity

Fruit were harvested at 3 maturities: H1 = 14.5-15lbf, H2 = 13lbf, and H3 = 12.5lbf.

RESULTS AND DISCUSSION

1. Elevated storage temperatures

1.1. Storability and ethylene production during cold storage

1-MCP treated fruit that were stored at 30°F developed non-measurable IEC, therefore, maintained FF and skin color with minimum reductions for 8 months of storage. 1-MCP treated fruit that were stored at 36°F lost FF, skin green color and TA quickly after 3-4 months of storage (data not shown). 1-MCP treated fruit at 34°F maintained FF for 7 months, however, decreased skin green color after 5 months and FF after 8 months of storage (Fig. 1). There was no significant difference in the changes of FF and skin color of control and 1-MCP treated fruit during cold storage between 2011 and 2012. 1-MCP treated fruit that were stored at 34°F started to synthesize ethylene after 4-5 months of storage, accompanied with an increased expression of *PcACS1* and *PcACO1* genes (Fig. 1).

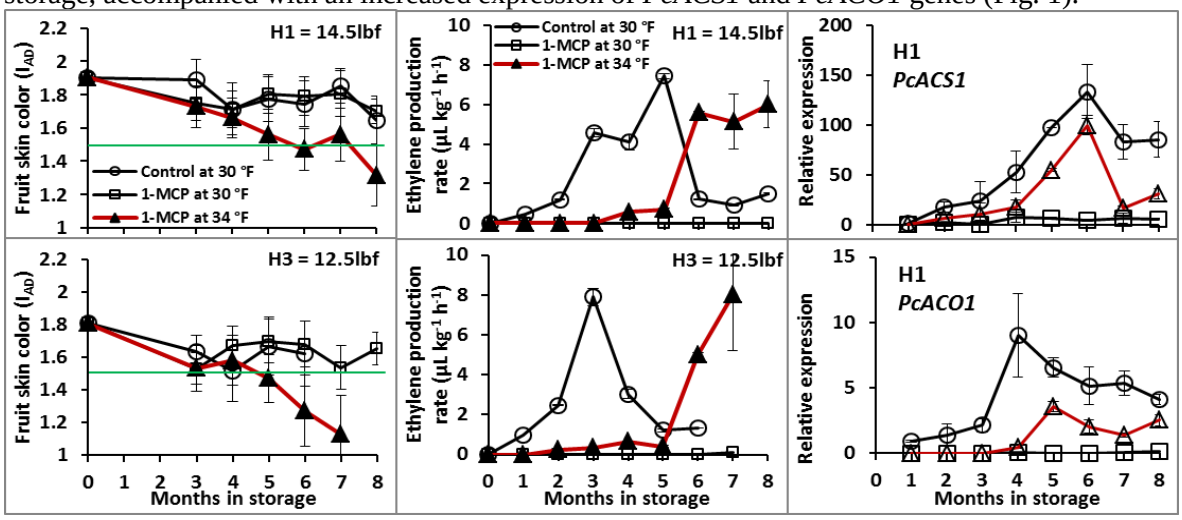


Fig.1. Effect of storage temperatures on storability of 1-MCP treated Anjou pears.

1.2. Ripening capacity and scald development at 68°F following cold storage

1-MCP treated fruit stored at 30°F did not develop ripening capacity at 68°F following 3-8 months of storage. 1-MCP treated fruit stored at 34°F did not develop ripening capacity at 68°F following 3-4 or 3-5 months of storage, but could ripen to below 4-6lbf within 7d at 68°F following 5-8 or 6-8 months of cold storage in 2011 and 2012, respectively (Fig.2). Interestingly, it took 2 and 3 months of cold storage to fulfill the chilling requirement for developing ripening capacity of the control fruit in 2011 and 2012, respectively. 1-MCP treated fruit that were stored at 34°F developed minimal severity and incidence of scald within 7d at 68°F following cold storage (Fig. 2)

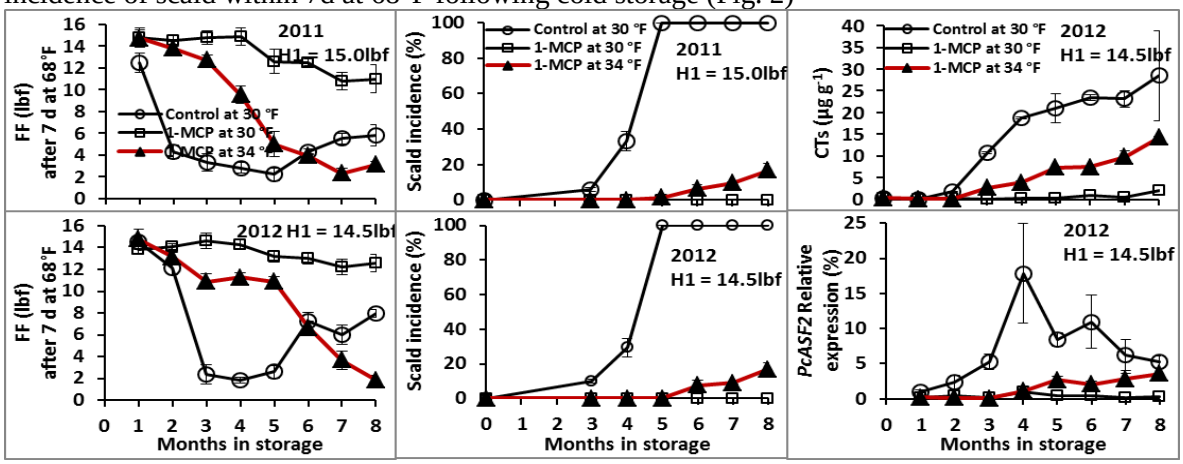


Fig. 2. Effect of storage temperatures on ripening capacity and scald incidence of 1-MCP treated Anjou pears after ripening at 68°F following cold storage.

2. Simultaneous exposure with 1-MCP + ethylene at 1:2 (150ppb:300ppb)

2.1. Storability and fruit internal ethylene concentration at 30°F

Simultaneous treatment with 1-MCP and ethylene maintained fruit firmness and skin green color for 8 months of storage at 30°F (Fig. 3). Fruit that were treated with 1-MCP + ethylene simultaneously started to develop internal ethylene after 4 months of cold storage (Fig. 3).

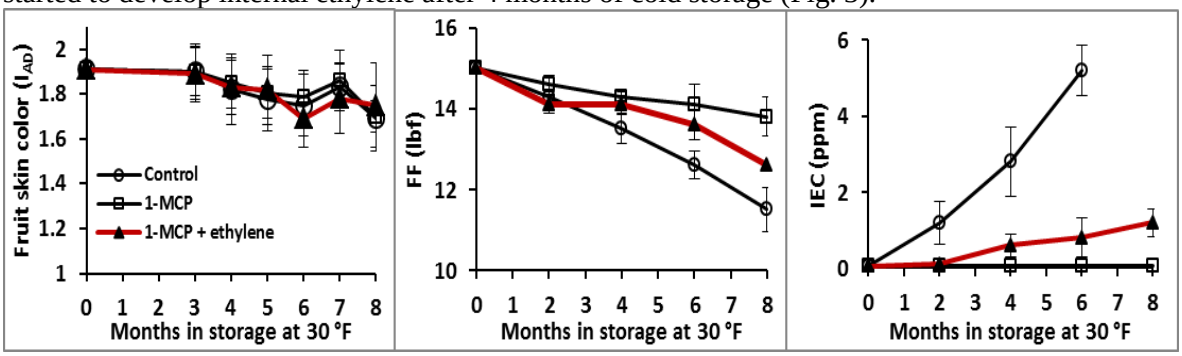


Fig. 3. Effect of simultaneous treatment with 1-MCP + ethylene on fruit skin color, FF, and IEC of 1-MCP treated Anjou pear during storage at 30°F.

2.2. Ripening capacity and scald development at 68°F following cold storage

Fruit that were treated with 1-MCP + ethylene simultaneously developed ripening capacity with minimal amount of scald within 7d at 68°F following 5-8 months of cold storage at 30°F (Fig. 4).

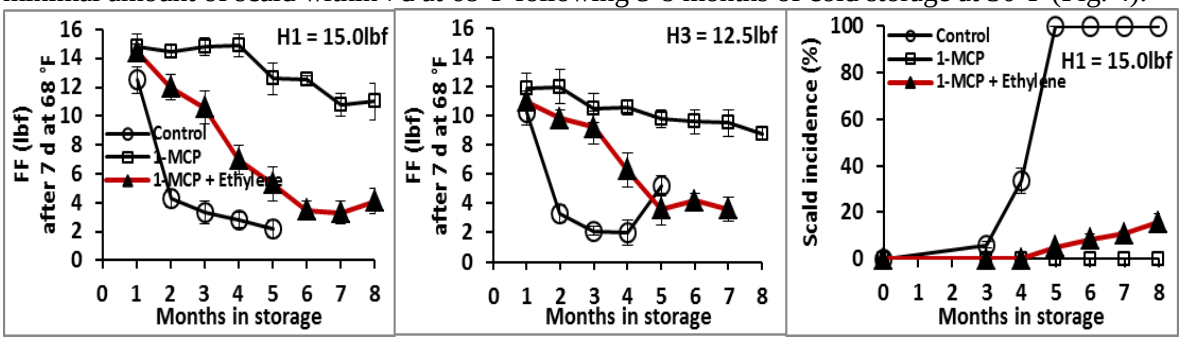


Fig. 4. Effect of simultaneous treatment with 1-MCP + ethylene on ripening and scald development of 1-MCP treated Anjou pears at 68°F following cold storage at 30°F.

3. Post-storage ethylene and temperature conditionings

3.1. Post-storage ethylene conditioning

Post-storage ethylene conditioning could ripen 1-MCP treated fruit that were stored at 30°F following 7-8 and 8 months of cold storage in 2011 and 2012, respectively. The post-storage conditioning could ripen 1-MCP treated fruit that were stored at 34°F following 4-5 or 4-6 months of cold storage in 2011 and 2012, respectively (Fig. 5).

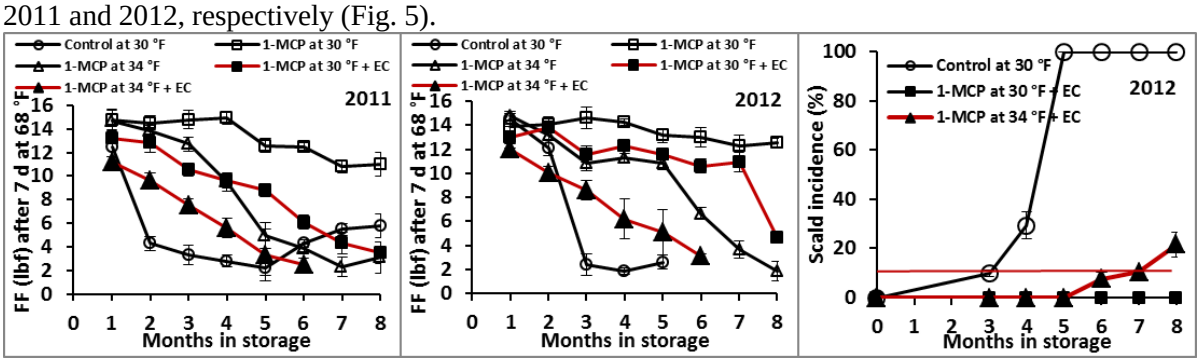


Fig. 5. Effect post-storage ethylene conditioning on ripening capacity and scald development of 1-MCP treated Anjou pears that were stored at 30°F and 34°F following cold storage.

The post-storage ethylene conditioning at 100ppm for 2d at 68°F significantly reduced FF and skin green color of 1-MCP treated fruit that were stored at 34°F, especially after 6 months of cold storage. FF was reduced below 10lbf after the ethylene conditioning follow 6 months of cold storage (Fig. 6).

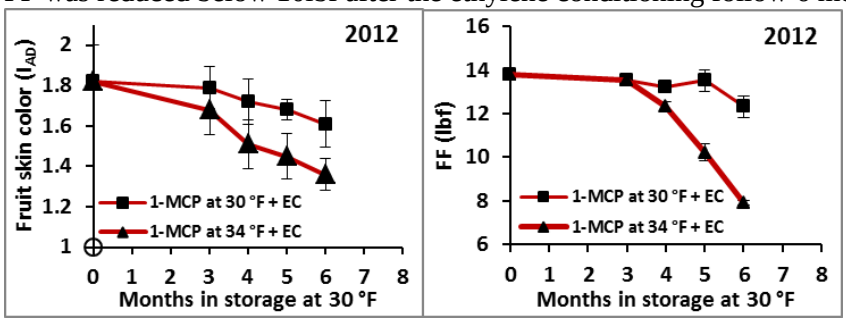


Fig. 6. Effect of post-storage ethylene conditioning on fruit skin green color and fruit firmness (FF) of 1-MCP treated Anjou fruit that were stored at 30°F and 34°F following cold storage.

3.2. Ripening compounds

All the ripening compounds tested could not ripen the 1-MCP treated fruit that were stored at 30°F for 3 or 5 months (Fig. 7).

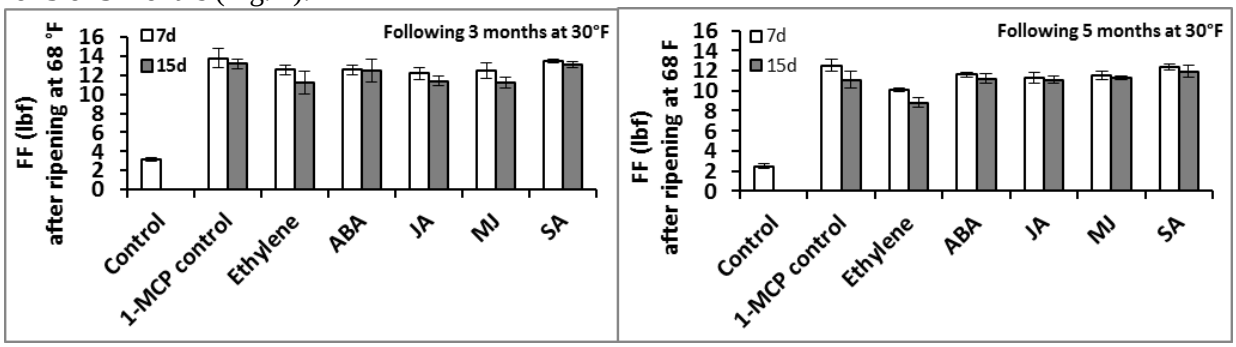


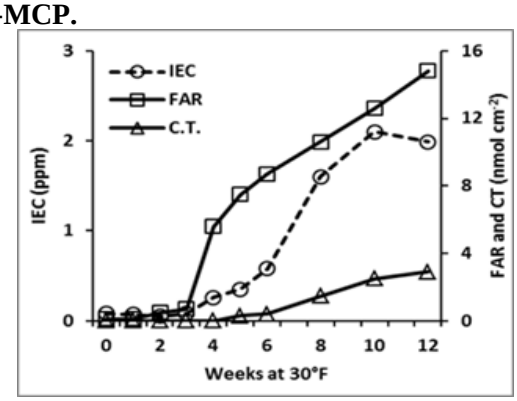
Fig. 7. Effect of ripening compounds: abscisic acid (ABA), jasmonic acid (JA), methyl jasmonate (MJ), salicylic acid (SA) on ripening capacity of 1-MCP treated Anjou that were stored for 3 or 5 months at 30°F.

3.3. Post-storage temperature conditioning

Post-storage temperature conditioning (for 15d at 50°F) could ripen 1-MCP treated fruit that were stored at 30°F following 7-8 and 8 months of cold storage in 2011 and 2012, respectively (data not shown). However, fruit skin green color lost significantly after the temperature conditioning, especially following 6-8 months of cold storage.

4. Dynamics of IEC, FAR, and CTs in fruit peels after harvest and effect of delayed treatments after harvest on the responsiveness of Anjou pear to 1-MCP.

The oxidation products (CTs) of FAR damage the hypodermal tissue of fruit and cause superficial scald of pear and apple. Ethylene enhances FAR synthesis. 1-MCP controls scald of d'Anjou pears by inhibiting ethylene production, therefore, reducing productions of FAR and its oxidation products, CTs. Within the initial two months of cold storage at 30°F, Anjou pears developed IEC, FAR and CTs in a dynamic manner. IEC and FAR were determined to increase significantly after 3 weeks and CTs started to increase after 6 weeks.



In the year-1research of this project, we arbitrarily set two delayed treatments: 2-weeks-delay and 4-weeks-delay. Results indicated that fruit treated with 1-MCP at 2-weeks-delay did not develop ripening capacity during a period of 15d at 68°F following 3-8 months of storage at 30°F. In contrast, fruit treated with 1-MCP at 4-weeks-delay did not control superficial scald during ripening following 4-8 months of storage. In the year-2 research, we treated Anjou with 1-MCP at 2-weeks-delay, 3-weeks-delay, and 4-weeks-delay after harvest and stored them at 30°F. Results indicated that fruit treated at 2 or 3-weeks-delay did not develop ripening capacity following 3-8 months of cold storage, in contrast, fruit treated at 4-weeks-delay developed unacceptable scald during ripening following cold storage (Fig. 8).

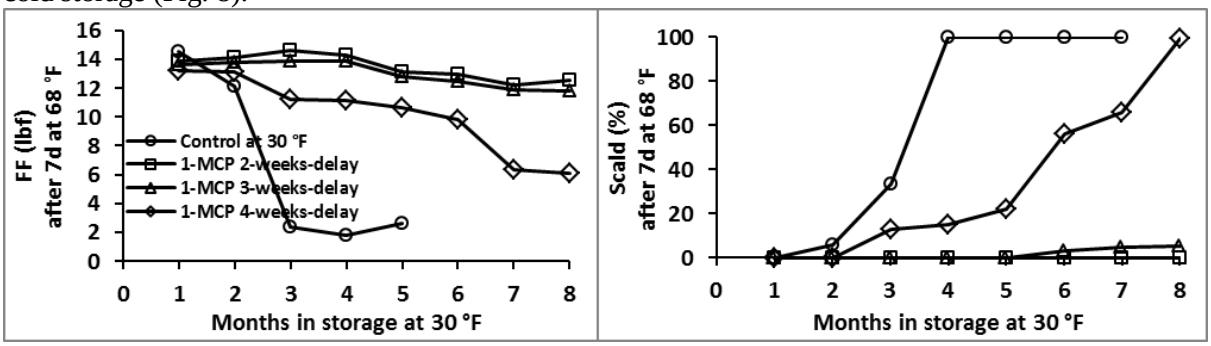


Fig. 8. Effect of delayed 1-MCP treatments after harvest on ripening capacity and scald development of 1-MCP treated Anjou following cold storage 30°F.

5. Effect of harvest maturity on storability, ripening capacity and superficial scald of 1-MCP treated Anjou pear.

There were no differences of 1-MCP efficacy on storability, ripening capacity and scald control of Anjou fruit between H1= 15lbf (early commercial harvest maturity) and H2 = 13lbf (late commercial harvest maturity). 1-MCP treated Anjou that were harvested at H3 = 12.5lbf developed ripening capacity following 7-8 months of cold storage at 30°F. However, the 1-MCP treated H3 fruit did not developed ripening capacity following 6-8 months of cold storage at 30°F in 2012. 1-MCP did not hold the green color of H3 fruit during cold storage.

LITERATURE CITED:

1. Argenta L.C., Fan, X., Mattheis, J.P. 2003. Influence of 1-methylcyclopropene on ripening, storage life and volatile production by d'Anjou cv. Pear fruit. J. Agr. Food Chem. 51:3858-3864.
2. Bai, J., Mattheis, J.P., Reed, N. 2006. Re-initiating softening ability of1-methylcyclopropene-treated 'Bartlett' and d'Anjou' pears after regular air or controlled atmosphere storage. J. Hort. Sci. Biotechnol. 81:959-964.
3. Mattheis, J.P. 2004. Manipulation of pear fruit ripening by control of ethylene action. http://jenny.tfrec.wsu.edu/wtfrc/core.php?rout=catdisp&start=151
4. Mattheis, J.P. 2007. Harvest and postharvest practices for optimum quality. http://jenny.tfrec.wsu.edu/wtfrc/core.php?rout=catdisp&start=151
5. Sugar, D. 2011. Advances in postharvest management of pears. Acta Hort. 909: 673-678.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-12-108

Project Title: Physiological genomics of 1-MCP use in pear

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Organization: Washington State University
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Address: PO Box 646414
City/State/Zip: Pullman, WA 99164-6414

Cooperators: Bob Gix, Blue Star Growers; A. Nathan Reed, AgroFresh, Inc.

Total Project Request: **Year 1:** $11,000 **Year 2:** $43,392 **Year 3: $44,788**

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

Item	2012	2013	2014
Salaries[1]		32736	34045
Benefits		2156	2243
Wages			
Benefits			
Equipment			
Supplies[2]	8500	7500	7500
Travel[3]	2500	1000	1000
Miscellaneous			
Plot Fees			
Total	$11,000	$43,392	**$44,788**

Footnotes: [1] Salaries for agricultural research assistant for performing physiological and genomic profiling and all molecular work. The increase in salaries for year three reflects a 4 % rate increase.
[2] Supplies include funding for ethylene gas, compressed air, two proprietary chemistries, fruit sampling, RNA isolation products, quantitative reverse transcription PCR and consumables.
[3]Travel includes funding for fruit retrieval at BlueStar and AgroFresh.

OBJECTIVES
We aim to gain an understanding of pear genetic responses to 1-MCP treatment, and test approaches to induce optimal fruit quality in response to 1-MCP treatment through the following objectives.

1. (Years 1 and 2) Measure activity of genes responsive to cold-treatment, 1-MCP exposure, and the putative ripening-regulatory gene.

We have identified ripening-regulatory genes, and have utilized two ripening-stimulating compounds (RCs) to chemically stimulate ripening activity. We have used 1-MCP(SmartFresh)-treated and untreated Bartlett and Anjou fruit harvested at commercial maturity, then placed in typical commercial storage conditions at Blue Star Growers. 1-MCP was applied to Bartlett and Anjou fruit at current industry standards (300 and 100 ppb, respectively). Early and late-season Anjou fruit were used in RC trials for the 2013-2014 season. Fruit were held at 40°F at Blue Star Growers until retrieved, and used in RC experiments. Fruit firmness and soluble solids were measured from multiple 1-MCP-treated and untreated Bartlett and Anjou fruit for each RC treatment, and subsequently incubated for 5-6 days at 66°F in 6.0 liter chambers of a flow-through respiration chamber in Pullman, WA. Peel tissue was collected from several replicate fruit of each unique variety-treatment combination after RC exposure and incubation.

2. (Years 2 and 3) Correlate ripening-regulatory gene pathway activity, fruit ripening phenotypes, and chemical approaches to address controlled induction of pear ripening in 1-MCP treated fruit.

To build on initial findings, we have tested four additional proposed RCs (in addition to RCs 1 and 2) on Bartlett and Anjou fruit from the 2013-2014 harvest. We will conduct tests with two additional RCs with remaining experimental fruit in our inventory through February of 2014. Additionally, we will conclude testing of RC combinations to determine if ethylene production, fruit softness, and soluble solids can be further enhanced. This work will be concluded by March, 2014. Physiological data was collected for additional RC experiments as described above. Upon completion of all RC experiments in SmartFresh-treated Anjou and Bartlett fruit, data from years 1 and 2 of the project will be collated. Statistical analysis will be applied to identify additional significant ethylene and ripening responses caused by RCs beyond RC1 and RC2. This work will be completed by April, 2014.

Genetic material (RNA) from peel tissues was obtained in RC1 and RC2-experiments in Bartlett and Anjou for quantitative gene expression analysis during February - April, 2014. This approach will allow for the capture of gene expression intensity across the pear genome. We will compare this intensity on a gene-by-gene basis to develop a genetic context for the ripening chemistry. Finally, we will be able to develop a detailed model addressing the genetic variability in pear ripening in response to 1-MCP application. We will complete full gene expression profiling and model development of 1-MCP induced ripening variability in RC1 and RC2-treated Bartlett and Anjou fruit by September - December, 2014.

SIGNIFICANT FINDINGS (Objectives 1 and 2)

- We have identified a unique ripening-related regulatory pathway in 1-MCP-treated, unconditioned Bartlett and Anjou fruit.
- We are nearing completion of the initial testing for 8 proposed RCs, thought to selectively stimulate activity of this regulatory pathway in unconditioned and untreated Bartlett and Anjou fruit.

- We have demonstrated successful stimulation of ripening in 1-MCP treated fruit, and unconditioned fruit that had not received sufficient chilling. Responses are seen in both varieties in a dose-dependent manner, allowing reliable timed induction of ripening.
- RC1 may pose significant challenges for implementation in postharvest chains, and will be further pursued for gene expression-based studies only, in accordance with the final year objectives of this project.

Methods

Sample procurement, 1-MCP treatment, tissue collection: For this study, mature pear fruit treated with 300 and 100 ppb (Bartlett and Anjou, respectively) 1-MCP and untreated control fruit were obtained from Blue Star Growers in Cashmere, WA. Testing of experimental RCs on whole pear fruit were all done following the protocols outlined below.

Ripening-inducing chemical treatments, ripening phenotypes: Unconditioned 1-MCP-treated and untreated Bartlett fruit were exposed to RCs for 24 hours. Unconditioned 1-MCP-treated and untreated Anjou fruit were exposed to RC's for 12 and 24 hours and the ripening response measured by carbon dioxide and ethylene evolution determined by gas chromatography. All fruit were submerged in 8.0 liter aqueous solutions of one of four levels of each experimental chemical (high concentration, middle, low, and a control). Each RC solution was then covered during the exposure time with plastic sheeting to minimize evaporative water loss. Submerged fruit were incubated at room temperature (68°F) for 12 hours (Anjou) and 24 hours (Bartlett and Anjou). Following drenching, fruit were removed from solutions, dried with towels and placed 6.0L flow-through respiration chamber with a 100 mL/min dynamic flow rate. Four fruit of each unique treatment combination were placed into individual chambers. Fruit incubated in these chambers for 5-6 days at 66°F, during which headspace carbon dioxide and ethylene was measured. Gas sampling was performed in 8 hour intervals via gas chromatography. Flesh firmness, soluble solids measurements and peel tissue were obtained from a subsample of fruit from each unique 1-MCP treatment-variety-RC treatment combination after the 24 hour soak, and subsequent 5-6 day incubation. Additionally, peel samples were isolated and immediately frozen in liquid nitrogen to assess ripening-regulatory and ethylene-related gene expression in response to the RC treatments.

A

RC	Conc	*"Low"*	*"Middle"*	*"High"*
2	0	0.25	0.5	2.5
6	0.0% (v/v)	0.25% (v/v)	0.5% (v/v)	2.5% (v/v)

B

RC	Conc	*"Low"*	*"Middle"*	*"High"*
2	0	0.25	0.5	2.5
6	0.0% (v/v)	0.10% (v/v)	0.75% (v.v)	1.0% (v/v)

C

RC	Conc	*"Low"*	*"Middle"*	*"High"*
2	0	0.25	0.5	2.5
7	0	0.1	0.25	0.5

D

RC	Conc	*"Low"*	*"Middle"*	*"High"*
2	0	0.25	0.5	2.5
7	0	0.25	0.5	2.5

The methods described above will be followed for the remainder of the project. By March of 2014, we will complete testing of ethylene and ripening responses from RC 6 and 7 from the proposed list. We are completing tests with combinations of RC treatments this year (Figure 1), using RC2, and additional compounds. Varying ratios of RC2 and one additional compound will be completed by March, 2014. We hypothesize these combinations will further accelerate activity of genes repressed by 1-MCP treatment, and enhancing the observed ethylene response from exposure to RCs 1 and 2.

Figure 1. RC combination tests to be completed by March, 2014.

Gene expression analysis: Genetic material (RNA) from actively expressed genes in peel samples collected from 1-MCP-treated and untreated Bartlett and Anjou fruit exposed to RC's 1 and 2 is being extracted. This material will be measured for integrity, and prepared for high-throughput quantitative gene expression profiling using RNA sequencing. This technique will allow for study of expression of the proposed ripening-regulatory genes along with those of ethylene perception, production and fruit quality-related genes. This approach captures intensity of all genes expressed in a given tissue, under specific experimental conditions. It generates large

quantities of data, from an expansive list of gene targets, in a relatively short amount of time. Expression of these genes will be correlated to expression of both the proposed ripening master-switch gene previously identified in our lab, and the genes being stimulated by the RCs. This procedure is now a routinely-used laboratory procedure and is currently being applied toward other pear-ripening related projects. Using this method, we can directly compare intensity of gene expression between 1-MCP and untreated Bartlett and Anjou pears during storage and ripening. Additionally, this approach allows for advanced modeling techniques to be applied. Development of correlation between expression of genes can resolve complex regulatory relationships. We are completing this modeling approach now on this and additional gene expression-based work.

Results and Discussion

Significant costs are incurred in the production and transport of fresh pear to market. Maintenance of fruit quality is essential to minimize losses through the postharvest chain. In response, tools to gain greater control over ripening have been desired in the pear industry. Variable ripening of fruit in the postharvest chain can quickly lead to loss in quality. Losses can be compounded as unmarketable or marginal fruit or are outright rejected by consumers at points of retail sale. As a result, the pear industry has begun to adopt use of 1-MCP (applied as 'SmartFresh') throughout the storage and transport chain. 1-MCP inhibits ethylene responses by competing for the ethylene binding site in fruits, preventing downstream gene activity. SmartFresh and similar 1-MCP products have been used for some time in apple and other fruit industries with success. However, pear exhibit unique responses to 1-MCP application. Unlike other fruits, 1-MCP application in pear can result in variable rates of recovery and ripening. Fruit can also remain in a permanent, undesirable state of ripening inhibition (Bai et al., 2006; Chen and Spotts, 2005; Rudell et al., 2005; Chiriboga et al., 2011). Treated fruit can fail to ripen, soften, and develop flavor and aroma profiles (Argenta et al., 2003; Bai and Chen, 2005; Villalobos-Acuña et al. 2011). This introduces further potential for loss into the fresh pear market as fruit can remain in a 'locked' state.

Much of modern understanding of climacteric ripening comes from apple and tomato-based models. These systems not only do not require conditioning treatments to elicit ethylene production and ripening, but recover from 1-MCP treatments in regular, repeatable intervals (Watkins, 2006). Much of the modern postharvest storage and handling practices in fresh pear are derived from the work of Sugar et al. (2009). Use of ethylene is now commonly used to accelerate, but not replace, the chilling requirement of pear. Such capacity to eliminate chilling does not yet exist at the genetic or molecular level in pear. Some surveys of ethylene-related genes in 1-MCP-treated pear fruit in ripening conditions has been performed, but this information does not address causality of challenging pear ripening phenotypes. Considering this knowledge gap, there exists little to no gene-level understanding of why pears are unable to uniformly recover from application. The unique responses to 1-MCP in pear illustrate the limitations that exist in purely translational approaches from systems lacking pear's unique biology (Fischer, 1991; Lay-Yee et al., 1990). A broad and detailed analysis of ethylene-related and interacting genes can allow for detailed mechanistic context of this unique biology to be developed. The resulting model of recovery and ripening in 1-MCP-treated pear can provide a foundation for enhanced postharvest management protocols to be developed.

Initial work on this project identified a ripening-related genetic pathway which was hypothesized to enhance ethylene production, and overall ripening when stimulated. In response, we developed a set of proposed ripening-stimulating compounds (RCs). After exposure to RCs 1 and 2, 1-MCP-treated and untreated Bartlett and Anjou fruit exhibited significant increases in ethylene and carbon dioxide production rates, exemplified in Figure 2. While less drastic, ethylene ripening responses in Anjou agreed with expectations as it is more recalcitrant in induction of full ripening (compared to Bartlett). The work in year 2 of this project has tested 3 additional RCs among Bartlett and Anjou fruit. Overall, responses were not as strong as seen in those of RCs 1 and 2 of the prior year of the project. An included repetition of RC2-treatments again yielded increases in ethylene production in excess of 2-fold after exposure after 24 hour submergence (Figure 3). Initial data from the "A" combination scheme did not show a significant increase in carbon dioxide production. Interestingly,

the "High" RC2 and RC6 combination did result significantly enhanced ethylene production rates over untreated control Anjou fruit (Figure 4).

We have found ethylene responses to be greater in fruit of both varieties which underwent longer storage treatment. Generally, responses were also greater in Bartlett than Anjou, and fruit not exposed to 1-MCP. These trends agree with expectations. Based on these findings, we are advancing with quantitative gene expression analysis from tissues exposed to RCs 1 and 2 to resolve the gene-level underpinnings of this response. We will be using a technique that allows for faster, broader and more detailed capture of gene expression information from these tissues.

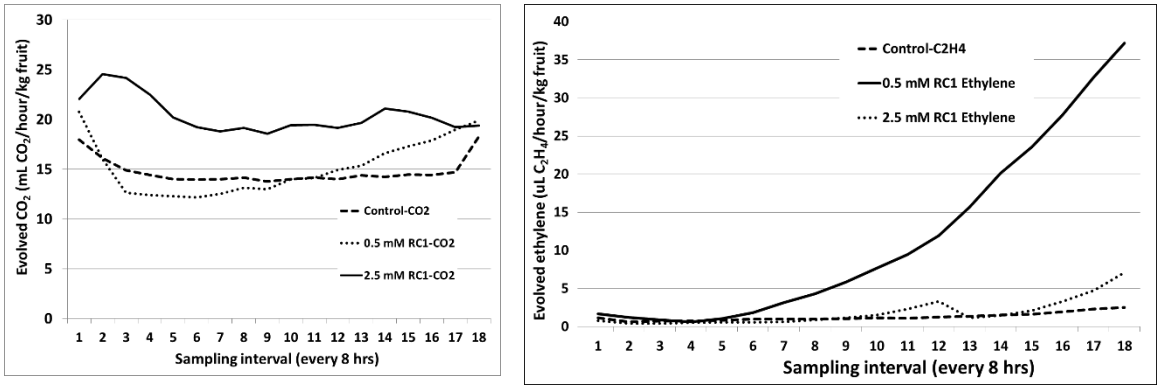


Figure 2. Carbon dioxide (left) and ethylene (right) production from RC1 exposure from year 1 of the project.

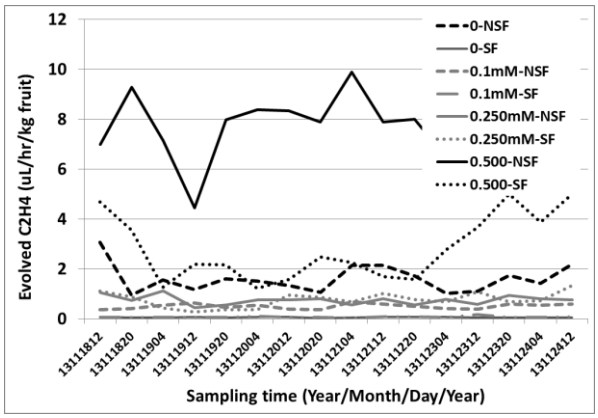


Figure 3. Ethylene production from repeated 24 hour RC2 exposed Anjou fruit. Results are similar to data obtained in year 1 of this project.

Data of RCs 3,4,5 and 6 indicate lower ripening and ethylene release rate increases relative to those obtained from RCs 1 and 2. This is exemplifed by carbon dioxide and ethylene-response to RC4, shown in Figure 5. These compounds also presented significant challenges for use in a postharvest chain and experimentation by leaving significant tissue damage, a residual odor, and disruption of our gas chromatography-column. These products remain to be evaluated in Anjou. Repeated RC1 and RC2 treatments to Bartlett and Anjou fruit demonstrate similar results to that from the 2012-2013 harvest season. Early season Bartlett fruit exposed to RCs 1 and 2 exhibit slightly reduced responses, suggesting maturity at harvest may influence the strength of RC1 and 2 response. Similar to data from year 1, ripening appears to progress over a longer time frame in both Bartlett and Anjou fruit in response to RC2 exposure. Similar results have been seen with RC2, which appears to induce its ripening-

stimulation effects over a longer time frame. Considering this and data from the 4 hour exposure, the penetration and mobility of the RCs toward the interior of the fruit to significantly influence ethylene and ripening responses.

Future research and Conclusions:

As a whole, our research from years 1 and 2 of this project have demonstrated environmental, temporal and genotypic factors involved in the acceleration of ethylene production and ripening rates in pear tissue. We will use the gene expression data to develop gene correlation networks to develop a detailed understanding of this novel mechanism of increasing ethylene release in pre-climacteric, unconditioned, 1-MCP-treated pear. This model will address the causal factors affecting ethylene and ripening responses from RC treatments. To advance understanding of these responses in RC2, we have initiated collaboration with Dr. Yan Wang at OSU-MCAREC to replicate initial findings. Years 1 and 2 of the project suggest RC2 may serve as a useful companion product to the 1-MCP product SmartFresh in the pear industry, providing a comprehensive tool set to manage fruit quality through the postharvest chain although its application needs to be standardized in a commercial context. Further testing is essential to advance our knowledge of RC responses. Upcoming gene expression analysis and subsequent modeling work should provide a biological context and understanding to this novel mechanism of ethylene-stimulation. Collaborative work with partners will accelerate the foundational knowledge needed for commercial deployment of this technology.

Data from year 2 of this project has added to our physiological understanding of RC-induced ethylene and ripening responses in fresh pear. Objectives of year 3 will provide a gene-level understanding of this novel mechanism, and will be used for publication of data. Additional work will seek to enhance fruit ripening responses to RCs 7 and 8 and enhance RC2 responses with combination treatments.

As the project evolves we continue to build upon the knowledge needed to effectively use RCs in a postharvest chain. We expect this work to further increase ethylene and ripening stimulation seen in data from years 1 and 2 of the project. Upon completion we expect to provide optimized protocols for maintenance of superior fruit quality in storage and transit operations of the pear industry. This will finally allow synchronization of ripening in a variety and time-specific manner, which remain goals of the pear industry (Ing et al., 2002).

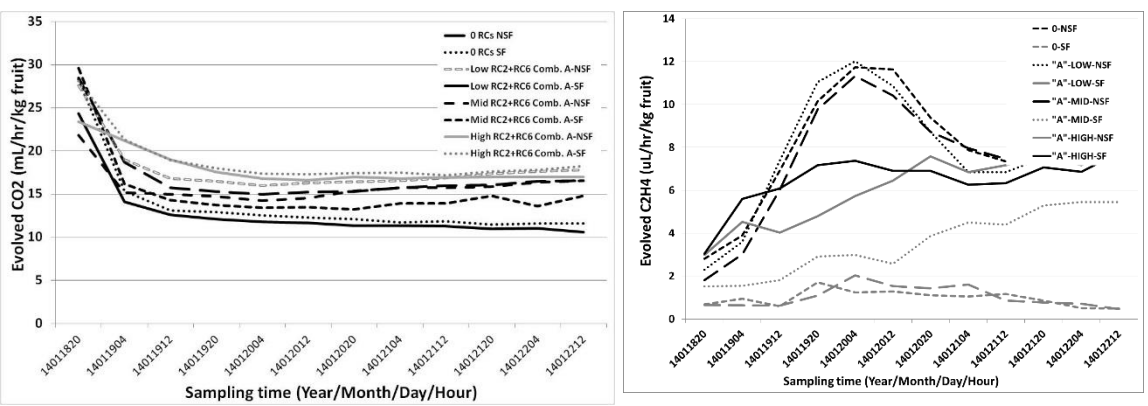
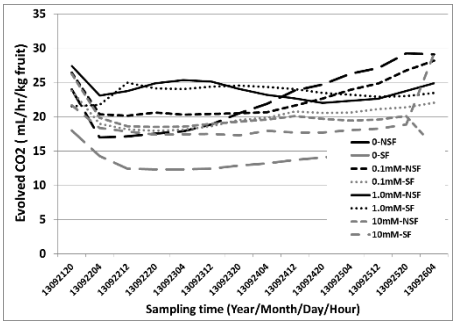


Figure 4. Carbon dioxide (left) and ethylene production (right) from initial combination of RC2 and RC6 exposure to Anjou. The High treatment to SmartFresh-treated and untreated fruit produced a significant increase in ethylene production. Similar results were not seen in carbon dioxide data.



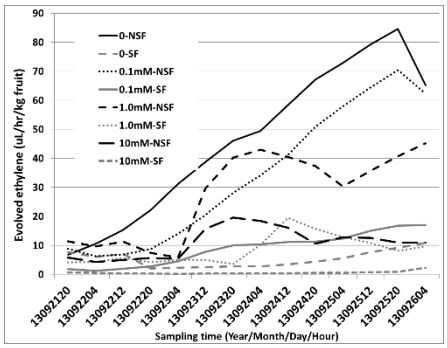


Figure 5. Carbon dioxide (top) and ethylene production (bottom) from RC4 exposure in SmartFresh-treated and untreated Bartlett fruit. Results clearly indicate no significant increases in ethylene or carbon dioxide production over untreated controls. This exemplifies similar responses from RCs 3, 5, and 6.

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CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: PR-13-106

Project Title: Miticide resistance in spider mite pests of pears

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Collaborators: None

Total Project Request: **Year 1:** $23,696 **Year 2:** **$24,614**

Other funding sources: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509-335-4564/509-663-8181 x221 **Email:** carriej@wsu.edu/joni.cartwright.wsu.edu

Item	2013	2014
Salaries[1]	12,000	12,480
Benefits	4,666	4.853
Wages[2]	5,720	5,949
Benefits	555	577
Equipment	0	0
Supplies[3]	500	500
Travel[4]	255	255
Miscellaneous	0	0
Plot Fees	0	0
Total	$23,696	**$24,614**

Footnotes:
[1] Research Intern
[2] Temporary employee
[3] Publications
[4] 500 miles per year @$0.51 per mile

Objectives
1. Survey resistance status of spider mite populations on pear to key miticides.
2. Examine population genetics of resistance in spider mites.
3. Develop recommendations for effective control of spider mites and a resistance management plan.

Significant Findings
- Resistance ratios (LC_{50}[R]/LC_{50}[S]) for FujiMite ranged from 22-88x.
- The LC_{99} for the RR of 22 was 128 mg AI/liter, equivalent to 34 fl oz/acre (max label is 32 fl oz).
- Resistance ratios for Agri-Mek ranged from 370,788 – 1,592,381x.
- To achieve 99% mortality with Agri-Mek SC required 5.3 gal/acre (max label is 4.25 fl oz).

Methods
1. Survey resistance status of spider mite populations on pear to key miticides.
Our first objective is to characterize levels of resistance in spider mite populations in pear orchards of Washington State. This is essential for understanding which miticides offer the most potential for sustainable and effective mite control in the short and long-term, and for identifying which miticides are least likely to be effective due to resistance. Screening populations for resistance will also allow us to explore whether failures of miticides to provide control in certain orchards is due to a genetically-based decrease in susceptibility rather than other factors such as inadequate coverage or poor timing of miticide applications. Finally, screening populations from various locations of Washington State will aid in identifying the extent of resistance to key miticides. Such information will be useful for developing targeted miticide resistance management strategies for various regions of Washington State.


Potter spray tower

To assess resistance in spider mite populations, populations of twospotted spider mites will be collected from pear orchards experiencing mite outbreaks, with emphasis on those that have already applied one or more miticides. We will collect a total of eight populations from throughout Washington State, focusing on orchards in the major pear-growing districts, with emphasis on those that are experiencing problems with mite control. The mites will be transferred to bean plants, and kept in a colony in growth rooms, taking care to isolate the populations. After densities have built to a sufficient level in the colony, the population will be screened with commonly used tree fruit miticides (see list), including one representative from each IRAC Mode of Action group (MOA).

Bioassays will be conducted by exposing individuals from each field-collected mite colony, and a susceptible reference colony that has not been exposed to miticides, to a series of miticide rates. The rates tested will be selected using historic data, or where none exist, an initial screening with 0.1x, 1x, 10x and 100x the field rate will be used. Once an appropriate rate range is established, a dose-response curve will be developed using five-six rates (plus an untreated check), with five replicates at each rate. All bioassays will be conducted on bean leaf disks (3 cm diam) with the lower surface facing up in a plastic portion cup with cotton and water. Adulticide bioassays will use 20 adult female mites/disk and be evaluated after 48 h. For ovicidal bioassays, 10 adult females will be transferred to the disks and allowed to lay eggs for 24 h (resulting in about 50 eggs/disk). Eggs will

be counted, and their positions marked with a felt-tip pen, and the females removed. Eggs will be held at 25° C (68 °F) in a growth room until egg hatch is complete in the check, and then evaluated for treatment mortality (unhatched eggs). These methods are essentially the same as have been used historically in collecting information on mites from Washington tree fruits, allowing for comparisons across time.

The dose-response curves will be used to calculate the LR_{50} (the rate needed to kill 50% of mites) and associated 95% confidence intervals for each field-collected mite colony and for the susceptible laboratory colony. Statistically-significant resistance will be indicated when the 95% confidence interval of the LR_{50} of a field-collected colony with a history of miticide exposure is higher and does not overlap with the 95% confidence interval of the susceptible laboratory colony. Based on these values, we will also calculate the resistance ratio (RR) for each colony by comparing the LR_{50} of the field-collected and susceptible strains. Resistance ratios > 10 typically indicate high-levels of resistance that could result in field failures of miticides. For each miticide examined, variation in resistance ratios from various locations will indicate which areas of Washington State face the greatest threat of miticide resistance. Both Beers and Crowder have extensive experience conducting bioassays on this scale; however, the common pitfalls are weather conditions in a given year that do not promote mite populations and contamination of colonies with predators. Our work will be conducted over two years to minimize the potentially confounding effects of weather or predators in any single year.

List of Miticides for bioassays of twospotted spider mite

Trade name	Common name	Group	MOA	bioassay type
Agri-Mek	Abamectin	avermectins	6	adulticide
Acramite	bifenazate		unknown	adulticide
FujiMite	fenpyroximate	METI	21A	adulticide
Envidor	spirodiclofen	tetronic/tetramic acid derivatives	23	ovicide
Onager	hexythiazox	mite growth inhibitors	10A	ovicide
Zeal	etoxazole	mite growth inhibitors	10B	ovicide

2. *Examine population genetics of resistance in spider mites.*
Understanding the population genetics of resistance is essential for developing miticide resistance management programs. Resistance is expected to evolve more rapidly in natural populations when genetic mutations conferring resistance provide large fitness advantages for individuals with these mutations compared to individuals without them. Additionally, resistance is expected to evolve more rapidly when it is inherited from parents to offspring as a dominant genetic trait. For example, if resistance is controlled by a single locus with two alleles (S for susceptibility and R for resistance), then susceptible individuals have genotype SS, resistant individuals have genotype RR, and heterozygous individuals have genotype SR. When resistance is dominant, heterozygous SR individuals have the same survival as resistant RR individuals, and the rate of resistance evolution is expected to be very rapid (i.e., within a few generations if miticides are intensively used). However, when heterozygous SR individuals have the same survival as susceptible SS individuals, resistance is recessive and resistant management strategies are more likely to be effective. Both the selective advantage provided by resistant mutations, and the degree of dominance, can vary widely between populations and for different miticides. Additionally, both factors can be affected by the rate of miticides used. Thus, understanding relationships between miticides and genetics of resistance can provide the basis for developing resistance management strategies. Fortunately, both factors can be characterized relatively simply using bioassays of natural populations.

For a subset of the field-collected resistant mite colonies (up to four), and for each miticide tested, the selective advantage provided by resistance alleles will be determined using the bioassays described in Objective 1, by comparing the LR_{50}s of field-collected resistant colonies to the susceptible colonies. To determine the degree of dominance in resistant mite colonies, reciprocal crosses will be conducted between resistant and susceptible mite colonies. For this objective, we will only test colonies that have significant resistance (as identified in Obj. 1) in order to maximize our use of resources. For each colony identified as resistant, reciprocal crosses will be conducted by allowing virgin females (susceptible or resistant) to mate with males of the alternative colony for 3 days. Heterozygous female offspring from these matings will be reared to adulthood and then subjected to bioassays. Bioassays will be conducted as in objective 1 with adult heterozygous females (or eggs from the reciprocal crosses), with individuals from the field-collected and susceptible colonies tested simultaneously. For each colony, miticide, and rate tested, we will estimate the degree of dominance by comparing survival of the heterozygous females to mortality of females from the resistant and susceptible colonies. For each colony, miticide, rate combination, and the degree of dominance (*h*) will be calculated as follows:

$$h = \frac{\text{(Survival of heterozygous females – survival of susceptible females)}}{\text{(Survival of resistant females – survival of susceptible females)}}$$

Values of *h* vary from 0 to 1, with 0 indicating recessive resistance (i.e., heterozygous and susceptible individuals have the same survival) to 1 indicating dominant resistance (i.e., heterozygous and resistant individuals have the same survival). Values of *h* can vary with miticide rate and, therefore, for each colony we will test 5-6 rates with each miticide tested.

Through this objective we will determine how dominance of resistance varies across field-collected colonies with varying rates of different miticides. Our goal is to identify rates of particular miticides that effectively control mites while also promoting recessive resistance, which will be used to inform development of a resistance management plan.

3. Develop recommendations for effective control of spider mites and a resistance management plan. Data from Objectives 1 and 2 will be used to develop more effective resistance management plans for miticides in Washington State pear orchards. As described above, resistance to miticides is expected to evolve more rapidly when resistant colonies have significantly greater survival when exposed to miticides compared to susceptible colonies (estimated in Obj. 1), and when resistance is inherited as a dominant trait (estimated in Obj. 2). To predict the rates at which resistance will evolve, data from our bioassays will be incorporated into a population genetics model developed by PI Crowder in similar insecticide-pest systems to predict the rates of resistance evolution and under a range of management scenarios commonly used by pear growers. Using models to predict the spread of resistance to insecticides and miticides has been effective in many pest systems, and generally forms the basis for sound resistance management programs.

For each miticide, models will predict the rate of resistance evolution under various initial conditions, which will reflect typical management strategies used by pear growers. In a sensitivity analysis, we will vary three factors: 1) miticide rates, 2) the frequency of miticide application, and 3) the percentage of acreage treated with each miticide. The output of models will be the frequency of resistant individuals in populations, and will estimate how varying rates of particular miticides, and/or the frequency and intensity of applications might be modified to reduce the potential for resistance. For example, we expect to be able to predict which rates of miticides, and which rotations of

miticides, might provide the foundation for long-term miticide control while limiting the potential for resistance evolution.

Results from this objective will be disseminated to growers through talks at tree-fruit grower and consultant meetings, and in the development of an article in *Orchard Pest Management Online* (http://jenny.tfrec.wsu.edu/opm/). Details in these talks and this publication will report on the combinations of miticides, and rates of these miticides, which are expected to be most successful in controlling mite populations while limiting the potential for resistance evolution. Because data from Objectives 1 and 2 will be collected from many populations, these predictions can be targeted to growers from particular regions based on the characteristics of populations collected from those regions. PI Crowder has developed such models for other pest insects, which have effectively predicted rates of resistance evolution in the field. Thus, we expect this objective will provide the basis for an effective resistance management plan.

Results & Discussion

Four populations of twospotted spider mite were collected from pear-growing regions in eastern Washington. Two were from the Dryden area, one from east Cashmere, and one from Wapato (Table 1). Mite populations were relatively easy to find during the 2013 season. Mites collected from the orchards were placed on uninfested bean plants, and placed in four separate rooms scattered around the TFREC campus to ensure isolation. In addition to the four field-collected populations, an additional population was obtained from a long-term culture kept at the New York State Experiment Station in Geneva, NY, which serves as a second susceptible population. Where available, data from previous experiments in eastern Washington were used for a temporal comparison of resistance status.

Table 1. Sources and collection dates of four colonies of twospotted spider mites collected from pear orchards used for bioassays

Colony #	Source	Collection Date
1	East Cashmere	17 June 2013
2	Dryden	20 June 2013
3	Dryden	28 June 2013
4	Wapato	9 August 2013

Based on results from 2012, the high rate of Agri-Mek in bioassays was increased from 5 ppm to 30 ppm, a 6-fold increase. The first bioassay using colony #1 failed to produce acceptable levels of mortality after 72 h (data not shown), thus an LC_{50} could not be calculated. A non-probit bioassay using the three adulticides (Agri-Mek, FujiMite, and Acramite) was conducted to better determine the high end of the rate range needed to generate an effective dose-response curve. From this bioassay, we concluded that a much higher rate of Agri-Mek was needed to produce high levels of mortality (>3000 ppm) than had been tested previously, and 1,000 ppm would serve as a starting point for FujiMite and Acramite (Table 2).

Table 2. Mortalities of twospotted spider mites produced by high rates of three adulticidal miticides, 2013

Colony	Trt. #	Pesticide	mg AI/liter (ppm)	N	% Mortality	SEM
Col. 1	C1	Agri-Mek	3,000	5	91.10	0.98
Col. 1	C2	FujiMite	1,000	5	95.10	1.58
Col. 1	C3	Acramite	1,000	5	92.05	4.07

Lab (S)	C4	Agri-Mek	3,000	5	99.00	1.00
Col. 1	C5	Check	0	5	7.00	2.55

The Agri-Mek probit bioassay was repeated, with the high rate of 4,500 ppm. The rate range used produced useable levels of mortality at all doses, and an LC50 with 95% confidence limits (CL) was generated from probit analysis (Table 3).

Table 3. Agri-Mek probit bioassay with TSM Colony #1, Cashmere.

Miticide	Colony	ppm	N	% Mortality	SEM
Agri-Mek	Col. 1	4500	5	99.00	1.00
Agri-Mek	Col. 1	2250	5	88.95	3.98
Agri-Mek	Col. 1	1125	5	88.13	4.69
Agri-Mek	Col. 1	562.5	5	58.00	8.60
Agri-Mek	Col. 1	281.25	5	35.00	5.70
Agri-Mek	Col. 1	140.62	5	33.00	5.15
Agri-Mek	Col. 1	0	5	4.90	2.18

Slope=1.83 (±0.16); LC_{50}=389 (277.54-508.77 95%CL))

Using a higher rate range, the Agri-Mek bioassay was repeated using Colony #3 (Table 4). The high rate was lowered slightly in order to provide more data in the central part of the probit line (25-75% mortality), however, because this used a different colony, the spread of mortalities was similar to Colony #1.

Table 4. Agri-Mek probit bioassay with TSM Colony #3, Dryden

Pesticide	Colony	ppm	N	% Mortality	SEM
Agri-Mek	Col. 3	3,000	5	99.00	1.00
Agri-Mek	Col. 3	1,500	5	45.48	4.89
Agri-Mek	Col. 3	750	5	26.00	4.30
Agri-Mek	Col. 3	375	5	35.00	10.37
Agri-Mek	Col. 3	188	5	16.00	4.00
Agri-Mek	Col. 3	94	5	5.00	1.58
Agri-Mek	Col. 3	0	5	8.00	2.55

Slope=8.77 (±1.46); LC_{50}=1,672 (1369-1998 95%CL)

This bioassay was repeated with a slightly different set of rates (data not shown), and produced an LC_{50} more similar to that in Table 3 (Slope 1.39 (±0.20); LC50 459 (147-883 95%CL).

Bioassays were also performed with FujiMite (Table 5) and Acramite (Table 6). Despite the preliminary test indicating that 1,000 ppm would be a suitable high dose, 100% mortality occurred at the three highest doses. However, an LC_{50} with confidence limits was obtained. Conversely, the Acramite bioassay produced slightly lower than expected mortalities, although still providing an LC_{50} with confidence limits.

Table 5. Probit bioassay with FujiMite, Colony #1, Cashmere.

Pesticide	Colony	mg AI/liter	N	% Mortality	SEM
FujiMite	Col. 1	1,000	5	100	0
FujiMite	Col. 1	500	5	100	0
FujiMite	Col. 1	250	5	100	0
FujiMite	Col. 1	125	5	98.05	1.2
FujiMite	Col. 1	62.5	5	94.05	1.88
FujiMite	Col. 1	31.25	5	69.19	7.04
FujiMite	Col. 1	0	5	2	1.22

Slope 2.95 (±0.49); LC_{50}=20.8 (13.7-26.3 95%CL)

Table 6. Probit bioassay with Acramite, Colony #1, Cashmere

Pesticide	Colony	mg AI/liter	N	% Mortality	SEM
Acramite	Col. #1	1000	5	74.29	2.92
Acramite	Col. #1	500	5	32.08	4.26
Acramite	Col. #1	250	5	13.83	4.27
Acramite	Col. #1	125	5	4.74	2.10
Acramite	Col. #1	62.5	4	10.08	3.07
Acramite	Col. #1	31.5	5	4.05	1.88
Acramite	Col. #1	0	5	4.00	1.87

Slope=3.5 (±0.48); LC50=687 (600-790 95%CL)

In addition to the adulticide bioassays shown here, two preliminary tests were done for the ovicidal bioassays. The first was an egg hatch curve in the controlled temperature room, which indicated that an interval of 10 days should be sufficient for optimal egg hatch in the checks. The second was a test of the high rates of the three ovicides (Envidor, Onager, and Zeal,), which indicated that a maximum dose of 300, 300, and 100 ppm, respectively, caused near 100% mortality, and would serve as a starting point for the bioassays.

Discussion: The resistance ratios (LC_{50}[R]/LC_{50}[S]) for FujiMite for TSM populations from pear were 88 and 22 when compared to the most susceptible population found in a 2011 resistance survey for this miticide. The LC_{99} generated from the latter bioassay was 128 ppm, which is equivalent to 34 fl oz/acre at 100 gpa for this material. For reference, the maximum rate for this material is 32 fl oz/acre. These RR are indicative that TSM populations from pear are gaining extremely high levels of resistance. The situation with Agri-Mek is far more severe, with calculated RR ranging from 370,788-1,592,788 in comparison to LC_{50}s found in 1989, early during the use period in eastern Washington. While bioassays are generally used to delineate changes in susceptibility, they also present a 'best-case' scenario for mortality – 100% exposure of all mites at a specified dosage. These data predict field failure for TSM is probable in some pear orchards, and Agri-Mek should only be used for other target pests (pear psylla and rust mites).

Plans for 2014-2015: Challenges with growing uninfested beans and rearing mites slowed progress in 2013. We plan to finish the bioassays on the current populations by June of 2014, and begin field collections of four new populations. Work on Objective 2 will begin in the fall of 2014, and Objective 3 in early winter of 2015.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-12-107

Project Title: Pear scion trials in the Pacific Northwest

PI: Kate Evans
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Telephone: 509 663 8181 x 245
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Co-PI (2): Todd Einhorn
Organization: OSU-MCAREC
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Address: 3005 Experiment Station Drive
City/State/Zip: Hood River/OR/97031

Co-PI(3): Richard Bell
Organization: USDA-ARS
Telephone: 304 725 3451 x 353
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Address: Appalachian Fruit Research Station
Address 2: 2217 Wiltshire Road
City/State/Zip: Kearneysville/WV/25430

Cooperators: Tim Smith, WSU; Rachel Elkins, CA; Tom Auvil, WTFRC; grower cooperators – Chuck Peters, Ray Schmitten, Jim Koempel

Total Project Request: **Year 1:** 4,220 **Year 2:** 8,891 **Year 3: 22,800**

Other funding sources: None

Budget 1 – Kate Evans
Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509-335-4564/509.663.8181 **Email**: carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2012	2013	2014
Wages[1]	0	1000	1,040
Benefits[1]	0	149	180
Supplies[2]	1350	0	0
Travel[3]	500	500	1,000
Trees[4]	0	0	5,000
Plot Fees	0	500	0
Total	1,850	2,149	**7,220**

Footnotes:
[1]Non-student temporary at $10/hr for 10 wks.; benefits 17.3%
[2]Planting supplies and fumigation.
[3]Travel to trial sites.
[4]Trees ordered from C & O.

Budget 2 – Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** L.J.Koong
Telephone: 541-737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Wages	0	750	772
Benefits[1]	0	518	534
Supplies[2]	1350	0	0
Travel	0	250	250
Plot Fees	0	3104	3104
Total	1,350	4,622	**4,660**

Footnotes:
[1]Salary is 1 week of Technician time in 2013 and 2014
[2]Fumigation and irrigation system

Budget 3 – Tom Auvil
Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
Telephone: 509-665-8271 x2 **Email address:** kathy@treefruitresearch.com

Item	2012	2013	2014
Salaries & benefits	0	1000	5,150
Travel	0	120	620
Total	0	1,120	**5,770**

Budget 4 – Grower reimbursement
Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
Telephone: 509-665-8271 x2 **Email address:** kathy@treefruitresearch.com

Item	2012	2013	2014
Grower reimbursement	0	1000	5,150
Total	0	1000	**5,150**

Budget 5 – Richard Bell
Organization Name: USDA-ARS **Contract Administrator:** Stephanie Kreger
Telephone: 304-725-3451 x332 **Email address:** stephanie.kreger@ars.usda.gov

Item	2012	2013	2014
Supplies – Trees[1] **& Freight**	1020	0	0
Total	1,020	0	**0**

Footnotes:
[1]Trees produced at Adams County Nursery

OBJECTIVES

1. To test five new scion selections from the USDA-ARS pear breeding program in small scale replicated plantings in Washington and Oregon.
2. To test two new pear scions from Prevar, Australia, in medium scale plantings in Washington and Oregon.

SIGNIFICANT FINDINGS

- Randomized replicated plantings of the USDA-ARS pear scion selections were established in two sites in WA (Wenatchee Valley and Wapato) and one site in OR (MCAREC, Hood River).
- Suitable trial planting sites have been found and prepared for the plantings of the new Coregeo Australian scions.
- The Coregeo Australian Trees have been lifted and are ready to be shipped.

METHODS

1. Identify and prepare suitable trial planting sites (research orchards and grower sites).
2. Establish randomized replicated plantings of five trees of each of five new scion selections from the USDA-ARS pear breeding program. Standard comparison trees will also be included. Two sites (a warm and a cooler one) will be chosen in WA.
3. Establish 75-100 tree plantings of the two new Coregeo Australian pear selections (on OHF 87) in grower sites in WA (two sites) and OR.
4. Maintain sites as appropriate, establish harvest protocols and harvest and assess fruit as available.
5. Provide opportunities for grower visits as necessary (note: grower visits may be premature within the timescale of this project).

Modern training systems and planting designs will be utilized at all sites; however, each grower-collaborator will select a unique training system based on their experience. The objective is to evaluate the performance of new pear genotypes under diverse, commercially relevant training systems.

RESULTS & DISCUSSION.

Objective 1:

The five new USDA-ARS scion selections were planted in two sites in Washington and one site in Oregon. Trees were planted in Wapato (Chuck Peters), in the Wenatchee Valley (Josh Koempel) and at MCAREC, Hood River.

Trees in the Wenatchee Valley planting are spaced at roughly 3 ft in row and 12’ between rows in a randomized block design (Fig 1). They will be alternately angled to 70º in about year 4. All the limbs will be tied down horizontally, with only a heading cut to stimulate branching about four times per growing season. The planting should end up looking like a V trellis but without the trellis. Trees in the Wapato planting are at 4 ft in row and 12’ between rows in a randomized complete block design (Anjou was added in an adjacent block). Again the trees are angled into V trellis (Fig 2).

In Oregon, a site at MCAREC was fumigated and planted 14-May, 2014. Trees were planted 5 ft in-row and 12' between rows in a randomized complete block design (Fig 3). Trees and limbs were left unheaded at planting. Limbs were removed only if their size exceeded ~ half the size of the trunk. Remaining limbs were spread to widen branch angels (between 30-45 degrees). Irrigation was provided at a frequency of two to three times per week for 4 hours per set. Urea was applied (side-dressed) at a rate of 10 lbs per acre every 10-14 days beginning June 1. Excellent, uniform tree growth was realized. Trees will be tied to the wire in spring of 2014 and trained to a 10° V whereby each tree is tipped in the opposite direction.

Figure 1. USDA-ARS pear selection evaluation trial in the Wenatchee Valley

Figure 2. USDA-ARS pear selection evaluation trial in Wapato

Figure 3. USDA-ARS pear selection evaluation trial at MCAREC

Trunk diameters were recorded fall/winter 2013 and cross-sectional areas calculated (Table 1).

Table 1. Trunk cross-sectional area of scion selections following first year of growth at all three sites.

Scions	*Trunk cross-sectional area (cm^2)*		
	Wapato	Wenatchee Valley	MCAREC
US-69426-038	1.9ab	1.8b	2.8b
US-71655-014	2.1ab	2.1b	-
US-84907-069	1.9ab	1.8b	2.6b
US-84907-078	2.1ab	2.9ab	3.5ab
US-84907-166	1.6b	2.8ab	3.5ab
Anjou	0.8b	2.0b	4.9a
Bartlett	3.1a	3.9a	5.1a

Note: Each site mean analyzed independently

Trees will be monitored throughout the coming year and standardized fruit testing protocols will be determined for the expected 2014 crop.

Objective 2:

Sites have been prepared in the Wenatchee Valley (Koempels), Wapato (Peters) and at the MCAREC ready for the planting of 100 trees of each of the two new Coregeo Australian pear selections, spring 2014. (Fig. 4)

Figure 4. MCAREC site prepared to receive two new Coregeo Australian pear selections.

CONTINUING PROJECT REPORT **YEAR**: 2 of 2 (extension)
WTFRC Project Number: PR-12-109

Project Title: Genotype work for pear

PI: Kate Evans
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Co-PI(1): Amit Dhingra
Organization: WSU
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City/State/Zip: Pullman/WA/99164

Cooperators: Richard Bell (USDA-ARS, Kearneysville, WV); Todd Einhorn (OSU); Rachel Elkins (UCD); Stefano Musacchi (WSU/Univ. of Bologna); Feli Fernández (EMR, UK); Joan Bonany (IRTA, Spain); François Laurens & Marie-Hélène Simard (INRA, France)

Total Project Request: **Year 1:** $25,000 **Year 2:** $0

Other funding sources
None (although SCRI proposal in preparation)

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509.335.4564/509.663.8181 **Email address**; carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2012	2013
Salaries	0	0
Benefits	0	0
Wages	0	0
Benefits	0	0
Equipment	0	0
Supplies	0	0
Travel	0	0
Plot Fees	0	0
Miscellaneous[1]	25,000	0
Total	25,000	0

Footnotes:
[1]To import accessions into U.S. and clear quarantine

OBJECTIVES

- To import new pear rootstocks and pear rootstock selections into the U.S. through the Clean Plant Center for testing.

This project was extended at no additional cost for an extra year due to problems experienced in moving the imported germplasm through quarantine.

SIGNIFICANT FINDINGS

- Material Transfer agreements (MTA's) have been drafted by WSU and sent to INRA- France, IRTA - Spain, University of Bologna - Italy and EMR-UK.
- MTA has been approved by EMR.
- MTA has been approved by University of Bologna.

METHODS

1. MTA's will be drafted and sent to the collaborators for approval. Once approved, the PI's and the collaborators will work together to prioritize a list of germplasm to import into the U.S.

2. Dormant propagating wood will be sent to the Clean Plant Center to start quarantine testing.

RESULTS & DISCUSSION

A Material Transfer Agreements (MTA) was drafted by WSU's Office of Grant and Research Development and sent to EMR UK, INRA France, IRTA Spain and the University of Bologna Italy. EMR has approved and signed their MTA as has the University of Bologna. Approval from the others is pending.

Due to the impending departure of Dr. Musacchi from the University of Bologna, the decision was made to concentrate on accessing this germplasm first. Thirteen accessions in tissue culture were sent from Bologna by Dr. Musacchi in July. Unfortunately due to a miscommunication, we were unaware that APHIS and the Clean Plant Center were unable to deal with germplasm in tissue culture and the material did not survive. If possible following the changes in the Clean Plant Center policy, dormant wood will be accessed this winter. We are currently considering bringing wood in to the US through APHIS Beltsville instead.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2

Project Title: ABA chemical fruit thinning of Bartlett Pears

PI: Todd Einhorn
Organization: OSU-MCAREC
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todd.einhorn@oregonstate.edu
Address: 3005 Experiment Station Drive
City: Hood River
State/Zip: OR 97031

Cooperators: Mike Sandlin, Mateus Pasa, Matthew Arrington (MS student), Gorham Blaine

[1]Budget: **Year 1**: $10,483 **Year 2**: **$10,871**

Other funding sources: None

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2013	2014
Salaries[1]	6,944	7,152
Benefits	745	745
Wages[2]	1,800	1,800
Benefits	198	198
Equipment	0	0
Supplies	0	0
Travel[3]	200	200
Miscellaneous[4]	776	776
Total	10,483	**10,871**

Footnotes: [1]Salaries are calculated as 4 months of a 0.49 FTE Graduate Student Research Assistantship at the monthly rate of $1,736. The increase in salary in year 2 reflects a 3% rate increase. Graduate student benefits for the period equate to $745. [2]Wages are for one part-time employee to work 150 hours ($12/hr) to aid in weekly plant measurements, bloom count, fruit set, harvest, and postharvest fruit quality assays. Part-time employee benefits are calculated at an 11% rate. [3]Travel includes trips to and from one regional PNW research site. [4]Miscellaneous costs account for MCAREC plot fees at a rate of $3,103/acre, prorated to ¼ acre (= $776) for field on-site field trials.

Objectives:

1. Identify the appropriate application timing of ABA for thinning Bartlett pears.
2. Identify the appropriate rates of ABA for thinning Bartlett pears.
3. Compare and contrast the response of Bartlett pears following ABA applications over multiple sites using meteorological data generated from individual test sites.

Significant Findings 2013:

- In 2013, ABA (ProTone®, Valent Biosciences Corp.) applications were performed at two trial sites to whole canopies of Bartlett pear trees.
- In Hood River (MCAREC), 17-year-old trees were either treated at petal-fall, or when fruits reached 10 mm with a step-wise ABA concentration gradient. Only small reductions in fruit set were recorded, irrespective of timing. Rates were equivalent to those applied in 2012 which resulted in significant fruitlet abscission/thinning.
- In Parkdale, ABA applications were made to ~15-year-old whole canopies at 10mm fruit size. Thinning response was highly significant and rate responsive, reproducing results obtained in 2012. At rates of 100 and 200 ppm, ~50% and 20% of Control fruit set was attained, respectively.
- We showed that ABA reduces photosynthesis, indirectly by reducing stomatal conductance (partial closure of stomates, which in turn proportionally reduces CO_2 uptake by the plant necessary for photosynthesis). This action persists in the plant for a relatively short period of 7-14 days after application, with diminishing effect each day from application.
- We hypothesized that ABA alone does not produce enough stress to induce an adequate thinning response when high-light conditions occur the week after application (i.e., the Hood River 2013 trial). However, when combined with cloudy, overcast weather (prevalent during early spring in Oregon, and observed for the 2012 Hood River and 2013 Parkdale trials), the thinning effect was consistent and pronounced. Daily solar radiation (light) data supported this hypothesis when analyzed for all three trials.
- Higher rates can compensate, somewhat, for high-light conditions; however, rates exceeding 400 ppm were phytotoxic to leaves resulting in significant leaf burn and abscission.

Methods, Results and Discussion:

Site 1. At the MCAREC, single-tree replicates of 17-year-old Bartlett trees were sprayed to drip with ABA (ProTone, Valent BioSciences Corp.). Treatments comprised five solution concentrations: 0 (water), 50, 100, 150, and 200 ppm. Rates were based on results from our 2012 experiments. To each concentration, 0.1% surfactant was added and tank mixed. Applications were made using a pressurized handgun. Each treatment was replicated 4 times throughout the orchard. Two timings were evaluated: 1) Petal-fall, and 2) 10 mm fruit size. For each replicate tree, a minimum of 200 flower clusters were counted and tagged on scaffold limbs. Just prior to hand thinning timing (~35 days after bloom), fruit were counted on tagged sections of scaffolds and fruit set was determined and expressed as the total number of fruit per cluster. Scaffolds were roughly chest-height and adequately represented the condition of the lower and mid-canopy, but fruit set, in general, was greater in the top portion of the canopy. Twenty fruit per tree were tagged and measured weekly beginning 30 days after full bloom. Photosynthesis measurements were intended to begin one day prior to each of the two treatment timings and continue every couple of days until the effect disappeared; however, our photosynthesis chamber malfunctioned, necessitating shipment to the East coast for repairs. Consequently, photosynthesis was not analyzed for the petal fall treatments. We received the instrument 5 days after the 10 mm treatments were applied and measurements began the next day. Fruit set was only slightly and inconsistently reduced by ABA, irrespective of application timing (Figure 1). Though it appears from the figure that fruit set was higher in the 50 ppm, 10 mm application timing compared to the control (0 ppm), these treatments did not significantly differ (i.e.,

the variability in the data can be seen by the overlapping standard error bars). The lack of sufficient thinning by ABA was in stark contrast to our 2012 results, where the magnitude of thinning increased with increasing ABA rate. Six days after the 10 mm application timing, photosynthesis had recovered for all rates except 200 ppm, which was significantly lower than the controls (i.e., 72% of control levels; Figure 2). By day 13, the effect had disappeared in the 200 ppm trees. These results suggest that only a limited period of stress occurred for the higher ABA rates, implying that fruit drop due to insufficient carbon gain to support fruit growth would be unlikely. Moreover, fruit set was relatively low in the orchard (typical of the lower Hood River valley in 2013) and very little thinning was required for control trees. Further, fruit growth did not differ for any of the rates (data not shown). Therefore, no differences in yield were evident among treatments at harvest (data not shown).

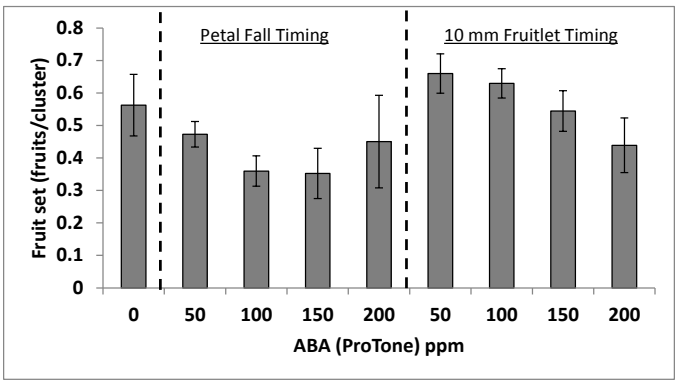

Figure 1. Fruit set following applications of ABA at two timings and four rates, compared to a control (0 ppm ABA).

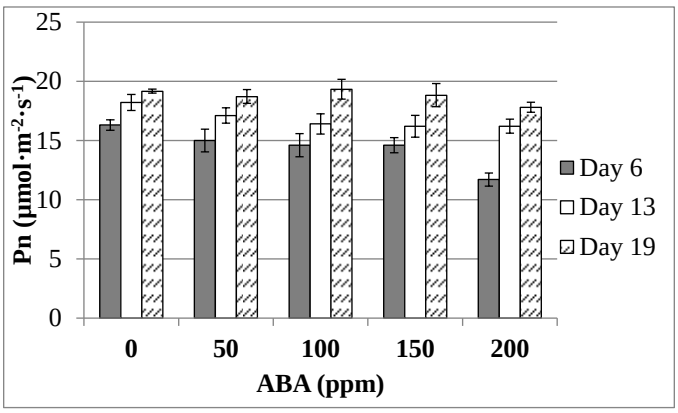

Figure 2. Photosynthesis of pear leaves 6, 13 and 19 days after applications of ABA at 10 mm fruitlet timing. Data are means of 4 replicates (n=4).

Site 2. We hypothesized that the fairly low concentrations of ABA in combination with the high light (sunny) conditions during the treatment period at MCAREC did not produce the necessary carbon deficit to thin fruit. The high thinning levels achieved by ABA in 2012 were commensurate with several weeks of cloudy, overcast weather. Given that development in Parkdale is typically ~3 weeks delayed relative to Hood River, we identified a second trial site in upper Parkdale. At this time fruits were ~10 mm in size. Trees were ~15-year-old Bartlett. Four, two-tree replicates were assigned to each ABA concentration: 0 (water), 50, 100, 200, and 400 ppm. Treatments were sprayed as outlined above, but only at the 10 mm timing. Flower clusters were counted and tagged as described previously.

Intermittent clouds and low light conditions were prevalent throughout the first 10 days from application. A pronounced, rate-responsive thinning effect was observed (Figure 3). Results were nearly identical to those achieved in 2012. Rates of 400 ppm resulted in a fairly high degree of phyto-toxicity to foliage (necrotic spots and defoliation of ~25% of the canopy). Leaf area of the canopy recovered, but the combined effects of 400 ppm ABA on photosynthesis and defoliation led to a drastic thinning response.

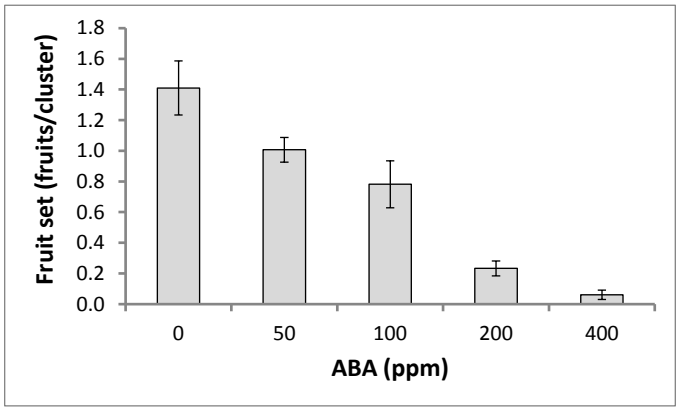

Figure 3. Fruit set following applications of ABA at 10 mm timing at four rates and compared to a control (0 ppm ABA). Data are means of 4 replicates.

Thinning was associated with a significant increase in the number of blank spurs (i.e., spurs failing to set any fruit; Figure 4). For control trees, ~50% of the spurs were void of any fruit following natural 'June' drop, compared to 94% in trees sprayed with 400 ppm ABA.

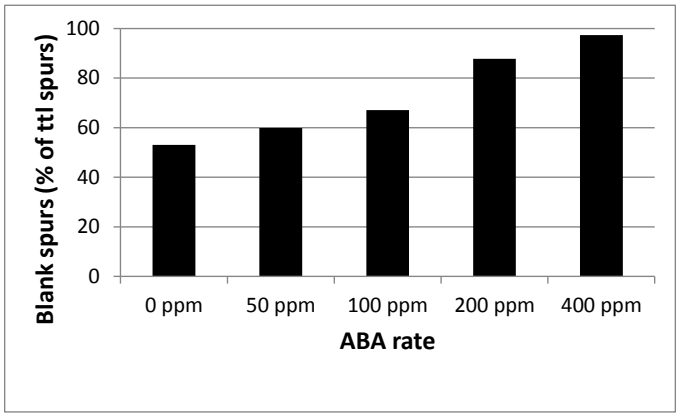

Figure 4. Percentage of total spurs on selected scaffold limbs that did not set any fruit (i.e., blank) following applications of ABA at 10 mm timing at four rates and compared to a control (0 ppm ABA). Data are means of 4 replicates.

Prior to hand thinning, a higher percentage of the total fruit on ABA-treated trees resided singly on spurs (Figure 5). In general, fewer fruit were set on multiple-fruit spurs for ABA-treated trees compared to control trees, irrespective of rate. However, for the concentration range best-suited for industry use (~100 ppm), ABA was fairly non-selective. It appears that fruit drop associated with ABA was quite random (i.e., spurs were not consistently thinned to 1, or even 2 fruits/spur). More work is required to understand ABA action on fruitlet abscission. We did not determine whether smaller (i.e., weaker) fruitlets were affected differently by ABA compared to larger fruitlets. In 2014

we intend to categorize the size disparity among fruits on spurs before and after ABA applications to establish if the thinning response is dependent upon fruit size.

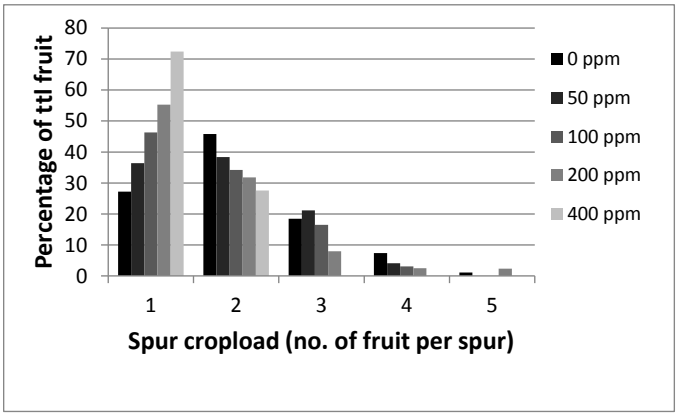

Figure 5. The percentage of total fruit per scaffold borne on spurs of discrete fruit densities (i.e., spurs carrying either one, two, three, four, or five fruit). Frequency distributions were done prior to hand-thinning, but after natural (and ABA induced) fruit drop occurred. Data were collected from selected scaffolds and are means of 4 replicates.

Photosynthesis was markedly reduced one day following ABA applications and remained so for ~ 1 week (Figure 6). The duration of the effect was related to dose, with 200 and 400 ppm treatments limiting photosynthesis for ~ 2 weeks after treatment applications. Photosynthesis measurements were made during breaks in precipitation near midday; unfortunately, cloudy/rainy conditions during much of the first two weeks precluded a higher measurement frequency. From these data (and photosynthesis measurements made at MCAREC), ABA activity in the plant has a short residency, being metabolized within 1-2 weeks, depending on dose. Therefore, to achieve an adequate thinning effect both natural fruit set and timing of application would appear to be critical factors.

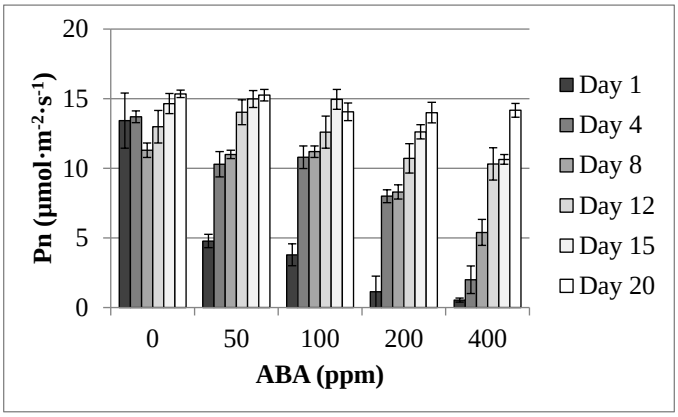

Figure 6. Photosynthesis of single pear leaves every three to four days following applications of ABA at 10 mm fruitlet timing. Data are means of 4 replicates (n=6).

Trees were hand-thinned according to commercial practices (i.e., our crew was trained by the grower as to the appropriate level of commercial thinning). All fruit removed during hand thinning were counted. Despite the 200 and 400 ppm treatments resulting in significant thinning, additional fruit were hand-thinned from these trees based on their size at thinning time and/or their position in the canopy (i.e., with increasing canopy height [>8 ft.], fruit set was markedly higher- likely the result of poorer coverage and/or higher vigor). At harvest, yield was reduced proportionately to ABA dose (Table 1). Fruit size was not adversely impacted by ABA, but there wasn't an observable size

advantage from the 50 or 100 ppm ABA treatments, despite fruit drop ~2 weeks prior to hand thinning (Table 1).

Table 1. Effect of ABA applications at 10 mm fruit size to whole canopies of ~15-year-old Bartlett trees.

Treatment ABA (ppm)	No. fruit before hand thinning (no.fruit/tree)	No. fruit after hand thinning (no.fruit/tree)	Tree yield (lbs)	Average fruit wt (g)
0	911 a	494 a	211 a	196 c
50	748 b	439 ab	183 b	194 c
100	570 c	383 b	170 b	205 c
200	336 d	225 c	106 c	238 a
400	293 d	184 c	83 cd	226 ab

data are means of 4 single-tree replicates

A comparison of solar radiation (light) incident on the orchard within the first 10 days from treatment for all three trial sites was illustrative of the low light requirement for ABA efficacy (Figure 7). Table 2 shows the thinning effect observed at each site. These results present somewhat of a challenge: ABA will not be broadly applicable. High-light conditions will preclude its use; although, sunny conditions are typically accompanied by higher temperatures and, therefore, conducive to Maxcel use.

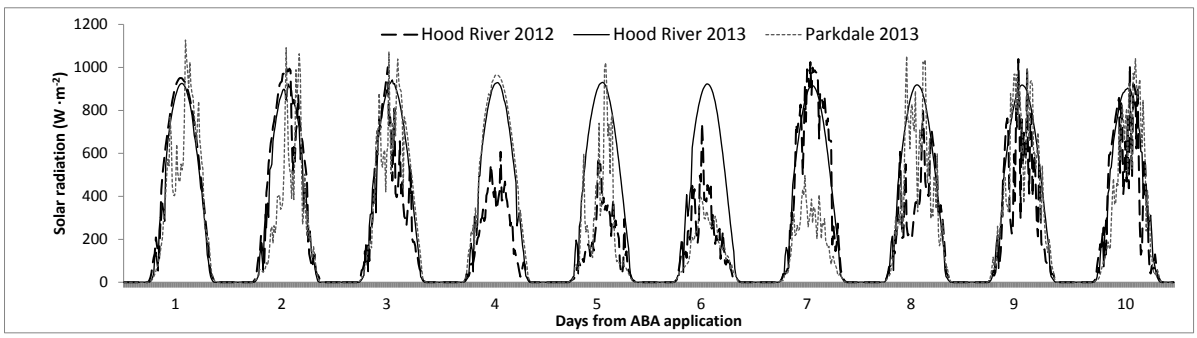

Figure 7. Daily solar radiation levels throughout the 10-day period immediately succeeding ABA applications at the 10 mm timing for all sites. Cloudy, low-light conditions in Hood River (2012) and Parkdale (2013) contrast the sunny period immediately following the Hood River 2013 experiment. Data were calculated from meteorological stations in Hood River and Parkdale.

Table 2. The percentage of fruit retained compared to control trees (i.e., 100%) after fruit drop for ABA rates applied at 10 mm fruit size over 2 years and 3 sites.

ABA (ppm)	2012 Hood River	2013 Hood River	2013 Parkdale
0	100	100	100
50		100	71
100		100	57
125	59		
150		96	
200		78	18
250	12		
400			7
500	2		

On the contrary, ABA used in combination with forecasted, low-light conditions should produce a fairly predictable response. We propose to focus efforts in the second and final year of this project on reproducing the results achieved in both 2012 and 2013 at Parkdale (i.e., by targeting ABA applications with the appropriate environmental conditions for efficacy). Further, to prove the correlative relationships shown by Figure 7 and Table 2, we intend to construct shade houses of varying percentages of shade over whole canopies treated with a range of ABA rates. We will aim for sunny conditions within the first 3 weeks from bloom for applications. If these environmental conditions are not met at MCAREC, we will locate an orchard in The Dalles or Parkdale that fits the criteria.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Improving fruit set, production efficiency, and profitability of pears

PI: Todd Einhorn
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Co-PI (2): Stefano Musacchi
Organization: WSU-TFREC
Telephone/email: (509) 663-8181
stefano.musacchi@wsu.edu
Address: 1100 N. Western Ave
City: Wenatchee
State/Zip: WA, 98801-1230

Cooperators: Growers: Mike Sandlin (WA), Don Kiyokawa (OR), Gorham Blaine (OR), Yan Wang

Total Project Request: Year 1: $75,151 **Year 2:** $72,278 **Year 3: $74,012**

Other funding sources: Match funding of $20,384 from DCA-UNIBO, Italy

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Salaries[1]	29,250	37,072	38,183
Benefits	20,183	20,788	21,411
Wages	7,040	7,040	7,040
Benefits	774	774	774
Equipment[2]	2,500	0	0
Supplies[3]	8,000	1,000	1,000
Travel[4]	4,300	2,500	2,500
Miscellaneous[5]	3,104	3,104	3,104
Total	75,151	72,278	**74,012**

Footnotes: [1] Salaries are calculated as 0.75 FTE of Full Time Technician's salary and OPE, for management of all experimental designs and field plots, operation of root pruner, PGR applications, plant measurements, and data management; 4 months of a 0.49 FTE Graduate Student Research Assistantship at the monthly rate of $1,736. The increase in salaries for years two and three reflects a 3 % rate increase. Wages are for 2 part-time employees to work a combined total of 640 hours ($11/hr) to aid in plant measurements, harvest, and training of field plots. [2]Equipment costs cover supplies and fabrication of root pruner. [3]Includes purchase of trees for new 'Bartlett' planting (funding for trellis supplies and irrigation is not being requested), PGR's, tags, flagging, and tree training supplies for field trials. [4]I am requesting the transfer of travel funds initially requested for Stefano Musacchi and his technician ($6,100 for 2013 and 2014) to support an MS student at OSU given Stefano Musacchi's new position and relocation to Wenatchee, WA. He will no longer have a technician in Bologna, Italy to travel to the States to participate in the project in 2013-2014. The remaining travel budget will be allocated to travel to and from regional PNW research sites, and to support travel of Musacchi to Hood River from Wenatchee, including per-diem, and lodging. [5]Miscellaneous costs are MCAREC per acre plot fees (3,104/acre), for a one-acre Bartlett planting.

Objectives

1. Develop plant growth regulator protocols for early and consistent fruit set. Test and adapt current protocols successfully utilized in Europe on PNW varieties. Characterize PGR effects on flowering, fruit set, and vegetative growth.

2. Apply current root pruning technologies available in the US to existing, and future, plantings. Test application timing, depth, and severity of root removal, and characterize the effect of these treatments on shoot growth, flower development, fruit set, fruit size and productivity.

3. Develop new plantings of competitive orchard systems. Develop demonstration orchards at MCAREC of single axe and bi-axe planar hedgerows. Work collaboratively with growers to establish planar commercial high-density blocks.

Significant Findings 2013

Objective 1:

- Four trials to evaluate ReTain on pear fruit set and production were implemented in 2013.
- Results were generally positive, though inconsistent. In three trials ReTain significantly improved fruit set relative to untreated control trees, but the magnitude differed according to the site (ranging from a two-fold increase to only modest numerical gains). In most cases, the response improved with rate. Bloom applications did not improve set (as previously observed in 2012). Applications coinciding with 10-14 days after full bloom had the greatest impact on fruit set (supported by 2012 data). In one trial no changes in fruit set, yield or fruit size were observed indicating that other factors influence the response.
- We monitored the rate of ethylene production in untreated and ReTain-treated flowers and fruitlets of Comice and Anjou throughout the treatment period. ReTain markedly reduced, but did not completely inhibit, ethylene production. Residency in the plant was long (lasting ~21 days after application and diminishing with time). Ethylene production was reduced relative to rate. Interestingly, ethylene production of untreated fruitlets was highest between 10-14 days after full bloom for both Comice and Anjou- a period coinciding with petal fall and natural abscission- then sharply reduced to ~ 0.

Objective 2:

- Root pruning was applied just prior to bloom to one orchard in 2013: Trellised 4th leaf Anjou/OH×F 87 (4 x 12 ft; 908 trees/acre) trained to a V. Root pruning was applied with and without ReTain to a depth of 45 cm to either one, or two sides of the tree row.
- The most severe root pruning treatment (two-sided) significantly reduced shoot growth of 4th leaf Anjou by ~20% compared to control trees. Single-sided root pruning produced intermediate effects.
- Fruit set and yield was not affected by root pruning (relative to controls). Fruit size, however, was reduced for both root pruning treatments from size 80 (control) to size 90.
- The combination of ReTain and root pruning proved effective (i.e., a two-fold yield improvement and vigor control compared to controls), but further reduced fruit size to 100s.
- A second orchard treated in 2012 was monitored for year 2 effects. Roughly half of the vigor control remained at the end of 2013. Return flowering was increased by root pruning treatments, but translated to only modest numerical increases in set and yield.

Objective 3:

- Acceptable growth accrued in 2013 following poor growth in year 1 (2012). Trees have reached ~75% the height of the trellis. There were no differences in tree size when comparing trees of bi-axis or single axis at any of the in-row spacings (2, 4, 6 or 8 ft.)

Results and Discussion

Objective 1:

Four experiments were performed to evaluate the effect of the ethylene inhibitor AVG (a.i. in ReTain, Valent Biosciences Corp.) on pear (Anjou and Comice) fruit set and production. Each trial was designed as a randomized complete block; replicates varied. In experiments 1, 2 and 4 whole trees were sprayed using a pressurized hand gun. For experiment 3, whole-rows were sprayed uisng an airblast sprayer. In all experiments a surfactant (Sylgard 309) was added to ReTain at 0.1% by volume and mixed.

1. 5th leaf Anjou/OH × F 97, Mt. Adams, WA (6 single-tree replicates)
Treatments: 1. Control; 2. ReTain 30 ppm applied at 80% of full bloom; 3. ReTain 60 ppm applied at 80% of full bloom; 4. ReTain 120 ppm applied at 80% of full bloom; 5. ReTain 30 ppm applied one week after full bloom; 6. ReTain 60 ppm applied one week after full bloom; 7. ReTain 120 ppm applied one week after full bloom; 8. ReTain 30 ppm applied two weeks after full bloom; 9. ReTain 60 ppm applied two weeks after full bloom; 10. ReTain 120 ppm applied two weeks after full bloom

2. 17-year-old Comice/OH × F 97, Hood River, OR (4 single-tree replicates)
Treatments: 1. Control; 2. ReTain 30 ppm applied at 50% of full bloom; 3. ReTain 60 ppm applied at 50% of full bloom; 4. ReTain 120 ppm applied at 50% of full bloom; 5. ReTain 30 ppm applied two weeks after full bloom; 6. ReTain 60 ppm applied two weeks after full bloom; 7. ReTain 120 ppm applied two weeks after full bloom

3. Three separate plots were treated in Odell, OR with the same treatment regime: ~30-year-old Anjou /unknown rootstock; 4th leaf Anjou/OH × F 87; and, 7th leaf Anjou /OH × F 87.
Treatments: 1. Control; 2. 1 pouch ReTain/acre (applied 10 d after full bloom); 3. 0.5 pouch ReTain/acre (applied 10 d after full bloom)

4. 4th leaf Anjou/OH × F 87, Dee Flat, OR
Treatments: 1. Control; 2. 1 pouch ReTain (applied 10 d after full bloom); 3. 1 pouch ReTain (applied 10 d after full bloom) + root pruning 1 side of tree row; 4. 1 pouch ReTain (applied 10 d after full bloom) + root pruning 2 sides of tree row

ReTain did not statistically improve fruit set (Figure 1) or production (data not shown) of 5th leaf Anjou (Mt. Adams, WA), irrespective of rate or timing. These results differed markedly from 2012 experiments, where ReTain produced a two- to three-fold increase in fruit set and yield at similar rates. Applications during flowering (~80% of full bloom) resulted in slightly lower fruit set, as observed in 2012.

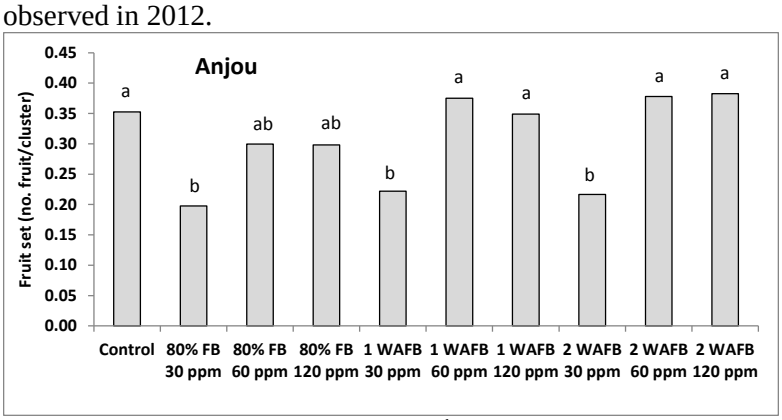

Figure 1. Fruit set (fruit per cluster) of 5th leaf Anjou trees treated with 3 doses of ReTain (30, 60 and 120 ppm) at three timings (80% full bloom, 1 week after full bloom [1WAFB], or 2 WAFB). Fruit set was determined on scaffold limbs (1 per replicate tree x 6 reps).

Fruit set, total yield and fruit number were significantly increased for Comice trees treated with 120 ppm ReTain two weeks after full bloom [WAFB] (Figure 2; Table 1). As similarly observed with Anjou, applications made during flowering were not effective, and in fact, had a significant negative effect on production. Fruit size was reduced by the most efficacious fruit-setting treatment (120 ppm applied 2 WAFB), likely an indirect effect of cropload.

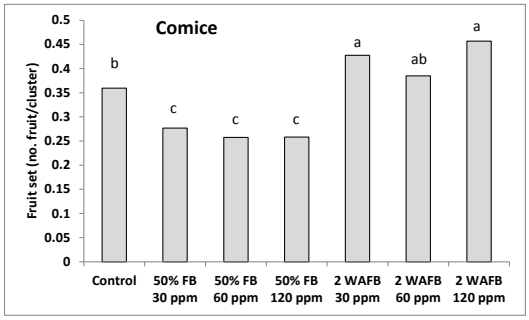

Table 1. Effect of ReTain treatments applied to 17-year-old Comice trees on tree yield and fruit size at harvest.

Treatments	Tree yield	Tree yield	Average fruit size	
ReTain rate/timing	(lbs/tree)	(no.fruit/tree)	(g)	(no./44lb box)
Control	182 b	405 b	206 a	100
30 ppm (50% FB)	151 c	348 c	197 a	100
60 ppm (50% FB)	148 c	362 c	185 b	110
120 ppm (50% FB)	161 bc	391 bc	191 ab	100
30 ppm (2 WAFB)	161 bc	391 bc	191 ab	100
60 ppm (2 WAFB)	195 ab	445 b	199 a	100
120 ppm (2 WAFB)	221 a	569 a	176 b	110

data are means of 4 single-tree replicates

Figure 2. Fruit set (fruit per cluster) of 17-year-old Comice trees treated with 3 doses of ReTain (30, 60 and 120 ppm) at two timings (50% full bloom and 2 WAFB).

Ethylene, produced in untreated Anjou and Comice flowers and fruitlets, steadily increased during early bloom (50%-80% of full bloom), peaked ~ 14 days after full bloom, and then declined to nearly zero by ~30 days after bloom (Figure 3). ReTain significantly reduced ethylene production in flowers and fruitlets, but did not completely eliminate it. Ethylene production was highly affected by rate. ReTain continued to have a significant effect on ethylene production of fruitlet tissues for ~30 days after applications, though the effect diminished with time.

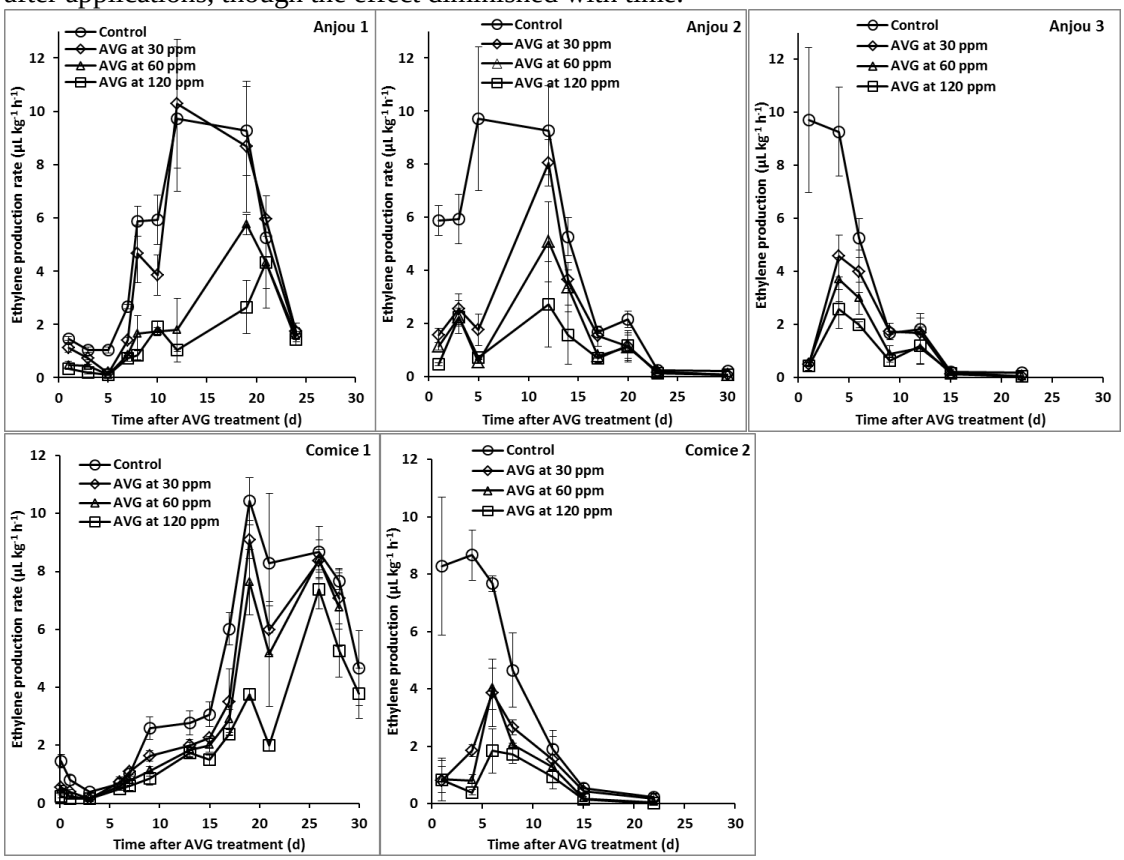

Figure 3. Ethylene production rate of flowers and fruitlets of Anjou (top panels) and Comice (lower panels) throughout the ReTain treatment periods outlined above [Experiments 1 & 2]. Ethylene was analyzed from headspace gas pulled from sealed tubes containing flowers or fruit and injected into a gas chromatography

instrument (GC) in collaboration with Dr. Yan Wang. The horizontal axis on the Anjou graphs from left to right represent: Anjou 1 (days from 80% full bloom application); Anjou 2 (days from 1 week after full bloom application); and, Anjou 3 (days from 2weeks after full bloom application). For the Comice graphs from left to right: Comice 1 (days from 50% full bloom application); and, Comice 2 (days from 2 weeks after full bloom application).

A positive relationship between ethylene and fruit abscission is well-established. Fruits retained by 2012 ReTain treatments showed no difference in seed number relative to control fruit (presented in 2012 report). These data suggested that a lack of fertilization was not the critical factor limiting fruit set potential. Based on ethylene production data (Figure 3), ReTain, when sprayed at 1 to 2 weeks after bloom, is likely effective because natural ethylene production in developing fruitlets is at its highest. Further, regulation of ethylene is strongest within the first few days from application, explaining why carryover effects on fruit set from bloom applications were not detected. It may be that a threshold level of ethylene triggers higher fruitlet abscission (>5 µl/kg/hr). The fact that 2013 fruit set was inconsistently increased by ReTain indicates that other factors (likely environmental) regulate the response. Resolving these factors will be the focus of our work in 2014.

Several blocks of Anjou (varying in tree age) were treated commercially with ReTain ~10 days after bloom (Table 2). In all cases fruit set was numerically improved by ReTain, though not always significantly. Treatments were applied to whole-rows at either 0.5 pouch, or 1 pouch per acre rate. Ten scaffolds per replicate were selected to evaluate fruit set. Fruit were harvested commercially and yield data were not recorded.

Table 2. Effect of ReTain at two rates on fruit set of Anjou. Applications were made ~10 days after bloom.

Anjou 2013 Trials	Fruit set (%)		
Odell, OR	7th leaf	30 year-old	4th leaf
control	11 b	22	5 b
ReTain (1/2 pouch)	18 ab	21	10 ab
ReTain (full pouch)	22 a	31	14 a

data are no. fruit/ttl clusters; means of 4 reps

The forth experiment was in combination with root pruning and will be described under Objective 2.

Objective 2:

We continued to evaluate carry-over effects from root pruning on 6th leaf Anjou/OH×F 87 trees, pruned in 2012. Entire rows were root-pruned to a depth of 1.5 feet and a distance of ~ 1.5 feet from the trunks on either one, or both sides of the tree row. Yield, fruit size and vegetative growth were all reduced significantly compared to untreated control trees (please refer to 2012 report). We did not impose root pruning in this orchard in 2013, but continued to evaluate treatment trees to determine the effects on return bloom, fruit set, productivity and vigor. Return bloom (measured on scaffold limbs at ~ chest height) was significantly improved by root pruning, but did not translate to significantly higher fruit set (Figure 4).

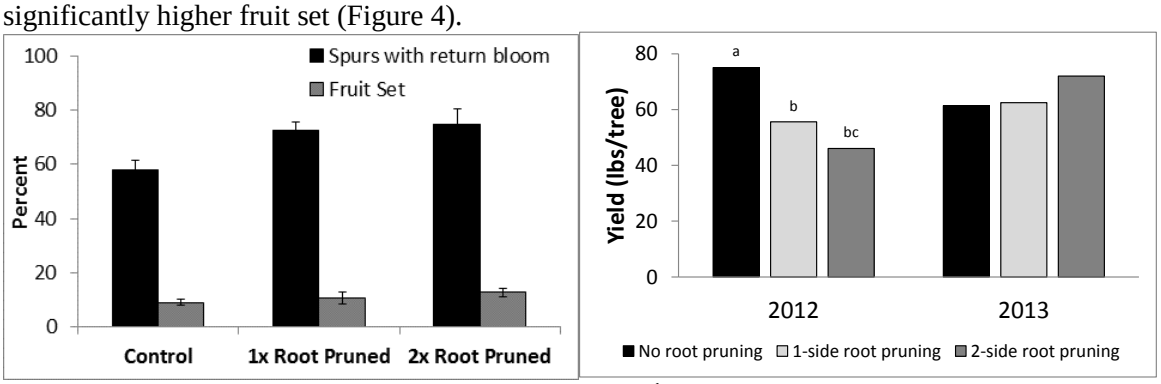

Figure 4. Return bloom and fruit set (left panel) in 2013 of 7th leaf Anjou trees root pruned in 2012 to two levels (1x= 1 side of tree row; 2x= 2 sides of tree row). 2012 and 2013 yield as affected by 2012 root pruning treatments. Data are means of 4 replicates (n=10).

The improved return bloom of root pruned trees indicated that high reductions in vigor observed in 2012 (~20% and 40% less than control trees for one and two-sided root pruning treatments, respectively) resulted in better light distribution within canopies and improved flower initiation. In general, poor pollination conditions limited fruit set in the orchard. Yield in 2013 was only slightly increased by the most severe root pruning treatment (both sides of the tree row), albeit non-significantly. Combined yield over the two years did not compensate for the reductions in year-one production (Figure 4). However, at the end of 2013, vegetative growth was still reduced by ~7% and 15% for one and two-sided root pruned trees relative to controls (Figure 5), indicating the potential for heightened flowering and fruit set in 2014. Effects were most pronounced in canopy tops.

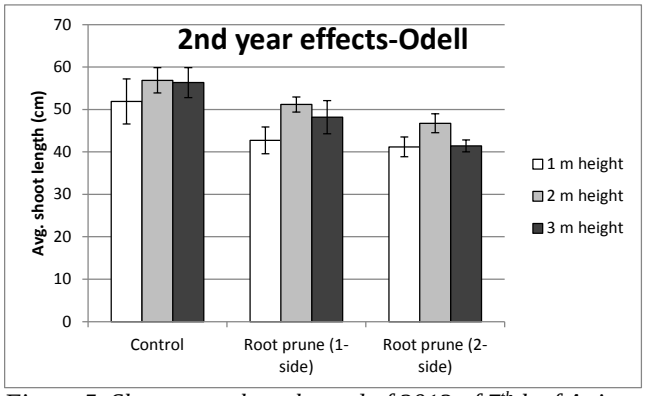

Figure 5. Shoot growth at the end of 2013 of 7th leaf Anjou trees root pruned in 2012 to two levels (1 side of tree row and both sides of tree row). Twenty limbs per replicate were randomly selected at three heights in the canopy (3, 6, and 9 ft) and measured.

In addition to monitoring carryover effects from our 2012 trial, we established a second root-pruning trial in a commercial, trellised block of 4th leaf Anjou/OH×F 87 (4 x 12 ft; 908 trees/acre) trained to a V in Dee Flat, OR. Trees had largely filled their space in 2012. Equivalent root pruning treatments as described above were applied; however, ReTain was superimposed on root pruning treatments. The major objective was to increase fruit set and reduce vigor in a young Anjou block, that would otherwise exhibit low fruitset and excessive vigor. Root pruning reduced shoot growth by ~10% to 20% for single and double sided trees, respectively, with the greatest effects observed in both lower and upper canopy positions (Figure 6). Unlike the 2012 trial, fruit set and yield were not affected by root pruning in the year of application (Table 3). Fruit size, however, was reduced by root pruning, but was still considered commercially acceptable.

Table 3. Effects of root pruning (one or both sides of the tree row) and ReTain on 5th leaf Anjou production. ReTain was applied one week after full bloom at 1 pouch/acre rate. Data are means of 4, 8-tree replicates

Treatment	Percent Fruit Set (no. fruit/100 cl.)	Yield (lbs/tree)	Fruit no. (per tree)	Avg Fruit Wt. (g.)	Avg. Fruit Sz. (# per 44lb box)
No Root Pruning	11.3 b	16.0 b	28.1 b	265 a	80
Single Sided RP	12.1 b	13.3 b	26.8 b	228 b	90
Double Sided RP	11.2 b	18.2 b	34.1 b	234 b	90
ReTain	23.9 a	27.4 a	60.0 a	202 bc	100
SS RP with ReTain	22.4 a	25.1 a	49.6 a	222 b	100
DS RP with ReTain	26.0 a	22.2 ab	51.7 a	198 bc	100

ReTain applications led to a ~100% increase in fruit set and number of fruit at harvest (Table 3) whether alone, or combined with root pruning treatments. Yield was significantly higher, but average fruit weight was reduced to size 100 in ReTain treatments, likely a result of source limitations due to higher croploads. The combination of double-sided root pruning and ReTain produced a good combination of vigor control and improved fruit set. Vegetative growth and production will be evaluated in the orchard in 2014. Half of the trees in each rep (4) will be root pruned again in 2014; the remaining 4 will be left unpruned.

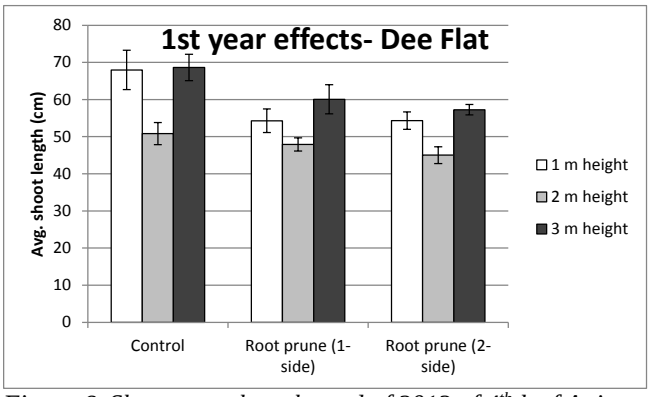

Figure 6. Shoot growth at the end of 2013 of 4th leaf Anjou trees root pruned just prior to bloom to two levels (1 side of tree row and both sides of tree row). Twenty limbs per replicate were randomly selected at three heights in the canopy (3, 6, and 9 ft) and measured.

An additional site (Bosc/ OH×F 97; 242 trees/acre and trained to a central leader) was root pruned to the same levels outlined above in October 2014. These treatments will be compared to equivalent treatments applied in the spring to determine the influence of timing on tree response. Trees will be entering their 'off' year of bearing.

Objective 3:

A planting was established at the MCAREC in 2012 to compare bi-axe and single axe training systems at several in-row tree spacings. Bartlett/ OH×F 87 in-row treatments are 2, 4 and 6 ft.; Anjou/ OH×F 87 are planted at 4 and 8 ft. Between-row spacing is 12 ft. for the entire block, and cultivars alternate every two rows to account for pollination. Each training system/spacing combination is planted in 10-tree replicates, and replicated five times in the block. Planting was delayed until the 3rd week of May, however, due to timing of spring fumigation resulting in relatively poor growth in 2012; however, trees have responded well and accumulated acceptable growth in 2013.

All trees were provided four nitrogen applications (granular urea) each 10 days apart, beginning the first week of June. Each application provided a rate of 10lbs per acre based on the tree density of the treatment to account for the different spacing treatments. Microsprinkler irrigation was provided three days per week for four hours per irrigation event. All urea applications occurred immediately before irrigation events to minimize nitrogen loss due to volatilization. Trunk size at the end of 2013 did not differ between bi-axis and single axis plants, or for trees at different in-row spacings, irrespective of cultivar (data not shown). On average, trees reached ~3/4 the height of the trellis and are well branched. Shoots will continue to be trained to wires in 2014.

'd'Anjou' scions were grafted to standard dwarfing quince rootstocks and OH×F in Italy by Stefano Musacchi prior to leaving for WSU in 2013. Trees will be monitored for graft incompatibility in 2014. This planting was a component of objective 3, covered by a funding match from DCA UNIBO, Italy, and designed to compare Anjou performance (precocity, fruit size and yield) on quince and Pyrus stocks in order to address the question of whether rootstock induced vigor control of 'd'Anjou' results in early fruit set and productivity.

CONTINUING PROJECT REPORT **YEAR**: **2 of 3**

Project Title: Cold hardy quince: propagation, rapid multiplication and field trials

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Co-PI: Richard Bell
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Telephone: 304-725-3451 ext. 353
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Cooperators: Stefano Musacchi

Budget: Year 1: $37,492 **Year 2:** $26,640* **Year 3**: **$39,430***

*We were approved for $26,640 for year 2 (2013); however, due to delays in developing and providing plant material (see report) several of the objectives were unachievable in 2013. As a result only $8,900 of the requested Year 2 budget was spent in 2013. This results in a surplus of $17,740. Of this amount, only $8,400 will be shifted to the Year 3 budget (2014); the remaining $9,340 will not be requested.

Other funding sources: None

Budget 1 – Barbara Reed & Joseph Postman
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: 510-559-5769 **Email address:** chuck.myers@ars.usda.gov

Item	2012	2013	2014
Wages	$29,400		
Benefits	$2,352		
Equipment			
Supplies[1]	$5,500	$1000	
Travel			
Miscellaneous			
Total	$37,252	$1000	$0

Footnotes: [1] rootstocks and greenhouse supplies to produce additional trees to fill gaps in Helios stool beds and Kearneysville plot and package and ship trees to Kearneysville for fire blight study.

Budget 2 - Richard Bell
Organization Name: USDA-ARS **Contract Administrator:** Stephanie Kreger
Telephone: 304-725-3451 ext. 332 **Email address:** stephanie.kreger@ars.usda.gov

Item	2012	2013*	2014
Salaries			$ 8095
Benefits			$ 648
Wages		$ 7,908	
Benefits		$ 632	
Equipment			
Supplies[1]		$ 800	$ 800
Travel			
Plot Fees[2]			$200
Miscellaneous			
Total	$ 0	$ 9340*	**$ 9743**

Footnotes: [1] supplies to produce *Erwinia amylovora* inoculum and maintain quince field plot
[2]plot fees of $200 were added to 2014.
*2013 budget was not spent and will not be requested in 2014

Budget 3 – Todd Erickson
Organization: Helios Nursery (owner; Tye Fleming) **Contract Administrator:** Todd Erickson
Telephone: 971-241-8116 **Email address:** toddaericksonsr@hotmail.com

Item	2012	2013*	2014*
Wages[1]	0	8,400	16,800
Benefits			
Supplies			
Travel			
Plot Fees			
Total	$0	$8,400*	**$16,800**

Footnotes: [1] 2013-2014 costs are to bud 3,500 rootstock liners (including ½ with interstems), and raise for one-year in nursery ($16,800). Costs are distributed over 2013-2014.
*2013 budget of $8,400 was not spent. These funds will be shifted to 2014.

Budget 4 – Yongjian Chang
Organization: North American Plants **Contract Administrator:** Yongjian Chang
Telephone: 503-474-1852 **Email address:** ychang@naplants.com

Item	2012	2013	2014
Wages	0	$7900	0
Benefits			
Supplies			
Travel			
Plot Fees			
Total	$0	$7900	$0

Footnotes: [1] 2013 costs are to produce 3,500 rootstock liners in vitro (250 plants for each of 12 quince selections plus smaller number of 6 additional), to be supplied to Helios Nursery for grafting.

Budget 5 – Kate Evans
Organization: WSU-TFREC **Contract Administrator:** Carrie Johnston & Kevin Larson
Telephone: 509.335.4564, 509.663.8181 **Email address:** carriej@wsu.edu ; kevin_larson@wsu.edu

Item	2012	2013	2014
Wages			$ 1,000
Benefits			$ 173
Supplies[1]			$ 2,750
Travel			
Plot Fees			$2,000
Total	$0	$0	**$5,923**

Footnotes: [1] to cover field preparation, fumigation and irrigation costs

Budget 6 – Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Wages			$ 1,000
Benefits			$110
Supplies[1]			$ 2,750
Travel			
Plot Fees			$ 3,104
Total	$0	$0	**$ 6,964**

Footnotes: [1]to cover field preparation, fumigation and irrigation costs

Three Year Project Objectives:

1) Determine effective propagation methods for quince with commercial nursery partners.
2) Test graft compatibility of cold hardy quince rootstocks and commercial pear cultivars.
3) Determine fire-blight resistance/sensitivity of cold-hardy quince rootstocks.
4) Deliver 10-12 rootstock clones grafted to Bartlett and Anjou for field trials in Wenatchee and Hood River.

Significant Accomplishments and Findings in 2013:

1. **Stoolbed Establishment –**Fifteen of the 22 cold-hardy accessions identified as potential candidates for the PNW were established in a stool bed at Helios Nursery (Todd Erickson, co-pi). The remaining seven accessions will be supplied by either N.A. Plants (rooted explants), or Joseph Postman (rooted cuttings) and delivered to Helios Nursery in 2014 for inclusion in the stooling trial. Trees were pinned down early summer of 2013 and generated a sufficient number of upright shoots for stooling. Heading and mounding processes will be performed in spring of 2014. Rooting will be assessed fall of 2014.

2. **In vitro multiplication-**
   a. 70% of the cold-hardy quince clones were successfully cultured *in vitro* at N.A.Plants. All accessions have been transplanted to media and are rooting (the final group scheduled for Feb 2014). In most cases, numbers of transplants per accession exceed the number required for field trials.
   b. Shoots are presently being forced in the greenhouse to re-initiate cultures of the 7 remaining clones to be transferred to North American Plants for multiplication.

3. **Production of quince trees for fire blight field trial** – Due to poor uniformity and small plant size for many of the rootstock clones delivered April, 2013, no artificial inoculations with the fire blight pathogen, *Erwinia amylovora*, were conducted this year. Liners of thirty-four quince rootstocks including 18 of the 22 cold-hardy clones were established in an orchard plot to increase plant size in an effort to reduce the variability of the inoculation test. The planting has a total of 274 plants, with each clone having ~10 replicates. For those clones with fewer than 10 replicates, additional trees were made in 2013 for shipment in February of 2014. In addition, 34 OH×F 87 rootstocks were planted as resistant controls. No natural fire blight was observed in these plants during 2013, in spite of thin, vigorous shoot growth.

RESULTS AND DISCUSSION

In our previous efforts (2009-2011) we identified 22 quince taxa that showed 50% or less browning following exposure to -22 °F. These accessions had equal or greater cold hardiness than our currently used Pyrus rootstocks (OH × F 87, OH × F 97). Our main objectives for this phase of the project are to develop propagation knowledge/protocols for these accessions, and to produce an adequate volume of trees for field evaluations. The three propagation techniques under evaluation are in-vitro (tissue culture and rooting of explants), cutting (hard- and soft-wood), and stooling.

In vitro initiation was highly successful at N.A. Plants. Fifteen of the 22 clones have been cultured and transplanted for root initiation and development (Table 1 and photos). Slight alterations

to tissue culture media were made to optimize in-vitro production of the different genotypes. Despite being delayed from our initial timeframe due to loss of cultures to thrips infestation in October/November of 2012, N.A. Plants has rapidly multiplied a sufficient number of transplants for liner production. In fact, all clones are maintained in culture permitting rapid production of additional plants should the need arise. N.A. Plants will supply liners to Todd Erickson (Helios Nursery) spring of 2014. Erickson will transplant liners and culture for maximum growth. Growth accrued during 2014 will be the key determinant to base the decision to bud interstems (Beurre Hardy) and scions (Anjou and Bartlett) this summer. The decision to bud will be made with input from the team. Plant tissue and/or cultures of the seven genotypes not represented in the in-vitro collection will be provided to N.A. Plants from USDA-NCGR in 2014. Some of these are clones had difficulty in multiplying.

Propagation results from hardwood and softwood cutting trials were reported last year. A second hardwood cutting trial was initiated in November, 2013 and a summary of those results has been combined with earlier results and presented in Table 2. Previous trials showed that both hardwood and softwood cuttings rooted significantly better when rooting hormone was used. Some clones had superior rooting from softwood and others from hardwood cuttings. Only one clone (the hardiest of the population) did not root easily from either soft or hardwood cuttings. Although the proportion of cuttings that rooted was relatively low, many of the quince accessions were observed to root more efficiently than OH × F clones.

As described above (under significant findings), a decision was made to delay fire blight inoculations one year. All but three clones were established in a replicated field trial in Kearneysville, WV in 2013. Clones with inadequate propagation success were grafted on Quince A rootstocks to provide sufficient replication. In addition, 61 grafted trees are ready for shipment in February 2014 to bolster replication (Table 3).

Initial Goals for Year 3 (2014 at Wenatchee, WA; Hood River, OR; Kearneysville, WV) with Revisions:

- **Assess graft-compatibility of Bartlett and Anjou as they develop into finished trees.**
  (Schedule is modified as follows: initial graft compatibility evaluation will occur beginning summer 2014 through 2015).
- **Second year of fire blight testing at Kearneysville.** (Schedule is modified as follows: First year of inoculations trials and evaluation will occur in 2014).
- **Finished trees completed at Helios Nursery.** (Schedule is modified as follows: Liner rootstocks will be received by Helios Nursery spring 2014. Depending upon tree growth at Helios during spring and summer 2014, trees will either be budded summer of 2014 or spring 2015. Trees are expected to be finished in 2015 for delivery to Wenatchee and Hood River for field trial establishment spring of 2016).

METHODS to achieve year 3 goals (2014):

1. **Field test self-rooted quince clones for fire blight /resistance/susceptibility.**
   a. Shoots, including the leader scaffold shoots on each plant, will be inoculated with a local isolate (AFRS 581) of the fire blight pathogen (*Erwinia amylovora*). Shoot length at the time of inoculation will be recorded.
   b. At weekly intervals until infection ceases to progress, the lesion length of infection will be recorded.
   c. Resistance/susceptibility will be assessed on the basis of the final lesion length, percentage of current seasons shoot length infected, length of older wood infected, and the area under the disease progress curve (AUDPC).

d. The data will be appropriately analyzed and clones ranked according to their susceptibility/resistance.

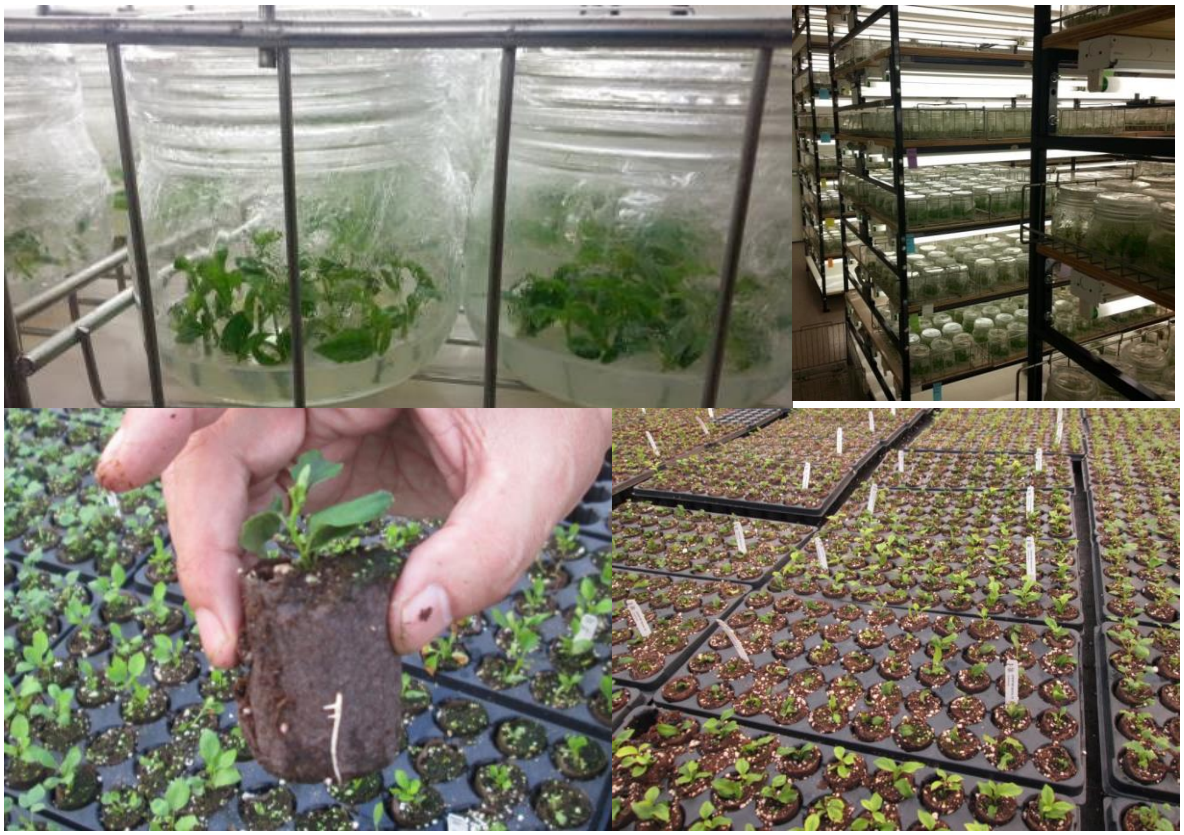
2. Produce adequate plant numbers of cold-hardy quince plants with and without interstems to be grown on for grafted field trials.

a. Selected clones to be multiplied in vitro (North American Plants). Need 160 trees for the hardiest 12 quince clones for replicated field trials with and without interstems at Wenatchee and Hood River, (4 reps of 10 trees with and without interstems) at each site for each rootstock selection). Half of the quince liners to be worked to Beurre Hardy interstem and Anjou or Bartlett scion following the 2014 growing season. The other half budded to Anjou or Bartlett (without interstems).
b. OHxF 87 and OHxF 97 (no interstems) will also be budded to 'Anjou' and 'Bartlett' as controls.
c. Virus tested sources will be used for all 'Anjou','Bartlett', and 'Beurre Hardy' budwood.

Photos: Fourteen cold-hardy quince accessions prepared (pinned down) for stooling at Helios Nursery. The goal is to produce ~100 rooted plantlets from each accession. Photos taken Oct. 24, 2013.

Table 1. Fifteen of the of twenty-two clones previously determined to possess adequate cold hardiness for the PNW have been successfully tissue cultured by Dr. Yongjian Chang at N.A. Plants. Explants have been planted in three stages (October, 2013; January, 2014; and, February, 2014). Plantlets transplanted in October have successfully rooted (see photos). All plants will attain adequate size to ship to Todd Erickson (Helios Nursery) in March, 2014.

Variety	Accession	Number of tissue-cultured explants transplanted		
		Planted in October	Planted in January	Ready for planting in Feb
CYD 118.001	C. oblonga - Seghani	**563**		
CYD 65.001	Quince C7/1	**520**		
CYD 23.001	WF-17	**466**		
CYD 22.001	W-4	**430**		
CYD 57.001	Quince S	**400**		
CYD 99.002	Kashenko no. 8	**307**		
CYD 67.001	Akhtubinskaya O.P. seedling 1		**500**	
CYD 67.004	Akhtubinskaya O.P. seedling 4		**500**	
CYD 68.002	Krukovskaya O.P. seedling 2		**500**	
CYD 70.001			**500**	
CYD 128.001	C. oblonga - Babaneuri			**500**
CYD 29.001	Quince W			**400**
CYD 123.001	Trentholm			**300**
CYD 32.004	Tashkent AR-232 seedling 4			**300**
9.001	Pyronia Veitchii			**200**



Photos: Quince accessions in tissue culture at NA Plants (above images); rooted explants planted into media in October, 2013 (lower). All photos taken Oct. 24, 2013.

Table 2. Summary of percent rooting success following three separate cutting propagation trials. Dates signify when cuttings were produced. Results were evaluated after 6 weeks and 6 months for soft-wood and hard-wood cuttings, respectively.

Hardiness Rank	Inventory No.	Accession	hardwood cuttings 01/2012	softwood cuttings 06/2012	hardwood cuttings 11/2012
1	120.001	C. oblonga - Arakseni, Armenia	-	0.0	0.0
2	104.001	Aiva from Gebeseud	0.0	33.3	0.0
3	67.004	Akhtubinskaya O.P. sdlg. 4	0.0	33.3	0.0
4	32.004	Tashkent AR-232 sdlg. 4	4.8	4.2	0.0
5	70.001	Skorospelka O.P. sdlg. 1	-	12.5	0.0
6	57.001	Quince S	23.8	8.3	28.6
7	29.001	Quince W	42.9	29.2	52.4
8	126.001	C. oblonga - Megri, Armenia	4.8	12.5	4.8
9	118.001	C. oblonga - Seghani, Armenia	9.5	25.0	4.8
10	32.002	Tashkent AR-232 sdlg. 2	0.0	37.5	0.0
12	128.001	C. oblonga - Babaneuri, Georgia	14.3	8.3	9.5
13	68.002	Krukovskaya O.P. sdlg. 2	-	45.8	0.0
14	22.001	W-4	0.0	12.5	33.3
15	123.001	Trentholm	0.0	12.5	0.0
16	23.001	WF-17	28.6	25.0	47.6
17	75.001	Bereczki [Beretskiquitte]	-	29.2	0.0
18	99.002	Kashenko No. 8	-	12.5	0.0
19	65.001	Quince C7/1	57.1	8.3	23.8
20	9.001	Pyronia veitchii	-	-	23.8

Base of freshly made cuttings dipped in rooting hormone (0.8% indole-3-butyric acid; Hormex No. 8 powder).

Table 3. Cold hardy quince rootstock candidates propagated and shipped to R. Bell in Kearneysville, WV for fire blight evaluations.

Hardiness Rank	Inventory No.	Accession	Sent* 04/2013	Ready ** 01/2014
1	120.001	C. oblonga - Arakseni, Armenia	10	
2	104.001	Aiva from Gebeseud	11	
3	67.004	Akhtubinskaya O.P. sdlg. 4	26	
4	32.004	Tashkent AR-232 sdlg. 4	6	5
5	70.001	Skorospelka O.P. sdlg. 1	8	9
6	57.001	Quince S	12	
7	29.001	Quince W	14	
8	126.001	C. oblonga - Megri, Armenia	3	8
9	118.001	C. oblonga - Seghani, Armenia	11	
10	32.002	Tashkent AR-232 sdlg. 2	21	
12	128.001	C. oblonga - Babaneuri, Georgia	2	5
13	68.002	Krukovskaya O.P. sdlg. 2	10	
14	22.001	W-4	6	7
15	123.001	Trentholm		14
16	23.001	WF-17	8	4
17	75.001	Bereczki	11	
18	99.002	Kashenko No. 8	3	9
19	65.001	Quince C7/1	16	
20	9.001	Pyronia veitchii	7	
		total	185	61

* self-rooted trees; ** grafted trees

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Horner rootstock grower evaluation trials

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Cooperators: Growers: Mike McCarthy and Eric Von Lubken (Hood River Trial), Chuck Peters (Wapato Trial), Bob Foyle and site manager Garrett Znan, (Bridgeport Trial), Mark Stennes (Methow Trial).

Total Project Request: **Year 1:** $14,335 **Year 2:** $16,134 **Year 3: $16,663**

Other funding sources: None

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Salaries[1]	3,142	3,236	3,333
Benefits	2,168	2,233	2,300
Travel[2]	500	1,300	1,300
Total	$5,810	$6,769	**$6,933**

Footnotes: [1] Salaries are calculated as 2 weeks of a Full Time Technician's salary and OPE, for oversight of field plots, plant measurements, and data management. The increase in salaries for years two and three reflects a 3 % rate increase. [2] Travel includes 1 trip to WA sites/year beginning in year 2 (2013) at 0.51 cents per mile, one night lodging and two days per diem for PI and technician, and visits to OR orchard sites for data collection and support.

Budget 2: Tom Auvil
Organization Name: WA Tree Fruit Research Comm. **Contract Administrator:** Kathy Schmidt
Telephone: 509-665-8271 **Email address:** Kathy@treefruitresearch.com

Item	2012	2013	2014
Salaries[1]	3,000	3,500	3,600
Benefits[1]	1,050	1,225	1,260
Wages[1]	2,675	2,800	2,900
Benefits	800	840	870
Travel[1]	900	900	1,000
Miscellaneous	100	100	100
Total	$8,525	$9,365	**$9,730**

[1]Salary and benefits include WTFRC internal program's time for supervision, planning, logistics and data management for pear projects.

Objectives:

1. Determine the influence of Horner 4 and 10 on tree growth, flowering, fruit size, yield (both annual and cumulative) and quality for the cultivars, 'Bartlett', 'Golden Russet Bosc' and 'd'Anjou'. OH×F 87 will be used as the standard.

2. Compare rootstock/scion interactions among orchards at different geographic locations.

Significant Findings 2013:

- OH×F 87 is, by far, the superior rootstock for yield, tree size, and yield efficiency when compared to Horner 10 and Horner 4 over three cultivars and four sites.
- Depending on the cultivar, Horner 4 has performed relatively well (yield and yield efficiency) and in nearly every case produced the largest fruit.
- Horner 10 had the lowest yields, yield efficiency and fruit size. Tree size was similar to OH×F 87.
- Tree mortality differed from site to site and does not appear to be influenced by rootstock genotype.
- 'Bartlett' trees at Wapato produced high yields in the fifth leaf on OH×F 87 (projected per acre yield of 55 bins) followed by Horner 4 (48 bins). Horner 10 yields (44 bins) were significantly less than OH×F 87. Tree size was not influenced by rootstock. Fruit size, however, was significantly smaller on Horner 10 (100s) compared to OH×F 87 and Horner 4 (90s).
- 'Bartlett' yields at Methow were lower than Wapato, but trends were similar. Fruit size, however, was small in general, though, largest on Horner 4.
- Tree yield of 'GR Bosc' was 20% higher on OH×F 87 compared to either Horner 4 or 10. Projected production was 43 bins per acre for OH×F 87. Tree size was slightly larger on Horner 4, but not significantly.
- 'd'Anjou' yields were characteristically low relative to Bartlett and Bosc for 5th leaf trees, but differed between sites. In Hood River, OH×F 87 and Horner 4 produced two-fold the yield (~ 10 bins per acre) as Horner 10. Yields in Parkdale were less affected by rootstock and nearly double those in Hood River; however, significant fire blight injury has reduced the number of measurement trees and limited valid comparisons among rootstocks.
- 'D'Anjou' tree size at both sites was ~40% larger on Horner 4 than OH×F 87 or Horner 10.

Results and Discussion:

1. Sites.
Wapato (Bartlett and Bosc) had roughly 40% of the planting affected by fire blight. There did not appear to be any interaction between rootstock and fire blight susceptibility (i.e., trees on all rootstocks were similarly affected). The Parkdale, OR 'd'Anjou' site continued to suffer significant damage due to a combination of cold injury and pseudomonas infection (from 2010/2011 freeze events) and fire blight infection (both 2012 and 2013) and will likely be eliminated from the trial in 2014. Methow (Bartlett) had no additional tree mortalities or issues in 2013. When averaged across all sites (and cultivars), OH×F 87 outperformed both Horner rootstocks in production (Table 1). Horner 4 produced the largest fruit (compared to other rootstocks), but also produced the largest trees (however, differences in tree size were highly influenced by inclusion of 'd'Anjou' data). Horner 10 has similar vigor to OH×F 87, but with significantly less yield; a situation described by its low yield efficiency.

Table 1. Cumulative performance of rootstock selections averaged over 5 sites since planting establishment in 2009. Percent mortality includes trees which were diseased and not used for data collection.

Rootstock **All cultivars**	Yield (lbs/tree)	Proj. production (bins/a)	Fruit wt. (g)	TCA (cm^2)	Yield effic. (kg/cm^2 TCA)	Mortality/disease (%)
OH×F87	63.8 a	53	211 b	35.1 b	0.99 a	33
Horner 4	54.2 b	45	224 a	43.2 a	0.81 b	25
Horner 10	47.2 b	39	206 b	34 b	0.77 b	29

Cumulative average over 5 years from 5 sites (2 Bartlett; 2 Anjou; 1 Bosc)

Details pertaining to the existing trial sites are provided below:

Hood River
- Spacing: 17' x 6' (427 trees per acre)
- Scion: 'd'Anjou'
- Rootstocks: OH×F 87, Horner 4, Horner 10
- System: Modified central leader/three wire support
- Replicates: Six, five-tree reps

Parkdale
- Spacing: 12' x 6' (605 trees/acre)
- Scion: 'd'Anjou'
- Rootstocks: OH×F 87, Horner 4, Horner 10
- System: In-line "V" fruiting wall/wire support
- Replicates: Six, five-tree reps

Wapato
- Spacing: 10' x 4' (1089 trees per acre)
- Scion: 'Bartlett' and 'Bosc'
- Rootstocks: OH×F 87, Horner 4, Horner 10
- System: Tall spindle fruiting wall/wire support
- Replicates: Five, five-tree reps

Methow
- Spacing: 12' x 4' (907 trees per acre)
- Scion: 'Bartlett'
- Rootstocks: OH×F 87, Horner 4, Horner 10
- System: Tall spindle/wire support
- Replicates: Five, five-tree reps

2. Rootstock effects

Effects of rootstocks are organized according to cultivar.

D'Anjou'.

Anjou tree growth continued to be antagonized by Horner 4 producing trees markedly larger than those on either Horner 10 or OH×F 87. The effects were nearly identical at both sites indicating a conserved response from this combination on tree vigor (Tables 2 & 3). Notable differences in fruit set between the two sites (~35% at Parkdale vs. ~12% on average at Hood River) translated to tree

yield. Moreover, the markedly higher tree density at Parkdale (~40% greater than Hood River) produced per acre yields nearly double those observed at Hood River. As expected, yield efficiency of Anjou was drastically lower when compared to Bosc and Bartlett but was positively influenced by OH×F 87.

Table 2. 2013 Hood River Anjou fruit set, average tree yield, projected production, average fruit weight, trunk size (TCA), Yield Efficiency (YE), and mortality as affected by rootstock. Percent mortality includes trees which were diseased and not used for data collection.

d'Anjou Hood River	Fruit set (%)	Yield (lbs/tree)	Proj. production (bins/a)	Fruit wt. (g)	TCA (cm^2)	Yield effic. (kg/cm^2 TCA)	Mortality/disease (%)
OH×F87	12.1 ab	26 a	10	215 b (90)	48 b	0.24 a	7 b
Horner 4	16.3 a	23 a	9	241 a (80)	69 a	0.15 b	0 c
Horner 10	8.5 b	14 b	5	193 c (100)	47 b	0.13 b	27 a

5th leaf production; 427 trees/acre

Table 3. 2013 Parkdale Anjou fruit set, average tree yield, projected production, average fruit weight, trunk size (TCA), Yield Efficiency (YE), and mortality as affected by rootstock. Percent mortality includes trees which were diseased and not used for data collection.

d'Anjou Parkdale	Fruit set (%)	Yield (lbs/tree)	Proj. production (bins/a)	Fruit wt. (g)	TCA (cm^2)	Yield effic. (kg/cm^2 TCA)	Mortality/disease (%)
OH×F87	33	37	20	188 (110)	46	0.37	77 a
Horner 4	36	25	14	197 (100)	65	0.18	37 b
Horner 10	38	33	18	175 (110)	49	0.31	23 c

5th leaf production; 605 trees/acre

‘Golden Russet Bosc’.

‘Bosc’ trees attained good 5th leaf yields with excellent fruit size (Table 4). OH×F 87 outperformed both Horner selections with respect to yield and yield efficiency. Horner 4 and Horner 10 produced similar tree yields, but fruits were larger on Horner 4. Fruit size was excellent, however, for all rootstocks. Horner 4 produced the largest tree, albeit nonsignificantly. Projected ‘GR Bosc’ 5th leaf production was 43 bins per acre on OH×F 87. Roughly 40% of the trees, irrespective of rootstock, required corrective pruning to remove fire blight infected wood; a large proportion of the bearing canopy was removed, hence these trees were not factored into the data analysis.

Table 4. 2013 Wapato-site ‘GR Bosc’ tree yield (lbs per tree), projected production (bins/acre), average fruit weight (g), trunk cross-sectional area (TCA), yield efficiency (kg per cm^2 of TCA), and mortality as affected by rootstock. Percent mortality includes trees which were diseased and not used for data collection.

Bosc' Wapato	Yield (lbs/tree)	Proj. production (bins/a)	Fruit wt. (g)	TCA (cm^2)	Yield effic. (kg/cm^2 TCA)	Mortality/disease (%)
OH×F87	43 a	43	301 b (70)	32	0.63 a	40
Horner 4	32 b	32	322 a (60)	35	0.43 c	44
Horner 10	34 b	34	295 b (70)	28	0.55 b	52

5th leaf production; 1089 trees/acre

‘Bartlett’.

Wapato ‘Bartlett’ production exceeded 50 bins per acre on OH×F 87, following a 50 bin per acre crop in 2012 (Table 5). Fruit size peaked on 90s and yield efficiency was significantly higher than either

Horner 4 or Horner 10. Tree size was not affected by rootstock. The cumulative yield efficiency of OH×F 87 through the 5th leaf (2.01 kg/cm^2 TCA) is roughly two-fold that achieved in comparable rootstock evaluation trials in the US (Elkins et al., 2011). A second year of high production was associated with good fertigation and irrigaton practices in the formative years. Horner 10 had the lowest yields, yield efficiency and fruit size.

Table 5. 2013 Wapato-site 'Bartlett' tree yield (lbs per tree), projected production (bins/acre), average fruit weight (g), trunk cross-sectional area (TCA), and yield efficiency (kg per cm^2 of TCA) as affected by rootstock. Percent mortality includes trees which were diseased and not used for data collection.

Bartlett' Wapato	Yield (lbs/tree)	Proj. production (bins/a)	Fruit wt. (g)	TCA (cm^2)	Yield effic. (kg/cm^2 TCA)	Mortality/disease (%)
OH×F87	55.5 a	55	219 a (90)	26	0.98 a	40
Horner 4	48.6 b	48	216 a (90)	24	0.92 ab	40
Horner 10	44.5 b	44	204 b (100)	25	0.81 b	44

5th leaf production; 1089 trees/acre

Methow 'Bartlett' followed a similar trend as observed at Wapato, but yields were ~1/3rd lower than those recorded at Wapato (Tables 5 & 6). Horner 4 produced significantly larger fruit than Horner 10 or OH×F 87 at Methow. As observed at Wapato, Bartlett tree size was not significantly influenced by rootstock. These data contrast recent results from Elkins et al. (2011) showing significantly larger Bartlett trees on Horner 4 relative to those on 6 alternative rootstock selections.

Table 6. 2013 Methow-site 'Bartlett' tree yield (lbs per tree), projected production (bins/acre), average fruit weight (g), trunk cross-sectional area (TCA), and yield efficiency (kg per cm^2 of TCA) as affected by rootstock. Percent mortality includes trees which were diseased and not used for data collection.

Bartlett' Methow	Yield (lbs/tree)	Proj. production (bins/a)	Fruit wt. (g)	TCA (cm^2)	Yield effic. (kg/cm^2 TCA)	Mortality/disease (%)
OH×F87	37.4 a	31	178 b (110)	24	0.72	0
Horner 4	32.9 ab	27	200 a (100)	24	0.63	4
Horner 10	28.3 b	23	174 b (110)	21	0.62	0

5th leaf production; 907 trees/acre

Plan for 2014.

We propose to complete the evaluation of rootstock performance in all remaining grower-cooperator orchards, with the exception of Parkdale given the high incidence of fire blight and lack of sufficient replication for statistical purposes. 2014 will be the final year of the evaluation.