Northwest Pear Research Review
Hood River Best Western
Tuesday, February 19, 2013

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FINAL PROJECT REPORT

Project Title: Systems approach for ensuring superior pear fruit quality

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Other funding sources – none

Total Project Funding: Year 1: 113,861 Year 2: 114,759 Year 3: 118,045

Budget 1 Amit Dhingra
Organization Name: WSU **Contract Administrator:** ML. Bricker
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Item	2010	2011	2012
Salaries[1]	55,002	46,765	43,871
Benefits	10,523	3,337	10,143
Wages	7,546	7,847	8,160
Benefits	724	753	783
Supplies	8,000	8,000	8,000
Travel	5,000	9,000	2,000
Consumer panel			5000
Miscellaneous – 454 sequencing		11,000	11,000
Total	86,795	86,702	88,957

Footnotes: [1] Salaries for agriculture research assistant (PhD-12 months) and agriculture research assistant (MS-9 months @ 65% of 0.50FTE) and visiting scholar for performing physiological and genomic profiling, all molecular work; sanitization platform and robotics respectively. The increase in salaries for years two and three reflects a 4 % rate increase.

Budget 2 Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton
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Item	2010	2011	2012
Salaries[1]	$21,662	$22,529	$23,430
Benefits[2]	$2,484	$2,608	$2,738
Wages[3]	$2,000	$2,000	$2,000
Benefits[4]	$170	$170	$170
Equipment			
Supplies			
Travel[5]	$750	$750	$750
Miscellaneous			
Total	$27,066	$28,057	$29,088

Footnotes:
[1]Salary is for a 0.49 FTE M.S. candidate calculated based on a 1.0 FTE salary rate of $44,208.
[2]MS OPE rate is $567/term * 4 terms/academic year
[3]Hourly wages for time-slip labor (~200 hours @ $9/hour) to assist with data collection and cultural practices
[4]Benefit rate for part-time employee is 8.5 %
[5]Travel includes transportation to off-station sites in OR, and one trip per year to WA sites at 0.59 cents/mile

OBJECTIVES

Summary Statement: This multi-investigator project represented multi-disciplinary activities aimed at ensuring superior pear fruit quality. Thanks to the vision of the PNW pear industry, over 68 scientists from US, Europe and South America representing diverse disciplines continue to work together with their respective industries on several aspects initiated as part of this project. A draft roadmap has been developed from our collective activities in collaboration with NW Hort Council. Further funding is being sought from USDA and NSF to build upon the foundation developed as part of this project.

Objective 1: Training systems: Evaluate, devise, and plant efficient orchard systems that are amenable to mechanized pruning and harvest using labor assist platforms. These will be located on both research station and grower cooperator sites.

Years 1-3	Years 1-3	Years 1-3	Year 3
Todd Einhorn	Todd Einhorn, Amit Dhingra	Kate Evans, Amit Dhingra	Qin Zhang, Todd Einhorn
1a.Develop cropload indices for the optimum productivity of target fruit.	1b.Plant progressive, high-density pear systems using both the physiological thresholds identified from objective 1a, and experience gained from recent high-density PNW pear plantings.	1c.Identify genotypic sources of dwarfing in rootstocks and collate information from Co-PIs project on potential rootstocks for pear.	1d.Assess the potential of mechanized pruning in high density, vertical trellis or inclined UFO pear orchards.

Objective 2: Vigor Control: Assess the effectiveness of vigor-retarding mechanical and chemical techniques.

Years 1-3	Years 1-3	Year 1-3
Todd Einhorn	Todd Einhorn, Amit Dhingra	Todd Einhorn
2a. Identify optimal limb orientation on vigor (shoot growth) precocity, fruit size and fruit quality in planar trellis systems.	2b. Perform a comparative analysis on the effect of vigor control chemistries on apple and pear.	2c. Assess different chemistries for vigor control and develop timing and rate recommendations for effective vigor control in pear.

Objective 3: Fruit Quality

Years 1 and 3	Years 1-3	Year 1-3
Amit Dhingra	Amit Dhingra	Amit Dhingra, Ray Schmitten, Josh Koempel, Nate Reed
3a. Study the impact of cuticle or fruit skin on fruit quality.	3b. Understand cork spot and russet using microscopy and genomic profiling under physiologically inductive conditions.	3c. Test the impact of chlorophyll stabilizing chemistries on scuffing and fruit quality.

Objective 4. Evaluate alternative fruit sanitization platforms

Years 1-3	Years 1-3	Year 1-3
Shyam Sablani and Karen Killinger	Qin Zhang	Shyam Sablani and Carolyn Ross

4a. Test alternate fruit sanitization methods to reduce pathogen load.	4b. Identify alternate methods of processing fruit on processing lines to prevent skin damage.	4c. perform a consumer preference study to assess consumer experience with alternately sanitized or processed pears.

SIGNIFICANT FINDINGS

Objective 1:

New plantings:

- One rootstock trial was established spring 2012 (Bartlett; Chuck Peters, Wapato, WA). A second (Anjou) will be planted in spring 2013 at MCAREC. Both sites will compare three training systems (single axe, bi-axe, and steep V). Each system will be evaluated on different rootstocks (OHxF 87, OHxF 69 and Pyro 2-33) and at three different in-row spacings (2, 4, and 6 ft.).

Rootstocks:

- More than 200 accessions of promising Pyrus rootstock material have been identified in Spain, France, Italy, UK and Argentina and are currently being imported in small groups. DNA based population structure analysis will be initiated shortly.
- In coordination with the Pyrus Crop Germplasm Committee, U.S. nurseries and national and international collaborators a selected list of desirable pear rootstocks and rootstock selections has been compiled.

Mechanized Pruning:

- Work with vertical trellis system in sweet cherry bodes well for its application in pears. Challenges for implementation of mechanized pruning in pears have been identified.

Objective 2:

Limb Training:

- 'Bartlett' and 'Anjou' scaffolds were initiated and trained to 0, 30 or 45 degree angles (from horizontal) in 2009 on an eight wire vertical trellis (18 scaffolds per tree). In 2011, Bartlett 3rd leaf scaffolds trained to 30 degrees from horizontal were significantly more precocious than those trained to 45 or 0 degrees from horizontal.
- Average fruit set per tree was highest on 30 degree limbs (216 fruit), intermediate on 45 degree limbs (141), and lowest on horizontal (0 degree) limbs (75).
- Total length of scaffolds decreased as the angle decreased. Heavy fruit loads of the 30 degree limbs significantly reduced the number and length of offshoots per scaffold relative to the other angles. Total canopy leaf area for the 30 degree trees was half that of the other two angles.
- In 2012, there were no significant differences among limb angles for Bartlett fruit set, yield (42 to 46 bins per acre projected) or fruit number (~130 fruit per tree). Fruit size at harvest was similar on 30 and 45 degree limbs (100s), and slightly smaller on 0 degree limbs (110s). In the dormant season (Feb, 2012) all scaffolds were pruned to 10 fruiting spurs, irrespective of their limb angle.
- Despite profuse bloom in 2011 and 2012 (130-240 clusters per tree), Anjou fruit set and yield was poor, and unaffected by limb angle treatments.

PGR Vigor Control:

- The plant growth regulator abscisic acid (ABA) showed limited value for controlling shoot growth of pear due to its rapid metabolism (i.e., ~2 weeks after application).
- Apogee was extremely effective in controlling Anjou and Starkrimson shoot and tree vigor over nine separate trials between 2010 and 2012. Apogee markedly reduced shoot length (~50%) in all years compared to untreated controls.
- In 2012, we refined our spring application timing to occur when shoots were ~2 inches long.
- 250 ppm was the most effective Apogee rate for controlling shoot growth, and typically only required one application per year; however, in a few trials treated shoots resumed growth needing a second application (250 ppm) ~60 days after the first.

- This second flush of growth was not observed at any of the upper valley trial sites possessing shorter, cooler seasons, or in cooler seasons at lower elevations (Hood River).
- Apogee did not negatively affect yield or individual fruit size of 'd'Anjou' and 'Starkrimson' in any year. In fact, in 2012 whole tree Apogee applications significantly improved fruit set and yield (+70%) over controls.
- Apogee was shown to have a strong localized effect on shoot growth in a hedgerow planting. Protected, untreated shoots arising from the same scaffold as treated shoots showed ~2-fold more growth at the end of the season than treated shoots.
- Apogee had stronger control over growth from un-headed shoots compared to dormant headed shoots.
- Return bloom of Anjou spurs was reduced by ~20% on average from 2010 and 2011 trials. Starkrimson return bloom was not affected by Apogee applied in 2011. 2012 return bloom will be evaluated spring of 2013. Despite the reduction in Anjou bloom, fruit set and yield the year after application was not significantly different than controls, implying that reduced return bloom did not adversely affect fruit set.

Objective 3:

Fruit quality:

- Freeze fracture method was found to be an efficient method for determination of cuticle structure
- A standardized model to correlate cuticle thickness and fruit quality as it exists for apple could not be established for pear. This is primarily due to the separation of maturity and ripeness in pears.
- The cuticle thickness was highly variable within a fruit and also within fruit collected from different areas.
- There was some difference observed in amount of cuticular waxes however no correlations could be established between the site of collection and amount of wax.

Russet and Cork spot

- Physiological induction of cork spot and russet using published protocols was not successful.
- The pear homolog of apple bitter pit-related gene has been cloned and its expression will be tested in cork tissue in 2013 growing season to establish any correlations.

Pigment stabilization and fruit quality

- Pigment stabilizing chemistry has a positive effect on fruit storage quality as it maintains its firmness throughout the storage process.
- Expanded field tests were performed in Year 3. Fruit is currently under storage and will be analyzed from Feb –April 2013.

Objective 4:

Alternate fruit sanitization to reduce pathogen load:

- UV-C was effective in reducing generic *E. coli* and blue mold populations on intact and wounded pear surfaces.
- Efficacy of UV-C treatment was dependent on the type of microorganisms and fruit surface morphological profiles, for example generic *E. Coli* bacteria were more UV-C resistant than blue mold, and higher UV-C doses were required to reduce microorganism population on wounded surfaces compared to intact fruit surfaces.

Alternative fruit handling

- 1-MCP treated pear fruit appearance does not seem to be affected by processing line components.
- If 1-MCP can be utilized successfully in pears, any damage on the processing line can be countered.

Consumer preference study

- Sensory study is underway and will complete on February 11. Soon after the data will be analyzed and results will be reported.

RESULTS AND DISCUSSION

Objective 1 and 2 (combined for simplicity of presentation and overlap of horticultural issues)

PGR vigor control. ABA proved to be ineffective at controlling vegetative vigor of pear trees (data not shown). Apogee®, on the other hand, was very effective at controlling vigor of Anjou and Starkrimson. Previous research demonstrated that Bartlett fruit size was directly limited by Apogee® in the year of application, while Bosc return bloom and yields were markedly reduced the year following application; Anjou fruit growth and return bloom, however, were not similarly affected (Elfving, Sugar and Mielke). Between 2010 and 2012 we conducted 9 Apogee experiments; six Anjou trials and three Starkrimson trials. In each trial we observed an approximate 50 percent reduction in the annual growth of shoots relative to untreated trees. The strongest response occurred when applications were made in early spring when shoots were ~2 inches (5cm) in length at a rate of 250 ppm (Figs 1 and 2). In 2012 we also combined Apogee with Ethrel based on previous research with sweet cherry showing a synergistic effect of these compounds on vegetative growth (Elfving and Lang). The combination did lead to slightly greater growth control than Apogee alone (Fig 1). Interestingly, Ethrel alone did not reduce vegetative growth (Fig 1). In most cases only one application was required to control Anjou shoot growth for the entire season, but in several cases a second application at the same rate was needed ~ 60-80 days after the first (Fig 1). This application coincided with a marked increase in the rate of shoot growth (Fig 1), presumably due metabolism of Apogee® in the plant. Favorable environmental conditions, however, likely play an important role in stimulating this regrowth, since we did not observe it in most years or trials. Strakrimson trees did not require multiple applications of Apogee® (Fig 2). In all years, total tree yields of Anjou and Starkrimson were either slightly improved on trees sprayed with Apogee® or similar to untreated trees (Tables 1 and 2). Strarkrimson fruit size was unaffected by Apogee®; Anjou fruit were smaller, though we considered this to be an indirect effect of the significantly higher croploads on Apogee® treated trees (Table 1). In years when yields were unaffected by Apogee, fruit sizes were equivalent to those of control trees (Table 2). In the seasons following applications, Anjou return bloom was on average 15 percent reduced (Fig 4), but this did not translate to similar reductions in yield. Return bloom of Starkrimson trees was not reduced by Apogee® (Fig 4). In 2012, Ethrel was applied ~60 days from bloom (corresponding to the flower induction period for pear) to determine if Ethrel at this timing could lead to improved return bloom in 2013.

In a separate trial, Apogee® was applied in early spring to individual Anjou shoots of a planar, hedgerow system that were either dormant headed or left unpruned. Strong control of growth was achieved for Apogee® treated shoots while growth of adjacent untreated shoots, often originating on the same scaffold as their treated counterparts, was unaffected, indicating limited transport within trees (Fig 3). The localized effect of Apogee® is notable since it offers the ability to precisely manage portions of the canopy that are imbalanced, such as is often observed with increasing canopy height, or in the tops of trees that have been headed during the dormant season. Good control of Anjou growth from dormant heading cuts to tops of mature Anjou trees has been previously shown (Elfving). Apogee® was more efficacious when applied to unheaded shoots, but significantly reduced shoot length of headed shoots relative to untreated headed shoots as well. Although Apogee® is not presently labeled for pear we have contacted the manufacturer to discuss the next steps to achieving a label for Anjou and possibly Starkrimson.

Limb training. In 'Bartlett', training scaffolds to 30° from the horizontal markedly improved precocity (2011 flowering, fruit set, and yield of 3rd leaf limbs) compared to scaffolds trained to 45° or 0° from horizontal (Table 3). Scaffolds trained to 30° also had the least vegetative growth relative to other branch angles (data not shown). The high cropload associated with the 30° angle resulted in smaller fruit size in 2011. The high early fruit set, and higher yields were effective at controlling vigor, but perhaps the balance was shifted too much in favor of fruit. In 2012, scaffolds were pruned in the dormant season to 10 fruiting spurs removing thin wood with weak fruiting buds on the ends of scaffolds. Pruning to 10 spurs also maintained scaffolds in their allotted canopy space (trees are planted at 4 ft. in-row, so each scaffold has ~2 ft. to develop). Some overlap from scaffolds of

adjacent trees was permitted. Yield was not affected by angle of scaffold in 2012; all trees had relatively good yields averaging 53 lbs per tree (projected production of ~45 bins per acre). Horizontal branch angles produced smaller fruit in 2012, presumably because the wood was markedly weaker. The situation was not the same for Anjou trees. As is typically observed with Anjou, profuse bloom in third and fourth leaf limbs did not translate to significant fruit set or yield, irrespective of limb angle treatment (data not shown).

New Plantings. One new Bartlett planting was successfully established in Wapato Washington (Chuck Peters) spring 2012; an identical planting of Anjou will be planted in Hood River (OSU-MCAREC) spring 2013. The trials were designed to evaluate Bartlett and Anjou performance on OH × F 87, OH × F 69 and Pyro 2-33 trained to three different systems: Tall spindle/single-ax; bi-ax (parallel to the row); and, a steep, perpendicular V (each side ~10-15° from the vertical). For the V, each tree is bent to the opposite side of the tree row. For each rootstock/training system combination, three within row spacings will be evaluated: 2ft.; 4ft.; and, 6ft. Between row spacing is 12ft. Rootstocks were raised from tissue culture (North American Plants, LLC.) and delivered to Willow Drive Nursery spring of 2011. Rootstocks were budded to Anjou late summer 2011. Double budding to establish bi-axe trees was performed in the nursery. The bi-ax system has the advantages of splitting vigor over two axes, and provides a larger proportion of future bearing surface at planting compared to single leader trees, or trees that are headed at planting to create V systems. Finished trees will be delivered to MCAREC spring of 2013 and planted in fumigated ground.

Figures and Tables:

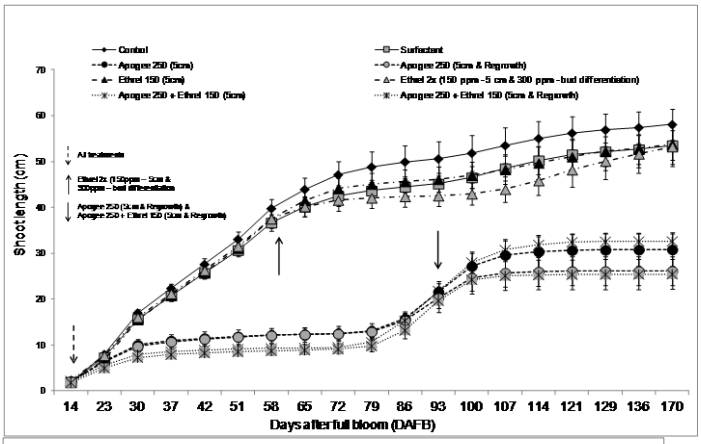

Figure 1. Shoot growth [length (cm)] of 'd'Anjou' pear trees sprayed with plant growth regulators, either alone or combined, when shoots were ~5cm long. Treatments were applied to whole trees (6 replicates) of similar trunk circumference (n=12 shoots per tree). MCAREC, 2012.

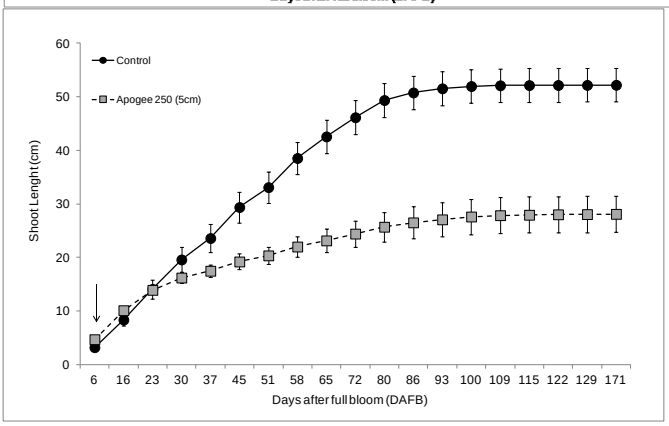

Figure 2. Shoot growth [length (cm)] of 'Starkrimson' pear trees sprayed with Apogee (250 ppm) when shoots were ~5cm long. Treatments were applied to whole trees (5 replicates) randomized within blocks (n=14 shoots per tree). MCAREC, 2012.

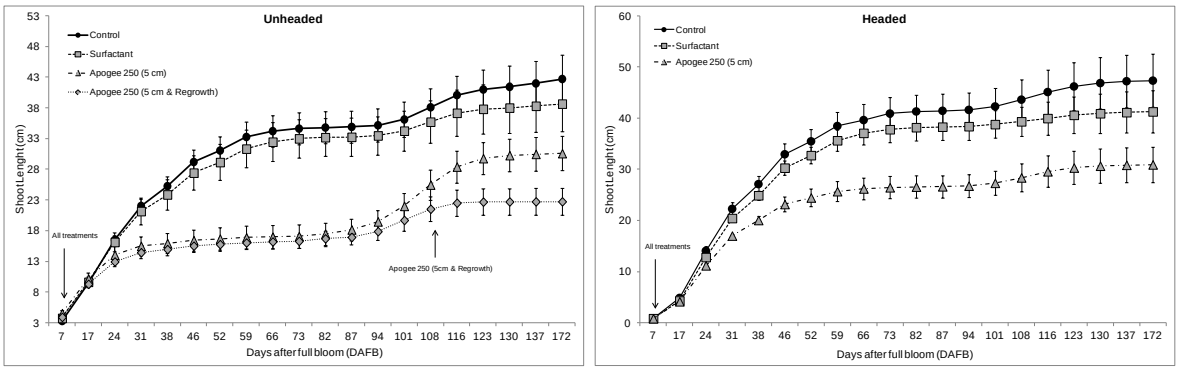

Figure 3. Shoot growth [length (cm)] of 'd'Anjou' single shoots (unheaded & headed at dormancy) following application of Apogee (250 ppm) when shoots were ~5cm long. Shoots were randomly selected in five-tree plots (5 replicates; n=10 shoots). Plots were selected in a high-density (906 trees/acre) planar system at MCAREC, 2012.

Table 1. The effect of plant growth regulators on 'd'Anjou' fruit number, yield and average fruit size. Treatments were applied to whole trees when shoots were ~5cm long and for certain treatments, again when a second growth flush was observed (data are means of 6 replicates). MCAREC, 2012.

Treatment	Yield		Avg. Fruit size
	(No. Fruit)	(lb per tree)	(g)
Control	266.8 c	160.7	273.0 a
Control + surfactant	296.8 bc	178.6	273.8 a
Apogee 250 ppm at 5 cm	447.8 a	229.5	234.5 cd
Apogee 250 ppm + Ethrel 150 ppm at 5 cm	396.8 ab	214.9	247.7 bcd
Ethrel 150 ppm at 5 cm	357.5 abc	200.1	257.3 ab
Apogee 250 ppm + Ethrel 150 (at 5 cm + regrowth)	349.2 abc	186.9	242.9 bcd
Apogee 250 ppm (at 5cm + regrowth)	345.3 abc	188.6	250.2 bc
Ethrel 150 ppm at 5 cm + 300 ppm at bud differentiation	323.0 bc	159.3	228.3 d

Within columns means with different letters are significantly different at P<0.05

Table 2. The effect of Apogee (250 ppm) on 'Starkrimson' fruit number, yield and average fruit size. Treatments were applied to whole trees when shoots were ~5cm (data are means of 5 replicates). MCAREC, 2012.

Treatment	Yield		Avg. fruit size
	(no.fruit per tree)	(lbs per tree)	(g)
Control	125	54.2	199.4
Apogee 250 ppm at 5cm	118.2	53.3	206.1

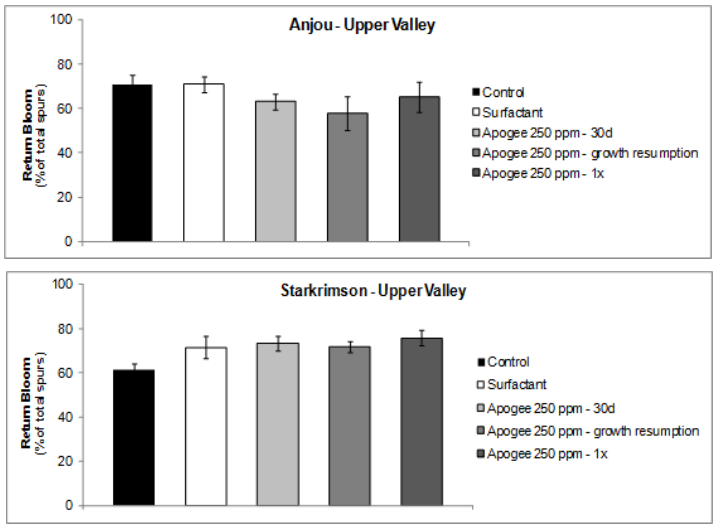

Figure 4. 'd'Anjou' and 'Starkrimson' return bloom in 2012 following 2011 Apogee applications. In 2011, Apogee was applied at a rate of 250 ppm when shoots were <10 cm long either once over the entire season (1x), twice (when shoots were <10 cm long and again when shoot growth resumed) or every 30 days, beginning when shoots were <10 cm long.

Table 3. Effect of primary scaffold branch angle (from the horizontal) on fruit set, yield, and average fruit size at harvest of Bartlett.

Limb Angle	**Fruit Set**	**Yield**				**Avg. Fruit Size**			
(° from Horiz.)	Fruit per tree (# before Thinning)	Per Tree (lb)		Per Acre (1,100 lb bins)		weight (g)		Box Size (# per 44 lbs)	
	2011	2011	2012	2011	2012	2011	2012	2011	2012
45[#]	141	38	51	31	42	196	198	100	100
30	216	50	55	41	46	179	194	110	100
0	75	27	56	22	47	202	183	100	110

[#]2011 and 2012 are 7th and 8th leaf for trees, but all previous scaffolds were removed in 2009. New, angled scaffolds were initiated in 2009. Tree spacing is 4 ft. x 12 ft. (906 trees per acre). System is an 8-wire vertical trellis, with a max height of 13 ft.

Objective 3:
Chlorophyll stabilizing chemistry:
The chlorophyll stabilizing chemistry shows an affect in d'Anjou pears which is a repeat of what was observed last year (Figure 5). Lower brix levels after CA storage can be exploited for delivering better pears. Since this chemistry does not interfere with the ethylene pathway it may provide an alternative to MCP in maintaining firmness in pears.

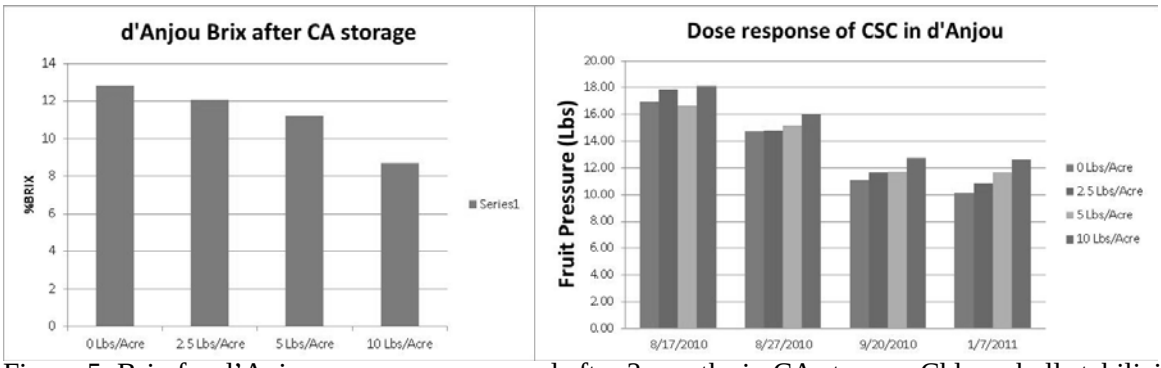

Figure 5: Brix for d'Anjou pears was measured after 3 months in CA storage. Chlorophyll stabilizing chemistry shows a clear dose response in maintaining fruit pressure similar to at harvest levels (right panel). Pears were stored in McDougal and Sons CA storage rooms.

Objective 4:

Alternate fruit sanitization with UV-C:

Maximum reductions of 3.70±0.13 log CFU/g were achieved for generic *E. coli* on intact pear surfaces, with lesser reduction on wounded pear (3.10±0.329 log CFU/g) after 4 minutes UV-C exposure at 7.56kJ/m^2. The time required for a 90% reduction in *E. coli* cell numbers for intact pear surfaces (0.019±0.009 min) was smaller than for wounded pear (0.062±0.013 min), suggesting that the wounds on pear surfaces helped to shield and protect microorganisms from UV-C radiation. Results indicated that blue mold inactivation on pear surface required lower UV-C doses than generic *E. coli* to reduce similar level of population (Figure 6). Fourier transform infrared (FT-IR) spectroscopy indicate that bacterial membrane damage (phospholipids, protein secondary structures and polysaccharides) and changes to DNA/RNA in *E. coli* resulted from UV-C treatment. UV-C can reduce microorganism populations on fresh pear but the efficacy of UV treatment is dependent upon the type of organism and morphological properties of the fruit and surface integrity.

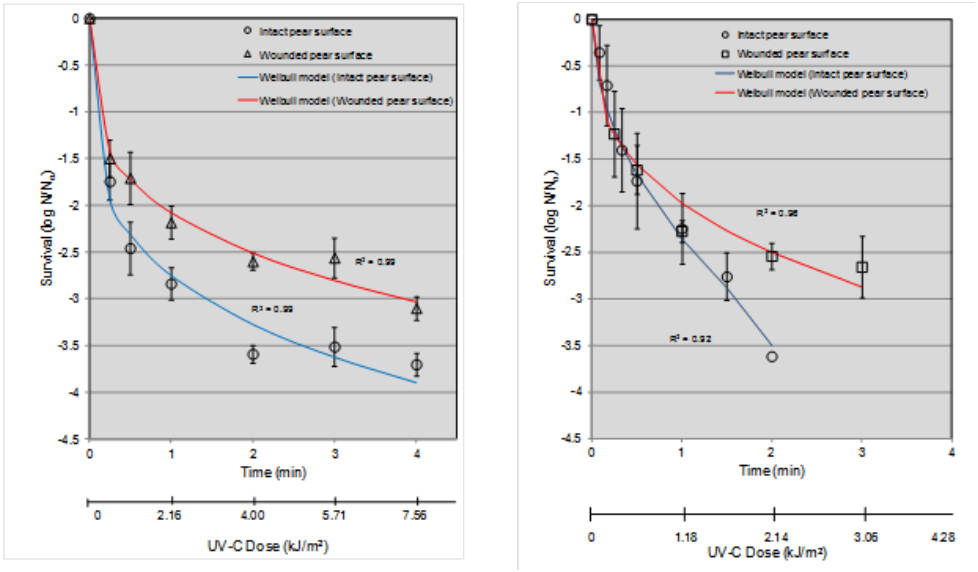

Figure 6. UV-C inactivation of generic *E. coli* (left figure) and blue mold (right figure) on intact and wounded pear surfaces

EXECUTIVE SUMMARY

This aim of this project was to conduct coordinated research in using a systems approach to ultimately improve fruit quality. In pears such an approach was needed to connect the sparse researchers and establish a core community. A network of researchers has been established that has contributed to the drafting of a pear research roadmap.

Significant progress has been made towards better understanding of horticultural management of the crop to impact fruit quality. A global network of pear breeders is already exchanging information, DNA and plant material that can be immediately implemented in the PNW in particular for rootstock improvement. A chemical has been identified to improve fruit quality along with promising results for alternate sanitization of fruit.

Summary of finding

Leaf scaffold angle regulated precocity in the new training systems. ABA was found not to be very effective in regulating plant vigor. However, Apogee was found to be highly effective not having any negative impact on yield or fruit size.

Chlorophyll stabilizing pigment continues to be promising in improving fruit quality and use of UV-C in sanitizing fruit has shown promising results.

Future directions
Some aspects of this research will be continued by individual investigators. In particular the impact of vigor controlling chemicals will be pursued further. Also, the efforts are ongoing to bring pigment stabilizing chemistry to market in collaboration with industries already working in this space. Additional funds are being obtained to continue research with UV-C. (Ultraviolet Light based Hybrid Technologies to Control Foodborne Pathogens on Fresh Produce, USDA AFRI Food Safety Program, $424,907, (Sablani, Rasco, Killinger and Syamaladevi, Pending**).**

FINAL PROJECT REPORT

Project Title: Physiological genomics of pear ripening

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Other funding sources: *None*

Budget History:
Budget 1
Organization Name: Washington State University **Contract Administrator:** ML Bricker
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Item	2010	2011	2012
Salaries[1]	29,255	30,426	31,643
Benefits			
Wages	6,500	6,760	7,030
Benefits	310	322	335
Equipment			
Supplies	6000	7000	7000
Travel	2000	1,000	2,000
Miscellaneous – 454 sequencing		11,000	
Total	$40,065	$56,508	$48,008

Footnotes: [1] Salaries for agriculture research assistant for performing physiological and genomic profiling and all molecular work. The increase in salaries for years two and three reflects a 4 % rate increase.

Budget 2
Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton
Telephone: 541 737 3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	2010	2011	2012
Salaries[1]	4,140	4,306	4,478
Benefits[2]	2,857	2,971	3,089
Wages			
Benefits			
Equipment			
Supplies	1,000	1,000	1,000
Travel			
Miscellaneous			
Total	$7,997	$8,277	$8,567

Footnotes: [1] Salary is based upon as 0.15 FTE Technician for harvest, cold storage and ethylene room maintenance, fruit quality attribute measurements, and data management. The increase in salaries for years two and three reflects a 4 % rate increase. [2] OPE rate is 69 %. Supplies largely include overnight shipping costs.

OBJECTIVES

This project was aimed at identify the genetic underpinnings of the chilling-requirement for ripening in European pear varieties and establish information for short and long term improvement of pear fruit quality.

With a range of variability in conditioning requirements among PNW pear varieties, identifying genetic causes of chilling-induced ripening and System 2 ethylene production will provide the foundational knowledge required for physiological management in the short term and in the future use breeding for adequate variety development. The physiological conditioning model implemented in the lab (Figure 1A, 1B, 1C), has established a reliable system for pear research at the physiological and genetic level. This infrastructure, although archaic, will be employed for further pear-focused research at WSU, including another ongoing project focusing on physiogenomics of 1-MCP use in pear.

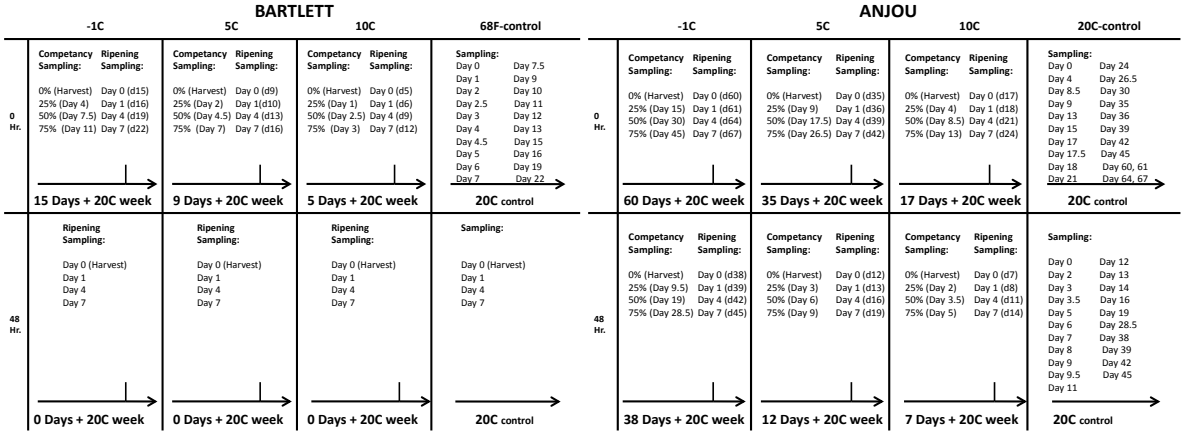

Figure 1. **(Top left)** Bartlett treatment and sampling scheme following conditioning protocols of Sugar and Kupferman. 1,920 fruit were divided into 8 groups of 24 each. These were subjected to one of 6 treatments, with 2 groups held at a constant 20°C. **(Top right)** D'D'Anjou treatment and sampling scheme following the conditioning protocols of Sugar and Kupferman. 1,920 fruit were divided into 8 groups of 240 each, then treated in the same manner as described for Bartlett. **(Lower left)** Flow-through respiration chambers inside climate-controlled room during D'Anjou conditioning. Where applicable, ethylene was injected into the system through a port on the rear of each chamber, to a concentration of 100 ppm (verified by gas chromatography). Outflow was set at 5 ml/min.

Objectives of this project were:

1. (Year1) Test the correlated activity of all ethylene, ripening-related and proposed regulatory genes along with the proposed cold-induced ripening master switch gene.

In this approach, peel tissue was sampled at regular intervals during conditioning, and subsequent ripening from Bartlett, Comice, and D'Anjou varieties. This work was performed both at OSU-MCAREC (Comice) and WSU-Pullman (Bartlett and D'Anjou). RNA, representing the active genes in the tissue, was then isolated from this tissue and used for quantitative real-time PCR (qPCR) analysis. This robust technique allows quantitative comparison of individual gene activity levels, and can help identify correlations between physiological processes and individual genes. In our work, we analyzed expression of 90 candidate ripening and System 2 ethylene biosynthetic regulatory genes that correspond to 6 major hormone and stress signaling pathways in pear. All genes examined via qPCR were related to one or more of the major regulatory pathways reported to control the onset of climacteric ripening in pear (Figure 2). These include a novel cold-induced gene (MIP, membrane integral protein identified in our lab from previous experiments) in cells and may play a critical role in integrating many of the signals reported to be involved in induction of climacteric ripening, and System 2 ethylene production in fruits. Also included are genes of a pathway which has been targeted in stimulation of ripening in 1-MCP treated fruit, under work for a related project. At the time of submission of this report, technical replicates of PCR tests are being performed for comprehensive data analysis and subsequent publication.

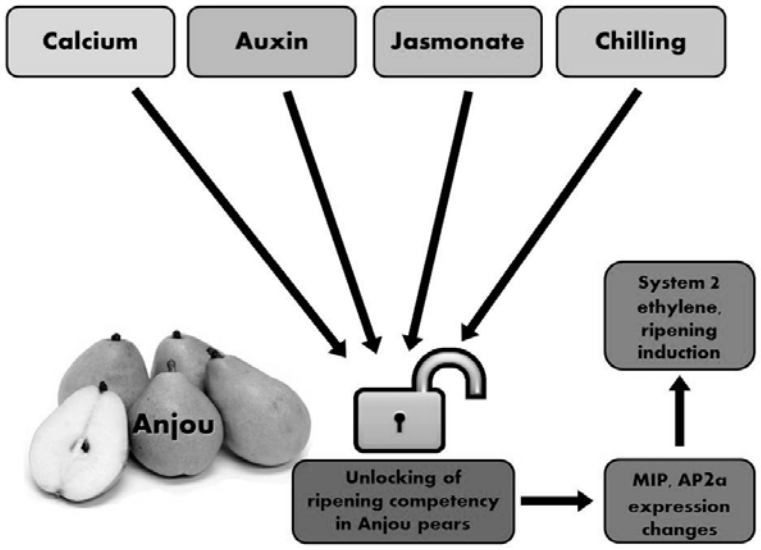

Figure 2. Model of physiological factors implicated in chilling-induced ripening and System 2 ethylene production in pear. Over 90 genes corresponding to these pathways were evaluated in this study providing a comprehensive insight into cold-induced ripening in pears.

2. (Year 2 and 3) Establish a relationship between ripening in winter pear and activity of the master switch regulator gene(s):

Tissues collected in Objective 1 were also be subjected to a gene-level comparative analysis to identify other genes involved in this phenomenon during ripening inductive conditions with ethylene and cold treatment. Among the genes tested, we applied statistical tests to determine which genes exhibited differential activity between Bartlett and D'Anjou samples during conditioning and ripening (Figure 3A, 3B, 3C below). Genes identified through this rigorous test serve as high-confidence elements in the overall regulatory mechanism governing the onset of ripening in conditioned pear fruit. Progress on this has yielded perhaps the most robust set of target genes involved in the chilling requirement for climacteric ripening and System 2 ethylene induction in pear available. Additionally, determination of when these genes are actively expressed in the fruit can offer in-field clues to growers toward fruit maturity and potential harvest windows.

3. (Year 2 and 3) Establish genetic diversity of the cold-induced ripening genes in pears:

Among the genes tested in Objectives 1 and 2, were the 1-aminocyclocarboxylic acid synthase (ACS) genes ACS1 and ACS2, which catalyze production of the immediate ethylene precursor, prior to and during the System 1-to-System 2 ethylene biosynthetic transition in pear (El-Sharkawy et al., 2004). This approach sought to isolate and sequence the unique MIP1, ACS1 and ACS2 sequences among PNW-specific varieties. This technique is useful in generating foundational knowledge to appropriately catalog ripening behavior and use by breeders to screen for desirable phenotypes during future pear improvement efforts.

SIGNIFICANT FINDINGS

Significant findings for Objective 1

- Auxin, jasmonate, ethylene, calcium, and a variety of cold-signaling pathway genes appear to be closely involved in chilling induced ripening and System 2 ethylene production. Many of these genes have powerful effects on the activity of several other genes, which in turn confers the traits of a ripened climacteric fruit, such as a 'burst' in ethylene production.
- Much of the same genetic machinery in other climacteric fruits is present in pear, aiding identification of novel genes which are not present, or whose activity is different than that reported in other species.
- The master-switch ripening regulatory gene (MIP) appears to be more heavily expressed in D'Anjou then in Bartlett fruit, where it may serve to suppress the activity of ripening-related genes.

Significant findings for Objective 2

- Statistical analysis of gene expression data identified nearly 20 of the 90 genes to be differentially expressed during the course of fruit conditioning and ripening (between Bartlett and D'Anjou). These represent candidate genes regulating activity of the proposed master-switch gene (MIP) and numerous downstream ripening-associated genes, including those associated with System 2 'burst' of ethylene production.
- Overall, there is ample evidence showing fruit of each variety respond differentially to calcium, auxin, jasmonate, and abscisic acid (ABA) in early stages of conditioning at the gene level. This work identifies an important phenological window where manipulation of ripening can be tested in future work.
- Some differentially active genes (between Bartlett and D'Anjou) illustrate completely novel avenues of research in ripening regulation in tree fruit.

Significant findings for Objective 3

- Established full gene sequence for D'Anjou and Bartlett MIP master-switch gene.
- ACS 1A/B and ACS2A/B genes have been amplified from D'Anjou and Bartlett.
- Work to establish the genetic diversity information is ongoing and expected to be complete by April 2013.

RESULTS & DISCUSSION

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

Time Frame	*Objectives*	*Progress*	*Milestones*
January 2010 – December 2012	1. Test activity of chilling and ripening-related genes in conditioning pear fruit	Over 90 genes of 6 major hormone and stress signaling pathways examined using quantitative real-time PCR. Performing tests in triplicate which are expected to be complete by April 2013.	Completed the first comprehensive examination of activity among genes in pathways implicated in controlling chilling-induced ripening.
	2. Correlate expression of master-switch regulator to ripening in winter pear	Completed statistical analysis of gene activity. Identified set of about 20 candidate genes differentially expressed among winter pear regulating chilling-induced ripening.	Identified cold, auxin, and calcium signaling pathway members as candidate genes for the differential conditioning requirement between Bartlett and D'Anjou.
	3. Establish genetic diversity of cold-induced ripening genes in pear varieties.	Genes have been amplified. They are currently being cloned and being sequenced.	Expect to complete this aspect by April 2013.

Overall, results illustrate numerous gene-level and physiological differences between conditioning Bartlett and D'Anjou fruit. The experimental infrastructure utilized and implemented at WSU-Pullman was effective in providing a physiological model of conditioning in Bartlett and D'Anjou pear.

1. *Gene expression analysis*: We based our gene analysis on previous research on physiological models that trigger ripening and System 2 ethylene induction pear and numerous other climacteric fruits. Overall results illustrate numerous gene-level and physiological differences between conditioning of Bartlett and D'Anjou fruit. At equal stages of conditioning and ripening, significant differences in gene activity are seen in members of the cold-signaling pathway (Figure 3A, 3B). Figure 3 is a heat map that indicates relative expression based on color. (Pardon the grayscale presentation in the written report). The different of individual gene activity is clearly visible amongst comparable samples from Bartlett and D'Anjou. Typically these genes have powerful downstream effects including activation of genes from nearly all other pathways probed in this work, including those of ABA, ethylene, calcium, and general stress responses. This suggests critical differences in varietal capacity to respond to prolonged chilling exposure during conditioning. Similar differences are seen for auxin-signaling pathway genes in the fully conditioned and fully ripened samples. With internal auxin accumulation being one of the primary physiological clues preceding the onset of ripening and System 2 ethylene production in chilling-dependent tree fruits (El-Sharkawy et al., 2008; El-Sharkawy et al. 2010), these differences also suggest powerful differences in the fruits' capacity to produce and respond to auxin, which could obstruct ripening progression. There is likely a relationship between the altered ability (between Bartlett and D'Anjou) of the fruit to respond to cold, and the accumulation of auxin in the fruit. Characterizing this relationship in greater detail will be a subject for future research efforts in the lab. Such work will need to include an in depth examination of genetic variability in sequence and regulation of genes comprising these pathways. The recently published apple (Velasco et al., 2010), Chinese pear (Wu et al., 2012) and European pear (Dhingra lab) genomes may help in this regard, highlighting the importance of foundational genomics resources. Interestingly, this work showed many aspects of ethylene-signaling which are similarly active during progression of conditioning and ripening, supporting the presence of an ethylene-independent but cold and/or auxin-dependent mechanism underlying differences in fruit competency for ripening as they undergo conditioning treatments. Finally, this work has yielded the first reported instance of differential expression among genes which confer signaling ability to ethylene receptors.

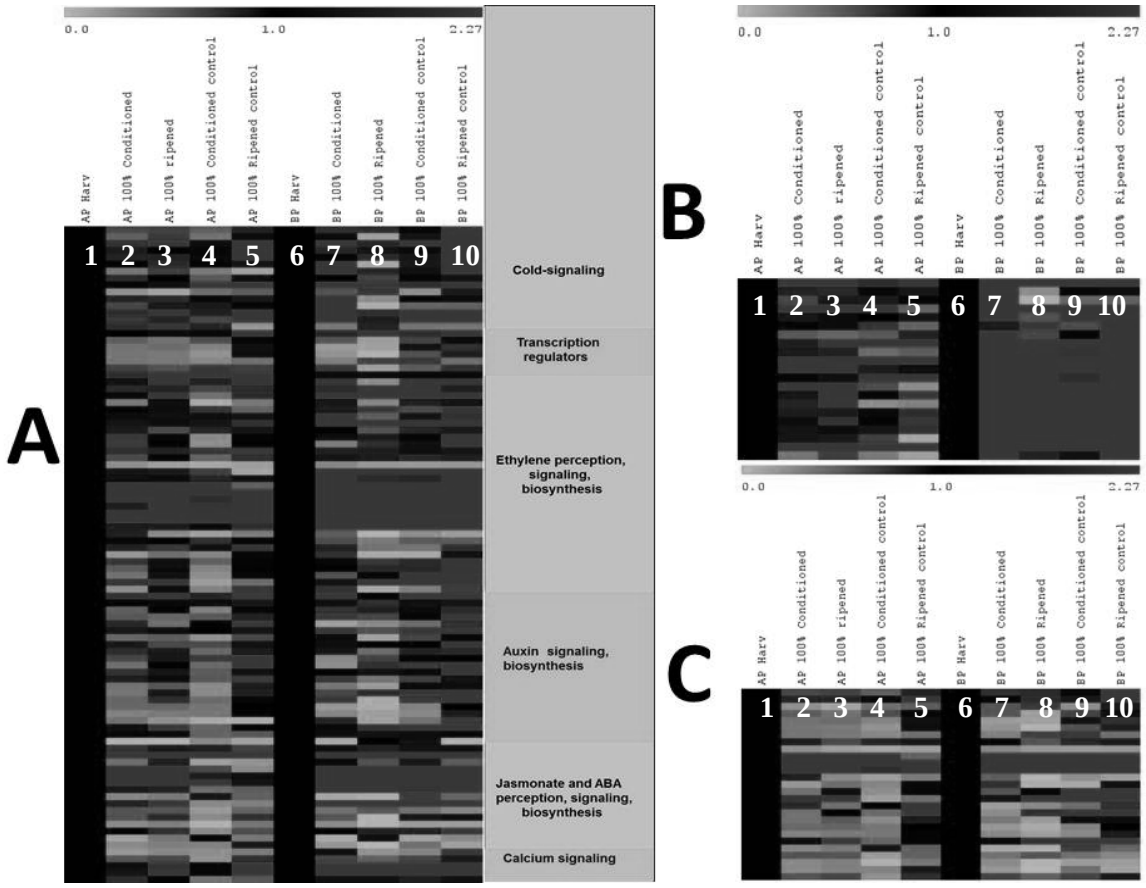

Figure 3. **(A)** Overall heat map indicating relative gene activity among 6 pathways containing selected genes regulating chilling-induced ripening and System 2 ethylene production in harvested D'Anjou and Bartlett (lanes 1 and 6, respectively), fully conditioned D'Anjou and Bartlett (lanes 2 and 7, respectively), fully ripened D'Anjou and Bartlett (lanes 3 and 8 respectively), unconditioned D'Anjou and Bartlett (lanes 4 and 9, respectively), and unconditioned D'Anjou and Bartlett controls at (conditioned) ripening dates (lanes 5 and 10, respectively). Darker colors represent higher activity, lighter colors represent lesser activity (relative to harvest date standards). Data represents quantitative real-time PCR C_t-values after 2-log transformation. **(B)** Genes exhibiting significantly differential expression (between D'Anjou and Bartlett during fruit conditioning and ripening) by variety using the Significance Analysis for Microarray (SAM). **(C)** Genes exhibiting differential expression by variety during ripening progression using the Bayesian Estimation of Temporal Regulation. The SAM approach clearly illustrates significant divergence in gene activity at equal conditioning stages in comparison of Bartlett and D'Anjou fruit. Heatmaps were generated using the MultiExperiment

2. *Correlate expression of ripening-regulatory genes to ripening in winter pear*:

Flesh softening correlated well with observed gene activity of ripening-related genes, with increases in expression of late-stage ethylene production genes corresponding with significant reductions in flesh firmness in both varieties, consistent with the results of Sugar and Kupferman (Figure 4). The cold-requirement can be supplemented with warmer conditioning temperatures, and ethylene to produce ripening competent fruit. Ethylene treatment of Bartlett fruit reduces the time required to reach marketable firmness- with only a 7 day treatment at 20°C needed. Interestingly, ungassed fruit stored at 10°C appear to soften more rapidly than fruit held at -1°C, illustrating ethylene-independent mechanisms at work in pear ripening. However, after over a month at -1°C, D'Anjou fruit retained most of its firmness. In Bartlett, a clearly decreasing flesh firmness is already apparent in the ungassed (no exogenous ethylene during conditioning) 10°C-stored fruit. Gene expression analysis in

these same sample tissues illustrates clear differences at this early stage. Results shown here demonstrate the powerful and rapid effects these genes may have in regulating the ripening process.

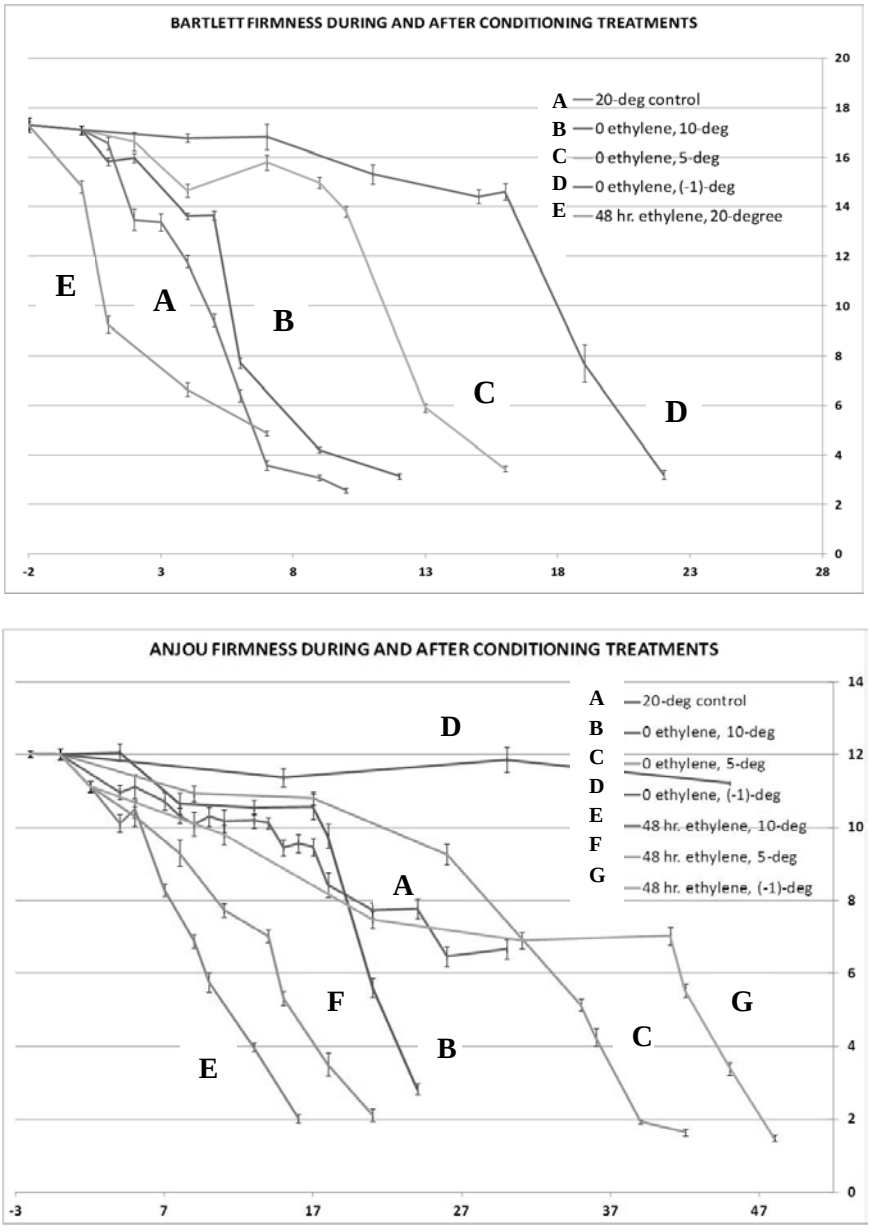

Figure 4. (Top) Flesh firmness in sampled conditioned (at -1, 5, and 10°C) and unconditioned (20°C-constant) Bartlett fruit in the presence and absence of use of a 48 hour 100 ppm ethylene dosing. (Bottom) Flesh firmness in sampled conditioned (at -1, 5, and 10°C) and unconditioned (20°C-constant) D'Anjou fruit in the presence and absence of use of a 48 hour 100 ppm ethylene dosing.

3. Establish genetic diversity

Among differentially expressed genes during fruit conditioning and ripening, were the European pear System 1-to-System 2 ethylene production transition-specific (El-Sharkawy et al., 2004) genes ACS1 and ACS2. Prior work suggests only two allelic forms of these genes (ACS1a/1b, and ACS2a/2b). Cloning and sequencing of Bartlett and D'Anjou ACS1 and ACS2 genes is ongoing and is expected to be accomplished by April 2013.

References

1. Velasco et al., 2010. The genome of the domesticated apple. Nature Genetics. 42:833–839.

2. Wu et al., 2012. The genome of pear (*Pyrus bretschneideri* Rehd.). Genome Research. doi: 10.1101/gr.144311.112.

3. Dhingra 2012. The DH Pear Genome. http://genomics.wsu.edu/pear-research/ (accessed Jan 22, 2013).

3. El-Sharkawy et al., 2004. Differential regulation of ACC synthase genes in cold-dependent and -independent ripening in pear fruit. Plant, Cell and Environ. 27(10):1197–1210.

4. El-Sharkawy et al., 2008. Differential regulation of four members of the ACC synthase gene family in plum. J. Exp. Bot. 59(8):2009-2027.

5. El-Sharkawy et al., 2010. Regulation of two germin-like protein genes during plum fruit development. J. Exp. Bot. 61(6):1761-1770.

EXECUTIVE SUMMARY

The aim of this project was to produce the foundational gene-level knowledge required to better understand the underpinnings of the chilling-requirement for ripening and System 2 ethylene induction in PNW pear varieties. This information is critical for much-needed improvement of pear varieties to meet evolving market needs. Among these needs is greater control over the fruit ripening process. With unique ripening characteristics, PNW pear varieties require customized post-harvest management. Despite this, asynchronously ripened and damaged fruit can lead to unacceptably high amounts of unmarketable fruit progressing through the fruit production, storage, and transport and sale chain. Results of this project have identified hormone and stress-signaling pathways which respond differently through the course of fruit conditioning and ripening. Variability in sequences reported to be uniquely expressed near the onset of the ethylene 'burst' can be used to appropriately catalog varieties, use as predictors of ripening and serve as molecular markers in pear variety breeding efforts to select for desirable conditioning-requirement phenotypes. As a whole, this work established the foundation required for short and long-term improvement of pear fruit quality.

Summary of findings

This work has identified the control points in auxin, calcium and cold-signaling pathways in Bartlett and D'Anjou tissue during conditioning and ripening while also confirming the presence of much of the genetic elements common to climacteric fruits. Variability in gene sequences and their expression behavior in the two varieties can be useful in predicting conditioning levels in the short term to predict fruit quality. These genes can also serve as useful markers in gene-assisted selection to advance desirable conditioning requirements into progeny. We have arrived at these findings by establishing a robust physiological conditioning model following protocols of Kupferman and Sugar, and employing a gene-level analysis of the inherent differences in conditioning-requirements between PNW pear varieties. This approach allows direct interrogation of causal underpinnings of this complex phenomenon.

Future directions

This is one of the most comprehensive examinations of the genetic underpinnings of this unique ripening phenomenon in climacteric fruits performed to date. Identification of these candidate genes provides critical clues to understand how such genetically similar pear varieties can differ so greatly in their conditioning requirements. The MIP gene identified in the lab through this work may serve to integrate many of the phytohormone and environmental stress signals preceding the trigger of ripening and System 2 ethylene induction in pear. We will further explore the mechanism behind these interactions to understand which are the essential genetic differences responsible for impaired ripening in winter pear. This information can prove to be critical for post-harvest management of existing pear varieties.

SPECIAL PROJECT REPORT **YEAR**: 1 of 1

Project Title: Chemical thinning of Bartlett with BA and NAA

PI: Tory Schmidt
Organization: WTFRC
Telephone: (509) 665-8271
Email: tory@treefruitresearch.com
Address: 1719 Springwater Ave.
City: Wenatchee
State/Zip: WA 98801

Cooperators: Felipe Castillo, Ines Hanrahan, Manoella Mendoza, Jim McFerson

Total Project Request: **Year 1:** 12,000

Other funding sources

All chemicals donated by companies

Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
Telephone: (509) 665-8271 **Email address:** kathy@treefruitresearch.com

Item	2012
Salaries	3000
Benefits	1000
Wages	6000
Benefits	1500
Equipment	
Supplies	
Travel	500
Miscellaneous	
Grand Total	**$12,000**

NOTE: Project was approved out-of-cycle by unanimous email vote of advisory committee members in April 2012; funding was drawn from $50,000 contingency fund for WTFRC projects recommended by the research subcommittee in February 2012 and approved by the fresh and processed pear committees in March 2012.

Objective:

Evaluate combinations of benzyladenine (BA) and naphthaleneacetic acid (NAA) for chemical thinning and fruit sizing in Bartlett pear.

Significant findings:

- Nearly all chemical thinning treatments significantly reduced fruit set in both trial sites
- Mean harvest fruit size was notably larger in all treatments than untreated controls, but not consistently enough for statistical significance
- Inclusion of NAA in tank mixes with BA did not produce clear effects on fruit set or size
- Tank mixes of BA+GA (Promalin) and prohexadione calcium (Apogee) did not improve fruit set of D'Anjou in a small pilot study (data not shown)

Introduction:

In light of the diminishing supply and increasing cost of labor in Northwest orchards, pear growers continue to seek out crop load management strategies which can reduce the need for expensive green fruitlet thinning, as well as promoting fruit size for better financial returns. The efficacy of BA products to achieve those objectives in Bartlett is well established, but many growers are interested in chemical programs that might offer greater reductions in fruit set than those provided by BA alone. Tank mixes of BA and NAA have recently proven successful in numerous apple thinning studies. At the request of the pear research subcommittee, the Internal Program of the WTFRC agreed to evaluate similar combinations to determine if addition of NAA might improve thinning efficacy of BA in Bartlett pears.

Methods:

Two trial sites were established utilizing identical treatment protocols in a randomized complete block design with four replicates: an 11th leaf V-trellis Bartlett/OHxF.87 block near Sawyer, WA spaced at 5' x 16', and a 14th leaf central leader Bartlett/OHxF.97 block spaced at 10' x 17' near Cashmere, WA. Initial bloom counts were recorded for representative sample branches from each plot. Both trials were successfully treated at approximately 10mm mean fruitlet size using 100gal water/acre. Conditions in Sawyer were favorable for good thinning response, with temperatures in the high 60s to low 80s for a week after treatment; conditions in Cashmere were even better, with temperatures in the 70s and 80s over the same period. Fruit set counts were made on sample branches after June drop, but before green fruit hand thinning. Representative fruit from each plot were sampled within a few days of commercial harvest and evaluated in the WTFRC lab for size, firmness, sugar levels, acidity, and fruit finish. Only one of four replicates could be sampled at harvest from the Cashmere site after the grower started commercial harvest in the trial block several days prior to the start date that had been communicated to WTFRC staff. Data from that single replicate are reported below, but those harvest results were unsuitable for statistical analysis.

Results and discussion:

Previous work by our group and others has clearly established that BA products effectively reduce fruit set and promote fruit size in Bartlett pears; in fact, BA has significantly improved fruit weight in more than half of our trials through the years, a success rate that surpasses the response of any plant growth regulator we have applied to apple or cherry for any purpose. Our earlier studies demonstrated: 1) little benefit of split applications of BA (as opposed to a single application in good

conditions) and 2) the addition of other products to the spray tank such as carbaryl, oil, abamectin (Agri-Mek), or calcium phosphite (Sysstem-Cal) did not significantly improve the performance of BA alone. NAA has been used historically as a stand-alone chemical thinner of pears, and based on the success of BA+NAA programs as postbloom thinners of apple, they merited investigation in pear.

Our 2012 results, however, offer no clear evidence that addition of NAA to standard BA programs improved thinning efficacy. All treatments in both sites significantly reduced fruit set except the full rate of BA in the Sawyer trial (Table 1); in the broader context of the success of other treatments, including a lesser rate of BA, we suspect that specific result to be an outlier that may have been confounded by unique conditions to trees in those particular plots. NAA tends to be most effective as a thinner in the same temperature range as BA (65-80 degrees); while conditions may have been slightly cool (low-mid 60s) during application at both trial sites, temperatures warmed to a near ideal range for several days following, so poor performance by either product is not likely related to poor weather conditions.

Harvest fruit weight was improved by 1-2 box sizes by all treatments in the Sawyer trial, although the results were not statistically significant (Table 1). Likewise in the Cashmere trial, harvest fruit weights tended to be improved by thinning treatments, but unfortunately, the results reported in Table 1 reflect the unanalyzable mean values of only one replicate due to confusion over timing of commercial harvest of that block.

Clearly, a more robust set of trial results would be necessary to draw firm conclusions about the addition of NAA to standard BA programs in Bartlett, but we did not see any indication from these two studies that strategy would improve thinning results.

Table 1. Crop load effects of benzyladenine (BA) and Fruitone L (NAA) applications at 10mm fruitlet size on Bartlett pears. WTFRC 2012.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
Bartlett/OHxF.87	128 oz BA	50 ab	55 bc	39 a	186 ns	107
- Sawyer	128 oz BA + 4 oz NAA	35 c	68 a	30 b	201	99
	96 oz BA	37 c	65 ab	34 ab	188	106
	96 oz BA + 4 oz NAA	40 bc	64 ab	32 ab	174	115
	96 oz BA + 6 oz NAA	38 c	66 a	30 b	183	109
	Control	52 a	55 c	39 a	163	123
Bartlett/OHxF.97	128 oz BA	21 b	81 a	18 b	207*	97
- Cashmere	128 oz BA + 4 oz NAA	23 b	79 a	19 b	230*	87
	96 oz BA	20 b	82 a	17 b	251*	80
	96 oz BA + 4 oz NAA	24 b	79 a	18 b	227*	88
	96 oz BA + 6 oz NAA	22 b	79 a	20 b	255*	78
	Control	40 a	66 b	27 a	214*	93

* Values reflect plot means of a single replicate and are not valid for statistical analysis

FINAL PROJECT REPORT
WTFRC Project Number: PR09-905

Project Title: Pear rootstock breeding

PI: Kate Evans
Organization: WSU Tree Fruit Research and Extension Center
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Address: 1100 N. Western Ave
City/State/Zip: Wenatchee/WA/98801

Cooperators: Timothy Smith, WSU Wenatchee; Amit Dhingra, WSU Pullman; Todd Einhorn, OSU MCAREC; Gennaro Fazio, USDA-ARS

Total Project Funding: Year 1: $4,500 **Year 2:** $12,300 **Year 3:** $3,500

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget History

Item	2009	2010	2011	2012
Travel	1,000	2,500	500	0
Propagation	3,500	8,800	2,000	0
Plot Fees	0	1,000	1,000	0
Total	4,500	12,300	3,500	0

Objectives:
1. Establish a pear rootstock advisory committee.
2. Review literature and search national and international collections for pear rootstock accessions.
3. Initiate propagation and planting of a new pear rootstock collection in Washington State.
4. Develop strategy for pre-selection of seedling populations.

Significant Findings:
1. Rootstock germplasm was selected at the pear collection in Corvallis; propagated trees were planted in the parental collection at Sunrise orchard in spring 2012.
2. Rootstock germplasm was selected at the Westwood interspecific *pyrus* collection at WSU Puyallup; propagated parental trees should be available to plant at Sunrise orchard in spring 2013.
3. Pear rootstocks and selection techniques have been highlighted as the focus for a new SCRI rootstock proposal.

Methods:
1. A pear rootstock advisory committee made up of industry and research experts will provide input on the objectives, activities and future planning for a pear rootstock research project.
2. Use internet searches, literature and informed contacts to review wide-ranging pear germplasm to identify possible accessions for a new rootstock parental collection.
3. Access germplasm for propagation from collections and other breeding programs, arrange for importation and propagation at commercial nursery.
4. Meet with Gennaro Fazio (apple rootstock breeder, Geneva, NY) and other experts to discuss possible methods of pre-selection of pear rootstock progenies and develop strategies for handling progenies in a cost-effective, efficient manner.
5. Establish a pear rootstock parental germplasm collection with at least two standard trees of each selection to facilitate future crossing programs.

Results & Discussions:
Literature reviews focused principally on conference proceedings from the most recent ISHS pear conferences as well as the ISHS Integrated Canopy and Rootstocks conferences. Other journal articles were either already on file or were accessed on line. Several popular press articles and websites also proved to be useful for example, http://extension.oregonstate.edu/catalog/pdf/pnw/pnw341-e.pdf, and of course, reports of previously-funded PNW pear rootstock trials.

Although there are certainly a number of interesting rootstock selections available for import into the U.S. with a view to establish trials, there is also a wide range of possible parental germplasm already present in the U.S. that should be very suitable for establishing a crossing program in the PNW. The USDA pear repository in Corvallis, Oregon has numerous *pyrus* species as well as several selections from the Westwood program. After visiting with Dr. Joseph Postman and viewing the collection, 17 accessions were selected from the repository in July 2010.

Budwood was supplied in August 2010 to Willow Drive Nursery where the trees were propagated onto OHF 87 rootstock.

The parents selected include *Pyrus communis* 'Old Home', 'Farmingdale', OHF87 and 333 as well as other dwarf and compact *P. communis* scion varieties. Three of the Oregon series of 'P' fire blight resistant dwarf and semi-dwarf rootstocks were also accessed. A diverse collection of other *pyrus* species were also selected to include characteristics such as resistance to fire blight, tolerance to pear decline, resistance to *phytopthora*, resistance to woolly pear aphid, cold hardiness, ease of propagation and a range of different vigors. Five replicates of each tree were planted in the WSU Sunrise orchard in spring 2012.

Further germplasm was assessed during a visit to WSU Puyallup in June 2011. A large collection of interspecific *pyrus* hybrids, originally produced by Westwood, was established at the research and extension center in Puyallup for assessment and possible selection of urban ornamental trees by Dr. Rita Hummel. A subset of 16 accessions from this germplasm was propagated in 2011. Characters for selection focused on dwarfing habit and a diverse genetic background, thus enabling a wide range of potential disease and pest resistance characters to be included in future crosses.

Further germplasm will be accessed from non-U.S. sources as part of project PR-12-109. Initial material transfer agreements (MTAs) are being negotiated for testing of the material only. Once germplasm is in the U.S. and on its way through quarantine, further negotiations regarding using this material for breeding can be addressed.

Discussions remain on-going regarding protocols for rootstock selection in seedlings. Currently there are very few molecular tools that can be used to select for important pear rootstock characters in seedlings, however this may change over the next 5 years so any protocol developed needs to be flexible. A visit to Stellenbosch, South Africa, in November 2010 provided the opportunity to discuss selection strategies with apple rootstock breeder Ken Tobutt. Pear rootstock breeding selection techniques have also been discussed with U.S. apple rootstock breeder Gennaro Fazio. One possible strategy involves planting rootstock seedlings at a fairly close spacing and budding them with a compact standard scion variety. Such a planting allows for the selection of precocity as well as tree vigor within the first few years of growth. Promising seedlings can then be propagated from root cuttings for further trials.

An SCRI proposal is being prepared, led by Dr. Kate Evans, which will include the development of some key selection tools for pear rootstocks, for example DNA markers for dwarfing, using micro-grafting to test for scion incompatibility and the initiation of a pear rootstock breeding program here in the PNW.

Executive Summary

Flowering parental germplasm is essential in order to establish a pear rootstock breeding program in the PNW. We have made the first steps in establishing a parental collection at the WSU Sunrise orchard.

1. Rootstock germplasm was selected at the pear collection in Corvallis; propagated trees were planted in the parental collection at Sunrise orchard in spring 2012.
2. Rootstock germplasm was selected at the Westwood interspecific *pyrus* collection at WSU Puyallup; propagated parental trees should be available to plant at Sunrise orchard in spring 2013.
3. Pear rootstocks and selection techniques have been highlighted as the focus for a new SCRI rootstock proposal.

Non-US germplasm will be added to the collection following the completion of Material Transfer Agreement negotiations and quarantine. Open-pollinated seed or importing seed from non-US pear breeding programs would be one way of establishing progenies; our aim now is to apply for SCRI funding to enable these options to be fully explored.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number:

Project Title: Ripening capacity and decay control in winter pears

PI: David Sugar
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Cooperators: Yan Wang

Total Project Request: Year 1: 34,955 **Year 2**: **34,955**

Other funding sources:
Agency Name: Syngenta Corp.
Amt. awarded: 7,000
Notes: Support for postharvest fungicide trials with Syngenta products.

Budget
Organization Name: Oregon Agricultural Research Foundation
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228 **Email address:** charlene.wilkinson@oregonstate.edu

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

This project proposes two overall 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 °F, and of Comice pears after 2, 4, and 8 weeks of storage at 30 °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.

In the NW Pear Research Priority Survey of 2011, Pear Conditioning (ripening) and Postharvest Pathogens ranked as the third and fifth highest priorities, respectively. Research in the first objective of this proposal addresses methods of conditioning, the amount of time required by each conditioning method, and the resulting shipping firmness and eating quality, measured after various lengths of time in cold storage. Research in the second objective specifically addresses postharvest decay management.

SIGNIFICANT FINDINGS

Objective 1 (Ripening Capacity):

1. When conditioning was applied after 1 month storage at 30°F, Anjou pears receiving 24 or 48 hours in ethylene did not ripen within 7 days at 68°F. However, after 24 or 48 hours in ethylene + 5 or 10 days at 50°F, Anjou pears ripened completely.

2. Combinations of ethylene and 50°F treatments resulting in sufficient firmness for shipping (> 8 lbf) and complete ripening (< 4 lbf) were identified for Anjou and Comice pears that had been stored at 30°F for 1, 3, and 5 months (Anjou, in progress) and for 2, 4, and 6 weeks (Comice).

3. Conditioning treatments with ethylene and 50°F interacted with harvest maturity in influencing the capacity to ripen and the shipping firmness.

Objective 2 (Postharvest Decay):

1. A postharvest fungicide treatment of pre-mixed Difenconazole and Scholar at 20 oz. per 100 gallons controlled gray and blue mold decays at levels equivalent to Penbotec or Scholar alone. The different modes of action in the pre-mix may retard resistance development in the pathogens.

2. Pears inoculated with major decay pathogens were placed in commercial CA rooms and treated with Penbotec as a thermofog, either alone or in sequence with Smartfresh. Control fruit were stored in RA. Results expected in late winter 2013.

3. Preharvest resistance stimulants were applied prior to harvest to trees that either had been treated with calcium chloride sprays or without calcium. Postharvest Penbotec was applied 0, 3, 6, or 9 weeks after harvest. Decay to be evaluated in late winter 2013.

METHODS

Within the objectives described above, experiments will be organized around the sub-objectives and/or questions listed below:

Objective 1: The experimental variables in this study will be:

(1) harvest maturity (0, 7, and 14 days after fruit in the orchard first reach the appropriate firmness to begin harvest);
(2) duration of cold storage prior to conditioning (1, 3, 5 months at 30°F for Anjou; 2, 4, and 8 weeks of storage at 30°F for Comice);
(3) duration of ethylene conditioning (0, 24, 48, and 72 hours);
(4) duration of 50°F conditioning (0-12 days, depending on variety and length of ethylene treatment).

Standardized treatment factors will be: return to cold (30-31°F) after conditioning for 7 days prior to initiating ripening to simulate commercial management, and 7 days ripening time at 68°F. Fruit firmness at harvest, after storage, after conditioning, after conditioning + 7 days in cold, and after ripening time will be the primary measures of fruit condition. Firmness measurements will be indicators of both shipping potential and ripening potential. Fruit that successfully ripen within 7 days will be evaluated for eating quality.

Objective 2: New treatment options for postharvest decay control will be evaluated, with emphasis on preparing fruit for long-term field-run storage. Primary experimental variables will be:

(1) Potential new pre-harvest fungicide treatments;
(2) Sequential combinations of summer calcium sprays and new pre-harvest fungicides:
(3) Pre-storage drench treatments of harvested fruit in bins.

Standardized treatment factors will be: artificial fruit wounding after harvest with 2-mm finishing nails to simulate stem punctures occurring during harvest and handling, postharvest line-spray treatments with either water (control) or Scholar fungicide, and storage in regular atmosphere at 30-31 °F for 4-5 months prior to decay evaluation. Decay-causing pathogens will be identified.

This project will use the series of research-size CA-ready rooms at the Southern Oregon Research and Extension Center for controlled temperature and ethylene treatments, as well as storage of fruit from postharvest decay treatments until decay evaluation. All experiments will be replicated four times, with replication based in the orchard; that is, replicate lots of fruit will come from distinct areas in the orchard to account for variability among orchard locations. Fruit firmness for maturity, shipping firmness, and storage quality measurements will be determined using a Fruit Texture Analyzer. Ethylene will be introduced from a compressed ethylene cylinder and concentrations verified using a gas chromatograph. Studies of the interaction of duration of cold storage, fruit maturity, ethylene exposure, and temperature conditioning, including follow-up factors of shipping firmness and storage life require detailed scheduling of the movement of fruit and the measuring of firmness and evaluation of quality. A technician supported by this project will have daily responsibilities for fruit tracking and firmness measurements. The Principal Investigator will be responsible for ethylene treatments, temperature management, weekend fruit measurements, and quality evaluations.

RESULTS & DISCUSSION

Objective 1 (Ripening Capacity):

In previous research, we established that after 24 hours in ethylene, 'Anjou' pears needed an additional 25-40 days at 30°F to develop ripening capacity, depending on maturity at harvest. After 48 hours in ethylene, 'Anjou' needed 15-30 days at 30°F. After 72 hours in ethylene, 'Anjou' pears without further temperature conditioning would ripen to nearly 4 lbf in 7 days at room temperature. When the post-ethylene conditioning temperature was 50°F, induction of ripening capacity proceeded significantly faster. Typically, 5 days at 50°F following 24 or 48 hours in ethylene was sufficient to complete induction of ripening capacity.

In the current project, ethylene + temperature conditioning treatments are being applied to Anjou pears after 1, 3, and 5 months at 30°F, and Comice pears after 2, 4, and 6 weeks at 30 °F. With Anjou pears, after 1 month at 30°F, further temperature conditioning was needed after ethylene treatment for 0, 24 or 48 hours for the fruit to develop ripening capacity (Fig. 1). This further conditioning need was satisfied by 5 days at 50°F after 24 or 48 hours of ethylene or 10 days at 50°F after 0 days of ethylene. After 3 months at 30°F, Anjou pears receiving 0 or 24 hours of ethylene still needed 5 days at 50°F to develop full ripening capacity (Fig. 1). For Anjou fruit stored 1 month at 30°F, detailed results of the interaction of harvest maturity, ethylene duration, and post-ethylene temperature conditioning duration at 50°F are shown in Table 1. In all cases, Anjou pears conditioned for 24 or 48 hours in ethylene followed by 5 days at 50°F maintain shipping firmness values > 10 lbf.

In Comice pears stored for 1 month at 30°F prior to conditioning, ethylene and 50°F treatments generally enhanced ripening and eating quality, although many treatment combinations resulted in low shipping firmness values. For Comice fruit stored 1 month at 30°F, details of the interaction of harvest maturity, ethylene duration, and post-ethylene temperature conditioning duration at 50°F are shown in Table 2.

Objective 2 (Postharvest Decay):

The widely-used postharvest fungicides Penbotec and Scholar can be highly effective when applied promptly after harvest, but efficacy can be compromised by late application timing and by selection for fungicide resistance in the pathogen population. A pre-mixed combination of Difenconazole and Scholar is in development for postharvest use on pome fruit. Difenconazole efficacy was modest on pears inoculated with gray mold and slight with blue mold (Fig. 2). The pre-mix at 20 oz. per 100 gallons controlled gray mold decay at levels equivalent to Penbotec or Scholar alone. The different modes of action in the pre-mix may retard resistance development in the pathogens.

An important objective of this project is to reduce decay in pears stored long-term field-run, in addition to or without the use of on-truck drenches. Several pre-harvest fungicides have been identified which can reduce decay during storage. In 2012 preharvest resistance stimulants were applied prior to harvest to trees that were either treated with calcium chloride sprays or without calcium. Postharvest Penbotec was applied 0, 3, 6, or 9 weeks after harvest. Decay incidence is to be evaluated in late winter 2013. In a separate experiment, pears were inoculated with major decay pathogens, then placed in commercial CA rooms and treated with Penbotec by thermofogging, either alone or in sequence with Smartfresh. Control fruit were stored in RA. Results are expected in late winter 2013.

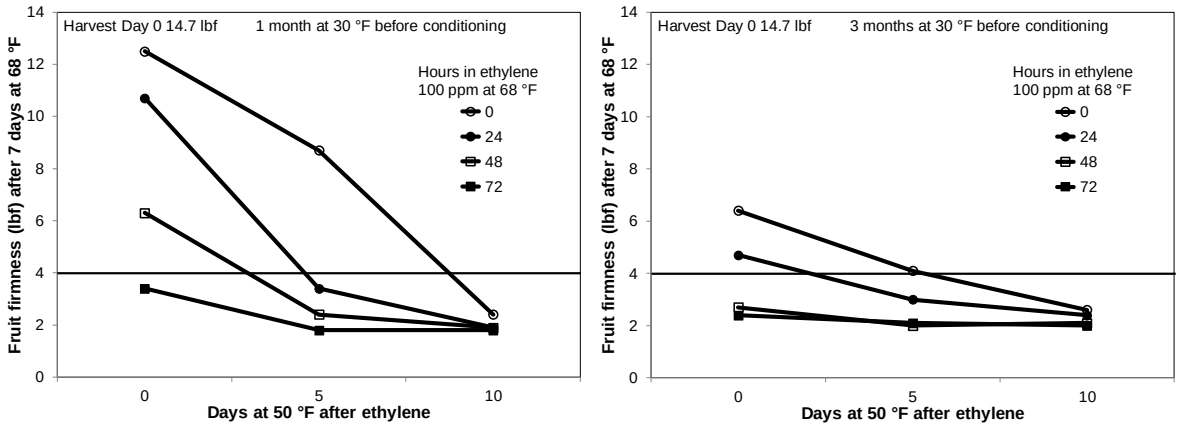

Fig. 1. Combined effects of ethylene conditioning and temperature conditioning at 50 °F on ripening capacity of 'Anjou' pears harvested at 14.7 lbf and stored at 30 °F for 1 month (left) or 3 months (right) prior to conditioning.

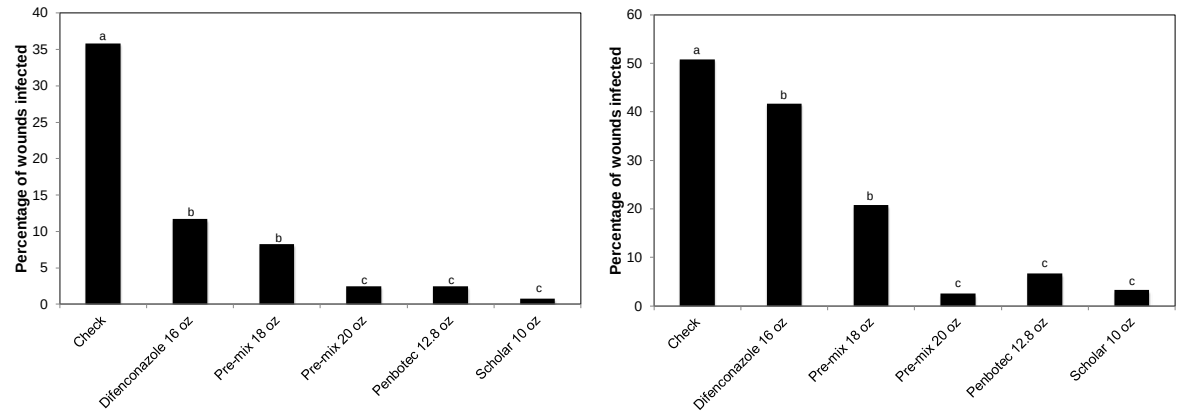

Fig. 2. Gray mold (Botrtyis) decay (left) and blue mold (Penicillium) decay (right) in inoculated Bosc pears treated by line-spray with experimental and standard fungicides. Rates are in fluid ounces per 100 gallons of water. "Pre-mix" is a combination product containing Difenconazole and Scholar.

Table 1. Firmness after conditioning (shipping firmness) and after 7 days ripening time in 'Anjou' pears stored at 30 °F for 1 month prior to conditioning. Shaded rows indicate successful treatments, defined as resulting in shipping firmness values > 8 lbf and ripened fruit firmness values < 4 lbf. Harvest day 0 = 14.7 lbf; day 7 = 13.5 lbf; day 14 = 13.1 lbf.

Harvest day	Ethylene treatment (hours)	Post-ethylene conditioning at 50°F (days)	Shipping firmness (lbf)	Firmness after 7 days at 68 °F (lbf)	Ripe fruit eating quality
0	0	0	14.0	12.5	
0	0	5	13.8	8.7	
0	0	10	12.0	2.4	EXC
0	24	0	13.3	10.7	
0	24	5	13.1	3.4	F
0	24	10	10.1	1.9	EXC
0	48	0	14.4	6.3	
0	48	5	11.2	2.4	
0	48	10	8.0	1.9	EXC
0	72	0	13.1	3.4	G
0	72	5	8.3	1.8	EXC
0	72	10	4.4	1.8	
7	0	0	13.2	10.3	
7	0	5	13.3	6.0	
7	0	10	11.0	2.1	EXC
7	24	0	13.2	8.2	
7	24	5	12.0	2.7	VG
7	24	10	8.4	2.0	EXC
7	48	0	12.7	4.8	
7	48	5	9.9	2.3	EXC
7	48	10	6.8	1.8	
7	72	0	11.6	3.0	G
7	72	5	8.2	2.0	EXC
7	72	10	4.1	1.8	
14	0	0	12.8	7.6	
14	0	5	12.7	4.8	
14	0	10	10.4	2.3	VG
14	24	0	13.3	7.4	
14	24	5	12.0	2.8	VG
14	24	10	8.9	2.1	EXC
14	48	0	12.4	4.5	
14	48	5	10.5	2.3	VG
14	48	10	6.6	2.1	
14	72	0	11.4	3.1	G
14	72	5	7.4	2.1	
14	72	10	4.2	1.9	

Table 2. Firmness after conditioning (shipping firmness) and after 7 days ripening time in 'Comice' pears stored at 30 °F for 1 month prior to conditioning. Shaded rows indicate successful treatments, defined as resulting in shipping firmness values > 8 lbf and ripened fruit firmness values < 4 lbf. Harvest day 0 = 12.4 lbf; day 7 = 11.0 lbf; day 14 = 10.8 lbf.

Harvest day	Ethylene treatment (hours)	Post-ethylene conditioning at 50°F (days)	Shipping firmness (lbf)	Firmness after 7 days at 68 °F (lbf)	Ripe fruit eating quality
0	0	0	11.5	4.0	F
0	0	5	11.1	2.4	G
0	0	10	5.0	1.0	
0	24	0	11.7	3.5	G
0	24	5	6.5	1.3	
0	24	10	4.9	1.0	
0	48	0	11.7	3.0	G
0	48	5	5.4	1.1	
0	48	10	3.5	1.0	
0	72	0	7.7	1.4	
0	72	5	3.7	1.0	
0	72	10	2.0	1.0	
7	0	0	10.6	3.8	VG
7	0	5	10.5	1.7	EXC
7	0	10	5.5	1.1	
7	24	0	10.7	2.2	VG
7	24	5	6.2	1.0	
7	24	10	3.5	1.0	
7	48	0	10.4	2.2	VG
7	48	5	5.2	1.1	
7	48	10	2.6	1.0	
7	72	0	6.2	1.3	
7	72	5	3.8	1.0	
7	72	10	2.8	0.9	
14	0	0	10.2	3.7	G
14	0	5	10.1	1.9	G
14	0	10	3.7	1.1	
14	24	0	10.6	2.7	G
14	24	5	6.1	1.0	
14	24	10	3.1	1.0	
14	48	0	10.2	1.9	VG
14	48	5	5.8	1.1	
14	48	10	3.6	1.3	
14	72	0	7.2	1.4	
14	72	5	3.4	0.9	
14	72	10	1.8	0.9	

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

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

PI: Yan Wang
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Co-PI (2):
Organization:
Telephone:
Email:
Address:
Address 2:
City/State/Zip:

Cooperators: David Sugar, 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 and production elevation (500 vs. 2000ft) 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. The year-1 data indicated that α-farnesene (FAR) concentration in fruit peel started to increase after 3 weeks of storage at 30°F. Treating with 1–MCP at 3-weeks-delay after harvest will be added to the delayed treatments (2, 3, and 4 weeks) in year-2 research.
2. Research on storage temperature, delayed treatment, simultaneous treatment with 1-MCP and ethylene, and post-storage conditioning will be repeated by using fruit from different production elevation (500ft).
3. Continue to evaluate effects of GRAS compounds and PGRs on waking-up ripening capacity of 1-MCP treated d'Anjou after storage.
4. Study the effect of combinations between late harvest maturity, delayed treatment, elevated storage temperature, and post-storage conditioning on storability, scald control, and ripening capacity of 1-MCP treated fruit after storage (year2-3). The purpose is to find a strategy to allow 1-MCP treated fruit to ripen to a better texture quality (e.g., 2-3lb).

SIGNIFICANT FINDINGS (year 1)

Ripening capacity referred in this report was defined as the capability of 1-MCP treated d'Anjou fruit to soften below 6lb within 15 days at 68°F.

1. Storage temperatures (30°F, 34°F, 36°F) influenced storability, superficial scald and ripening capacity of 1-MCP treated d'Anjou pear after storage. D'Anjou pear treated with 1-MCP and stored at 30°F did not develop IEC and ripening capacity for 8 months. 1-MCP treated fruit stored at 34°F developed measurable IEC and ripening capacity after 4 months of storage while maintaining storage quality and controlling scald for 6 months. 1-MCP treated fruit stored at 36°F lost storage quality (color, TA, and FF) significantly after 4 months of storage.
2. Within the initial two months of cold storage at 30°F, d'Anjou pears developed internal ethylene (IEC), α-farnesene (FAR) and conjugated trienes (CTs) in a dynamic manner. IEC and FAR increased significantly after 3 weeks and CTs started to increase after 6 weeks. Fruit treated with 1-MCP at 2-weeks-delay did not develop IEC and ripening capacity during 8 months of storage. 1-MCP treatment at 4-weeks-delay lost its ability to control scald after 4 months of storage.
3. Simultaneous exposure of d'Anjou fruit with 1-MCP + ethylene at 1:2 allowed treated fruit to develop IEC and ripening capacity while controlling scald for 4-6 months of storage.
4. Ethylene conditioning (100ppm for 72h at 68°F) after storage for 4-8 months rendered the 1-MCP treated fruit to develop ripening capacity while controlling superficial scald.

5. Harvest maturity influenced responsiveness of d'Anjou fruit to 1-MCP. 1-MCP treated fruit of late harvest with measurable increased IEC started to produce IEC after 4 months of storage at 30°F and developed ripening capacity while maintaining storage quality and controlling scald for 4-6 months.

METHODS

1. Storage temperature, delayed treatment, and simultaneously ethylene exposure.

D'Anjou fruit were harvested randomly at commercial maturity (FF = 14.6lb) from an orchard production lot in Pakdale, OR (~2000ft elevation). 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.

1.1. 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 3,4,6,8 months of cold storage, fruit IEC and storability [FF, skin color (*h*°), SSC, and TA] were evaluated after 1day and fruit ripening capacity (FF) and superficial scald were evaluated after 7 and 15 days at 68°F.

1.2. Delayed treatment

Fruit were exposed to 1-MCP at 150ppb at 2-weeks-delay and 4-weeks-delay 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 1.1. IEC, FAR, and CTs of control fruit were measured every week until 12 weeks at 30°F.

1.3. Simultaneously exposure fruit with 1-MCP and ethylene at 1:2

Immediately after exposure of fruit to 1-MCP at 150ppb, a calculated amount of ethylene (300ppb) 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 1.1.

1.4. Ethylene and temperature conditioning after storage

1-MCP treated fruit in 1.1 were stored at 30°F. After 4,6,8 months of storage, fruit were moved to an air-tight ethylene ripening room with ethylene concentration at 100ppm at 68°F for 3 days, or transferred to an ethylene-free room at 50°F for 15days. Then, fruit were transferred to 32°F for 2 weeks. Fruit evaluations were the same as described in 1.1.

2. Harvest maturity

To monitor harvest maturity, 30 d'Anjou fruit were sampled randomly every 3 days during the maturity window from an orchard production lot from MCAREC, Hood River, OR (~500ft elevation). FF and IEC were determined. For studying the effect of harvest maturity on 1-MCP treatment, fruit were harvested at 3 maturities: H1 = 14.6lb, H2 = 13.1lb, and H3 = 12.5lb. Defect-free fruit were packed into 20kg wooden boxes with perforated polyethylene liners. Packed fruit were immediately stored at 30°F. Fruit were treated with 1-MCP and then stored at 30°F as described in 1.1. Fruit evaluations were the same as described in 1.1.

4. Experimental design. Experimental units were boxes and there were three replications per treatment at each evaluation period. The experimental design was completely randomized.

RESULTS AND DISCUSSION

1. Effect of storage temperatures on storability, ripening capacity and superficial scald of 1-MCP treated d'Anjou pears.

1-MCP treated fruit stored at 30°F developed non-measurable IEC, therefore, maintained FF, skin color and TA with minimum reductions for 8 months of storage. 1-MCP treated fruit stored at 34°F started to develop measurable IEC after 4 months of storage (0.5ppm) and reached at 1.2ppm after 8 months of storage (Fig.1). Storage temperature of 34°F maintained fruit FF and TA with minimum reductions for 8 months, however, skin color reduced significantly after 8 months of storage. 1-MCP treated fruit stored at 36°F lost FF, skin color and TA quickly after 4 months of storage.

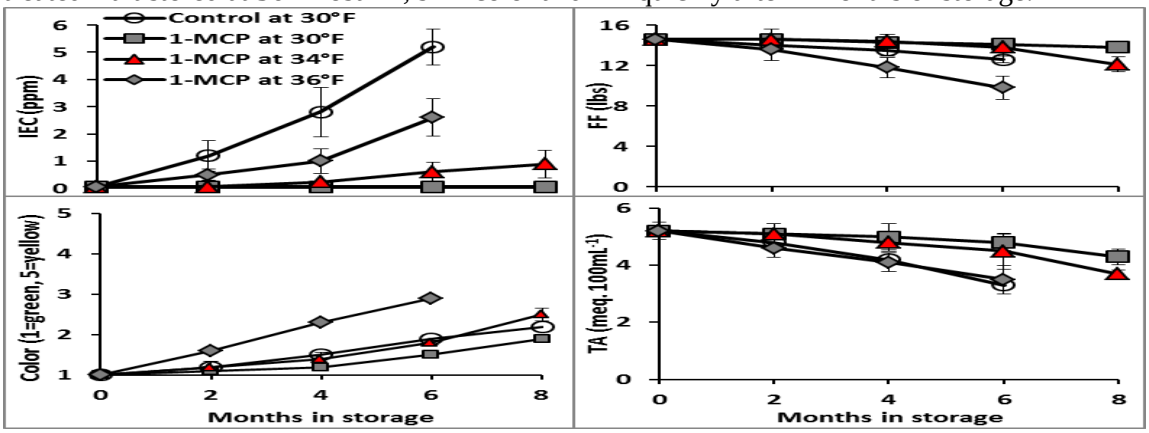


Fig.1. Effect of storage temperatures on storability of 1-MCP treated d'Anjou pear.

1-MCP treated fruit stored at 30°F did not develop ripening capacity during a period of 15d at 68°F after 3-8 months of storage. 1-MCP treated fruit stored at 34°F did not ripen within 15 days at 68°F after 3 months of storage, however, could ripen to 6lbs within 15d at 68°F after 4 months of storage and within 7d at 68°F after 6-8 months of storage (Fig.2). Storage temperature at 36°F did not render 1-MCP treated fruit to develop ripening capacity during a period of 15d at 68°F after 3 months of storage, but allowed fruit to ripen within 7d at 68°F after 4 months of storage.

1-MCP treated fruit stored at 30°F did not develop superficial scald during ripening after 4 and 6 months storage, but developed scald incidence lower than 5% after 8 months of storage. 1-MCP treated fruit stored at 34°F did not develop superficial scald during ripening after 4 months of storage, but developed scald incidences lower than 10% after 6 and 8 months of storage (Fig.2). 1-MCP treated fruit stored at 36°F developed scald incidence higher than 10% after 4 months of storage.

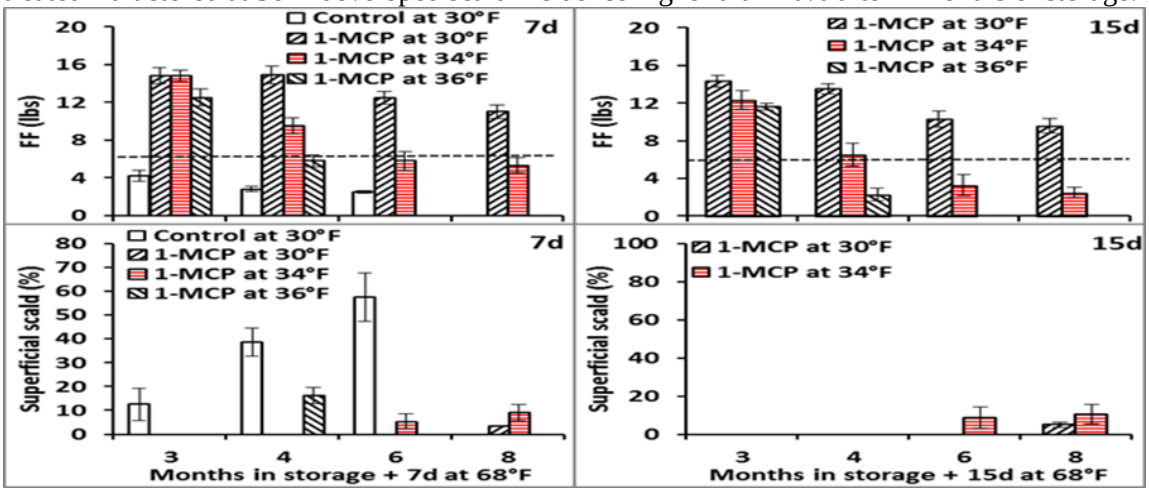
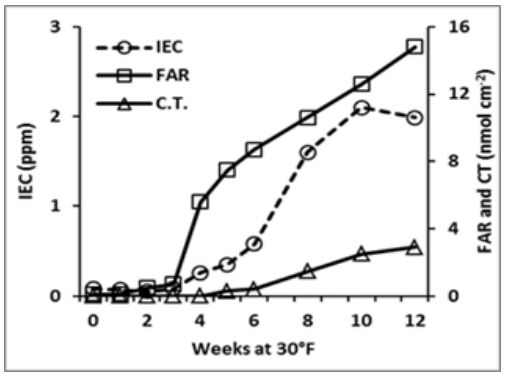


Fig.2. Effect of storage temperatures on ripening capacity and superficial scald of 1-MCP treated d'Anjou fruit after storage.

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

It was thought that the oxidation products 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, d'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. Unfortunately, fruit treated with 1-MCP at 2-weeks-delay did not develop ripening capacity during a period of 15d at 68°F after 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 after 4-8 months of storage. According to the developmental dynamic of IEC and FAR, a 3-weeks-delay of 1-MCP treatment after harvest will be added into the delayed treatments (2, 3, and 4 weeks after harvest) in next year research.

3. Effect of simultaneous exposure with 1-MCP + ethylene at 1:2 on storability, ripening capacity and scald development of 1-MCP treated d'Anjou pear.

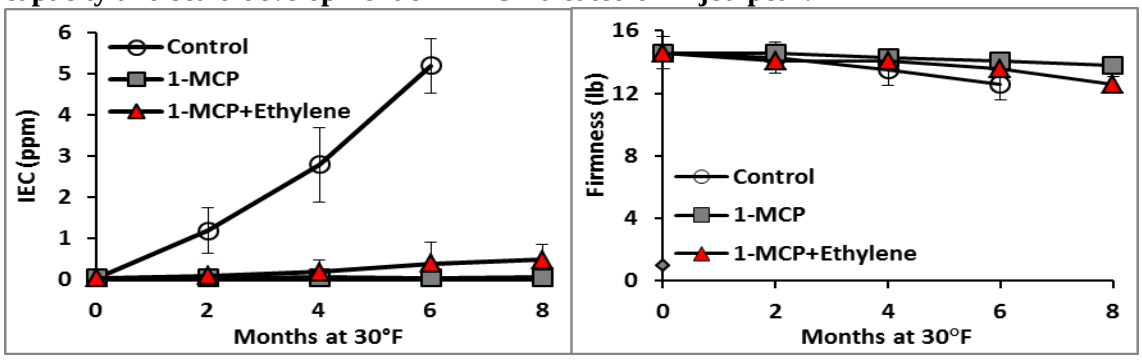


Fig.3. Effect of simultaneous treatment with 1-MCP + ethylene on IEC and FF of 1-MCP treated d'Anjou pear during storage at 30°F.

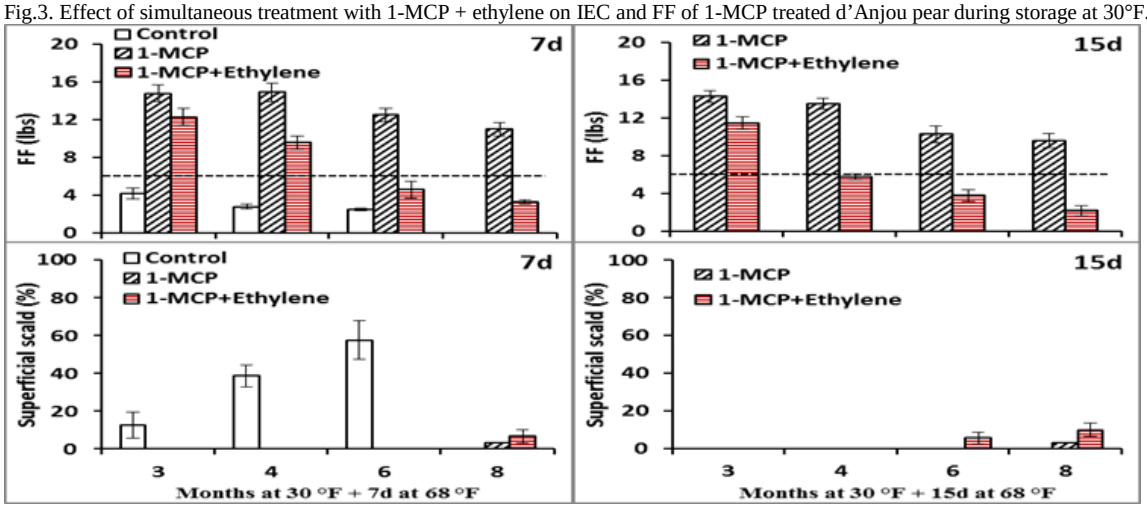


Fig.4. Effect of simultaneous treatment with 1-MCP + ethylene on ripening and scald of 1-MCP treated d'Anjou pears.

Fruit exposed to 1-MCP + ethylene (1:2) simultaneously after harvest started to produce IEC and to reduce FF after 4 months of storage at 30°F (Fig.3), developed ripening capacity within 15d at 68°F after 4 months and within 7d at 68°F after 6 and 8 months of storage at 30°F (Fig.4). The simultaneous treatment with 1-MCP + ethylene totally controlled scald for 4 and 6 months, and controlled scald incidence under 10% after 8 months at 30°F (Fig.4).

4. Effects of post-storage ethylene and temperature conditionings after storage on ripening capacity and superficial scald of 1-MCP treated d'Anjou pears.

Neither temperature at 50°F for 15 days nor ethylene at 100ppm for 3 days could ripen 1-MCP treated d'Anjou after 4 months of storage. However, both conditionings ripened fruit <6lb within 15 days at 68°F after 6 and 8 months of storage. Both conditionings did not generate scald after 4 and 6 months of storage, however, increased scald but < 10% after 8 months of storage (Fig.5).

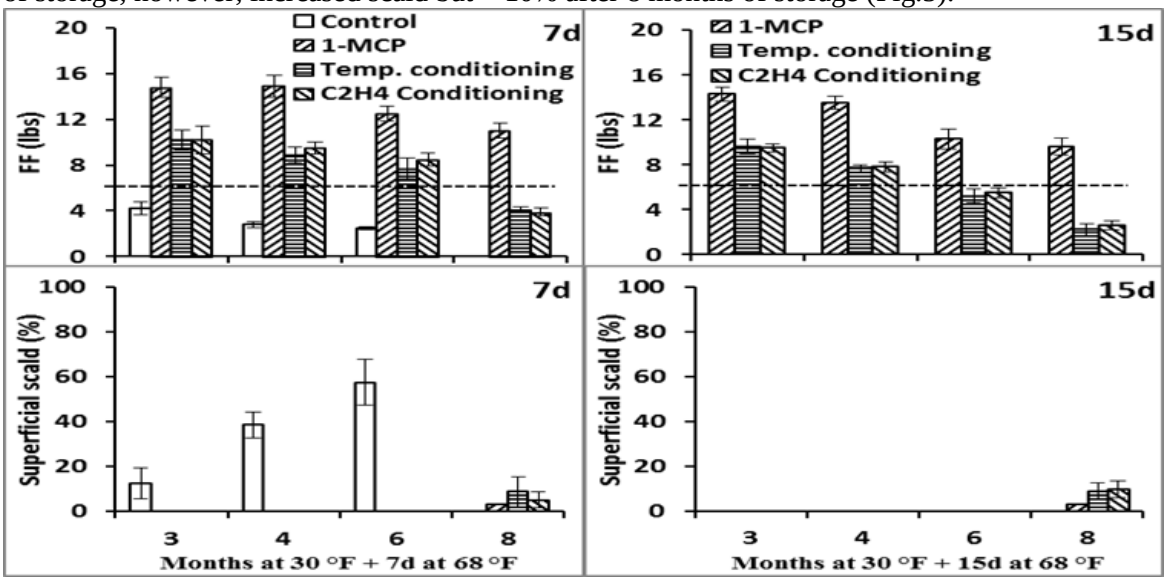


Fig. 5. Effects of temperature and ethylene conditionings on ripening and scald of 1-MCP treated d'Anjou pear.

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

Fruit IEC was measured from 3 harvest maturities H1 = 14.8lb; H2: = 13lb; H3 = 12.5lb. Only H3 fruit produced measurable IEC (Fig.6). There were no differences of 1-MCP efficacy on storability, ripening capacity and scald control of d'Anjou fruit between H1 and H2.

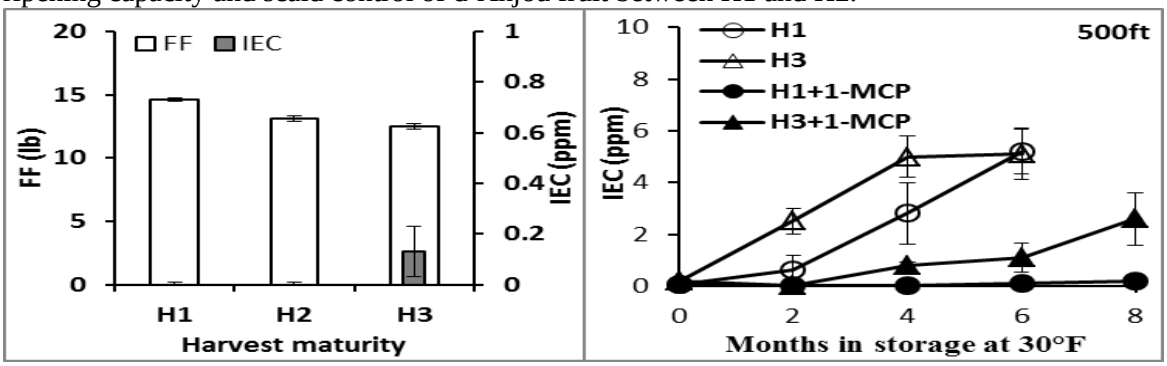


Fig.6. Effect of harvest maturity on IEC of d'Anjou pear at harvest and during storage.

1-MCP treated H3 fruit started to produce IEC after 4 months of storage (Fig.7) and maintained storability for 6 months at 30°F. Both FF and skin color were degraded significantly after 8 months of

storage at 30°F (Fig.8).

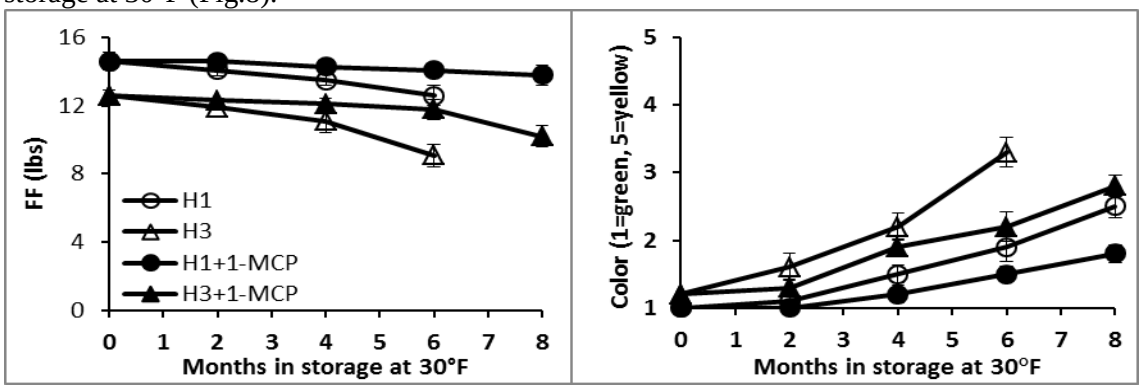


Fig.8. Effect of harvest maturity on FF and skin color of 1-MCP treated d'Anjou pear during storage at 30°F.

1-MCP treated H3 fruit developed ripening capacity within 15d after 4 months of storage and within 7d after 6-8 months of storage at 30°F (Fig.12). 1-MCP treated H3 fruit developed less than 5% superficial scald during ripening after 4 to 6 months, but developed scald higher than 10% after 8 months of storage (Fig.9).

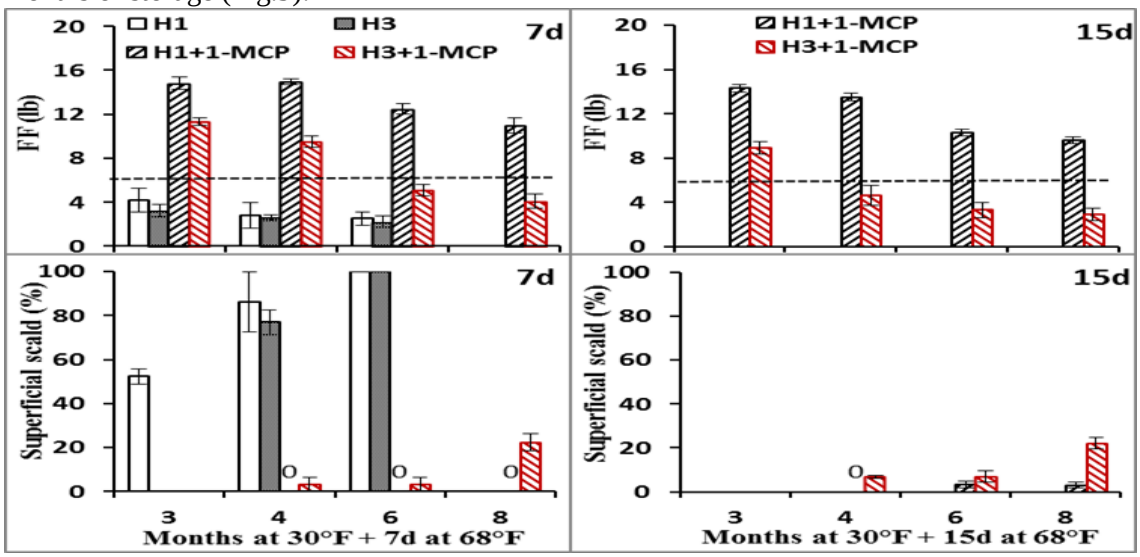


Fig.9. Effect of harvest maturity on ripening and scald of 1-MCP treated d'Anjou pear.

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CONTINUING PROJECT REPORT **YEAR**: 1 of 3 years
WTFRC Project Number: PR-12-108

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

PI: Amit Dhingra
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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

Other funding sources
None

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 agriculture 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 includes monies for ethylene gas, compressed air, two proprietary chemistries, fruit sampling, RNA isolation, quantitative reverse transcription PCR and consumables.
[3]Travel includes monies for fruit pick up at BlueStar and AgroFresh.

Objectives
We aimed 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) Test the activity of genes responsive to cold-treatment, 1-MCP exposure, and the proposed master-switch regulator gene.

We have identified master-regulatory genes which are amenable to chemical stimulation. Work is ongoing to further understand the genetic mechanisms that underlie the observed response of 1-MCP fruit treated with ripening chemistries (RCs).

We will use Bartlett and Anjou fruit harvested at commercial maturity, then subjected to 0, or 200 ppb 1-MCP treatment and stored at 3 storage temperatures for 14 days then ripened at 68°F (20°C) for 14 days. The treatments will be done by AgroFresh in-house or at BlueStar Growers facility. Peel and core samples will be collected from fruit of each treatment at regular intervals during the conditioning and ripening treatments, to establish a relationship between the 1-MCP treatment, and activity of the genes analyzed.

2. (Years 2 and 3) Establish a relationship between gene pathway activity, fruit ripening phenotypes, and chemical approaches to address controlled initiation of pear ripening in 1-MCP treated fruit.

To build on initial findings, we will conclude testing of all 6 pathway-targeted chemistries identified in year 1 to stimulate ripening in 1-MCP treated fruit. Following this, and results of gene expression work from Objective 1, we will begin optimization of ripening-induction on 1-MCP treated Bartlett and D'Anjou pear using various exposure times, concentrations and combinations of RCs.

Significant Findings (Objectives 1 and 2)
- We have identified a critical ripening-related master regulatory pathway in 1-MCP treated, inadequately conditioned Bartlett and D'Anjou fruit.
- We have identified 6 chemistries which specifically target and stimulate activity of this regulatory pathway in pear 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.

Methods
Sample procurement, 1-MCP treatment, conditioning and tissue collection: For this study, mature pear fruit treated with 200 ppb 1-MCP and controls were obtained from AgroFresh and Blue Star Growers in Cashmere. For initial testing of experimental ripening-stimulating chemistries, 1-MCP and control fruit were submerged in 12.0 liter tubs of one of three levels of each experimental chemical (high concentration, low, and a control). The tub was placed in room temperature (68°F) for 24 hours, and was covered tightly to minimize evaporative loss. Additionally, 1-MCP treated and untreated fruit were placed in a 180L flow-through respiration chamber in which a mixture of air and active RC (gaseous form) was fluxed (exchanged once per 2min with 5mL/min outflow). Subsampling of 1-MCP treated and untreated fruit was performed after each 24 hour exposure, upon which flesh firmness and peel tissue sampling was obtained. Peel samples will be used to assess ripening-regulatory and 1-MCP-related gene expression in response to the experimental chemistries. Following this, experimental fruit were transferred into chambers in which evolved carbon dioxide and ethylene were monitored at 8 hour intervals. Four 1-MCP and untreated fruit were each placed in (separate) chambers. This was replicated three times for each combination of 1-MCP and RC

treatments. After monitoring evolved gas from the fruit for seven days, all samples were removed. Soluble solids, flesh firmness and peel sampling was performed in all fruit upon removal from the chambers.

To better understand the ripening-stimulation we have obtained with the RCs, 1-MCP treated and untreated fruit will be subjected to conditioning treatments, based on recent protocols described by David Sugar, and Eugene Kupferman, and 1-MCP treatment schemes described by James Mattheis and A. Nathan Reed. Fruit treated with 1-MCP and control fruit will be conditioned at WSU using conditioning protocols recently described by David Sugar and Eugene Kupferman (*1*). A total of 160 fruit of each variety will be placed in climate-controlled rooms held at 31, 41, 50, and 68°F (-1, 5, 10 and 20°C) for 14 days. An additional 160 untreated fruit will be distributed among the chambers and rooms as a control. Peel and core samples will be collected from 10 Bartlett and Anjou fruit at 0%, 25%, 75% and 100% conditioning. The duration of condition has been broken down to percentages since the time to condition varies with the variety. Following conditioning, all fruit will be moved to 68°F (20°C) for a 14-day ripening period. Additional tissue sampling will occur at days 1, 4, 7 and 14 during this treatment. All tissue samples will be immediately frozen in liquid nitrogen for gene expression analysis. Finally, another 10 fruit will be subsampled at each sampling date (as described above) for measurement of flesh firmness with a fruit texture analyzer.

Gene expression analysis: On the existing and future peel samples collected from exposure to RCs we will use quantitative reverse transcription PCR to study the expression of ethylene perception, production and related genes known from prior research in the lab. Quantitative PCR assesses the level of a given gene's expression across several samples at once. It is a routinely used procedure in the lab and is currently being applied toward other pear-ripening related projects. 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. We can then directly compare intensity of gene expression between 1-MCP and untreated pears during storage and ripening.

Ripening-inducing chemical treatments: As previously described, fruit will be treated with 1-MCP and conditioned as described earlier, then placed in flow-through respiration chambers, held at 68°F (20°C) for 7-14 days. We will measure respiration and evolved ethylene at 8 hour intervals from 4 replicate groups of 5 fruit in response to 3 levels of RC dosage and combinations which we hypothesize will accelerate activity of genes repressed by 1-MCP treatment. Flesh firmness and peel tissue samples of all pears from one replicate group of each treatment level of a particular compound on days 1, 5, 9 and 14 for each variety will be obtained. Tissue will be immediately frozen in liquid nitrogen and finely ground for later gene expression analysis. From this work, confirmation of desired changes in ethylene perception, production and related gene activity will be completed using the quantitative PCR technique described above. We will then correlate induction of respiration burst associated with onset of climacteric ripening to changes in gene expression targeted each RC treatment.

Results and Discussion

Tools to gain greater control over ripening, and fruit quality loss in pear has been long-sought by the industry. Asynchronously ripened fruit leads to damaged and unmarketable fruit reaching retail sale which is thought to be a significant cause of diminished consumption. To combat these inevitable losses, the pear industry has sought use of 1-methylcyclopropene (1-MCP) throughout the storage and transport chain. 1-MCP is known to block ethylene perception, production, and subsequent ripening of pear fruit, but leads to inconsistent and unreliable recovery of fruit ripening capacity. Fruit can even remain 'locked' in a state in which ripening cannot occur (Bai et al., 2006; Chen and Spotts, 2005; Rudell et al., 2005; Chiriboga et al., 2011). Specifically, some have reported an inability of treated fruit to properly ripen, soften, and develop full flavor and aroma profiles (Argenta et al., 2003;

Bai and Chen, 2005; Villalobos-Acuña et al. 2011). From this knowledge, strategies to unlock ripening ability in recalcitrant 1-MCP treated pears can be developed using known properties of available chemical compounds, targeted to pear variety-specific responses to 1-MCP exposure.

Recent work by Sugar et al. (2009) has shown this conditioning requirement can be reduced by inclusion of an exogenous ethylene treatment in combination with chilling. This conditioning variability presents challenges in the development of post-harvest strategies such as 1-MCP use in prolonging pear fruit quality and retarding ripening induction. While the knowledge of the apple ripening model was built upon the framework of studies conducted using tomato, apple and Arabidopsis; unique responses to 1-MCP in pear illustrate the limitations that exist in adopting such treatments blindly (Fischer, 1991; Lay-Yee et al., 1990). Sparse gene-level analysis of 1-MCP treated pear fruit in ripening conditions has been performed, though not in a comprehensive and complete manner. Additionally, ethylene binding, and signaling genes such as CTR1, EIN2, EIN3, EIN5 and various ERFs have all been reported as being differentially expressed in various climacteric systems such as tomato, plum, pear and other climacteric fruit. As a whole, this research has demonstrated environmental, temporal and genotypic factors involved in specific expression of these genes. Comprehensive work of 1-MCP genetic responses in pear is still lacking in the majority of varieties grown. Considering this knowledge gap, there exists little to no gene-level understanding of why pears cannot recover from 1-MCP treatment and ripening retardation. An analysis and understanding of ethylene perception, signaling, production and related gene expression of 1-MCP treated pears can provide critical knowledge as to specifically what elements are altered, preventing System 2 ethylene induction and consequent ripening.

From prior work quantifying gene expression of a ripening-related regulatory pathway among Bartlett and Anjou tissue during conditioning, we hypothesized a previously unexplored pathway that may override the complex regulatory machinery of 1-MCP perception, and System 2 ethylene induction. We then investigated the possibility of targeted chemical stimulation of this pathway in pear. From initial tests, we have demonstrated what is (to our knowledge) the first and only chemical means of overcoming 1-MCP derived ripening inhibition. An invention disclosure and patent has been filed to protect this invention. Based on prior gene expression analysis conducted in the lab, we targeted a ripening-related regulatory pathway for chemical stimulation. We identified 6 RC specifically designed to affect two main pathways controlling chilling-induced ripening in pear. After 24 hour exposures to RC1 and RC2, 1-MCP treated Bartlett fruit achieved “eating quality” firmness, soluble solid content, and ethylene production consistent with a fully conditioned fruit, ready for final market sale (Figures 1 and 2). While less drastic, similar responses were also observed in 1-MCP treated D’Anjou fruit (Figure 3). These results are expected in D’Anjou, which is more recalcitrant in induction of full ripening (compared to Bartlett).

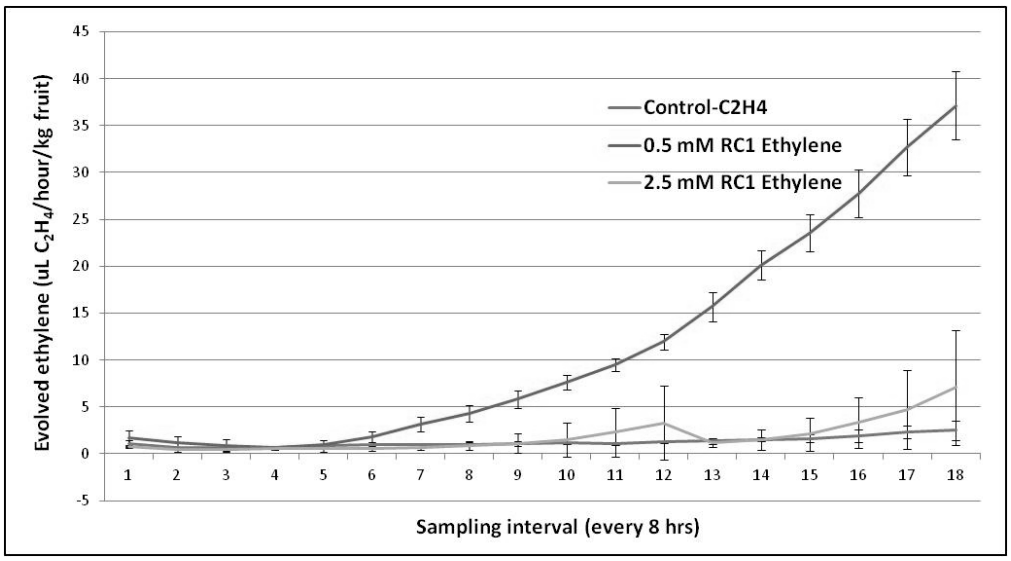

Figure 1. Evolved mean ethylene (uL ethylene/hour/kg fruit) among 3 replicate chambers containing 4 Bartlett fruit exposed to 300 ppb 1-MCP, and one of three levels of ripening chemistry 1. Error bars represent standard deviation from the mean.

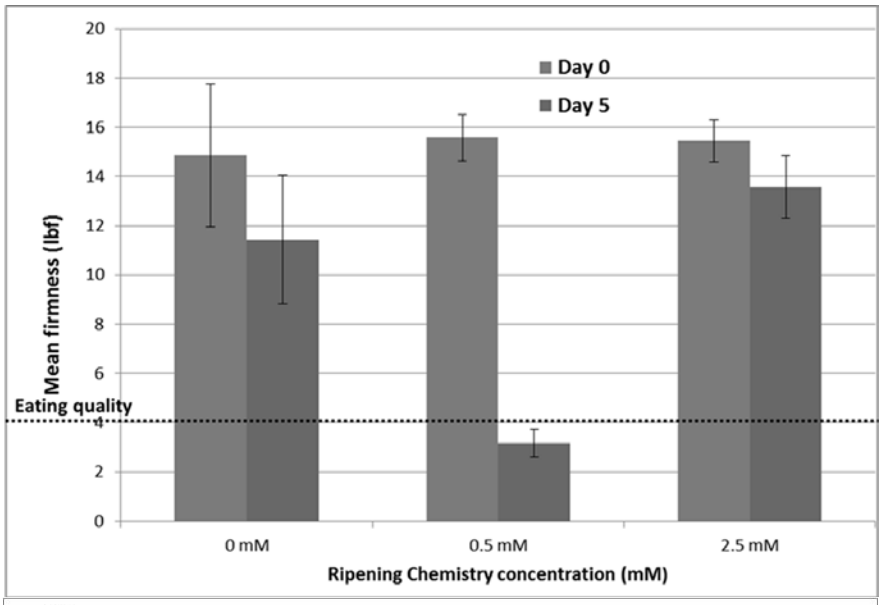

Figure 2. Mean fruit firmness as calculated on day 0 and day 5. Note the drop in fruit firmness in fruit treated with 0.5 mM of RC-1. The firmness of 4 lbf and below represents eating quality in pear fruit.

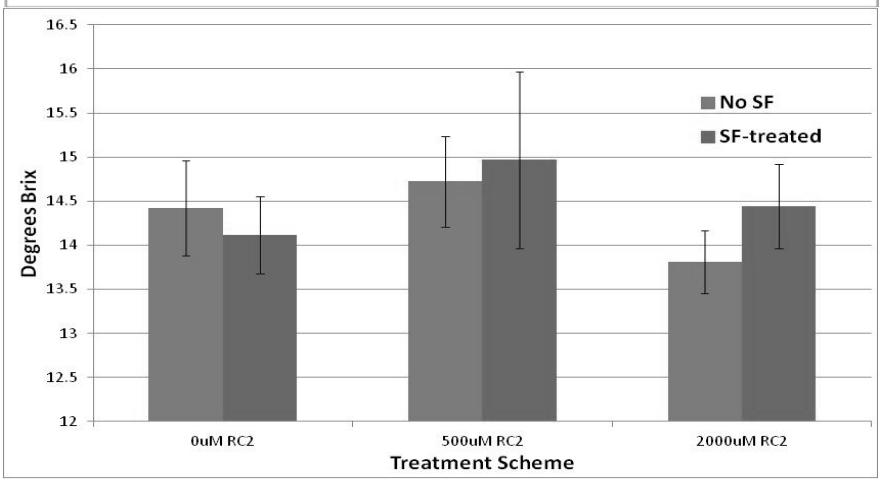

Figure 3. D'Anjou mean soluble solids (Brix) of 1-MCP treated (indicated by 'SF' for SmartFresh) and untreated fruit after exposure to 3 levels of ripening chemistry 1 (RC1). Error bars represent standard deviation from the mean.

Data clearly shows an initial stress response, followed by secondary response to the RC1 compound in nearly all trials conducted so far. Similar results have been seen with RC2, which appears to induce its ripening-stimulation effects over a longer time frame. We hypothesize this to be a reflection of mobility of the RCs toward the interior of the fruit.

The difficulties encountered in consistent use of 1-MCP in pear have prevented its widespread adoption in the industry. Within less than one year, results of this work have identified a pathway which can be exploited in the post-harvest management sector by chemical stimulation to overcome 1-MCP derived inhibition of ripening. While still preliminary, results clearly indicate rapid acquisition of eating quality in 1-MCP treated Bartlett and D'Anjou fruit as seen in Figure 4. Further work in this proposal will focus around gaining a better understanding of the genetic responses to the RCs among Bartlett and Anjou fruit. We expect this work to open possibilities for maintenance of superior fruit quality in storage and transit, and synchronized induction of ripening in a variety and time-specific manner. These have been long-sought goals of the industry for many years (Ing et al., 2002).

Figure 4. Ripened 1-MCP treated Bartlett fruit 1 week after ripening with RC1 exposure

References

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17. Fan et al., 1998. Planta 204(4):444-449.
18. Ing, G. 2002. Acta Hort. (ISHS) 596:61-65.

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

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

PI: Ken Johnson
Organization: Oregon State University
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City/ State/Zip: Corvallis, OR 97331-2902

Cooperators: Rachel Elkins (UC Cooperative Extension - Lake County, CA)
Steve Castagnoli (OSU Extension - Hood River County, OR)
Tim Smith, (WSU Extension - Chelan County, WA)

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

Other funding sources

Agency Name: California Pear Advisory Board
Amt. requested for 2012: $23K (Elkins $12K, Johnson $6K, others $5K)

WTFRC Collaborative expenses: None

Budget 1
Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Kelvin Koong
Telephone: (541) 737-4066 **Email address:** j.koong@oregonstate.edu

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 3 mo plus fringe, 150 hr undergrad labor, 2K M&S, 1K local travel & plot fee, 3% inflation

OBJECTIVES:

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

2a) Survey commercial orchards with a molecular scouting protocol designed to detect the fire blight pathogen in flower samples as affected by a copper sanitation treatment and weather.

2b) Evaluate and compare new LAMP technologies that will facilitate the use of molecular scouting protocols by individuals within regional fruit production districts.

SIGNIFICANT FINDINGS

- For a 2nd 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 Bosc pear.
- In apples, for a 2nd season, the addition of Actigard to antibiotic treatments significantly enhanced fire blight control.
- A non-crop destruct experimental use permit for Actigard has been obtained from EPA for the 2013-2014 seasons, which will allow for its continued evaluation in commercial orchards.
- For a 3rd season, molecular scouting during the bloom period detected and characterized the build-up of fire blight pathogen populations in pear flowers with the probability of pathogen detection being greatest near petal fall.
- The protocol for molecular detection of the fire blight pathogen was refined and adapted for use with machines designed to run the assay in the orchard.

METHODS

Objective 1 was addressed in a 4-yr-old block of Bosc pear located at the Oregon State University Botany and Plant Pathology Field Laboratory near Corvallis, OR. The experiment was arranged in a randomized complete block design with 10-15 replications. Flowers on trees were mist inoculated with the fire blight pathogen on 27 April. After running cankers were established in the trees, systemic acquired resistance (SAR)-inducing treatments (paints and a spray) were arranged onto the diseased trees. Fire blight cankers were cut on 6 June and SAR treatments were applied immediately after cutting. Pruning cuts to remove blight were made 6 to 8" below the visible canker margin. SAR paints were applied to the 30 cm (12") of symptomless branch tissue immediately below the fresh cut. Trees were monitored for re-ignition of disease symptoms; cuts were repeated on 27 June with SAR treatments applied to new cuts. After the second cut, fire blight infections, if not eliminated by cutting, were allowed to progress; the resulting disease severity was assessed on 4 October.

Objective 2a was addressed in cooperation with Rachel Elkins (UC ANR, Lakeport, CA) and Steve Castagnoli (OSU Extension, Hood River County, OR). Eight-acre sections of 7 orchard blocks in Lake County, 4 orchard blocks in Sacramento County, 6 orchard blocks in Yuba County, CA and 2 orchard blocks in Hood River Co., OR were divided into two 4-acre sections and either treated with 6 lbs. per acre of the fixed copper product Badge X_2 (Isagro USA) at bud swell or left untreated. Treatments were applied at 125 gallons per acre by cooperating growers using commercial air blast sprayers. In each plot on each sampling date, three samples of 100 flower clusters (a total of 300

clusters) were haphazardly collected into a 4-quart freezer bag from both treated and untreated sections along a W-shaped walking pattern. Plots were sampled at mid-bloom, full bloom, petal fall and rat-tail bloom for a total of 130 samples per treatment. Samples of flower clusters were analyzed at Oregon State University, Corvallis for the presence of *E. amylovora* with two techniques: loop-mediated isothermal amplification of DNA ('LAMP'), and to verify LAMP results, dilution plating.

For objective 2B, the LAMP protocol was refined for adaptation to new commercially-available 'LAMP machines', which have the potential to be used for molecular scouting in the orchard. New LAMP primers for *E. amylovora* (targeted to chromosomal DNA) were designed and evaluated to perform in conjunction with a commercial reagent master mix and a fluorescent 'assimilation probe' that creates a machine-readable signal when DNA of the target (fire blight pathogen DNA) is amplified.

RESULTS

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

4-yr-old Bosc pear. One 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 cut again on 27 June. Immediately after each cutting, the Actigard paints (rates in legend of Fig. 1) were applied to 30 cm (12") of symptomless branch below the cut. After cutting, running cankers re-ignited in about half of the trees. Compared to cut only and to a cut plus spray of Actigard, the Actigard paint treatments (and the combination paint of Actigard plus Apogee) significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers.

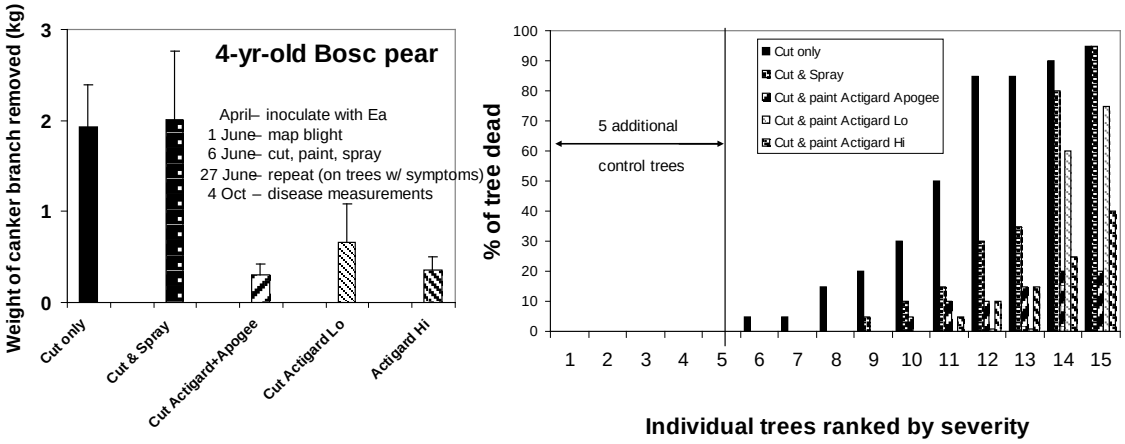


Fig. 1. Effect of branch paints and a spray of the SAR-inducer, Actigard, on re-ignited fire blight cankers in 4-yr-old 'Bosc' pear. Trees were inoculated with the fire blight pathogen on 27 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 6 and 27 June. Immediately after cutting, Actigard was applied by spray (0.45 g Actigard per L to runoff) or by paints (Actigard 30g/L in 2% Pentrabark (LO), Actigard 45g/L in 1% Pentrabark (HI), or Actigard 15g/L plus Apogee 15 g/L in 1% Pentrabark). Paints were applied to the 25-30 cm (10-12") of symptomless branch below the cut. Weight of cankered branches removed and percent of tree dead from fire blight and was assessed on October 4. A) Each bar is the mean and standard error of 10 trees (except untreated control, which was 15 trees. B) Ranked comparison of the disease severity on individual 'Actigard-treated' trees compared to individual 'cut only' trees.

Discussion of SAR. 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 the greenhouse, 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 report). Trunk paints of Actigard showed levels of 'disease resistance gene' (termed 'PR-gene') induction 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.

For the body of data collected from both pear and apple (see January 2012 apple and pear reports and January 2013 apple crop protection report), Actigard treatments applied by paint and spray 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 generally 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. For the reasons given above, Actigard could prove practical as aid to cutting blight in pear, either reducing severity of re-ignited cankers (as demonstrated) or perhaps reducing the incidence of re-ignition.

Failed SAR experiments in 2012: Two additional experiments also were conducted in 2012 to evaluate protection of pear trees with Actigard. One was a cutting experiment in Bartlett pear analogous to a 2011 experiment (see January 2012 Final pear report) and similar to the Bosc pear experiment shown above. The experiment failed because of a very poor success in an initial inoculation and then a month later after a follow-up pathogen inoculation into the fruitlets, the cankers failed to run. A shoot blight experiment on potted Concorde pear ended prematurely because an irrigation pump failed over a hot weekend in July. These experiments will be attempted again in 2013.

Obj. 2a) Survey commercial orchards with a molecular scouting protocol designed to detect the fire blight pathogen in flower samples as affected by a copper sanitation treatment and weather.

This is an ongoing study with similar surveys completed in 2010 and 2011. For 2012, as in previous seasons, very few detections of the fire blight pathogen were made in the mid- to full bloom period. Detections of the fire blight pathogen in flower samples began to increase near petal fall. As in previous years, the pathogen was detected most frequently in rattail samples (petal fall II in Fig. 2), which averaged 36% across all orchards). While the pattern of detection was similar to previous years, there was no overall difference between copper-treated and untreated plots, either in incidence (number of positive samples by LAMP) or from the positive samples, the number of pathogen colony forming units recovered on dilution plates. In the first two years of the survey, use of copper at bud

swell (just prior to green tip) delayed and reduced the detection of the fire blight pathogen in collected flowers samples (Fig. 2).

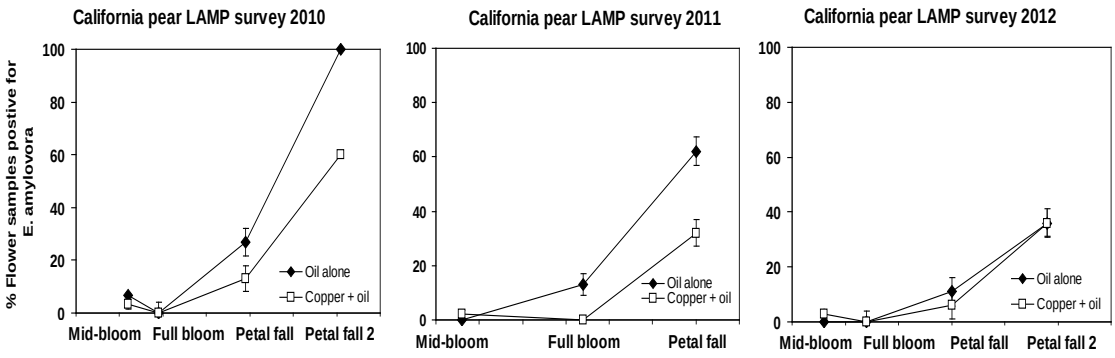
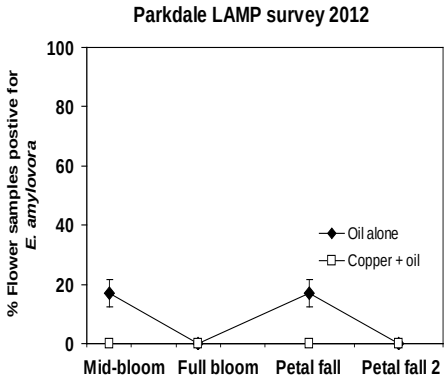


Fig. 2. Effect of delayed dormant timings of copper plus oil and oil only on the detection of *Erwinia amylovora* in 100-flower cluster samples from commercial pear orchards in Yuba, Sacramento and Lake Counties in California. Detection of the fire blight pathogen was based on a loop mediated isothermal 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.

For the two orchards surveyed in Parkdale (Hood River Co., OR) we did not see a pattern similar to what we have observed in the California surveys. In contrast, one of 12 flower cluster samples was LAMP positive in the first sample (mid-bloom) and one of 12 was LAMP positive in the first petal fall sample. Both of the positive samples were from the areas of the blocks that did not receive the delayed dormant copper treatments. Neither of the LAMP positive samples was confirmed by the dilution plating method.

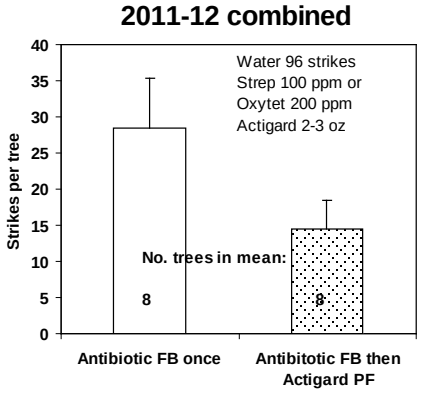
Fig. 3. Effect of delayed dormant timings of copper & oil and oil only on the detection of *Erwinia amylovora* in 100-flower cluster samples from commercial pear orchards in Parkdale (Hood River Co., OR). Each point is the mean of 12 100-flower cluster samples



Discussion of LAMP surveys. LAMP and dilution plate results from this third year of testing contrasted with 2010 and 2011, in that they failed to show that delayed dormant copper applications reduced the amount of *E. amylovora* inoculum. While the overall pattern of inoculum build up in flowers was similar to previous seasons, the overall frequency of pathogen detection was smaller. Because of a cool wet early spring, the period between the copper treatment and bloom was longer in 2012 (~ 7 weeks) than in the previous years (4 to 5 weeks). Thus, in 2012, the amount of residual copper remaining on the trees at the time of bloom was probably smaller than in the earlier years.

Whether LAMP-based scouting will have a place in commercial IPM programs remains to be seen. 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 an increased emphasis on the need for protective treatments near and after petal fall. Most seasons, primary bloom escapes fire blight, but strikes occur in secondary flowers in the period after petal fall. Antibiotics may not be cost effective when applied at petal fall but other materials could be effective during this period. One example is Apogee, a growth regulator used in apple but not pears. Another example is Actigard; orchard trials showing the effect of Actigard sprays at petal fall are shown in Fig 4. Potentially, induction of SAR at petal fall provides a longer residual protection from fire blight. Development of a data set that addresses this hypothesis is a primary goal of a 2013-2014 Crop Non-destruct Experimental Use Permit that has been obtained from EPA for the evaluation of Actigard treatments in commercial orchards.

Fig. 4. Effect of an Actigard treatment at petal fall on fire blight incidence in a Gala apple orchard located near Corvallis, OR in 2011 and 2012. *Erwinia amylovora* was inoculated onto the trees at full bloom, and an antibiotic treatment (max label rate of FireWall (streptomycin, 2011) or FireLine (oxytetracycline, 2012) was applied 1-3 days after inoculation. The Actigard treatment was sprayed 4-7 days after inoculation.



2b) Evaluate and compare new LAMP technologies that will facilitate the use of molecular scouting protocols by individuals within regional fruit production districts.

The rationale for this subobjective is to improve and simplify the LAMP technology on which molecular scouting is based so that other individuals can do the assay at the 'point-of-care', i.e., the orchard. In the last couple years, technology developed by Optigene, Ltd. (Horsham, West Surrey, England) has shortened reaction time from 45 to 20 minutes and their 'Mastermix' has eliminated the need for multiple reagents to perform the assay. Optigene and others have also developed 'LAMP machines' that run multiple assays at one time and potentially eliminate the need for DNA extraction from the floral wash.

Figure 5 shows the adaption of our fire blight pathogen LAMP protocol to the new Optigene technologies. To achieve these results, we designed and evaluated new primers (named 'Amy13', available on request) targeted at chromosomal DNA of *Erwinia amylovora*, and we developed a fluorescent 'assimilation probe' that creates a machine-readable signal when DNA of the target (fire blight pathogen DNA) is amplified. We have also streamlined the handling and preparation of flower samples for LAMP, such that multiple samples can processed in a shorter period of time.

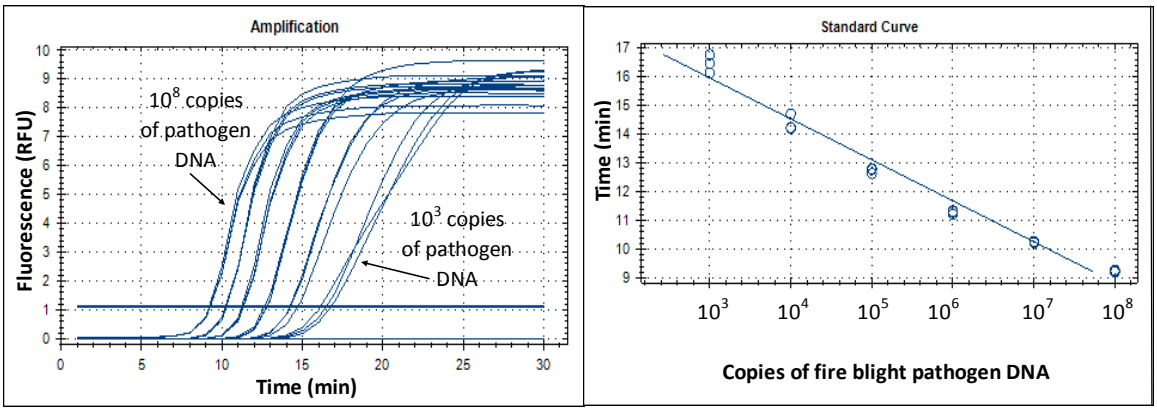


Fig. 5. Fluorescence-based loop-mediated isothermal amplification (LAMP) assay performed on DNA extracted from *Erwinia amylovora* cells grown in pure culture. Graph on left is cumulative fluorescence units emitted over time as influenced by a 1:10 dilution series of *E. amylovora* DNA (clustered groups of lines represent individual dilutions). Graph on right is time to achieve a 'positive' result as a function amount of *E. amylovora* DNA.

Discussion of new LAMP technologies. The new LAMP technologies have already simplified the process of performing a LAMP assay for specific detection of *E. amylovora*. Moreover, compared to the old 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 (Fig. 5). Compared to lab-grown pure cultures, samples of flowers from orchards are likely to 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 still have not purchased a LAMP machine because the companies have overpromised the availability of models designed for use in the field (e.g., Optigene stopped development of their model Genie III to initiate a re-design before it was ever marketed). We are now working with another company (diagenetix.com/product-and-technology/smart-dart-platform) about acquiring their field-based LAMP machine. The machine should arrive by spring. Currently we run fluorescence-based LAMP assays on a real-time PCR machine.

This next season we will save (freeze) all flower washes from surveyed orchards. Over the summer, by analyzing washes with the new machine, we will evaluate if the sample number subjected to LAMP assay can be reduced -- e.g. reduce to one 300-flower clusters samples -- without loss of information. Thus, the entire process of molecular scouting for *E. amylovora* -- assay technology, the handling of samples, and number of samples -- will be made more efficient.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: PR-12-101

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

PI: David Horton
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City/State/Zip: Wapato, WA 98951

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

Total Project Request: **Year 1:** $19,000 **Year 2: $19,000**

Other funding sources: None

Budget
Organization Name: USDA-ARS **Contract Administrator:** Janis Contento
Telephone: (510) 559-6007 **Email address:** Janis.contento@ars.usda.gov

Item	2012	FY 2013
Salaries[1]	$14,615	$14,615
Benefits	$ 4,385	$ 4,385
Total	$19,000	**$19,000**

Footnotes: [1]Partial support of GS-6 technician; benefits at 30%

OBJECTIVES

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

- **(A)** Determine whether SPLAT products developed by ISCA and shown to repel other psyllids can be used to disrupt re-entry by returning winterform psylla. **(B) New**: examine pear ester as repellent.
- **(A)** Determine whether saturation of atmosphere with 13-MeC27 pheromone slows mating by returning colonists. **(B) New:** examine synergism of newly identified pheromone component with previously identified 13-MeC27 compound.

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. **FY2013:** will conduct field test with product against returning winterforms.
- Pear ester shown to repel both winterform and summerform pear psylla in olfactometer trials. **FY2013:** will continue with these assays, including dose-response studies and possible interaction with sex pheromone (13-MeC27).

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. **FY2013:** examine potential synergism of the 11-Me compound with the previously identified 13-Me compound.
- Small cage studies were begun to examine whether saturation of the atmosphere with 13-MeC27 affects male winterform success at finding females. Data are currently being collected. **FY2013:** continue with these assays.

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). Having the repellent at that end of the cage did not overcome that light effect (Figure 1, bottom two

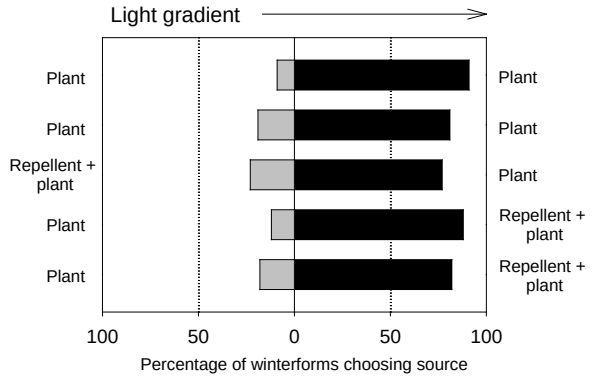

Figure 1. Large cage study with SPLAT-DMDS

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.

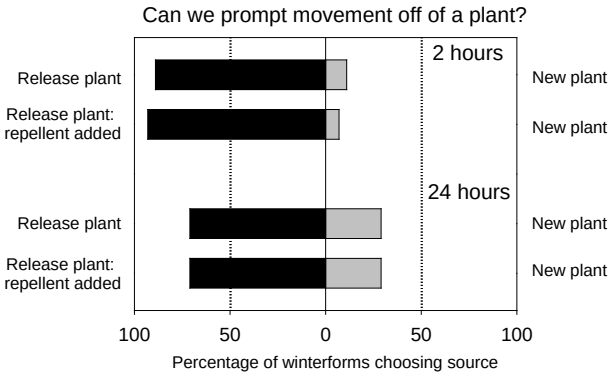

Figure 2. Cage study to examine whether SPLAT-DMDS prompts movement off of plants.

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

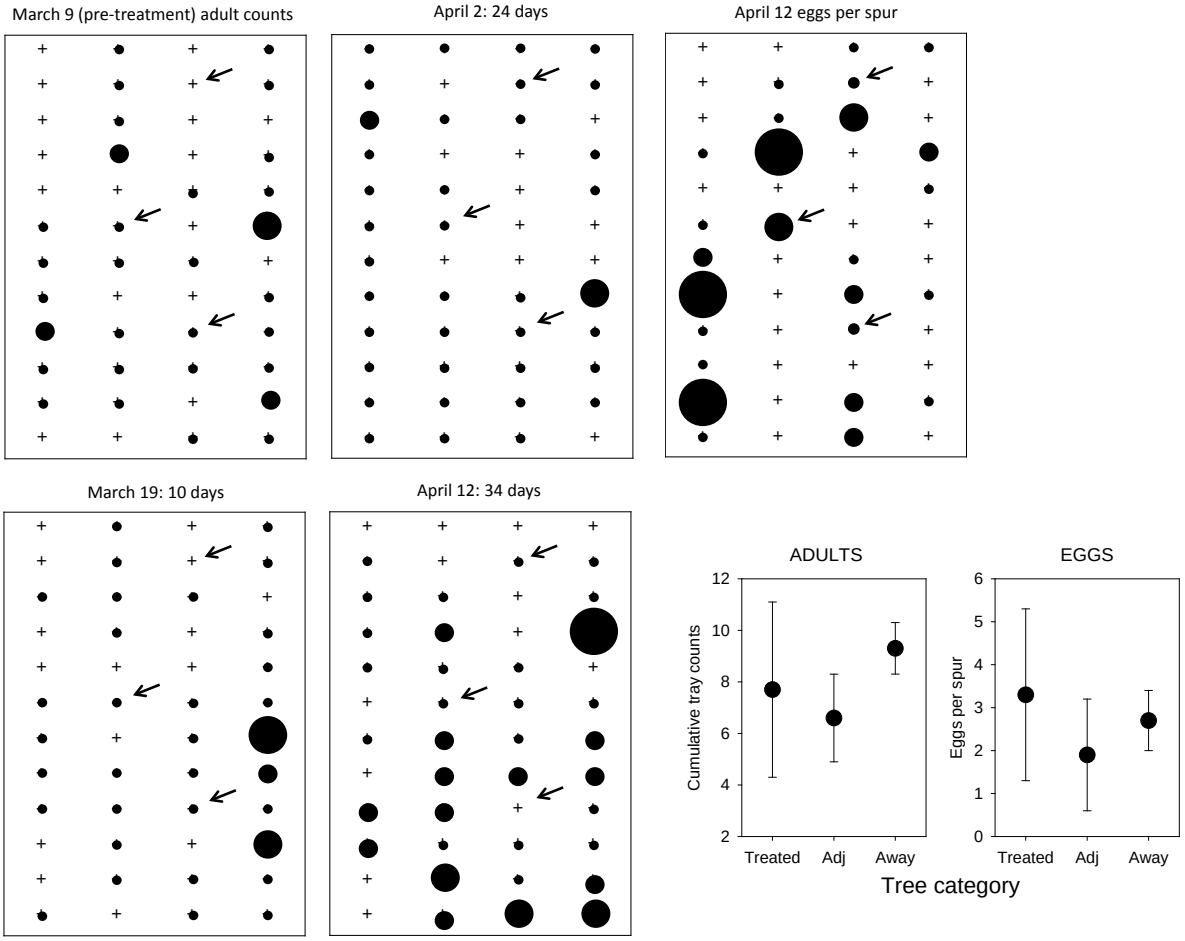

Figure 3. Field trial (48-tree orchard). Arrows: location of SPLAT-DMDS. Size of circle proportional to numbers. Four left-most panels: adults. Graphs in bottom right show count data summarized by whether tree was treated, adjacent to a treated tree, or away from treated tree. + indicates no psylla.

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.

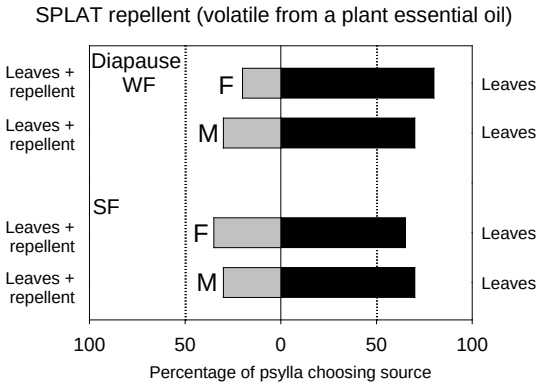

Figure 4. Olfactometer test of new SPLAT psyllid repellent (proprietary).

4. SPLAT-New product: olfactometer trials. Olfactometer trials have been initiated 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). Work with this compound will continue in 2013.

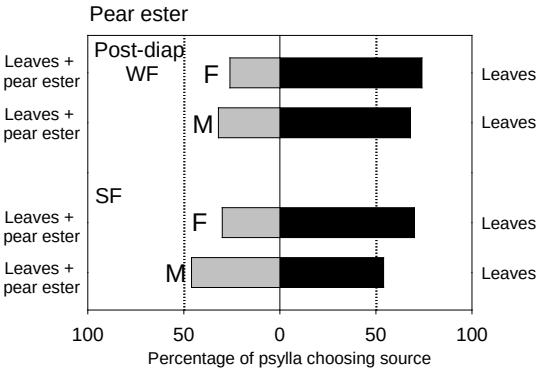

Figure 5. Repellency of pear ester to winterform and summerform psylla in olfactometer trials.

5. Pear ester: olfactometer trials. Olfactometer trials have been initiated with pear ester in efforts to find a new attractant for pear psylla. The olfactometer trial compared pear leaves alone vs pear leaves + pear ester. ***Results.*** Pear ester was actually repellent to both summerform and winterform psylla in olfactometer trials (Figure 5), rather than attractive. Work with this compound will continue in 2013.

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 6). Studies in 2013 will examine these compounds as winterform attractants, and will examine whether the 11-MeC27 compound acts synergistically with the previously identified 13-MeC27.

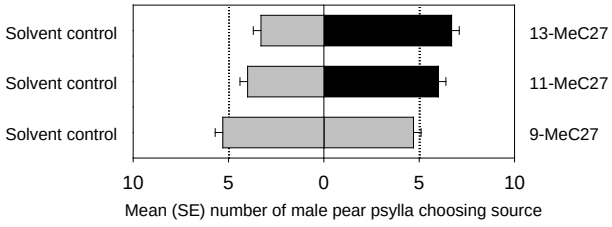

Figure 6. Olfactometer trial showing attractiveness of 11-MeC27 to male summerforms.

7. Pheromone, effects on mate-finding of male winterforms. Small cage studies are ongoing with field-collected winterforms to determine whether saturation of a cage with 13-MeC27 interferes with how rapidly males find and mate previously unmated female winterforms. ***Results.*** Trials are ongoing. Work on this topic will continue extensively in 2013, to include also trials with the newly discovered 11-MeC27.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-12-107

Project Title: Pear scion trials in the Pacific Northwest

PI: Kate Evans
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Co-PI (2): Todd Einhorn
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City/State/Zip: Hood River/OR/97031

Co-PI(3): Richard Bell
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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: 6,771** **Year 3:** 12,155

Other funding sources
None

Budget 1 – Kate Evans
Organization Name: 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	0	1000	1040
Benefits	0	149	155
Supplies[1]	1350	0	0
Travel	500	500	1000
Trees[2]	0	0	4,800
Plot Fees	0	500	500
Total	1,850	**2,149**	7,495

Footnotes:
[1]Planting supplies and fumigation
[2]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, ext. 2 **Email address**; kathy@treefruitresearch.com

Item	2012	2013	2014
Salaries & benefits	0	1000	5,150
Travel	0	120	620
Miscellaneous[1]	0	1000	5,150
Total	0	**2,120**	10,920

Footnotes:
[1]Grower reimbursement

Budget 4 – 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 being 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

- Suitable trial planting sites found for the first plantings of the new scion selections.
- Trees lifted and 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. Anjou, Bosc and Bartlett will be used as standard comparison trees. 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).

RESULTS & DISCUSSION.

Suitable trial planting sites have been found ready for the planting of the five new scion selections. In Washington, trees will be planted in Wapato (Chuck Peters) and in the Wenatchee Valley (Josh Koempel). In Oregon, a site at MCAREC was ripped but was not fumigated due to inadequate soil moisture conditions in the fall. Therefore, the site will be fumigated spring 2013 (Trident) and planted ~4 to 5 weeks later. 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.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2 (Extension)
WTFRC Project Number: PR-12-109

Project Title: Genotype work for pear

PI:	Kate Evans	**Co-PI(1)**:	Amit Dhingra
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Email:	kate_evans@wsu.edu	**Email**:	adhingra@wsu.edu
Address:	1100 N. Western Ave	**Address**:	Johnson 46
City:	Wenatchee	**City**:	Pullman
State/Zip:	WA 98801	**State/Zip**:	WA 99164

Cooperators: Richard Bell (USDA-ARS, Kearneysville, WV); Todd Einhorn (OSU); Rachel Elkins (UCD); Stefano Musacchi (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

Other funding sources: None (although SCRI proposal in preparation)

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Kevin Larson/Carrie Johnston
Telephone: 509.663.8181/509.335.4564 **Email address:** kevin_larson@wsu.edu/carriej@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 the time required to move 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

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. Where possible, germplasm will be sent in tissue culture to the Dhingra lab as quarantine restrictions do not apply to this material.

3. 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. Approval from the others is pending.

Lists of potential germplasm are being prepared by our collaborators and dormant propagating wood will be sent to the Clean Plant Center once the MTAs are approved.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

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

PI:	**Todd Einhorn**	**Co-PI (2):**	Stefano Musacchi
Organization:	**OSU-MCAREC**	**Organization:**	University of Bologna
Telephone:	**(541) 386-2030**	**Telephone:**	+390512096400/14
Email:	todd.einhorn@oregonstate.edu	musacchi@agrsci.unibo.it	
Address:	3005 Experiment Station Drive	**Address:**	Viale G. Fanin, 46
City:	Hood River	**City:**	40127 Bologna
State/Zip:	OR 97031	**State/Zip:**	Italy

Cooperators: Growers: Don Gibson (WA), Ken Goe (OR), Mike McCarthy (OR)

Total Project Request: Year 1: $75,151 **Year 2: $72,278** **Year 3:** $72,960

Other funding sources: Match funding of $20,384 from DCA-UNIBO, Italy (please refer to match letter included in this submission packet).

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**	73,982

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. Specifically, trial single axe and bi-axe planar canopy architectures, recently developed in Italy, using PNW cultivars planted in both OR and Bologna. Graft 'd'Anjou' to readily available, standard dwarfing quince rootstocks and OH×F in Italy, and compare tree performance, precocity, fruit size and yield to answer the question of whether rootstock induced vigor control of 'd'Anjou' results in early fruit set and productivity. Develop demonstration orchards at MCAREC of single axe and bi-axe planar hedgerows. Work collaboratively with growers to establish planar commercial high-density blocks.

<u>Significant Findings 2012</u>:
Objective 1:

- **Six plant growth regulator (PGR) trials were initiated in 2012.**
- **A European PGR protocol to improve pear fruit set was evaluated on Anjou and Comice. Anjou responded inconsistently. Comice yields were significantly increased.**
- **NAA and NAD applied during early bloom significantly reduced seed number per fruit.**
- **Seed number per fruit was poorly related to final fruit size of Anjou and Comice pears.**
- **ReTain markedly improved fruit set of Anjou and Comice when applied at 2 weeks after bloom. The effect was responsive to rate with 80 ppm resulting in the highest set.**
- **Whole tree Anjou yields (fruit number at harvest) were 2.2-fold greater than control yields when ReTain was applied 2 weeks after bloom.**
- **Fruit size of ReTain-treated trees was markedly reduced compared to control fruit, but the markedly higher croploads played a significant role in limiting fruit size.**

Objective 2:

- **Root pruning was applied during bloom to 2 orchards: Trellised 6th leaf Anjou/OH×F 87 (10 x 14 ft.) trained to a parallel V; and, free-standing 30-year-old Anjou/seedling (10 x 18 ft.) central leader. Root pruning was applied to one-side of the tree row, or both.**
- **One and two-sided root pruning significantly reduced shoot growth of 6th leaf Anjou by 20% and 35% compared to control trees, respectively. Trunk growth was similarly reduced.**
- **6th leaf Anjou yield was reduced by one and two-sided root pruning 20% and 40% compared to control trees, respectively. Fruit size was reduced by root pruning by one box-size.**
- **30-year-old Anjou yield was significantly greater for the two-sided root pruning treatment relative to control and one-sided treatment. Fruit size was not significantly reduced.**

Objective 3:

- **A one acre training planting of Bartlett and Anjou was established at MCAREC to compare bi-axe and single-axe high-density training systems. For Bartlett, each system was planted at 2, 4, or 6ft. in-row spacing. For Anjou, each system was planted at 4 or 8ft.**

Results and Discussion:

Objective 1:

We performed 6 experiments to evaluate individual plant growth regulators and combination sprays on pear fruit set. Each trial was designed as a randomized complete block comprising 4 single-tree replicates. In each experiment whole trees were sprayed using a pressurized hand gun.

1. 4th leaf **Anjou/OH × F 97**, Mt. Adams, WA
    a. Control
    b. PGR regime
    - Balloon flower stage- Promalin 125 ppm + NAA/NAD 750 ppm
    - 20% Bloom- Promalin 125 ppm + NAA/NAD 750 ppm
    - 80% Bloom- Promalin 125 ppm
    - Petal Fall- Apogee 250 ppm
2. 7th leaf **Anjou/OH × F 87**, Hood River, OR
    a. Control
    b. PGR regime
    - Balloon flower stage- Promalin 125 ppm + NAA/NAD 750 ppm
    - 20% Bloom- Promalin 125 ppm + NAA/NAD 750 ppm
    - Petal Fall- Apogee 250 ppm
    c. 125 ppm Promalin (80% bloom)
    d. 250 ppm Promalin (80% bloom)
3. 17-year-old **Comice/OH × F 97**, Hood River, OR
    a. Control
    b. PGR regime
    - Balloon flower stage- Promalin 125 ppm + NAA/NAD 750 ppm
    - 20% Bloom- Promalin 125 ppm + NAA/NAD 750 ppm
    - Petal Fall- Apogee 250 ppm
4. 12-year-old **Anjou/OH × F 97**, Hood River, OR
    a. Control
    b. 500 ppm NAA/NAD (20% bloom)
    c. 750 ppm NAA/NAD (20% bloom)
    d. 1,000 ppm NAA/NAD (20% bloom)
5. 10-year-old **Anjou/OH × F 97**, Hood River, OR
    a. Control
    b. 40 ppm ReTain (80% bloom)
    c. 40 ppm ReTain (2 weeks after full bloom)
    d. 80 ppm ReTain (80% bloom)
    e. 80 ppm ReTain (2 weeks after full bloom)
6. 17-year-old **Comice/OH × F 87**, Hood River, OR
    a. Control
    b. 40 ppm ReTain (80% bloom)
    c. 40 ppm ReTain (2 weeks after full bloom)
    d. 80 ppm ReTain (80% bloom)
    e. 80 ppm ReTain (2 weeks after full bloom)

In general, experiments 1-4 produced inconsistent results for fruit set (Fig 1), yield, and fruit size at harvest (Table 1). The PGR regime applied to Anjou in experiment 1 resulted in lower fruit set and yield compared to the control (Fig 1). A similar regime, however, did not adversely affect fruit set or yield in another Anjou block (Experiment 2). Experiments 2 and 3 intended to have an 80% Promalin application in addition to the 20% timing, however, bloom advanced too quickly (i.e., 20% to full in one day) to accommodate both application timings. The PGR regime was based on European

protocols to improve fruit setting of Abate Fetel and Comice. The Promalin treatments in Experiment 2 (125 vs. 250ppm) were an attempt to isolate the effect of Promalin from the PGR regime. Promalin, on its own, appeared to improve fruit set and yield of Anjou (Fig 1; Table 1). At higher rates Promalin has been shown to alter fruit shape of more round pear cultivars (Musacchi, personal communication), but this was not observed in our trials (Table 1). This potentially implicates the NAA/NAD component of the PGR regime as a limiting factor in fruit set. In a separate trial we applied several rates of NAA/NAD, but did not observe a reduction in fruit set (Fig 1). In fact, fruit set appeared to be higher for the 750 ppm rate (which was the equivalent rate of the PGR regime), but the variability in fruit set among treatments and reps was too high to observe statistical significance.

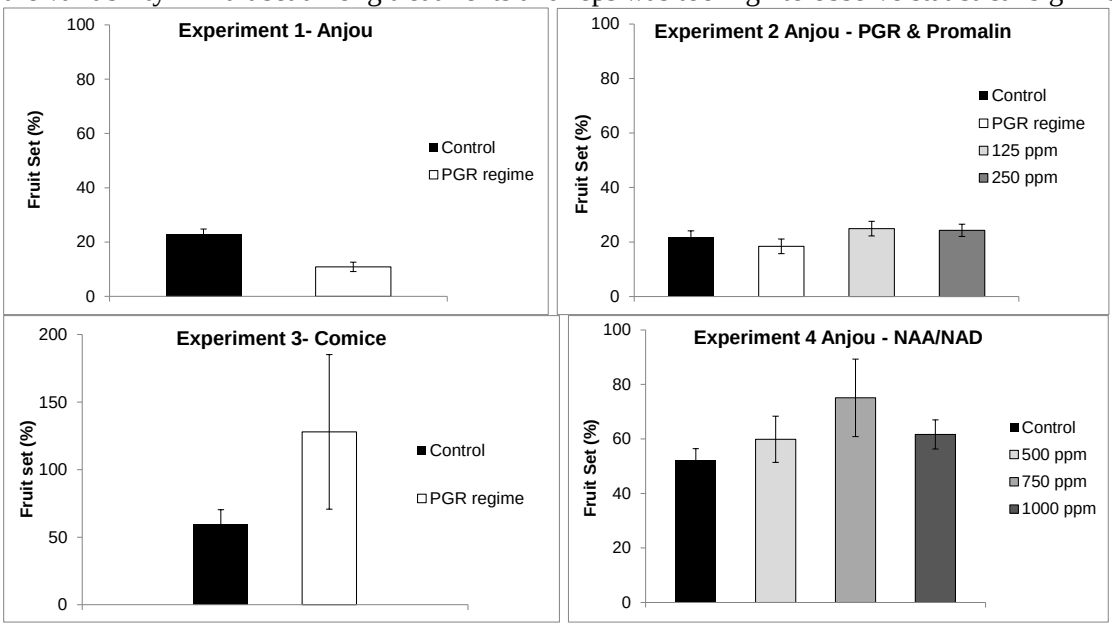


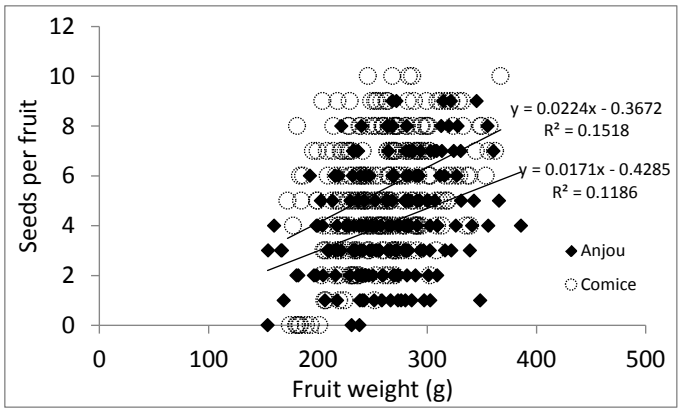
Figure 1. Fruit set (number of fruit per 100 clusters) for Experiments 1-4 (outlined on previous page). The large error bar for the PGR regime treatment in experiment 3 represents high variability in fruit set among the four single-tree replicates. Fruit set was determined on scaffold limbs. All limbs had a minimum of 100 flowers.

Table 1. Effect of PGR treatments on yield, fruit size, fruit shape (ratio of length:width) and number of seeds per fruit for Experiments 2-4. Experiment 1 data were collected but not analyzed at time of reporting; however, the PGR regime in that trial markedly *reduced* both yield and fruit size compared to untreated controls.

Experiment 2 Anjou	Yield (lb per tree)	Projected Yield (1100 bins per acre)	Fruit size (g)	Ratio length:width (avg. per fruit)	Seed count (avg. no. per fruit)
Control	48 b	12	199	1.27	5.1 a
PGR regime	50 b	12	172	1.24	2.7 c
125 promalin	60 a	15	232	1.31	4.4 ab
250 promalin	67 a	17	200	1.29	4.4 ab

Experiment 3 Comice	Yield (lb per tree)	Projected Yield (1100 bins per acre)	Fruit size (g)	Ratio length:width (avg. per fruit)	Seed count (avg. no. per fruit)
Control	93 b	26	260 a	1.16	5.5 a
PGR regime	119 a	33	215 b	1.08	2.6 b

Experiment 4 Anjou	Yield (lb per tree)	Projected Yield (1100 bins per acre)	Fruit size (g)	Ratio length:width (avg. per fruit)	Seed count (avg. no. per fruit)
Control	224	49	264	1.24	4.1 a
500 NAA/NAD	225	50	259	1.23	2.1 b
750 NAA/NAD	222	49	239	1.22	2.2 b
1000 NAA/NAD	206	45	241	1.22	2.5 b



Interestingly, seed number per fruit was markedly reduced by NAA/NAD when tested on its own, and when used in combination with other PGRs in the PGR regime (Table 1). Despite the accepted belief that seed count is positively related to fruit size, plotting the relationships for both Anjou and Comice control fruit suggests that while a positive relationship does exist, seed count only accounts for roughly 10-15% of the variability in fruit size (Fig 2). Regardless, NAA/NAD appears to have a negative effect on fertilization.

Fruit set of both Anjou and Comice was markedly improved by ReTain, an ethylene inhibitor, in a rate responsive manner (Fig 3). The greatest effect on fruit set occurred from applications timed at 2 weeks after bloom (Fig 3). In the case of Anjou, this fruit setting effect translated to a 2.3 fold increase in yield (in terms of total fruit per tree at harvest; Table 2). Comice yield also showed a rate response to ReTain, but positive effects on yield were also observed for the 80% bloom application (Table 2). Comice has been previously shown to possess a short ovule viability period. Our intent was to improve fruit set by inhibiting ethylene (a growth regulator that promotes senescence). The fact that the two-week after bloom treatment improved fruit set, however, does not entirely support the role of ovule longevity as the limiting factor to fertilization and set. For Anjou, the drastic

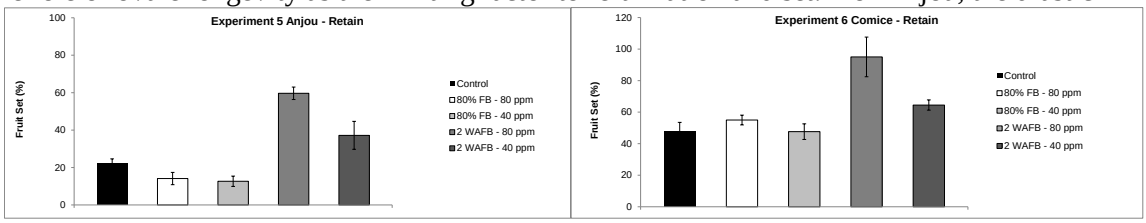


Fig. 3. Fruit set of Anjou (left) and Comice (right) following ReTain applications at 80% full bloom and 2 weeks after full bloom (2WAFB). At each timing two rates were applied; 40 and 80ppm.

Table 2. Average Anjou and Comice tree yield (weight and fruit number), fruit weight, shape and number of seeds at harvest following ReTain applications at 80% full bloom or 2 weeks after full bloom (2WAFB).

Experiment 5	Yield per tree		Fruit wt.	Fruit shape	Seed count
Anjou	(lbs)	(fruit no.)	(g)	(length:width	(no. per fruit)
Untreated Control	88 b	172 c	230 a	1.3	4.9 a
40 ppm ReTain® (80% FB)	57 c	118 cd	221 ab	1.3	3.5 ab
80 ppm ReTain® (80% FB)	52 c	111 cd	214 b	1.34	3.2 b
40 ppm ReTain® (2 WAFB)	160 a	409 b	177 c	1.32	4.0 ab
80 ppm ReTain® (2 WAFB)	198 a	558 a	160 c	1.34	4.0 ab

Experiment 6	Yield per tree		Fruit wt.	Fruit shape	Seed count
Comice	(lbs)	(fruit no.)	(g)	(length:width	(no. per fruit)
Untreated Control	77 c	138 c	251 a	1.17	5.4 a
40 ppm ReTain® (80% FB)	95 b	189 bc	226 bc	1.19	4.5 ab
80 ppm ReTain® (80% FB)	106 b	235 ab	203 c	1.22	3.9 b
40 ppm ReTain® (2 WAFB)	100 b	215 b	209 c	1.17	5.6 a
80 ppm ReTain® (2 WAFB)	127 a	269 a	213 c	1.16	5.8 a

effect on fruit set from 2-week-after-bloom ReTain applications indicates that the poor fruit-setting ability that characterizes younger Anjou trees is due to an excessively high fruit drop of fertilized fruitlets after bloom. This observation is supported by seed counts of Anjous from the 2-week-after-bloom treatment timing (Table 2). Fruit size from ReTain treatments was significantly reduced for both cultivars (Table 2); however, there is undoubtedly an indirect effect of cropload confounding fruit size, since Anjou fruit loads of both two-week-after-bloom treatments, for example, had double the fruit number of control trees (Table 2). More robust experiments, testing a range of rates and timings, will be performed in 2013. Vegetative growth was markedly reduced in these treatments as well; a desirable result of increasing the sink strength of fruit (data not shown). Stimulating fruit set in otherwise vigorous and unfruitful Anjou trees offers a potential strategy to control vigor and initiate early fruiting. Though we will not re-treat trees from Experiments 5 and 6, we will record return bloom, yield, and fruit size in 2013.

Objective 2:

We did not opt to purchase the commercial root pruner manufactured by Phil Brown Welding as we initially proposed, since we were able to collaborate with a pear producer, and fabricator in Hood River (special thanks to Herbie Annala for manufacturing and supplying the root pruner used in 2012 trials). We evaluated root pruning on a 6th leaf Anjou/OH×F 87 trellised planting in which trees had filled their allotted space. Entire rows were root-pruned to a depth of 1.5 feet and a distance of ~ 1.5 feet from the trunks on one or both sides of the tree row. Shoot length of one-sided and two-sided root-pruned trees was reduced by 20 and 35 percent of control trees, respectively (Fig 4). Trunk growth, an indicator of total vegetative growth, was similarly reduced over the season; however, yield was reduced by 20 and 40 percent for one and two sided root-pruned treatments, respectively, relative to the control (Table 3). Fruit size was also reduced by one box size for the root pruned treatments (size 100) compared to controls (size 90). Given the characteristically low yield potential of Anjou in the formative years, a yield sacrifice in the year of treatment is tolerable if trees settle into a bearing mode. Bloom, fruit set and yield characteristics will be determined in 2013 to assess the utility of root pruning young, established pear orchards. A second site comprised 30-year-old, widely spaced, free-standing Anjou trees on seedling rootstock. Rows were root-pruned as previously described, but at a distance of ~2.5 feet from the trunks. Tree yield was significantly increased for the two-sided root pruning treatment only (Table 4).

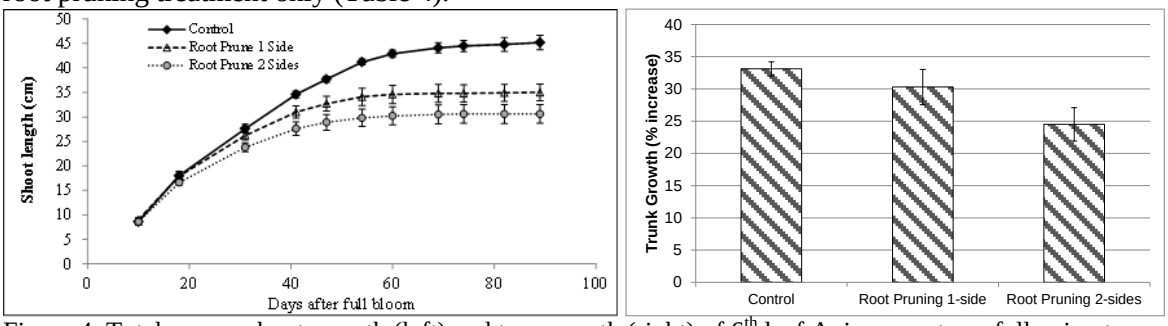


Figure 4. Total season shoot growth (left) and tree growth (right) of 6th leaf Anjou pear trees following two levels of root pruning (one side of the tree row, or both sides) compared to a control. Application timing was ~50% full bloom. A vertical root pruner was pulled at a depth and distance from trees of 1.5 ft.

Table 3. Fruit set, tree yield and average fruit size of 6th leaf Anjou following root pruning.

Treatment	Percent fruit set (no. fruit/100 clusters)	Yield (lb)	Fruit no. (per tree)	Avg. fruit wt. (g)	Avg. fruit sz. (# per 44 lb box)
Control	10.3 a	83.9 a	163 a	231 a	90
1-side Root Pruning	10.1 a	66.3 ab	144 ab	209 b	100
2-side Root Pruning	7 b	48.6 b	111 b	194 b	100

Table 4. Average tree yield and fruit size of 30-year-old Anjou trees following root pruning.

Treatment	Yield (lbs/tree)	Fruit weight (g)	Fruit size (#/44 lb box)
Control	273.4 b	199.4	100
1-side Root Pruning	245.2 b	205.8	100
2-side Root Pruning	306.1 a	190	100

Fruit size was not significantly reduced by root pruning. Vegetative growth was only slightly reduced. Greater tree and root reserves of older trees after root pruning were likely responsible for the different yield responses between the two orchards. Additional trial sites to evaluate young and old trees are scheduled for next season.

Objective 3:

We were able to establish a large experimental planting (~ one acre) of Bartlett and Anjou trees at MCAREC to evaluate bi-axe and single axe training systems at several in-row tree spacings. Both cultivars are on OH×F 87 rootstock. Bartlett spacings under evaluation are 2, 4 and 6 ft.; Anjous 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 good pollination. Each training system/spacing combination are planted in 10-tree replicates, and replicated five times across the block. Planting was delayed until the 3rd week of May, however, due to timing of spring fumigation. Trellis poles were installed prior to tree planting, and wire was installed afterward.

All trees were provided four nitrogen applications (granular urea) each 10 days apart, beginning the first week of July. 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. Trunks were measured 20 cm from the graft union at planting. 2012 trunk growth will be determined following a second set of trunk measurements made prior to 2013 bloom. In 2013, shoots will continue to be trained to wires with the aim of reaching the top wire of the trellis (8 ft.). Seasonal trunk growth will be determined in fall 2013.

In years 2 and 3, we will be unable to pursue the grafting of ‘d’Anjou’ to readily available, standard dwarfing quince rootstocks and OH×F in Italy. This is a function of Stefano accepting a new position at WSU requiring his relocation to Wenatchee, Washington. 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**: **1 of 3**

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

PI: Todd Einhorn
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Co-PI: Kate Evans
Organization: WSU-Wenatchee
Telephone: 509-663-8181 ext. 245
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Co-PI: Richard Bell
Organization: USDA-ARS
Telephone: 304-725-3451 ext. 353
Email: richard.bell@ars.usda.gov

Budget: **Year 1**: $37,492 **Year 2: $26,640** **Year 3**: $30,830

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			
Miscellaneous			
Total	**$ 0**	**$ 9340**	**$ 9543**

Footnotes: [1] supplies to produce *Erwinia amylovora* inoculum and maintain quince field plot

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

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

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.

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 2012:

1. **Hardwood Cuttings** (Table 2)
    - **a.** Rooting of hardwood cuttings made in January 2012 ranged from 0% to 62%.
    - **b.** Hormone significantly improved rooting.
    - **c.** Greenhouse grown quince trees resulting from hardwood cuttings were used as source plants for in vitro cultures.
    - **d.** Quince trees resulting from hardwood cutting trial were provided to Helios Nursery and were successfully established in layer beds.
    - **e.** A second hardwood cutting trial was initiated in November, 2012. Results will be collected in late winter/early spring 2013.

2. **Softwood Cuttings** (Table 2)
    - **a.** Rooting of quince softwood cuttings made in mid-June ranged from 0% to 46% after 6 weeks.
    - **b.** Hormone significantly improved rooting.
    - **c.** Our goal was to identify the most easily propagated clones, and results were therefore collected when fastest rooting samples had good root initiation. Many additional cuttings had callused and would have rooted with additional time.
    - **d.** Quince trees grown from softwood cuttings are ready to ship to Kearneysville for establishing field fire blight

3. **Stoolbed Establishment –** 29 quince clones were established in a stool bed at Helios Nursery. 15 of these are in the top cold-hardy group identified as potential candidates for grafted field trial (Table 1). Three additional quince clones and a *Pyronia* (pear x quince hybrid) are needed to complete the Helios stoolbed, and are being propagated at NCGR.

4. **In vitro multiplication** (Table 3)
    - a. Twenty eight quince clones and one Sorbus x Pyrus clone were established *in vitro*, and shoots were screened for bacterial and fungal contaminants. Clean cultures were grown on Pear 1 medium.
    - b. Multiplication rates ranged from 2x to 19x during the 4 month establishment and multiplication phase based on data taken at each transfer. However, most accessions were lost to a thrips outbreak before a properly replicated multiplication trial could be conducted.
    - c. Many of the clones grew well *in vitro* on improved Pear medium. Eight quince clones had multiplication rates >10 and twenty one had multiplication rates ≥ 6. Seven quince and one *Sorbopyrus* had low multiplication rates, which were possibly due to weak mother plants or later collection dates.

d. Shoots are presently being forced in the greenhouse to re-initiate cultures of the 18 quince and 1 *Pyronia* clone listed in Table 1, to be transferred to North American Plants for multiplication.

5. **Rapid production of interstem grafts –** Several dozen young quince trees from January hardwood cuttings were grown in the greenhouse and tip-grafted with actively growing shoots of the compatible cultivar Beurre Hardy in July. Poor survival of green interstem grafts (due in part to unusually hot weather and greenhouse conditions at the time) do not give us confidence that this method can be reliably used to generate interstem trees large enough to topwork at the end of the same growing season.

6. **Production of quince trees for fire blight field trial** – More than 10 self-rooted trees were produced for each of 15 clones, including nearly all of the top cold-hardy selections, to be shipped bare-root to R. Bell in March 2013 for establishing the fire blight field inoculation trial.

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 (OHxF 87, OHxF 97).

Our aim was to determine the ease of propagation of these individuals to several propagation methods. Propagation by softwood cuttings was more successful than hardwood cuttings for most selections (Table 2). Rooting hormone improved levels of rooting. Eight accessions had ≥ 19% rooting success from hardwood cuttings with hormone, and only two rooted at this rate with no hormone. Twelve accessions had > 25% rooting with hormone from softwood cuttings. Only one top selection did not root (Table 1). Softwood results were scored at 6 weeks to identify the most easily rooted clones. Although the proportion of cuttings that rooted was relatively low, many of the quince accessions were observed to root more efficiently than OHxF clones (Table 2).

In vitro initiation was successful on newly developed pear medium. Eight quince clones had multiplication rates >10 and twenty one had multiplication rates ≥ 6 during in vitro establishment (Table 3). A multiplication rate of 6 or higher is considered good. Experimental comparison of media, and rooting experiments were not accomplished due to loss of cultures to thrips infestation in October/November. We will, however, establish new cultures to be transferred directly to NA Plants for multiplication and liner production.

Testing of interstem grafts was not successful, due in part to unusually hot weather and greenhouse conditions at the time. The extra difficulties of this technique do not give us confidence that this method can be reliably used to generate interstem trees large enough to topwork at the end of the same growing season.

Twenty clones were selected to establish stool beds and to initiate *in vitro* cultures (Table 1) and fifteen clones were propagated for a replicated fire blight trial.

We estimate that we have lost one year of progress toward in-field rootstock trials due to contamination. However, our team has developed a strategy to re-focus our efforts. We have assigned the provision of in vitro cultures to our commercial collaborator (NA Plants) as our highest priority. We will no longer pursue our initially proposed in vitro rooting experiments. We remain on-schedule for fire blight testing. A list of modified objectives is provided below.

Initial Goals for Year 2 (2013 at Kearneysville, WV; Corvallis/McMinnville/Carlton, OR), with Revisions:

- **Establish fire blight susceptibility trial. (Kearneysville)**
- **in vitro multiplication of candidate quince clones. Produce adequate rootstock numbers for grafted field trials (North American Plants).**
- **Tip-graft rootstock liners with interstems (USDA- NCGR) –** modify objective: interstem trees to be produced by bench grafting, or shield budding at Helios Nursery.
- **Expand quince stoolbeds at Helios Nursery.**
- **Grow out rootstock liners with and without interstems, fall bud to commercial pear cultivars (Helios Nursery).** (modify schedule: quince liners will be planted in fall and bench grafted to interstem cultivar winter 2013-14 if large enough ; Anjou and Bartlett buds to be placed in fall 2014).

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. (modify schedule: initial graft compatibility evaluation will be following 2015 growing season)
- **Second year of fire blight testing at Kearneysville.**
- **Finished trees completed at** Helios Nursery. Delivery to Wenatchee and Hood River for field trials in spring of 2015. (modify schedule: finished trees to be available after 2015 season)
- **Prepare plots for replicated field trials -** modify schedule: finished trees to be available following 2015 growing season for spring 2016 planting in Hood River and Wenatchee.

METHODS to achieve Year 2 (2013) Goals

1. **Field test self-rooted quince clones for fire blight resistance.**
   a. Ten plants of each quince clone produced in Year 1 at NCGR will be field planted at Kearneysville, and grown under high fire blight pressure.
   b. Trees will be evaluated for disease severity and plant survival for two growing seasons.

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 each of 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 topworked to Beurre Hardy interstem prior to planting (fall 2013, or spring 2014). Anjou and Bartlett buds to be grafted following the 2014 growing season.
   b. Remaining in vitro generated liners from NA Plants will be planted at Helios Nursery and fall budded to ‘Anjou’ and ‘Bartlett’.
   c. OHxF 87 and OHxF 97 (no interstems) will also be budded to ‘Anjou’ and ‘Bartlett’ as controls.
   d. Virus tested sources will be used for all ‘Anjou’,’Bartlett’, and ‘Beurre Hardy’ budwood.

Table 1 - Quince clones ranked according to oxidative browning scores (mean mid-winter rating, 3 years). Lower number indicates less damage and greater cold tolerance. Percent rooting of cuttings based on means of 3 reps, 7-8 cuttings per rep (from tables 2 & 3). Multiplication rate in vitro after 4 months (i.e. 10 shoots initiated produce 60 = multiplication rate of 6; from table 4). Fifteen clones established in layer bed at Helios Nursery are marked.

			% rooting with hormone			
Hardiness Rank	Accession	Browning Score (1-6)	hardwood cutting 01/2012	softwood cuttings 06/2012	in vitro multiplic.	Helios Layer Bed
1	C. oblonga - Arakseni, Armenia	1.50	-	0.0	6.8	-
2	Aiva from Gebeseud	2.39	0.0	33.3	3.7	X
3	Akhtubinskaya O.P. seedling 4	2.42	0.0	33.3	6.9	X
4	Tashkent AR-232 seedling 4	2.75	4.8	4.2	7.0	X
5	Skorospelka O.P. seedling 1	2.86	-	12.5	19.0	X
6	Quince S	3.00	23.8	8.3	12.5	X
7	Quince W	3.00	42.9	29.2	8.6	X
8	C. oblonga - Megri, Armenia	3.03	4.8	12.5	5.1	X
9	C. oblonga - Seghani, Armenia	3.08	9.5	25.0	11.4	X
10	Tashkent AR-232 seedling 2	3.14	0.0	37.5	6.0	X
12	C. oblonga - Babaneuri, Georgia	3.61	14.3	8.3	10.5	X
13	Krukovskaya O.P. seedling 2	3.64	-	45.8	2.0	X
14	W-4	3.69	0.0	12.5	5.7	-
15	Trentholm	3.75	0.0	12.5	14.8	-
16	WF-17	3.75	28.6	25.0	4.6	X
17	Bereczki [Beretskiquitte]	3.78	-	29.2	6.0	X
18	Kashenko No. 8	3.81	-	12.5	15.1	X
19	Quince C7/1	3.86	57.1	8.3	8.7	X
20	Pyronia veitchii	3.89	-	-	-	-

Table 2 – Percent rooting of Quince cuttings, with and without hormone (mean of 3 reps, 7-8 cuttings/rep).

plantname	Hardwood Cuttings		plantname	Softwood Cuttings	
	with hormone	no hormone		with hormone	no hormone
Pigwa S-1 - Poland	61.9	42.9	Krukovskaya o.p. seedling	45.8	29.2
Quince C7/1	57.1	14.3	Teplovskaya O.P. seedling	45.8	33.3
Quince W	42.9	47.6	Tashkent AR-232 seedling 2	37.5	16.7
WF-17	28.6	9.5	Aiva from Gebeseud	33.3	16.7
Pillnitz 1	23.8	4.8	Akhtubinskaya O.P. seedling 4	33.3	12.5
Quince S	23.8	14.3	Pillnitz 1	33.3	12.5
Pillnitz 5	19.0	0.0	Pillnitz 5	33.3	20.8
Teplovskaya O.P. seedling	19.0	9.5	Bereczki	29.2	0.0
C. oblonga - Babaneuri, Georgia	14.3	0.0	Pillnitz 2	29.2	8.3
Pillnitz 2	14.3	4.8	Quince W	29.2	4.2
C. oblonga - Seghani, Armenia	9.5	4.8	C. oblonga - Seghani, Armenia	25.0	16.7
OHxF 97 *	7.1	0.0	WF-17	25.0	16.7
Akhtubinskaya O.P. seedling 2	4.8	0.0	Pigwa S-2 - Poland	16.7	4.2
C. oblonga - Megri, Armenia	4.8	0.0	C. oblonga - Megri, Armenia	12.5	8.3
Pigwa S-2 - Poland	4.8	0.0	Kashenko no. 8	12.5	8.3
Tashkent AR-232 seedling 4	4.8	0.0	Skorospelka o.p. seedling	12.5	12.5
Aiva from Gebeseud	0.0	0.0	Trentholm	12.5	0.0
Akhtubinskaya O.P. seedling 4	0.0	4.8	W-4	12.5	25.0
Fontenay	0.0	0.0	Akhtubinskaya O.P. seedling 2	8.3	0.0
Pigwa S-3 - Poland	0.0	0.0	C. oblonga - Babaneuri, Georgia	8.3	0.0
Provence (BA 29-C)	0.0	0.0	Provence (BA 29-C)	8.3	8.3
Sorbopyrus 'Smokvarka'	0.0	0.0	Quince C7/1	8.3	0.0
Tashkent AR-232 seedling 2	0.0	0.0	Quince S	8.3	8.3
Trentholm	0.0	0.0	OHxF 87 *	4.2	0.0
W-4	0.0	14.3	Pigwa S-1 - Poland	4.2	8.3
			Sorbopyrus 'Smokvarka'	4.2	0.0
			Tashkent AR-232 seedling 4	4.2	0.0
			C. oblonga - Arakseni, Armenia	0.0	0.0
			Fontenay	0.0	0.0
			OHxF 97 *	0.0	0.0
			Pigwa S-3 - Poland	0.0	0.0
			Pyronia veitchii	0.0	0.0
			Quince A	0.0	0.0

Table 3. Multiplication rates of cold hardy quince clones 4 months after initiation, on Pear medium.

local	name	multiplication
70.001	Skorospelka O.P. seedling	19.00
99.002	Kashenko no. 8	15.13
123.001	Trentholm	14.75
57.001	Quince S	12.50
61.001	Pigwa S-1	12.00
62.001	Pigwa S-2	12.00
118.001	C. oblonga - Seghani	11.42
128.001	C. oblonga - Babaneuri	10.50
71.001	Teplovskaya O.P. seedling	9.89
64.001	Quince A	9.57
60.001	Provence (BA 29-C)	9.50
65.001	Quince C7/1	8.67
29.001	Quince W	8.64
32.004	Tashkent AR-232 seedling 4	7.00
67.004	Akhtubinskaya O.P. seedling 4	6.88
120.001	C. oblonga - Arakseni	6.80
67.002	Akhtubinskaya O.P. seedling 2	6.56
63.001	Pigwa S-3	6.43
20.001	Pillnitz 5	6.36
32.002	Tashkent AR-232 seedling 2	6.00
75.001	Bereczki	6.00
22.001	W-4	5.67
126.001	C. oblonga - Megri	5.08
9.001	Pillnitz 1	4.75
23.001	WF-17	4.63
10.001	Pillnitz 2	4.50
104.001	Aiva from Gebeseud	3.67
68.002	Krukovskaya O.P. seedling 2	2.00
IGC 34.001	Sorbopyrus	2.00

CONTINUING PROJECT REPORT **YEAR**: 1 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
Wages			
Benefits			
Equipment			
Supplies			
Travel[2]	500	1,300	1,300
Miscellaneous			
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 Coffey
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
Equipment			
Supplies			
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'. OHxF 87 will be used as the standard.

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

Significant Findings 2012:

- Of the five original trial sites planted in 2009, the Bridgeport site (having both a Bosc and Anjou planting) was removed in 2012. Another site (Parkdale; Anjou) suffered significant damage due to fire blight infection. The other three sites are performing well.
- The mortality rate for all sites was 9%, but varied markedly among sites (e.g., range of 1% to 20%). Averaging across scion cultivars and sites, there were no significant difference among rootstocks in mortality rate; however, in Parkdale fire blight infection eliminated one complete replication of OH×F 87.
- 'Bartlett' at Wapato produced a good second crop on OH×F 87 and Horner 4 (i.e., ~ 50 lbs per tree). Considering the planting density (1,089 trees per acre), projected 4th leaf yields were 50 bins per acre. Horner 10 yields were significantly less than Horner 4 or OH×F 87. Fruit size was small (110 per box) for all rootstocks, but larger on Horner 4 (bordering 100s).
- At Wapato, yield efficiency of Bartlett on OH×F 87 was higher than on Horner 4, as a function of slightly higher yields on smaller trees. Bartlett/Horner 10 had the lowest yield efficiency.
- 'Bartlett' yields at Methow were much lower than Wapato. Rootstocks did not influence any of the performance attributes measured, with the exception of fruit size which was larger on Horner 4.
- For 'GR Bosc', yields were slightly higher on OH×F 87 (projected yield of ~28 bins per acre) compared to Horner 4 or 10, but not significant at the 95% probability level. Fruit size was good (80s and 90s) on all rootstocks, but slightly larger for OH×F 87.
- Fruit of OH×F 87 had significantly higher soluble solids at harvest than fruit from either of the two Bartlett/Horner combinations.
- There were no significant differences among rootstocks for tree size for Bosc or Bartlett.
- For 'd'Anjou' strong bloom was observed in 4th leaf trees, irrespective of rootstock; however, fruit set and final yield was characteristically poor. Precocity, in terms of fruiting, was not induced by either of the Horner selections at the Hood River site. Parkdale experienced several frost events during bloom that eliminated most of the crop.
- 'D'Anjou' tree size at both sites was significantly larger on Horner 4. OH×F 87 and Horner 10 produced trees of similar size and ~ 40 % smaller than trees on Horner 4.

Results and Discussion:

1. Sites.
The site at Bridgeport (possessing both Anjou and Bosc) was removed due to a change in ownership. The site was initially characterized as a low vigor site due to poor soil fertility and the presence of gravel bars throughout the profile, offering a good contrast to the two vigorous Anjou sites. Herbicide-induced phyto-toxicity, however, confounded our early results and limited the value of the data collected from this site. All other sites are intact. Wapato (Bartlett and Bosc), Methow (Bartlett), and Hood River (Anjou) had no additional tree mortalities in 2012; however, in Parkdale several low temperature frost events during bloom removed potential crop, and severe fire blight

infection resulted in significant tree injury and mortality (Table 1). One complete 5-tree replication of OH×F 87 was removed due to fired blight infection.

Table 1. Cumulative mortality rates of rootstock selections and sites since the projects inception in 2009.

Rootstock	Total trees Planted	Individual Tree Losses 2009-2011	Individual Tree Losses 2012	Mortality rate %
Horner 4	185	18	0	10
Horner 10	185	17	4	11
OH x F 87	185	14	9	12
Site				
Hood River	90	10	0	11
Parkdale	90	5	13	20
Bridgeport	150	18	0	12
Wapato	150	2	0	1
Methow	75	1	0	1

Details pertaining to the remaining 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 trees on Horner 4 were markedly larger than trees on either Horner 10 or OH×F 87 at both of the remaining Anjou sites (Tables 2 & 3). Significant bloom did not translate to high fruit set or yield on any of the rootstocks; a characteristic of Anjou, and one that needs to be altered if high-density plantings are going to be successful with this cultivar. Yield efficiencies of Bosc and Bartlett were ~5 to 10 fold greater than those of Anjou. It is notable that the high vigor associated with Anjou was augmented by Horner 4; a scenario that has not been observed for either Bosc or Bartlett in these trials and may preclude future adoption of Anjou/Horner 4. Parkdale yields were markedly reduced by frost events during bloom, and later by removal of fire blighted wood (via pruning).

Table 2. 2012 Hood River Anjou flowering (total clusters per tree), fruit set, average tree yield, average fruit weight, trunk size (TCA), and Yield Efficiency (YE) as affected by rootstock.

Rootstock	Total Flowers	Fruit set	Yield	Avg Fruit wt.	TCA	YE
	no. clusters	(%)	(lb per tree)	(g)	(cm^2)	(kg/cm^2 TCA)
Horner 10	198	6	6.9	242	31.2 b	0.1
Horner 4	229	5	6.4	225	45.9 a	0.06
OHxF 87	259	4	6.1	244	30.6 b	0.08
Statistical Significance	n.s.	n.s.	n.s.	n.s.	*	n.s.

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

Table 3. 2012 Parkdale Anjou flowering (total clusters per tree), fruit set, average tree yield, average fruit weight, and Yield Efficiency (YE) as affected by rootstock.

Rootstock	Total Flowers	Fruit set	Yield	Avg Fruit wt.	TCA	YE
	no. clusters	(%)	(lb per tree)	(g)	(cm^2)	(kg/cm^2 TCA)
Horner 10	149	14 a	8 ab	174	28.7 b	0.11 ab
Horner 4	152	7 b	4.1 b	192	40 a	0.06 b
OHxF 87	166	20 a	13.3 a	177	30.4 b	0.21 a
Statistical Significance	n.s.	*	*	n.s.	*	*

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

'Golden Russet Bosc'.
In general there were no significant rootstock effects on any of the performance attributes evaluated; however, OH×F 87 appeared to have a slight advantage in flowering, yield, fruit size, and yield efficiency. Interestingly, for 'GR Bosc', Horner 4 did not impart significantly greater tree vigor (Table 4), compared to the other rootstocks. Projected 'GR Bosc' yields on OH×F 87 were 28 bins per acre at the planting density of the orchard (1,089 trees per acre). Fruit size was excellent for all rootstocks; though, slightly larger fruit were harvested from OH×F 87 (nearly significant at the $P<0.05$ level).

Fruit quality at harvest was slightly improved for OH×F 87, with fruit possessing significantly higher soluble solids (Table 5). Total acids and fruit firmness (Table 5), and percent defects (data not shown) were not significantly affected by rootstock.

Table 4. 2012 Wapato-site 'GR Bosc' flowering (total clusters per tree), fruit set (per 100 clusters), trunk cross-sectional area (TCA), fruit weight (g), average tree yield (lbs per tree) and yield efficiency (kg per cm^2 of TCA) as affected by rootstock.

Wapato Bosc	Total flowers (no. of clusters)	Fruit set (fruit no./100 clusters)	TCA (cm^2)	Fruit wt. (g)	Yield (lb)	YE (kg cm^{-2})
OH×F 87	125.8	46.3	23.5	248	27.8	0.56
Horner 4	93.0	56.3	22.6	217	23.4	0.47
Horner 10	101.5	39.7	19.8	229	19	0.45
Statistical Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

Table 5. 2012 Wapato-site 'GR Bosc' soluble solids content (SSC), total acids (TA) and flesh firmness (FF) at harvest as affected by rootstock.

Rootstock	SSC (%)	TA (%)	FF (lbsf)
OHxF87	13.1 a	0.22	15.7
Horner 4	12 b	0.21	16.4
Horner 10	12.5 b	0.2	16.3
Statistical Significance	**	n.s.	n.s.

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

'Bartlett'.

High Bartlett yields were observed for OH×F 87 and Horner 4 trees in Wapato (i.e., ~ 50 lbs per tree). This was the second crop following minimal yields in 2011 (~20 lbs per tree). Considering the planting density (1,089 trees per acre), projected 4th leaf yields were 50 bins per acre. Subsequently, Bartlett/OH×F 87 had extremely high yield efficiency (1.5 kg yield per cm^2 of TCA). In fact, yield efficiency was double the highest cumulative yield efficiency reported after the first 5 years of a Bartlett rootstock trial that evaluated six rootstocks on three separate sites (Elkins et al., 2011). High Bartlett yields in Wapato were associated with good fertigation and irrigaton practices in the formative years. Trees reached the top trellis wire in the second year, showing good, early canopy development (i.e., sites for future fruiting). Improved precocity was not observed for Horner 10, despite their slightly smaller tree size compared to Horner 4. In fact, yields of Horner 10 were significantly less than either Horner 4 or OH×F 87 (Table 6).

Table 6. 2012 Wapato-site 'Bartlett' trunk cross-sectional area (TCA), fruit weight (g), average tree yield (lbs per tree) and yield efficiency (kg per cm^2 of TCA) as affected by rootstock.

Wapato Bartlett	TCA (cm^2)	Fruit wt. (g)	Yield (lb)	YE (kg cm^{-2})
OH×F 87	17.95	174.6	56.9 a	1.49 a
Horner 4	19.7	191	50.3 a	1.18 b
Horner 10	17.4	182.8	29.3 b	0.74 c
Statistical Significance	n.s.	n.s.	**	***

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

Fruit size was small (110 per box) for all rootstocks (Table 6). Fruit were slightly larger, however, on Horner 4 (bordering on 100s), albeit not significantly at $P<0.05$ (the P value for fruit size was 0.07). Fruit quality at harvest was also higher for OH×F 87; soluble solids and total acids were significantly higher than fruit from Horner 10 (Table 7).

Table 7. 2012 Wapato-site 'Bartlett' soluble solids content (SSC), total acids (TA) and flesh firmness (FF) at harvest as affected by rootstock.

Rootstock	SSC (%)	TA (%)	FF (lbsf)
OHxF87	11.4 a	0.41 a	18.7 ab
Horner 4	11 ab	0.43 a	18.5 b
Horner 10	10.7 b	0.38 b	19.3 a
Statistical Significance	*	*	*

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

Methow 'Bartlett' yields were much lower than those observed at Wapato (Tables 7 & 8). Rootstocks did not influence any of the performance criteria evaluated, with the exception of fruit size which was larger on Horner 4 (Table 8). As observed for 'GR Bosc', 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 8. 2012 Methow-site 'Bartlett' flowering (total clusters per tree), fruit set (per 100 clusters), trunk cross-sectional area (TCA), fruit weight (g), average tree yield (lbs per tree) and yield efficiency (kg per cm^2 of TCA) as affected by rootstock.

Methow Bartlett	Total flowers (no. of clusters)	Fruit set (fruit no./100 clusters)	TCA (cm^2)	Fruit wt. (g)	Yield (lb)	YE (kg cm^{-2})
OH×F 87	120.9	40	18.5	196.2 b	19.8	0.49
Horner 4	119.2	30.7	17.3	227.8 a	17.6	0.47
Horner 10	135.9	30	15.5	190.2 b	19.6	0.49
Statistical Significance	n.s.	n.s.	n.s.	**	n.s.	n.s.

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

Slight differences in Bartlett fruit quality were observed at harvest. It is not clear why soluble solids were lower in fruit on OH×F 87 (Table 9), though based on the firmness at harvest, OH×F 87 fruit appeared to be slightly less mature when harvested (Table 9).

Table 9. 2012 Methow-site 'Bartlett' soluble solids content (SSC), total acids (TA) and flesh firmness (FF) at harvest as affected by rootstock.

Rootstock	SSC (%)	TA (%)	FF (lbsf)
OHxF87	10.9 b	0.52	20.1 a
Horner 4	11.7 a	0.49	19.1 b
Horner 10	11.8 a	0.53	19 b
Statistical Significance	*	n.s.	*

Significance notation: n.s., not significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<.001$. Means followed by different letters within columns are significantly different.

Plan for 2013.

We propose to continue evaluations of rootstock performance in all remaining grower-cooperator orchards. Bloom and fruit set measurements will be made in Anjous and Bosc only. Fruit size, yield, yield efficiency, and tree size will be evaluated at all sites.