Northwest Pear Research Review

Columbia Gorge Hotel, Hood River, OR

Wednesday, 2/18/2015

Time	Page	PI	Title	Yrs
			Final Project Reports	
8:50		Schmidt	Introduction, housekeeping & WTFRC Internal Research	
9:00	1	Dhingra	Physiological genomics of 1-MCP use in pear	12-14
9:15	11	Dhingra	Enhancing shelf life and quality of 1-MCP treated sliced pears	14
9:30	18	Wang	Deliver 1-MCP treated d'Anjou pears w/predictable ripening capacity	12-14
9:45	29	Einhorn	Improving fruit set, production efficiency, and profitability of pears	12-14
10:00	41	Einhorn	ABA chemical fruit thinning of Bartlett pears	13-14
10:15			Break	
10:30	51	Evans	Pear scion trials in the Pacific Northwest & Genotype work for pear	12-14
10:45	61	Einhorn	Horner rootstock grower trials	12-14
11:00	68	Einhorn	Cold hardy quince: propagation, rapid multiplication and field trials	12-14
11:15	79	Postman	Improve electronic data collection/public access to USDA pear genebank	14
11:30		Hanrahan	Technology Committee: *See Reports in Appendix*	
1:30 - 3:00			Continuing Projects	
1	83	Horton	Tests of a sprayable pheromone formulation against winterform psylla	14-15
1	88	Cooper	Suppression of pear psylla using elicitors of host-defenses	14-16
1	95	Unruh	Pesticide resistance in pear psylla *No cost extension*	14
1	101	Beers	Miticide resistance in spider mite pests of pears *No cost extension*	13-14
2	109	Musacchi	Fall and summer pruning to control vigor and psylla in d'Anjou pear	14-16
2	116	Musacchi	Improving quality and maturity consistency of 'd'Anjou'	14-16
2	123	Dhingra	Establishing NW-acclimated Pyrus rootstock breeding material	14-16
3	127	Johnson	Optimizing use of Actigard for post-infection fire blight control	14-15
3	135	Neale	Development of marker-based breeding technologies *co-funded CPAB**	14-15
3	139	Wang	Controlling postharvest disorders of pears during storage and export	13-15
3	146	Moffitt	Health role of pear for Metabolic Syndrome	14-15

FINAL PROJECT REPORT

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

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

Total Project Funding: $99,180 (3 years)

Budget History:

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.

This project addresses the US Pear Industry's topmost priority of ensuring uniform fruit quality to enhance per capita consumption.

OBJECTIVES

The aim of this project was to gain an understanding of pear genetic responses to 1-MCP treatment, and test chemical approaches to obtain optimal fruit quality in response to 1-MCP treatment through the following objectives.

1. (Years 1 and 2) Test the activity of 35 genes seen to be activated in relation to the proposed cold-induced ripening master-switch gene in D'Anjou and Bartlett fruit.

As part of objective 1, we studied how the ripening-related genes behave in relation to each other and the proposed cold-induced ripening master-switch gene before, during and after 1-MCP treatment, under 3 storage temperatures. Further, we compared the activity of the selected subset of genes between two different varieties to correlate them with the fruit ripening phenotype. From this work, we identified potential genes and associated metabolic pathways which become non-functional in response to 1-MCP treatment making the fruit unsuitable for ripening.

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.

Based on the identification of potential pathways from the results obtained as part of Objective 1, we identified several candidate ripening compounds that could potentially reactivate the process of ripening in 1-MCP treated D'Anjou and Bartlett fruit. The analyses for reactivation of ripening with the chemical compounds involved monitoring of fruit quality, ethylene and respiration rates. With the chemical approach we have demonstrated reactivation of ripening in 1-MCP treated fruit. Comprehensive analysis of gene activity, fruit quality, and ethylene release and respiration rates in Bartlett and D'Anjou varieties revealed the differences in response to 1-MCP treatment.

All the experiments were performed with commercial grade fruit obtained in 2012 and 2013 from project cooperator Blue Star Growers (Bob Gix). D'Anjou and Bartlett fruit were harvested at commercial maturity and treated with 1-MCP at the current industry recommended rates of 100 and 300 ppb respectively.

SIGNIFICANT FINDINGS

- Based on the analysis of the activity of several genes related to metabolic pathways that facilitate ripening, specific pathways were identified that are blocked in unconditioned Bartlett and D'Anjou fruit treated with 1-MCP.

- A total of nine potential ripening compounds have been identified. Some of these 'ripening compounds' or RCs that were tested in this project have been shown to reverse the effect of 1-MCP in Bartlett and D'Anjou.

- The optimal concentration of RCs tested, duration of treatment and type of response elicited from the fruit for each RC were established.

- In experiments with three RCs, reactivation of ripening as measured by release of ethylene or carbon dioxide in 1-MCP treated Bartlett and D'Anjou fruit met or exceeded the proposed increase of 20% compared to the control.

- Interestingly, it was observed that RCs not only reversed the impact of 1-MCP, they also induced ripening in non 1-MCP treated control fruit that had not met the chilling requirement. Ripening response was consistently obtained in both varieties tested in a dose-dependent manner, allowing reliable timed induction of ripening.

- While all the experiments done in this project were performed by soaking the fruit in RC solutions for 24 hours, a few recent experiments were successfully performed by vaporizing one of the RCs. This is expected to open up avenues for application of RCs on fruit in bins via fogging.

RESULTS & DISCUSSION

Pre-harvest and post-harvest ethylene perception or production blocking chemicals are desirable products to regulate fruit production and postharvest storage. By inhibiting ethylene sensitivity, ethylene-responsive processes are slowed down in treated fruit. The responses include ethylene biosynthesis, fruit quality development and respiration. A complete fundamental understanding of enhanced sensitivity to 1-MCP treatment and variable recovery is yet to be developed in pears, although physiological approaches for postharvest management for non-1MCP treated fruit have been developed (Chiriboga et al., 2011; Chiriboga et al. 2014). Traditionally, respiration rates are considered to be tightly coupled to ethylene-pathway activity in climacteric fruits. Results from work performed in this project have identified additional factors that exert influence on respiration despite 1-MCP induced reduction in ethylene-sensitivity. Recently, similar results have been reported in 1-MCP treated tomato (Xu et al., 2012), suggesting that genetic components of respiration-related pathways may not be completely coupled to ethylene pathway activity in climacteric fruit. Inversely, chemical inhibition of this pathway has been shown to suppress the respiratory climacteric and alternative oxidase (AOX) pathway activity in tomato (Xu et al., 2011).

Respiration rate of the fruit closely correlates to the development of desirable fruit quality and thus marketability. In addition to 1-MCP products, controlled atmosphere storage is also used to slow down the respiration rates and delay ripening. However, total respiratory activity is a cumulative result of multiple inputs where metabolites and energy are utilized in different ways. Cytochrome (CYT) and alternative oxidase (AOX) pathways are two different avenues by which energy balance in achieved. Besides some other genes, an increased abundance of alternative oxidase genes was observed in Bartlett pear in response to conditioning treatments (Figure 1). In Bartlett fruit, this correlated to an approximate 4-fold increase in response to cold-exposure relative to unconditioned controls. A similar increase was not observed in D'Anjou samples, suggesting either a variably expressed cold-responsive pathway that may influence pears ripening profile or another AOX allele may be active that was not sampled. Generally, AOX activity serves to maintain oxidative and biochemical conditions needed to drive energy production in plant cells. Alternative oxidase activity is associated with ripening in fruits (Perotti et al., 2014; Huang et al., 2013), and various stresses in plant tissues (Vanlerberghe, 2013). This was one of the several genes that demonstrated differential expression in conditioned vs. non-conditioned controls.

A set of chemical compounds was identified which were selected as potential candidates that could activate blocked pathways. The impact of increased respiratory activity from these compounds was thought to increase ethylene production. Stimulating respiration in 1-MCP treated pear can enable the

reactivation of ripening at will. In this role, RCs could serve as a companion product to existing postharvest strategies utilizing 1-MCP products.

Sample fruit were obtained from Blue Star Growers (Cashmere, WA) from multiple grower lots harvested at commercial maturity. After initial room-temperature aqueous drenching of fresh whole 1-MCP treated Bartlett and D'Anjou fruit (at 300 and 100 ppb, respectively) for 12-24 hours (2012 and 2013 season fruit) significant stimulation of respiration, ethylene production and flesh softening was observed. Between 2012 and 2013 growing seasons, 20 experiments were performed using 9 RCs. Experiments with some RCs were not pursued due to high costs and safety concerns. Results indicate significant stimulation of respiration, ethylene production and fruits' softening in 6 experiments using 4 compounds (Table 1). Respiration was stimulated to or beyond the 20% threshold established for an effective RC (Figure 2). In summary, significant stimulation of respiration and ethylene production was observed in 12 experiments using 8 RCs in 1-MCP treated fruit. Fruit softening was accelerated in 10 experiments using 6 RCs. Additional RCs stimulating all parameters measured produced significant tissue damage to sample fruit.

Notably, not all RCs tested produced significant stimulation of these parameters together. For example, RC1 elicited stimulation of respiration and ethylene production in 2013 season 1-MCP treated D'Anjou, but failed to accelerate fruit softening. Similar results were observed in the 2013 trial of RC2 on 1-MCP treated Bartlett. While fruit softening was accelerated in these samples, analysis of available data indicated a lack of stimulation of respiration and ethylene production. However, data logging system failures likely impacted these results. Despite equipment failures, observed stimulation of 1 or more (but not all concurrently) parameters measured in this study supports that idea that respiration, ethylene production, softening and ripening-associated processes are influenced by more than just ethylene. Further, these results support findings from prior studies in other crop systems (Xiao et al., 2010; Pastore et al., 2001), suggesting observed responses from this work may not be specific to 1-MCP treated pear. Overall RC1, RC2 and RC3 significantly accelerated respiration, ethylene production and softening in 1-MCP treated Bartlett and/or D'Anjou sample fruit at concentrations less than 1.0 mM without undesirable tissue damage or loss of soluble solid content. Among all experiments conducted in this study, soluble solid content was significantly increased in 2012 Bartlett (1-MCP treated and untreated) only in response to RC2. The unaffected soluble solid content may indicate that accelerated respiration in response to RC treatments may result in an increased sugar and organic acid consumption to drive energy production, and other ripening-related processes. Together, these findings demonstrate that at effective concentrations, RCs can accelerate ripening in 1-MCP treated pears without sacrificing quality or marketability of the fruit.

As an indicator of ripening processes in pear, ethylene biosynthesis was measured in 1-MCP treated and untreated control pears after exposure to RCs. Extensive physiological studies have demonstrated the putative need for ethylene pathway activity for induction of many ripening-associated processes and fruit quality development (Villalobos-Acuna and Mitcham, 2008). European and Chinese white pears are unique among climacteric fruits, requiring variable conditioning treatments to gain ripening capacity and marketability. Without this conditioning, ethylene production is negligible in pears.

Current experimental equipment and infrastructure limited the number of samples that could be tested concurrently. Thus, sample fruit used in each experiment experienced variable amounts of storage at 2°C storage prior to RC exposure and subsequent monitoring (Table 2). Despite this, results of RC2 and RC3 experiments were obtained using Bartlett and D'Anjou fruit held in storage for a maximum of 1 month. Without 1-MCP treatment, conditioning treatments at this storage temperature span 4-8 weeks (in D'Anjou). These results indicate that RC responses observed in these experiments may also mitigate the cold conditioning requirements. By accelerating ripening-related processes in preclimacteric pears which have not received sufficient conditioning, RC responses may be further

leveraged to better control onset of ripening. Further controlled studies are needed to assess the impact of these RCs in unconditioned fruit. Observed stimulation of respiration, ethylene production and softening in both 1-MCP treated and partially conditioned pear supports the model of RC responses being independent of ethylene pathway activity. Together, these responses can allow for expansion of fresh pear market in which maintenance of optimal fruit quality can be managed over greater distances and timeframes from distribution hubs.

While effective, 24 hour aqueous drenching applications of RCs present challenges for commercial application. To identify minimum exposure periods required to induce significant responses, we tested alternative delivery methods and reduced exposure times. An experiment was conducted with 1-MCP treated D'Anjou pears from the 2012 growing season using two effective RCs identified from prior studies. Instead of an aqueous 24 hour exposure to RC2 (Table 1), sample fruit were exposed for 12 hours using identical experimental concentrations. In both the 24 and 12 hour exposure experiments, sample fruit were held in storage for up to 4 weeks at 2°C prior to RC2 exposure. Results in D'Anjou from 12 hour exposure to RC2 indicated significant stimulation of fruit respiration and softening, but not ethylene production. In Bartlett samples exposed for 12 hours neither respiration and ethylene production nor fruit softening was stimulated. While further experimentation is needed to clarify the role of fruit maturity at harvest, annual fluctuations, and length in storage prior to RC2 exposure, shorter duration of exposure was not reliable. As a result of these findings and prior observations of peel tissue damage upon exposure to higher RC2 concentrations (Figure 3), further experiments with shorter exposure times were not conducted. Interestingly, we performed one experiment recently where RC2 can be vaporized and applied to the fruit directly opening up the possibility of applying the product via fogging and estimating the duration of exposure.

To determine if ripening-responses could be further enhanced, additional combination RC experiments were conducted. RC3 was included in combination experiments as it known to induce oxidative stress in exposed tissues. RC4 was included in combination treatments as it is a known precursor to auxin production, a known physiological cue to ripening competency in climacteric fruits. Two combination experiments were conducted using RC2 and RC4. Interestingly inclusion of higher RC4 concentrations with RC2 failed to elicit accelerated ethylene production in 1-MCP Bartlett samples. Similarly, softening was not stimulated in Bartlett samples exposed to lower RC4 concentrations. These results failed to demonstrate a significant gain over RC2 treatments, indicating RC4 may not offer desirable ripening responses in 1-MCP treated pears. RC3 produced unacceptable peel tissue damage and necrosis in all experimental levels.

An additional experiment (Table 1) was conducted using a reduced exposure time to gaseous RC1. An initial experiment using 96 hour exposure of 10 ppm RC1 (mixed in compressed atmospheric air) indicated significant stimulation of 1-MCP treated Bartlett respiration and ethylene production. Only fruit respiration was stimulated with fruit failing to soften much at all over the course of the experiment, relative to 1-MCP untreated controls.

To apply RCs commercially, gaseous or misting applications need to be explored. Development of fogging applications with the use of adjuvants or penetrants (including dilute ethanol) is expected to enhance penetration of active compounds into fruits' interior, increasing efficacy. While the objectives of this project have been met, further work is needed to optimize RC delivery protocols. To best address these needs, reliable, accurate high-throughput experimental capacity is needed to best ensure RC experiments are performed on sample fruit from equal physiological states. Results from this work offer an expanded understanding of regulation of ripening and 1-MCP responses in pears. To disseminate this, a manuscript describing this work and its impacts has been submitted to Nature Horticulture and an article in Good Fruit Grower is forthcoming.

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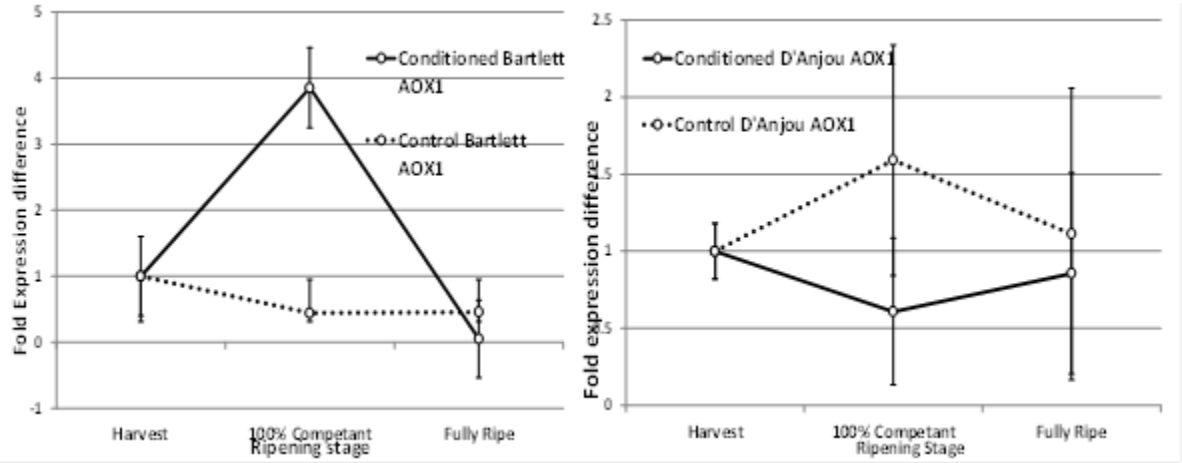

Figure 1. Relative gene abundance of alternative oxidase genes in Bartlett (left) peel and D'Anjou (right) peel tissues at harvest, fully conditioned, and fully ripened stages of postharvest management. Error bars represent standard deviation from the mean among 3 biological replicates. Note the nearly 4-fold increase in expression of AOX1 in Bartlett.

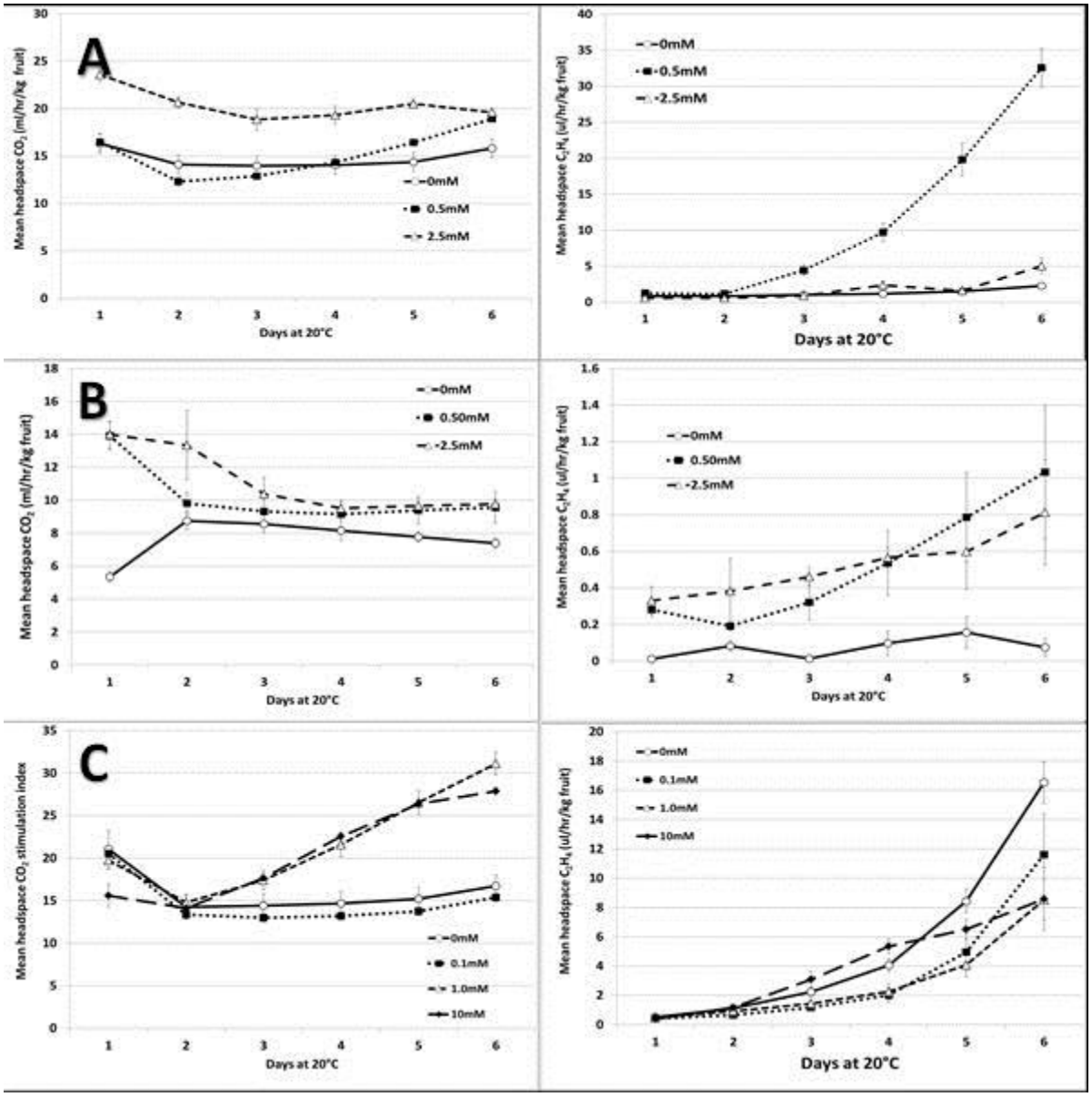

Figure 2. Mean respiration (left), and ethylene production (right) from sample pears in response to RC-1(A-Bartlett), RC-2 (B-D'Anjou) and RC-3 (C-Bartlett) in 1-MCP treated pears. Error bars represent standard error from the mean among 4 biological replicates.

Figure 3. Peel tissue damage and necrosis in 1-MCP treated Bartlett pears from highest level of RC2. Image of RC2 treated fruit was taken 6 days after 24 hour treatment.

Table 1. Significance of primary treatment factors (level of ripening compound, 1-MCP treatment and phenology) shown from 2-factor ANOVA and 3-factor ANOVA analysis in CO_2, ethylene evolution and fruit firmness (respectively). *- significant at α=0.05. **- significant at α=0.01. NS- no significance. !- indicates data was log transformed to meet normality assumptions of the ANOVA test.

Ripening compound	*Year*	*Cultivar*	*Hours exposure*	*Respiration*	*Ethylene production*	*Firmness*
RC1	2012	Bartlett	24	**	*!	**
RC1	2012	Bartlett	24†	*	NS	NS
RC1	2012	Bartlett	96†	**	**	NS
RC1	2012	D'Anjou	24	**	NS	**
RC3	2013	Bartlett	24	*	**	**
RC2	2012	Bartlett	24	**!	**	**
RC2	2012	D'Anjou	24	**	NS!!	NS
RC2	2013	Bartlett	12	NS	NS	NS
RC2	2013	Bartlett	24	NS!!	NS!!	**
RC2	2013	D'Anjou	12	**	NS	**
RC2	2013	D'Anjou	24	**	**	NS
RC2+RC4	2013	Bartlett	24	**	**	NS
RC2+ RC4	2013	Bartlett	24‡	*	NS	**
RC2+RC3	2013	D'Anjou	24	**	**	**
RC3	2013	Bartlett	24	**	**	**
RC3	2013	D'Anjou	24	**	**	NS
RC5	2013	Bartlett	24	NS	NS	NS
RC6	2013	D'Anjou	24	**	**	NS
RC7	2013	D'Anjou	24	*!	**!	NS
RC3	2013	Bartlett	24	**	*!	**

Table 2. Bartlett and D'Anjou mean maturity and soluble solid content at harvest, harvest dates, sample collection dates and experimental start dates from the 2012 and 2013 growing season. Upon harvest, fruit were placed in short-term storage at 5°C until receipt. Upon receipt, samples were placed into 2°C until experiment start date.

RC	*1-MCP treatment*	*Grower numbers*	*Pear cultivar*	*Harvest date*	*Maturity at harvest*	*Soluble solids at harvest*	*Sample collection date*	*Experiment start date*
RC1	+	1172	Bartlett	9/07/2012	16.96	12.8	9/13/2012	11/07/2012
RC1	-	740	Bartlett	9/02/2012	17.51	12.4	9/13/2012	11/07/2012
RC1	+	1172	Bartlett	9/07/2012	16.96	12.8	9/13/2012	12/20/2012
RC1	-	740	Bartlett	9/02/2012	17.51	12.4	9/13/2012	12/20/2012
RC1	+	1172	Bartlett	9/07/2012	16.96	12.8	9/13/2012	12/20/2012

RC1	-	740	Bartlett	9/02/2012	17.51	12.4	9/13/2012	12/20/2012
RC1	+	581	D'Anjou	9/23/2012	15.28	13.1	9/28/2012	10/16/2012
RC1	-	886	D'Anjou	9/19/2012	15.64	12.6	9/28/2012	10/16/2012
RC2	-	451	Bartlett	8/17/2013	17.94	11.9	9/11/2013	9/20/2013
RC2	+	581	Bartlett	9/23/2012	15.28	13.1	9/28/2012	10/31/2012
RC2	-	886	Bartlett	9/19/2012	15.64	12.6	9/28/2012	10/31/2012
RC2	+	581	D'Anjou	9/23/2012	15.28	13.1	9/28/2012	10/16/2012
RC2	-	886	D'Anjou	9/19/2012	15.64	12.6	9/28/2012	10/16/2012
RC2	+	1001, 591	Bartlett	8/17/2013	18.12	12.6	9/11/2013	10/20/2013
RC2	-	451	Bartlett	8/17/2013	17.94	11.9	9/11/2013	10/20/2013
RC2	+	23, 172	D'Anjou	9/14/2013	14.07	13.1	10/03/2013	10/31/2013
RC2	-	2128, 192	D'Anjou	9/10/2013	13.18	13.1	10/03/2013	10/31/2013
RC2	+	23, 172	D'Anjou	9/14/2013	14.07	13.1	10/03/2013	10/07/2013
RC2	-	2128, 192	D'Anjou	9/10/2013	13.18	13.1	10/03/2013	10/07/2013
RC2	+	1001, 591	Bartlett	8/17/2013	18.12	12.6	9/11/2013	11/07/2013
RC2	-	451	Bartlett	8/17/2013	17.94	11.9	9/11/2013	11/07/2013
RC3	+	1001, 591	Bartlett	8/17/2013	18.12	12.6	9/11/2013	9/13/2013
RC3	-	451	Bartlett	8/17/2013	17.94	11.9	9/11/2013	9/13/2013

EXECUTIVE SUMMARY

Variable ripening after 1-MCP application along with the conditioning requirements have hindered the adoption of 1-MCP use in pears. Therefore, strategies to reverse 1-MCP responses may allow for predictable ripening and control of fruit quality.

Analysis of 35 important genes related to 8 different ripening related pathways resulted in the identification of specific pathways that were blocked in unconditioned Bartlett and D'Anjou fruit treated with 1-MCP. A total of nine potential ripening compounds were identified and 3 have been shown to have the potential to reactivate the blocked pathways. It was interesting to find out that RCs not only reversed the impact of 1-MCP, they also induced ripening in non 1-MCP treated control fruit that had not met the chilling requirement.

One of the limitations of the experiment was that the RCs were applied by soaking the fruit in RC solutions for 24 hours. However, we have made a headway to apply the RCs via fogging. In one of the recent experiments RC2 was applied by vaporization. This is expected to open up avenues for application of RCs on fruit in bins via fogging.

This project demonstrated that the development of short term solutions for tree fruit improvement is feasible when a problem is addressed by using physiology-guided gene activity studies. We demonstrated that Bartlett and D'Anjou pear respiration can be stimulated by ripening compounds to reverse the impact of 1-MCP.

In summary, this project have identified a novel means of stimulating ripening processes in 1-MCP treated Bartlett and D'Anjou whole fruit. We are proceeding to continue the work with RC2 since it can be applied via vaporization and is stable at room temperature. RC2 is currently approved for use in the food chain through the FDA. RC2 exposure can be used to antagonize 1-MCP responses in fresh pear tissues and can be further developed as a companion product to 1-MCP in the postharvest management chain. These responses may be particularly effective in application to sliced pear products, where the increased flesh surface area can rapidly absorb the active compound. The impacts of this expanded toolset in postharvest management of fresh pear tissues will enable leveraging of the benefits of 1-MCP use, while mitigating its drawbacks of variable, unreliable recovery periods. Together this can allow for increased inventories, distribution and potential sales of pear products in expanding markets.

FINAL PROJECT REPORT

Project Title: Enhancing shelf life and quality of 1-MCP treated sliced pears

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Cooperators: Crunch Pak: Tony Freytag and Ozgur Koc; WSU: Christopher Hendrickson, Ph.D., Seanna Hewitt, Scott Mattinson and Frank Younce

Total Project Request: **Year 1**: $33,727

Other funding sources

Agency Name: Crunch Pak
Amt. awarded: $18,000
Notes: Based on estimates provided by Ozgur Koc at Crunch Pak, each experiment of sliced pear project costs them approximately $1500. The plan is to have 12 different samples/treatments resulting in a total in kind support of $18,000.

Agency Name: NIH Protein Biotech Training Program
Amt. awarded: $52,234
Notes: Support for Seanna Hewitt, Ph.D. student includes stipend, travel, medical, tuition and fees.

Total Project Funding: $33,727

Budget History:

Item	2014
Wages[a]	19,760
Benefits	7967
Supplies[b]	3500
Travel[c]	1500
Miscellaneous[d]	1000
Total	33,727

Footnotes:

a. Wages and benefits as partial support for a student to conduct the experiments.
b. Support for procuring chemical compounds (RCs) and experiment associated consumables
c. Support for travel to Crunch Pak facilities from WSU Pullman
d. Partial support for covering the cost of 1-MCP treated fruit

OBJECTIVES

This project addresses the following 2013-2014 priority: "Value added programs such as fresh sliced pears have great potential in getting more pears to our consumers with the convenience they demand" and near term priority: "MCP use and its associated effect on pear conditioning at the consumer level".

The objectives of the proposal were:

1. Evaluate the quality and shelf life of sliced pears derived from 1-MCP treated fruit with and without treatment with the ripening compounds and identify the optimal ripening compounds and corresponding concentrations.

One of the five ripening compounds, RC2, was found to be most effective. RC2 overcame the inhibition of ripening in1-MCP treated sliced pears. As part of the quality analysis, total soluble content, flesh firmness, physical appearance, ethylene and CO_2 release was measured over a period of 21 days.

2. Evaluate the optimized ripening compound concentrations in partnership with Crunch Pak.

This part of the work was performed by Crunch Pak. They added RC2 to their polymers directly and the fruit was packed and quality control was performed by their staff. A total of 4 trials have already been completed with several more trials planned in the next few months.

SIGNIFICANT FINDINGS

The goals of each objective were completed. While this was not a part of the original objectives, we conducted a non-trained consumer taste panel at the annual 2014 WSHA meeting with highly encouraging results.

- One of the five ripening compounds, RC2, was found to be most effective in inducing ripening in 1-MCP treated sliced pears as measured by release of ethylene and carbon dioxide. Visible compositional changes, including increased browning at high RC2 levels – an indicator of ripening, was observable.

- RC2 treatment of 1-MCP treated sliced pears resulted in immediate activation of aromatic pathways as evident from qualitative analysis. This could also have implications in sliced apple industry.

- Interestingly, prior treatment with 1-MCP ensured uniformity of the sliced product when RC2 was applied. When the non-1-MCP treated fruit was sliced and treated with RC2, the quality of the finished product was found to be highly variable.

- Very firm 1-MCP treated Bartlett fruit with an average firmness of 17 lb developed the characteristic pear aroma after RC2 treatment and softened to flavorful product within a few days while in the 2 oz. Crunch Pak bag.

- The shape of the D'Anjou pears is more amenable for slicing compared to Bartlett.

- Preliminary taste panel at the annual WSHA meeting in Kennewick indicated that RC2 treatment of even the unconditioned sliced D'Anjou pears enhanced the overall acceptance, flavor and texture of the product.

- A sliced pear product doesn't need to be soft and juicy as expected in the case of the whole fruit.

RESULTS & DISCUSSION

As the desire for convenience foods increases, so too does the demand for sliced fruit. The market for fresh, sliced pears is particularly promising; however development of suitable methods of shelf life extension of pear has proven challenging. The aim of our ongoing research is to provide a platform from which the sliced pear industry can better provide pear consumers access to the fruit with the convenience that they demand.

This project involved slicing of 1-MCP treated fruit and its subsequent treatment with the ripening compounds that were identified previously in the program. We also evaluated non-1-MCP treated fruit that had not met its conditioning requirement. Evaluation of fruit in the latter category was not part of the original plan due to limitation of resources however this experiment was driven by the commercial availability of fruit of desirable firmness. It was interesting to note that RC2 was able to compensate for lack of cold requirement for conditioning to ripen. It implies that the non-1-MCP treated fruit can be utilized for slicing soon after harvest. Further, 1-MCP treated fruit can be utilized for slicing late into the season extending the market window for producing sliced product. Crunch Pak cited this as a highly desirable factor for any commercial entity to get involved in producing sliced product.

In experiments conducted concurrently at WSU and Crunch Pak, 1-MCP-treated Bartlett and D'Anjou fruit were sliced and treated with RC2. Product quality analysis at Crunch Pak revealed that 1-MCP-treated Bartlett pears sliced at 17 lb firmness, treated with the appropriate concentration of RC2, and stored at 6 deg C (42.6 deg F) over a period of 21 days developed desirable flavor and softened while in a 2 oz. bag. The fruit met the quality standards established by McDonald's and the slices were able to outlast the necessary two weeks shelf life in bags (Table 1). The two week threshold is a market requirement for a sliced product to be successful. In fact, the sliced pears lasted well into the fourth week after being packaged in Crunch Pak 2 oz. bags. Crunch Pak is interested in performing large scale trials.

Release of ethylene and carbon dioxide evolution was measured using gas chromatography as well as with an on-loan CID Biosciences hand held instrument (Figure 1). Treatment of fruit with RC2 resulted in an overall increase in release of ethylene and carbon dioxide. There was a four-fold change in ethylene release which is both an indicator and inducer of ripening (Figure 1A). A 1 to 1.5-fold increase in carbon dioxide evolution was also observed. The experiment was replicated 3 times and each replicate consisted of six 2 oz. bags.

In addition to the analysis of ethylene and carbon dioxide levels, firmness and brix change was also measured (Figure 2). While the changes in these two parameters are not highly pronounced, they are effective in contributing to a favorable organoleptic experience as was evident from a non-controlled taste panel performed at the annual WSHA event in Kennewick, WA.

A total of 47 respondents participated in the sliced pear tasting panel (Table 2). Out of the 47 participants only one respondent reported no change in the organoleptic profile of the fruit. Treatment of fruit with RC2 at 2% enhanced the overall acceptance and taste/flavor of the product. Texture was most acceptable at 3% RC2 treatment, with 2% RC2 treatment following close behind. The controls

performed best in terms of the appearance. However, repeat purchase is not dependent on appearance alone as has been extensively documented in literature. There is a need to perform controlled and non-controlled taste panels. For this a collaboration has been established with Carolyn Ross and Karina Gallardo who perform such analysis and panels on a routine basis.

In summary, our data and preliminary taste panel survey has established the feasibility of the sliced pear product. With support from large scale market trials, economic analysis and controlled taste panel surveys enough data can be generated to attract the slicing entities to produce sliced pears for the larger market. This is also expected to increase the utilization of fruit that falls in the 120 to 135 box size range which is currently underutilized or culled.

Figure 1: Change in ethylene and carbon dioxide evolution in sliced pears treated with RC2.

A and B: measurements were performed on the gas chromatography equipment. Fold-change in ethylene and carbon dioxide levels is shown. The x-axis represents the percentage of RC2 used. The dry and wet annotation refers to the condition, where no (dry) or extra (wet) volume of non-browning-RC2 solution was included in the 2 oz.. bag. A four-fold change in ethylene release is discernible from this data.

C and D: measurements were performed with the on-loan CID Biosciences hand-held device.

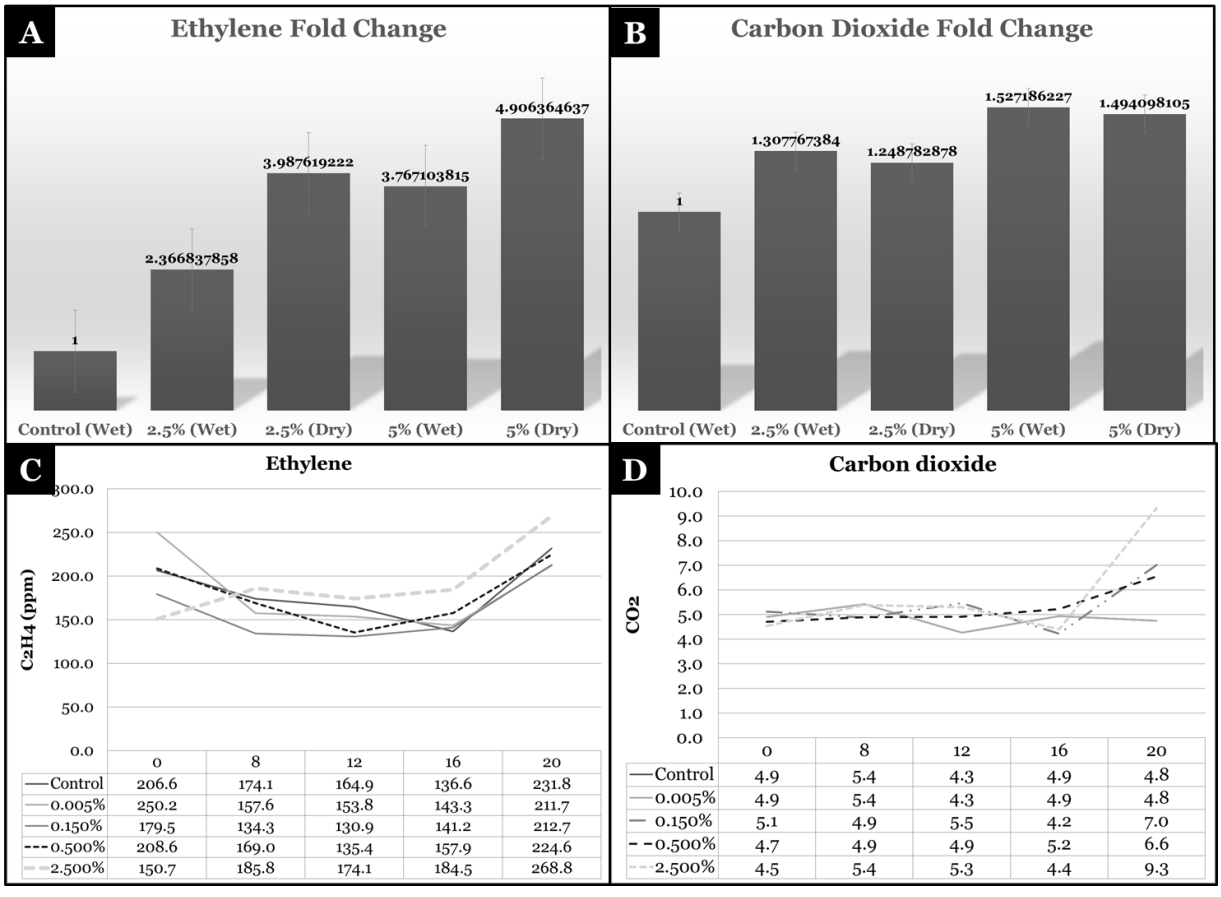
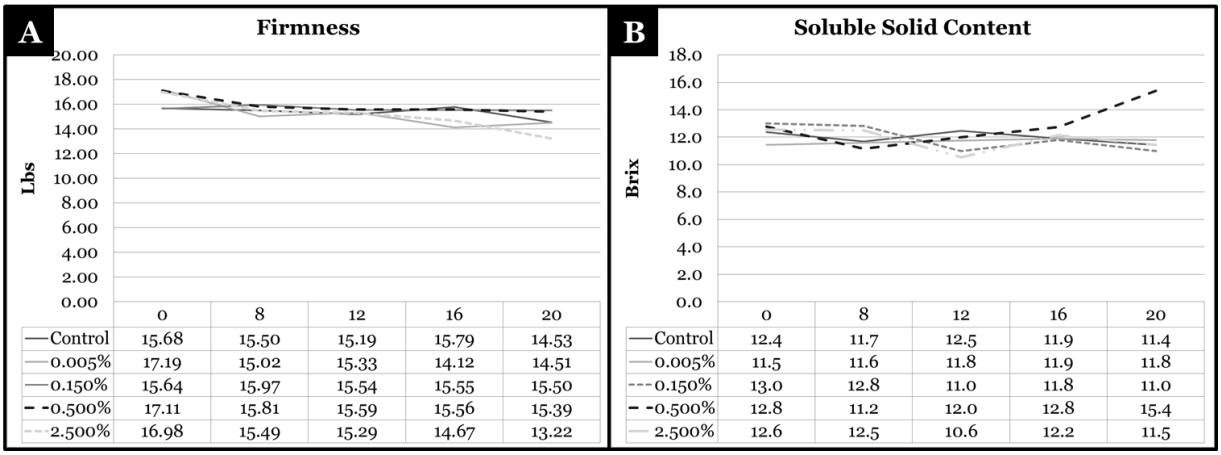


Figure 2: Change in fruit firmness and soluble solid content.



	0	8	12	16	20
Control	15.68	15.50	15.19	15.79	14.53
0.005%	17.19	15.02	15.33	14.12	14.51
0.150%	15.64	15.97	15.54	15.55	15.50
0.500%	17.11	15.81	15.59	15.56	15.39
2.500%	16.98	15.49	15.29	14.67	13.22

	0	8	12	16	20
Control	12.4	11.7	12.5	11.9	11.4
0.005%	11.5	11.6	11.8	11.9	11.8
0.150%	13.0	12.8	11.0	11.8	11.0
0.500%	12.8	11.2	12.0	12.8	15.4
2.500%	12.6	12.5	10.6	12.2	11.5

Table 1: Quality control data based on McDonald's standards generated at Crunch Pak. RC – Ripening compound.

	Control (No RC)		**0.5% RC**		**2.5% RC**	
Day	**Firmness**	**Flavor**	**Firmness**	**Flavor**	**Firmness**	**Flavor**
7	Very firm and crunchy	Little to none	Very firm and crunchy	Little to none	Very firm and crunchy	Little to none
10	Very firm and crunchy	Little to none	Still very crunchy; Less firm than control	No noticeable difference in flavor from control	Less firm than control; Still very crunchy	Slightly more flavor than control
14	Very firm and crunchy	Little to none	Less firm than control; Still crunchy	Slightly more flavor than control	Slightly less firm than 1.5%	Juicier than 0.5% RC; Slightly better flavor
17	Very firm and crunchy	Little to none	Less firm than control	Slightly more flavor than control	Still firm, but less so than control	Some flavor development
21	Very firm and crunchy	Little to none	Still firm and crunchy	Some flavor	Less firm than control; Still crunchy	Similar flavor to 1.5% RC; Moderate amount of juiciness

Table 2: Ranking for overall acceptance, appearance, taste/flavor, and texture of sliced pears. A total of 47 respondents comprised of this consumer survey. 46 individuals were able to distinguish a difference between treated vs. control samples.

	Ranking			
	Overall acceptance	**Appearance**	**Taste/Flavor**	**Texture**
Most acceptable	2%	Control	2%	3%
	Control	1%	3%	2%
	3%	2%	Control	Control
Least acceptable	1%	3%	1%	1%

EXECUTIVE SUMMARY

Application of 1-MCP has accrued large benefits to the apple industry however its application to pears has been hampered by the lack of predictable ripening post 1-MCP treatment. This confounds the existing issue of stagnant pear consumption. A boost in consumption of pears is urgently required to catalyze the reinvigoration of the pear industry. Pears are constantly competing against foods that offer convenience and 'on the go' consumption such as berries, sliced products, packaged ready to eat vegetables etc. More than ever there is a need to develop strategies to produce and deliver a consistent quality pear and if the factor of convenience can be added, that can push the static consumption line.

As part of ongoing research on topmost priorities in pear research, we first developed the genome information and then utilized the information on physiology guided activity of pear specific genes that are involved in ripening to identify potential ripening compounds that can reverse the impact of 1-MCP. The mode of application of these ripening compounds on whole fruit are currently being investigated. In the meantime, we have established that the benefits of these compounds can accrue in the area of sliced pears. Currently pears are valued at about $400 M and every 1% of the market share for sliced pears adds $4 M to the value of pears. With the increased demand for convenience foods, a market share of 10% for sliced pears is foreseeable in the near future. This can boost the consumption of pears which has been a long desired goal of the U.S. pear industry.

Commercial trials with Crunch Pak and preliminary taste panel surveys have met or exceeded the standard expectations. There is a need to perform market scale trials and also evaluate the economics of what prices sliced pears command in the market since they will be a novelty product. To continue this work additional support has been requested in the form of a new proposal.

FINAL PROJECT REPORT **YEAR:** 3 of 3

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

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Organization: OSU MCAREC
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Cooperators: Xingbin Xie, jiankun Song, David Sugar, Paul Chen, Nate Reed (AgroFresh Inc.)

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

Other funding sources: None

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

Item	2012	2013	2014
Salaries		15,450[5]	15,914[5]
Benefits		7,327	7,547
Wages	15,000[1]		
Benefits	7,113[2]		
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.
[5]Salaries for Postdoctoral Research Associate (Xingbin Xie)

OBJECTIVES

The goal of this project was 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 following strategies were investigated:

1. Storing 1-MCP treated 'Anjou' pears at elevated storage temperatures
2. Simultaneous application of 1-MCP and ethylene
3. Post-storage conditioning by ethylene, intermediate temperature, and other ripening compounds
4. Delaying 1-MCP application
5. Late harvest maturity and production elevation

SIGNIFICANT FINDINGS

1. Ripening capacity (RC), superficial scald (SS) inhibition, storage quality, ethylene production, and related gene expressions in 1-MCP treated 'Anjou' pears were affected by storage temperature, simultaneous application of 1-MCP and ethylene, post-storage ethylene conditioning (PSEC), post-storage intermediate temperature conditioning (PSITC), and orchard elevations.

2. 1-MCP treated 'Anjou' pears did not restore RC in 7d at 68°F following 8 months of storage at 30°F. The 1-MCP treated fruit that were stored at 34°F developed RC with minimal SS in 7d at 68°F following 5-8 or 6-8 months depending on production year. Low O_2 at 2% slowed down the losses of green color, flesh firmness (FF), and titratable acidity (TA) of the 1-MCP treated fruit during storage at 34°F. The 1-MCP treated fruit lost green color and FF quickly (i.e., after 3-4 months) during storage at 36°F.

3. Simultaneous application of 1-MCP and ethylene at 1:1-2 (i.e., 300:300-600ppb) allowed the 1-MCP treated 'Anjou' pears developing RC with minimal SS following 5-8 or 6-8 months (depending production year) of storage at 30°F.

4. PSEC and PSITC could ripen 1-MCP treated red (Columbia) 'Anjou' following 5-8 months of storage at 30°F. 1-MCP treated green 'Anjou' could be ripened by PSEC or PSITC only after 8 months of storage at 30°F. Ethylene is the most efficient ripening compound and other ripening compounds including abscisic acid, jasmonic acid, methyl jasmonate, salicylic acid … may not be effective on ripening 1-MCP treated 'Anjou' pears.

5. Delaying 1-MCP application after harvest may not be a useful protocol for ripening 1-MCP treated 'Anjou' pears following cold storage. Fruit treated with 1-MCP within 3 weeks after harvest did not develop RC for 8 months, but fruit treated with 1-MCP between 3-4 weeks after harvest developed unacceptable SS following 4-8 months of storage at 30°F.

6. Green 'Anjou' pears from high elevations are less responsive to 1-MCP than that from low elevations. 1-MCP treated green 'Anjou' pears that were from orchards at high elevations (i.e., 2000ft) could be ripened by PSEC and PSITC without affecting SS following 5-8 months of storage at 30°F.

7. Regarding RC, there were no differences in responsiveness to 1-MCP between harvest maturities (H1 = 15lb, H2 = 13lb, H3 = 12.5lb) of 'Anjou' pears following storage at 30°F.

METHODS

Defect-free fruit were packed into 20kg wooden boxes with perforated polyethylene liners. Packed fruit were immediately transported to MCAREC and stored at 30°F. 1-MCP (SmartFresh: AgroFresh, Spring House, PA, USA) treatment at 100-150ppb was carried out according to procedures provided by the manufacture in an air-tight $40M^3$ room at 32°F for 24h on the second day after harvest. An electronic fan was used to circulate the air in the treating room.

1. Storage temperature

After ventilation, 1-MCP treated fruit were transferred to storage rooms at 30°F, 34°F, and 36°F. Control fruit were included in each storage temperature. After each month of cold storage, fruit IEC (internal ethylene concentration), storability [fruit firmness (FF), skin color], superficial scald related chemistry [FAR (α-farnesene) and CTols (conjugated trienoles)], and ripening-related gene expression were evaluated after 1d at 68°F, and fruit ripening capacity [FF, EJ (extractable juice), SSC (soluble solid content), TA (titratable acidity)] and superficial scald were evaluated after 7d at 68°F. Transcript levels of ethylene biosynthesis and signal genes were analyzed. The aim was to determine the genes that regulate the recovery of ripening capacity in 1-MCP treated 'Anjou' pears.

2. Exposure fruit to 1-MCP and ethylene simultaneously

Immediately after exposure of fruit to 1-MCP at 300ppb, a calculated amount of ethylene (300, 600, 1500ppb) 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.

3. Post-storage conditionings

After each month of cold storage, 1-MCP treated green and red (Columbia strain) 'Anjou' fruit were moved to an air-tight ethylene ripening room with ethylene concentration at 100ppm at 68°F for 48h, or transferred to an ethylene-free room at 50°F for 15d, or dipped in solutions of abscisic acid (ABA), jasmonic acid (JC), methyl jasmonic (JA), salicylic acid (SA), RC-2…… at recommended concentrations for 1-24h following cold storage. Then, fruit were transferred to 32°F for 2 weeks. RC and SS were evaluated after 7d at 68°F.

4. Delayed 1-MCP treatment

Fruit were exposed to 1-MCP at 100-150ppb at 1, 2, 3, 4-weeks-delay-after-harvest in an air-tight $40M^3$ room at 32°F for 24h. After ventilation, treated fruit were transferred to a storage room at 30°F. Fruit evaluations were the same as described in 1.

5. Harvest maturity and production elevation

Fruit were harvested at 3 maturities: H1 = 14.5-15lbf, H2 = 13lbf, and H3 = 12.5lbf from two orchard elevations (500ft and 2000ft). The 1-MCP treated fruit were stored at 30°F. RC and SS were evaluated after 5-8 months of storage.

RESULTS AND DISCUSSION

1. Elevated storage temperatures

When stored at 36°F, 1-MCP treated 'Anjou' pears softened and yellowed quickly after 3-4 months in storage and developed dry-coarse texture after ripening. Regarding RC following 8 months of cold storage, fruit harvested at the late maturity (FF = 12.5lb) did not differ from the commercial harvest maturity (FF = 15-13lb) in responding to 1-MCP. Therefore, data of 1-MCP treated fruit stored at 36°F and 1-MCP treated fruit that were harvested at late maturity are not presented in this part.

1.1. Ethylene production and respiration rate

Control fruit started accumulating IEC after 2 months, and thereafter IEC reached the highest amount at 3 months, and maintained > 2ppm for 8 months at 30°F. 1-MCP treated fruit stored at 30°F had extremely low IEC (< 0.2ppm) throughout 8 months of storage. In contrast, the 1-MCP treated fruit that were stored at 34°F started accumulating IEC after 5 months, and thereafter increased continuously between 5 to 8 months of storage (Fig. 1). Ethylene production rate increased significantly after 2 months, reached a maximum value after 5 months, and thereafter decreased. 1-MCP treated fruit that were stored at 30°F showed no ethylene production following 1-8 months of storage. 1-MCP treated fruit stored at 34°F showed no ethylene production for 3 months, then started to produce ethylene at a low rate following 4 and 5 months, and produced a significant amount of ethylene after 6 months of storage (Fig. 1). The respiration rate of control fruit increased during 8 months of storage and was higher than that of 1-MCP treated fruit stored either at 30°F or 34°F during 1-8 months of storage. 1-MCP treated fruit stored at 30°F maintained the lowest respiration rate which remained stable during 8 months of storage. 1-MCP treated fruit stored at 34°F had a low but significantly higher respiration rate than 1-MCP treated fruit stored at 30°F in the first 5 months, and increased significantly after 6 months of storage (Fig. 1).

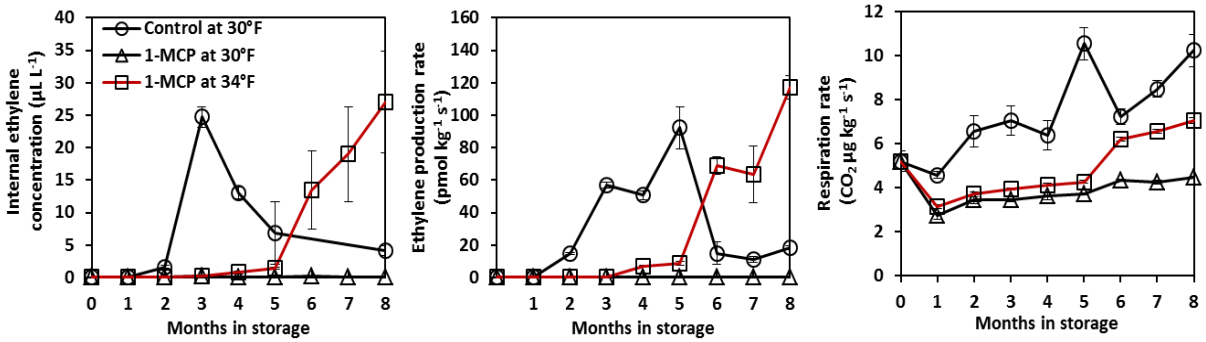


Fig. 1. Internal ethylene concentration, ethylene production rate, and respiration rate following storage of 1-MCP treated 'Anjou' pears at 30 or 34°F for 8 months.

1.2. Storability

1-MCP treated fruit that were stored at 30°F developed non-measurable IEC and ethylene production rate, therefore, maintained FF, skin color, and TA with minimum reductions for 8 months of storage. 1-MCP treated fruit stored at 34°F maintained FF for 7 months, however, decreased skin green color after 5 months and FF after 8 months of storage (Fig. 2).

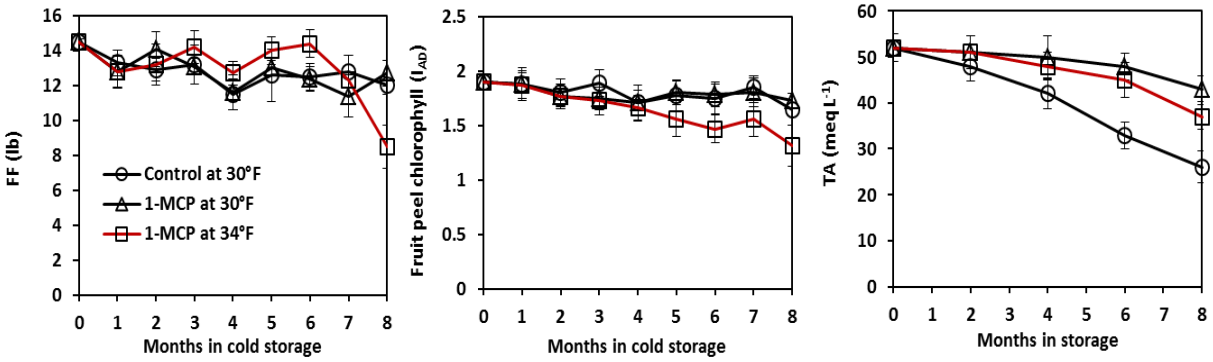


Fig.2. Effect of storage temperatures on storability of 1-MCP treated 'Anjou' pears.

1.3. Ripening capacity and superficial scald development in 7d at 68°F following cold storage

For 'Anjou' pears, we found that the buttery-juicy texture is related to fruit FF < 5-6lb with extractable juice (EJ) content on a fresh weight basis of < 650 mL kg^{-1}. 1-MCP treated fruit stored at

30°F developed neither RC nor SS in 7d at 68°F following 3-8 months of storage. The 1-MCP treated fruit stored at 34°F developed RC (FF < 5-6lb with EJ < 650 mL kg^{-1} in 7d at 68°F) after 5 and 6 months, in 2011 and 2012, respectively. It took 2 and 3 months of cold storage to fulfill the chilling requirement for developing RC of the control fruit in 2011 and 2012, respectively. 1-MCP treated fruit that were stored at 34°F developed minimal severity and incidence of SS in 7d at 68°F following 5-8 months of cold storage (Fig. 3).

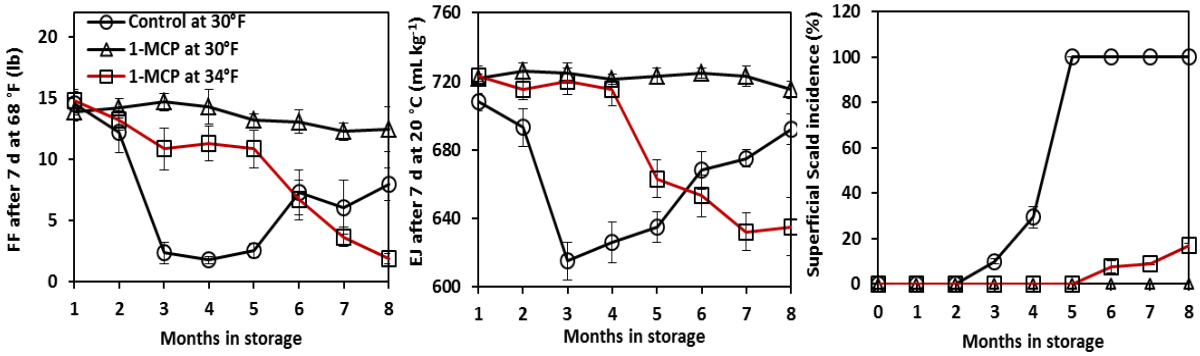


Fig. 3. Effect of storage temperatures on ripening capacity expressed as fruit firmness (FF) and extractable juice (EJ) and superficial scald (SS) incidence of 1-MCP treated 'Anjou' pears in 7d at 68°F following cold storage.

1.4. Ripening capacity related gene expressions

Compared to the control, the expression of ethylene synthesis (*PcACS1*, *PcACO1*) and signal (*PcETR1*, *PcETR2*) genes was stable at extremely low levels in 1-MCP@30°F fruit. In contrast, they increased expression after 4 or 5 months of storage in 1-MCP@34°F fruit. Other genes (*PcCTR1*, *PcACS2*, *PcACS4* and *PcACS5*) remained at very low expression regardless of fruit capacity to ripen. Therefore, the recovery of ripening capacity in 1-MCP treated 'Anjou' fruit was regulated by ethylene synthesis and signal genes *PcACS1*, *PcACO1*, *PcETR1 and PcETR2*.

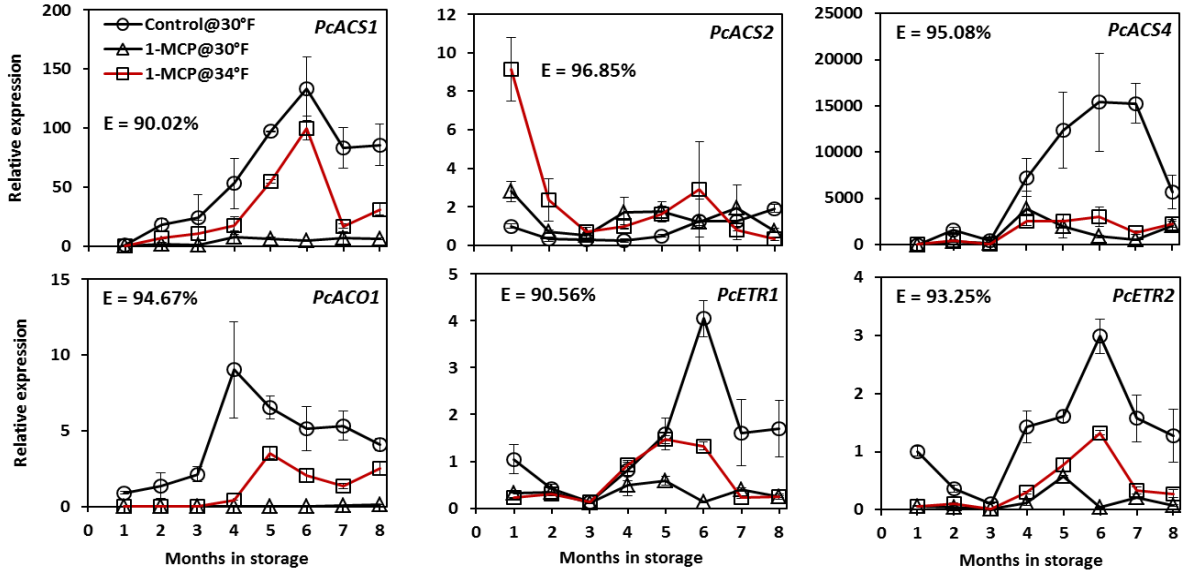


Fig. 4. Transcript levels of ethylene synthesis and signal genes in 1-MCP treated 'Anjou' pears stored at 30°F (1-MCP@30°F) and 34°F (1-MCP@34°F).

1.5. 1-MCP + Low O_2 (2%) storage

Low O_2 at 2% slowed down the losses of green color, FF, and TA without affecting RC (FF < 5-6lb with EJ < 650 mL kg^{-1} in 7d at 68°F) of 1-MCP treated fruit stored at 34°F for 8 months (Table 1). SS

incidence was 8.2% and 5.9% in RA and 2% O_2, respectively, after 8 months of storage at 34°F.

Table 1. Fruit storage quality [peel color, fruit firmness (FF), titratable acidity (TA)], ripening capacity [FF and extractable juice (EJ)], and superficial scald (SS) of 1-MCP treated 'Anjou' pears following 8 months of cold storage at 30 or 34°F in regular air (RA) or low O_2 storage.

	Peel color			after 7 d at 68 °F		
	(hue)	FF (lb)	TA (%)	FF (lb)	EJ (mL kg^{-1})	SS (%)
Control at 30°F in RA	91.4a	14.2a	0.20c	5.3b	686b	100a
1-MCP at 30°F in RA	92.0a	14.5a	0.27a	11.6a	710a	0c
1-MCP at 34°F in RA	88.3c	11.1c	0.24b	3.8c	640c	8.2b
1-MCP at 34°F in 2%O_2	90.8b	13.6b	0.26a	3.2c	639c	5.9b

Different letters indicate significant differences between treatments at each evaluation according to Fisher's protected LSD test at $p = 0.05$.

2. Simultaneous application of 1-MCP + ethylene at 1:1-5 (300:300, 600, 1500ppb)

2.1. Ethylene production and respiration rate

1-MCP at 300ppb totally shut down ethylene production and reduced respiration rate significantly during 8 months of storage at 30°F. Fruit received simultaneous application of 1-MCP + ethylene at 1:1-2 started to increase IEC, ethylene production rate, and respiration rate after 4 months of cold storage. Compared to control, simultaneous application of 1-MCP + ethylene at 1:5 had a little effect on IEC, ethylene production rate, and respiration rate of 'Anjou' pears during cold storage (Fig. 5).

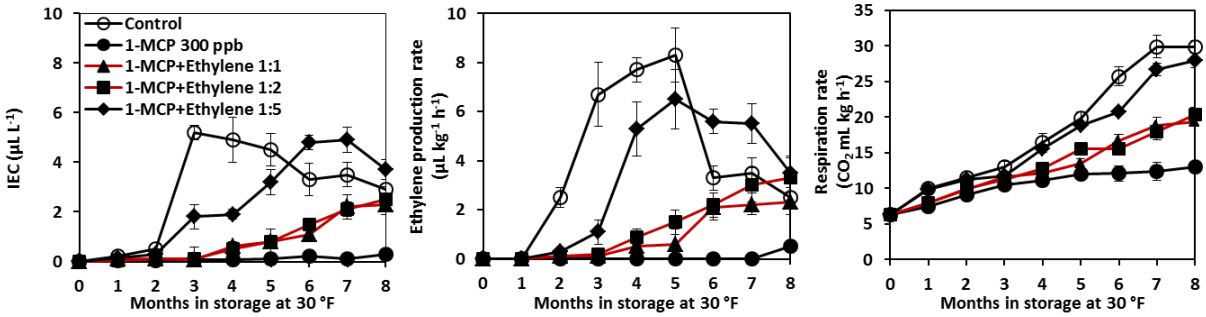


Fig. 5. Effect of simultaneous application with 1-MCP + ethylene on internal ethylene concentration (IEC) and respiration rate of 'Anjou' pears during storage at 30°F.

2.2. Storability

Simultaneous application of 1-MCP + ethylene at 1:1-2 maintained fruit firmness, skin green color, and TA the same as 1-MCP at 300ppb for 8 months of storage at 30°F. Compared to the control, the treatment of 1-MCP + ethylene at ratio of 1:5 did not affect fruit storability (Table 2).

2.3. Ripening capacity and superficial scald development in 7d at 68°F following cold storage

Fruit received simultaneous application of 1-MCP + ethylene at 1:1-2 developed RC with FF less than 5-6lb and extractable juice less than 650 mL kg^{-1} in 7d at 68°F following 5-8 months of storage at 30°F. Fruit received simultaneous application of 1-MCP + ethylene at 1:5 developed RC following 3-5 months, but developed dry-coarse texture indicated by EJ > 650 mL kg^{-1} following 6-8 months of cold storage. Fruit received simultaneous application of 1-MCP + ethylene at 1:1-2 produced higher amount of α-farnesene and CTols than 1-MCP treated fruit but much less than control, and therefore developed minimal amount and severity of SS in 7d at 68°F following 5-8 months of storage at 30°F. Fruit received simultaneous application of 1-MCP + ethylene at 1:5 developed unacceptable SS following 3-8 months of cold storage (Fig. 6).

Table 2. ‘Anjou’ fruit storage quality affected by simultaneous application of 1-MCP + ethylene at ratio of 1:1-5 after 5 and 8 months of storage at 30 °F.

	5 months				8 months			
	Peel color (hue)	FF (lb)	SSC (%)	TA (%)	Peel color (hue)	FF (lb)	SSC (%)	TA (%)
Control	96.3a	14.5a	12.4a	0.29b	91.4b	13.9b	12.3a	0.19b
1-MCP 300 ppb	97.1a	14.8a	12.5a	0.32a	92.5a	14.5a	12.5a	0.27a
1-MCP+Ethylene 1:1	97.0a	14.7a	12.6a	0.31a	91.9ab	14.5a	12.3a	0.25a
1-MCP+Ethylene 1:2	96.8a	14.6a	12.6a	0.31a	92.1ab	14.4a	12.4a	0.25a
1-MCP+Ethylene 1:5	96.4a	14.6a	12.4a	0.28b	91.5b	14.0b	12.5a	0.22b

Different letters indicate significant differences between treatments at each evaluation according to Fisher’s protected LSD test at $p = 0.05$.

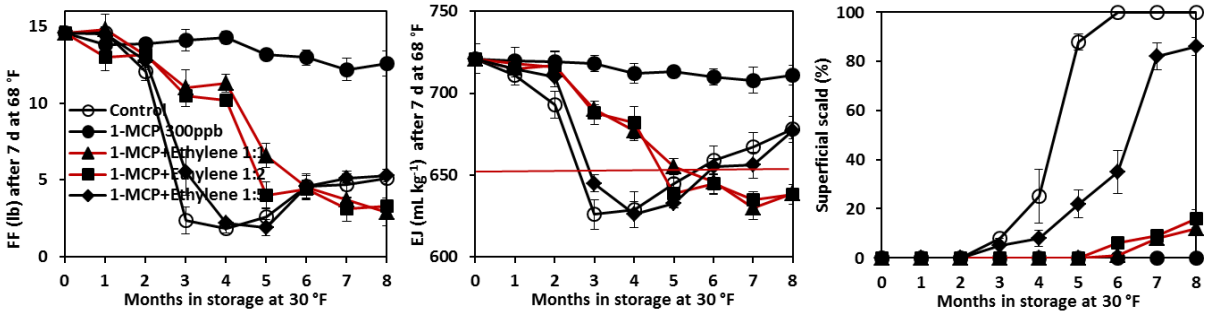


Fig. 6. Effect of simultaneous application of 1-MCP + ethylene on fruit firmness (FF), extractable juice (EJ), and superficial scald in 7d at 68°F following cold storage at 30°F.

3. Post-storage conditionings

3.1. Post-storage ethylene conditioning (PSEC)

Ethylene production rate of 1-MCP treated green ‘Anjou’ did not response to PSEC following 1-7 months, but increased following 8 months of storage at 30°F. In contrast, the ethylene production rate of 1-MCP treated red ‘Anjou’ (Columbia) increased in responding to PSEC following 5-8 months of cold storage. In consequence, PSEC could not ripen the 1-MCP treated green ‘Anjou’ following 1-7 months, excepting after 8 months of storage at 30°F. In contrast, the commercially standard PSEC could ripen 1-MCP treated red ‘Anjou’ following 5-8 months of storage at 30°F. 1-MCP controlled SS in both green and red ‘Anjou’ pears during 8 months of storage at 30°F. PSEC did not affect SS development in green and red ‘Anjou’ (Fig. 7).

PSEC up-regulated *PcACO1* and *PcETR2*, had no effect on *PcACS5*, and down-regulated the other genes in 1-MCP treated red ‘Anjou’, while it down-regulated all the genes in 1-MCP treated green ‘Anjou’. *PcACO1* may play an important role in initiating ripening capacity in 1-MCP treated red ‘Anjou’ pear upon PSEC treatment. The anthocyanin synthesis may influence ethylene metabolism in the response of pears to 1-MCP (Fig. 8).

3.2. Post-storage intermediate temperature conditioning (PSITC)

PSITC (for 15d at 50°F) had a similar effect with PSEC on ethylene production, RC, and SS of 1-MCP treated green and red ‘Anjou’ pears following storage at 30°F (data not shown).

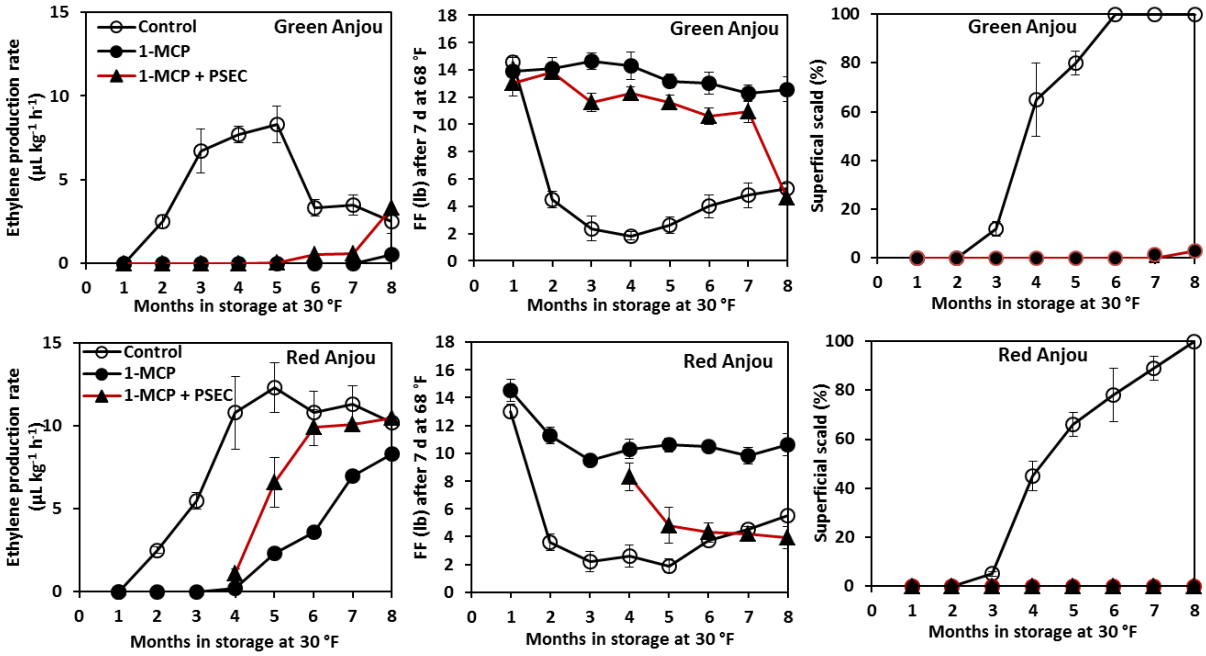


Fig. 7. Effect of post-storage ethylene conditioning (PSEC) on ethylene synthesis, ripening capacity and superficial scald of 1-MCP treated green and red 'Anjou' pears following storage at 30°F.

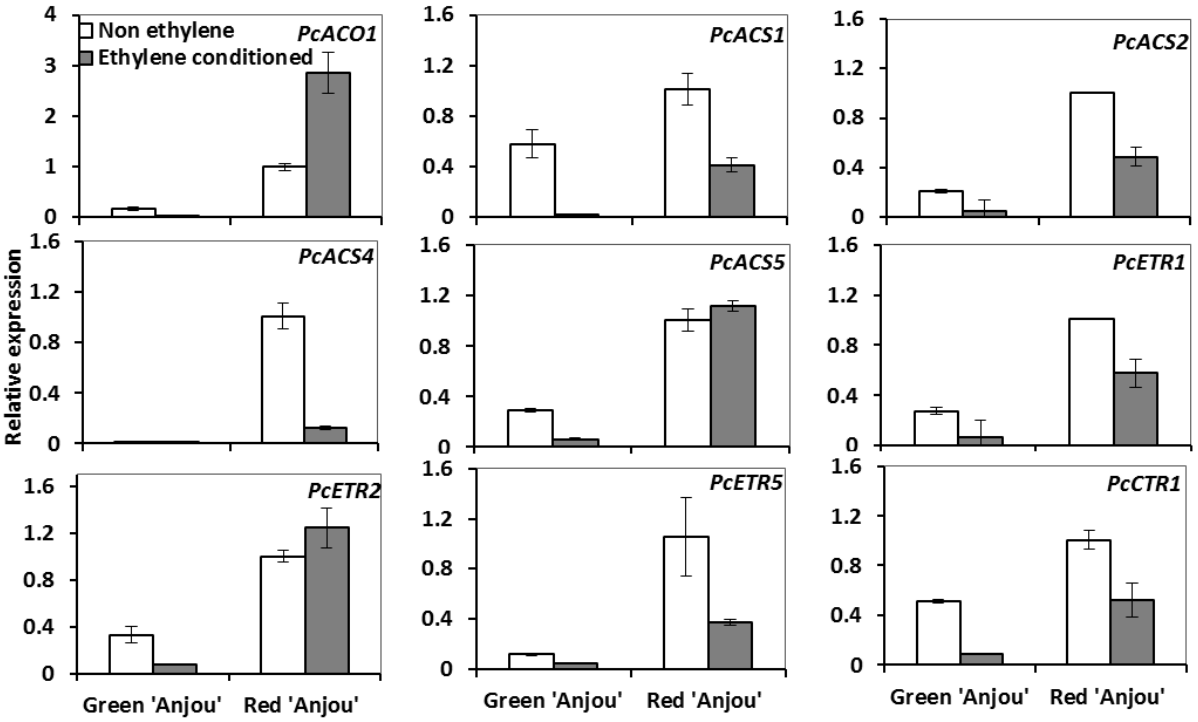


Fig. 8. Effect of post storage ethylene conditioning (PSEC) on transcript levels of ethylene synthesis (*PcACO1*, *PcACS1*, *PcACS2*, *PcACS4, and PcACS5*) and signal (*PcETR1*, *PcETR2*, *PcETR5*, and *PcCTR1*) genes in 1-MCP treated green and red 'Anjou' pears after 6 months of storage at 30°F.

3.3. Ripening compounds

The ripening compounds [abscisic acid (ABA), jasmonic acid (JA), methyl jasmonate (MJ), salicylic

acid (SA) ……] could not ripen the 1-MCP treated green ‘Anjou’ fruit to a buttery-juicy texture in 7d at 68°F following 3-8 months of storage at 30°F (Fig. 10). Ethylene was the most efficient compound to ripen the 1-MCP treated green and red ‘Anjou’ pears.

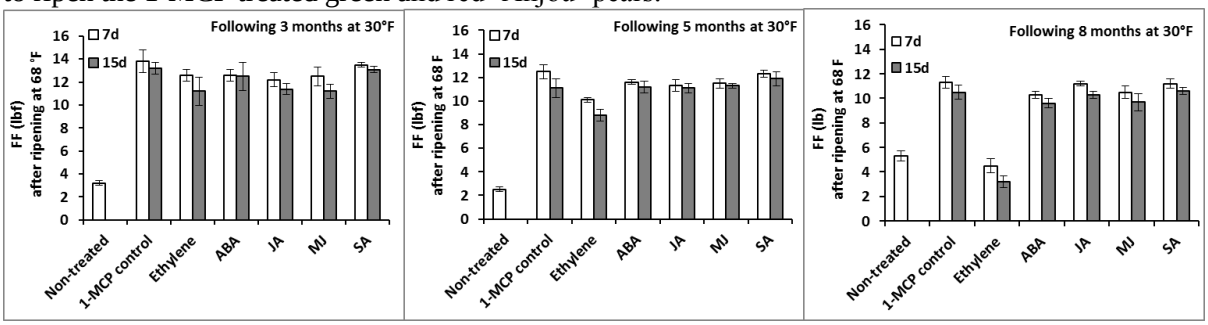
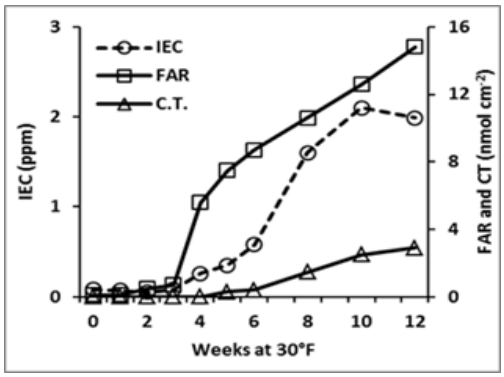


Fig. 10. Effect of ripening compounds: abscisic acid (ABA), jasmonic acid (JA), methyl jasmonate (MJ), salicylic acid (SA) on ripening capacity of 1-MCP treated ‘Anjou’ that were stored for 3, 5, or 8 months at 30°F.

4. Delaying 1-MCP treatment after harvest

The oxidation products (CTols) of FAR damage the hypodermal tissue of fruit and cause SS 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, CTols. Within the initial two months of cold storage at 30°F, Anjou pears developed IEC, FAR and CTols in a dynamic manner. IEC and FAR were determined to increase significantly after 3 weeks and CTols started to increase after 6 weeks.



In 2011, two delayed treatments: 2-weeks-delay and 4-weeks-delay were carried out. Results indicated that fruit treated with 1-MCP at 2-weeks-delay developed neither SS nor RC in 7d at 68°F following 3-8 months of storage at 30°F. In contrast, fruit treated with 1-MCP at 4-weeks-delay did not control SS during ripening following 4-8 months of storage. In 2012, we treated ‘Anjou’ with 1-MCP at 1, 2, 3, and 4 -weeks-delay after harvest and stored them at 30°F. Results indicated that fruit treated at 1, 2 or 3-weeks-delay develop neither SS nor RC in 7d at 68°F following 3-8 months of cold storage, in contrast, fruit treated at 4-weeks-delay developed unacceptable SS during ripening following cold storage (Fig. 11).

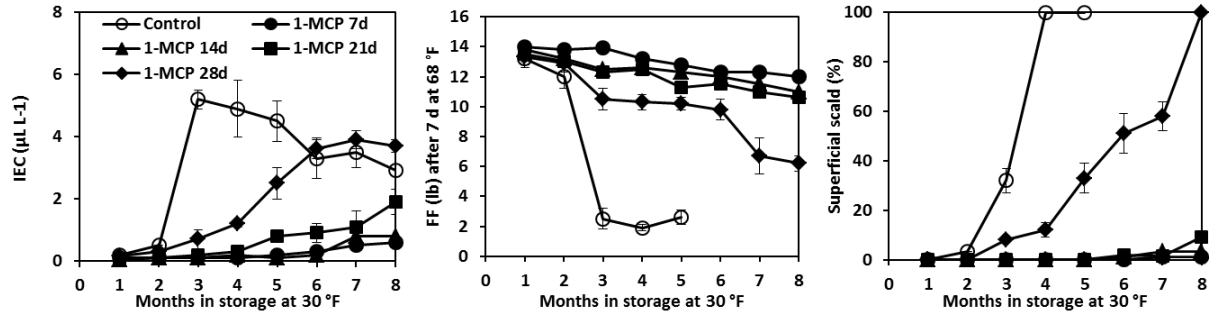


Fig. 11. Effect of delayed 1-MCP treatments after harvest on ripening capacity and scald development of 1-MCP treated ‘Anjou’ pears following cold storage 30°F.

5. Delaying harvesting

Regarding RC and SS, harvest maturity (H1= 15lb, H2 = 13lb) did not affect 'Anjou' pear' responsiveness to 1-MCP following storage at 30°F. 1-MCP treated 'Anjou' fruit that were harvested at H3 = 12.5lb developed RC following 7-8 months of storage at 30°F in 2011. However, the 1-MCP treated H3 fruit did not develop RC following 6-8 months of storage at 30°F in 2012 (Fig. 12).

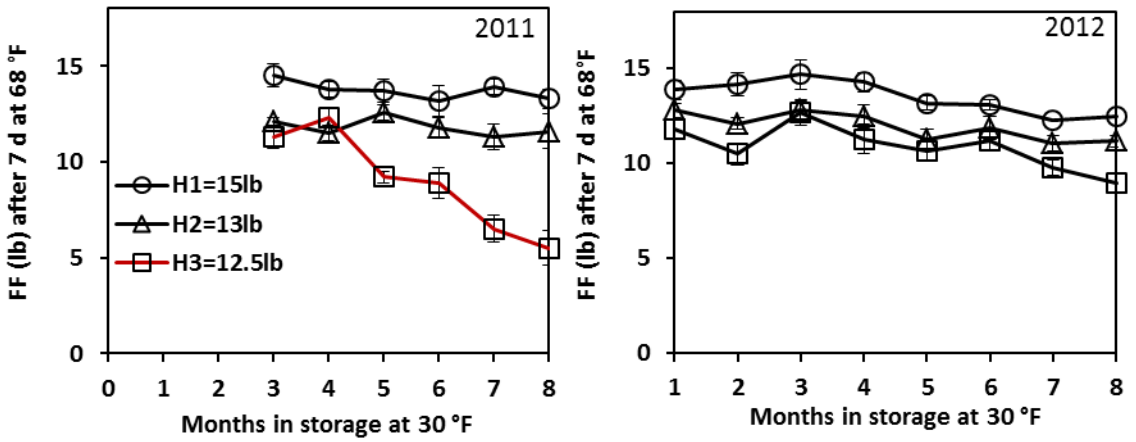


Fig. 12. Ripening capacity of 1-MCP treated 'Anjou' pears harvested at three maturities in two production years following 8 months of storage at 30 °F.

6. Production elevation

1-MCP treated 'Anjou' pears from orchard at 2000ft started to produce minimal amount of ethylene (< 0.1-1 µL kg^{-1} h^{-1}) after 5 months of storage at 30°F. However, the increased ethylene synthesis may not be high enough to trigger the RC. Both PSEC and PSITC enhanced ethylene production rate of the 1-MCP treated fruit following 5-8 months of cold storage. In consequence, the 1-MCP treated 'Anjou' pears developed RC upon PSEC or PSITC with minimal amount of SS (< 10%) following 5-8 months of storage at 30°F (Fig. 13).

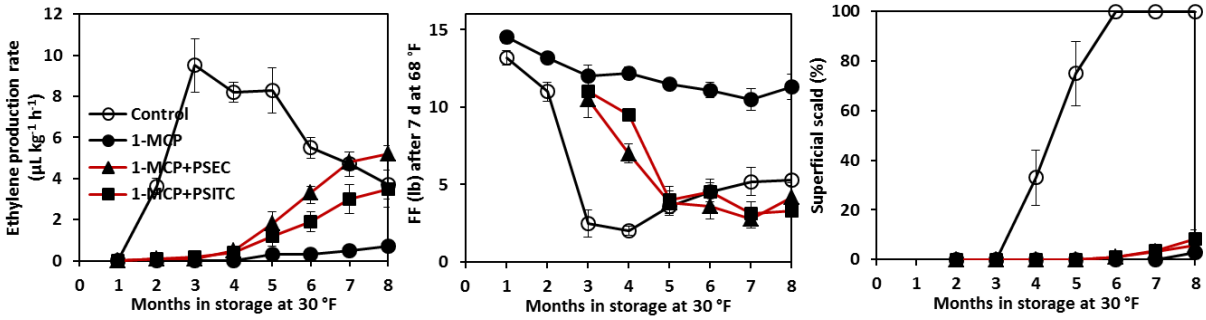


Fig. 13. Ethylene production rate, ripening capacity, and superficial scald of 1-MCP treated 'Anjou' pears from orchards at elevation of 2000ft affected by post-storage ethylene conditioning (PSEC) and post-storage intermediate temperature conditioning (PSITE) following 5-8 months of storage at 30°F.

EXECUTIVE SUMMARY

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

'Anjou' pear is the most produced pear cultivar in the Pacific Northwest of the US. It is enjoyed by consumers when fruit have ripened to a buttery and juicy texture at warm temperatures following cold storage. A peel disorder, superficial scald that appears during marketing following cold storage is a significant factor influencing postharvest management. The primary commercial control of scald on 'Anjou' pears at the present time is a postharvest treatment with the antioxidant ethoxyquin. In 2009, the European Union withdrew authorization for plant protection products containing ethoxyquin. Alternatives to ethoxyquin for controlling scald of 'Anjou' are needed. 1-Methylcyclopropene (1-MCP) is invaluable for controlling superficial scald and extending storage life of European pears, however it's initiation of ripening capacity in treated fruit following cold storage has been a challenge to overcome. We found the following protocols may control scald and extend storage life of 'Anjou' pears through postharvest application of 1-MCP at commercially manageable dosage (100-300ppb) while allowing ripening to outstanding eating quality.

Storing the 1-MCP treated 'Anjou' pears at 34°F instead of 30°F
While 1-MCP treated fruit stored at 30°F did not develop ripening capacity due to extremely low internal ethylene concentration and ethylene production rate for 8 months, 1-MCP treated fruit stored at 34°F produced significant amounts of ethylene during storage and developed ripening capacity with relatively low levels of scald within 7 d at 68°F following 6-8 months of storage. 1-MCP treated fruit stored at 36°F lost green color and fruit firmness quickly during storage. In conclusion, 1-MCP treatment of 'Anjou' pears followed by storing at an elevated temperature of 34°F instead of the traditional storage temperature of 30°F may be used as an alternative to ethoxyquin treatment for controlling scald while maintaining ripening capacity after long-term storage (i.e., 6-8 months). 'Anjou' pears that were stored at 34°F, however, lost green color, firmness and TA during long-term storage. Low O_2 at 2% slowed down the losses of green color, firmness, and TA of the 1-MCP treated fruit during storage at 34°F.

Simultaneous application of 1-MCP + ethylene at 1:1-2 (300:300-600ppb)
'Anjou' fruit received simultaneous application of 1-MCP + ethylene at 1:1-2 increased ethylene production and respiration rate after 4 months of storage at 30°F. Fruit treated with 1-MCP + ethylene at 1:1-2 maintained green color and TA, and developed ripening capacity with minimal superficial scald following 5-8 months of storage at 30°F.

Post-storage conditioning by ethylene or intermediate temperature
Post-storage ethylene conditioning (PSEC) and post-storage intermediate temperature conditioning (PSITC) could ripen red but not green 'Anjou' without increasing scald following 5-8 months of storage at 30°F. PSEC is the commercial standard method at 100ppm ethylene for 48h at 68°F. PSITC refers to conditioning at 50°F for 10-15d. Ethylene is the most efficient ripening compound and other ripening compounds may not be effective in ripening 1-MCP treated 'Anjou' pears.

Both PSEC and PSITC could ripen the 1-MCP treated green 'Anjou' that were from orchards at high elevations (i.e., 2000ft) following 5-8 months of storage at 30°F.

Delaying 1-MCP treatment after harvest and delaying harvesting
Delaying 1-MCP treatment after harvest and delaying harvesting may not be commercially feasible protocols to ripen 1-MCP treated 'Anjou' pears while maintaining its efficacy on storage quality.

FINAL PROJECT REPORT **YEAR**: 3 of 3

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

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

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

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

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

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

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

Objectives

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

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

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

Significant Findings

Objective 1:

- Eight trials were conducted to evaluate ReTain on pear fruit set and production; 60% of the trials resulted in significantly greater fruit set or yield, 40% did not. When effective, increased production ranged from a three-fold increase to only modest, numerical gains. For 'd'Anjou', ReTain was only effective when applications were made ~10 to 14 days after bloom at a rate of 1 pouch per acre. Applications near full bloom only improved fruit set for Comice in one of 3 trials, a cultivar purported to have a short ovule longevity period.
- Ethylene production rate of untreated 'd'Anjou' and Comice flowers steadily increased from bloom, peaking around 14 days after bloom, and declining to ~0 by 30 d after bloom. ReTain markedly reduced, but did not completely inhibit, ethylene production of flowers and fruitlets. Differences in the absolute rate of production were observed between 2013 and 2014. Applications of ReTain need to be applied just prior to this peak.
- Ethephon applications (300 ppm) ~45 d after full bloom significantly improved return bloom, fruit set and yield of 'd'Anjou' trees the year after application.

Objective 2:

- Root pruning was applied to orchards between 2012 (6th leaf d'Anjou') and 2014 (4th and 5th leaf 'd'Anjou'). In all trials, root pruning reduced vegetative growth (between 20% and 40%). Reduction in shoot length and trunk growth was positively related to tree age and the severity of pruning (two sides elicited a stronger response than one).
- Root pruning was too severe on 6th leaf trees, resulting in reduced yield and fruit size the year of application. Root pruning was not re-applied, but carry-over effects on vegetative growth and fruit size lasted through 2014. In contrast, shoot length of trees pruned in their 4th leaf recovered fully the year subsequent to application, but trunks remained significantly smaller.
- In all trials, double-sided root pruning consistently improved return bloom, fruit set, yield, and yield efficiency the year after application. In 2014, trees root-pruned in their 4th leaf (2013) had ~70% greater yield than controls (i.e., 41 bins per acre vs. 24 bins per acre) with no negative effect on fruit size or quality.
- Root pruning in consecutive years was also evaluated (4th and 5th leaf). 5th leaf trees pruned two years in a row had an equivalent reduction of shoot growth in both years (20%) in addition to producing similar yields as trees root pruned once in the 4th leaf (i.e., ~70% greater yield than untreated trees).

Objective 3:

- A training systems trial (single-axe vs. bi-axe) with d'Anjou' and 'Bartlett' on OH x F 87 was established in 2012. In-row tree spacing varied: 'Bartlett' was established at 2, 4, or 6 ft. and 'd'Anjou' at 4 or 8 ft. Trees required restarting in their 2nd leaf due to poor health. Bartlett tree size at the end of 2014 was slightly smaller at the closest spacing.
- In 2013, a rootstock trial was planted to evaluate the performance of 'd'Anjou' on OH x F 87, OH x F 69 or Pyro 2-33 at three different training systems (V, bi-axe, single-axe) and three

different in-row spacings (3, 4.5 and 6 ft.). After the 2nd leaf, trees on Pyro 2-33 were significantly smaller than OH x F 87 or OH x F 69. Individual axes of bi-axe trained trees were 40% smaller than single axe trees, irrespective of rootstock. Tree size was slightly smaller for trees planted at 3 ft., albeit nonsignificanlty.

Results and Discussion

Objective 1 (PGRs):

ReTain: Eight experiments were performed to evaluate the effect of the ethylene inhibitor AVG (a.i. of ReTain, Valent Biosciences Corp.) on pear (Anjou and Comice) fruit set and production. Each trial was designed as a randomized complete block; replicates varied. Whole trees were treated in all trials. Trees were sprayed to runoff with a pressurized handgun. In all experiments a surfactant (Sylgard 309) was added to ReTain at 0.1% (v:v). For Experiments 1-8 below please refer to Table 1 for supporting summary data.

Exp 1. 2012, 10th leaf Anjou/OH × F 97, MCAREC, OR (4 single-tree replicates)
Treatments: 1. Control; 2. ReTain 40 ppm applied at 80% of full bloom; 3. ReTain 80 ppm applied at 80% of full bloom; 4. ReTain 40 ppm applied two weeks after full bloom; 5. ReTain 80 ppm applied two weeks after full bloom.
Results: Fruit set and yield improved proportionately with rate. **Yield tripled** for highest ReTain rate (80 ppm) at 2 weeks after bloom; fruit size reduced (by cropload); 80% bloom applications reduced yield; seed counts of ReTain treatments similar to controls.

Exp 2. 2012, 17-year-old Comice/OH × F 97, MCAREC, OR (4 single-tree replicates)
Treatments: 1. Control; 2. ReTain 40 ppm applied at 80% of full bloom; 3. ReTain 80 ppm applied at 80% of full bloom; 4. ReTain 40 ppm applied two weeks after full bloom; 5. ReTain 80 ppm applied two weeks after full bloom.
Results: Fruit set and yield improved proportionately with rate. **Yield doubled** for highest ReTain rate (80 ppm) at 2 weeks after bloom; fruit size reduced (by cropload); 80% bloom applications increased yield; seed counts of ReTain treatments similar to controls.

Exp 3. 2013, 18-year-old Comice/OH × F 97, Hood River, OR (4 single-tree replicates)
Treatments: 1. Control; 2. ReTain 30 ppm applied at 50% of full bloom; 3. ReTain 60 ppm applied at 50% of full bloom; 4. ReTain 120 ppm applied at 50% of full bloom; 5. ReTain 30 ppm applied two weeks after full bloom; 6. ReTain 60 ppm applied two weeks after full bloom; 7. ReTain 120 ppm applied two weeks after full bloom.
Results: ReTain 120 ppm applied at 2 weeks after full bloom led to 40% higher fruit set and fruit number at harvest compared to controls. All other treatments had either similar or less (all 50% bloom timings) yield than controls; fruit size was reduced for ReTain 120 ppm applied 2 weeks after bloom.

Exp 4. 2013, 5th leaf Anjou/OH × F 97, Mt. Adams, WA (6 single-tree replicates)
Treatments: 1. Control; 2. ReTain 30 ppm applied at 80% of full bloom; 3. ReTain 60 ppm applied at 80% of full bloom; 4. ReTain 120 ppm applied at 80% of full bloom; 5. ReTain 30 ppm applied one week after full bloom; 6. ReTain 60 ppm applied one week after full bloom; 7. ReTain 120 ppm applied one week after full bloom; 8. ReTain 30 ppm applied two weeks after full bloom; 9. ReTain 60 ppm applied two weeks after full bloom; 10. ReTain 120 ppm applied two weeks after full bloom.
Results: ReTain treatments did not significantly increase yield relative to controls; a numerical increase in fruit number and yield was observed at the 2 weeks after full bloom timing for 60 and 120 ppm ReTain applications.

Exp 5. 2013, three separate plots were treated in Odell, OR with the same treatment regime: ~30-year-old Anjou /unknown rootstock; 4th leaf Anjou/OH × F 87; and, 7th leaf Anjou /OH × F 87.

Treatments: 1. Control; 2. 1 pouch ReTain/acre (applied 10 d after full bloom); 3. 0.5 pouch ReTain/acre (applied 10 d after full bloom).
Results: ReTain **significantly increased fruit set** for 2 of 3 trials in a rate responsive manner. The other trial had numerically increased fruit set. Trees were not harvested from these trials.

Exp 6. 2013, 4th leaf Anjou/OH × F 87, Dee Flat, OR
Treatments: 1. Control; 2. 1 pouch ReTain (applied 10 d after full bloom); 3. 1 pouch ReTain (applied 10 d after full bloom) + root pruning 1 side of tree row; 4. 1 pouch ReTain (applied 10 d after full bloom) + root pruning 2 sides of tree row.
Results: **Yield (fruits per tree ~doubled** for all ReTain trts; fruit size was reduced.

Exp 7. 2014, 12-year-old 'd'Anjou'/OH × F 97, Hood River, OR (4 single-tree replicates)
Treatments: 1. Control; 2. ReTain 30 ppm applied at two weeks after full bloom; 3. ReTain 60 ppm applied two weeks after full bloom; 4. ReTain 120 ppm applied two weeks after full bloom.
Results: **ReTain treatments did not significantly increase yield** relative to controls; yield was numerically higher for 120 ppm rate.

Exp 8. 2014, 19-year-old Comice/OH × F 97, Hood River, OR (4 single-tree replicates)
Treatments: 1. Control; 2. ReTain 30 ppm applied at two weeks after full bloom; 3. ReTain 60 ppm applied two weeks after full bloom; 4. ReTain 120 ppm applied two weeks after full bloom.
Results: **ReTain treatments did not significantly increase yield** relative to controls.

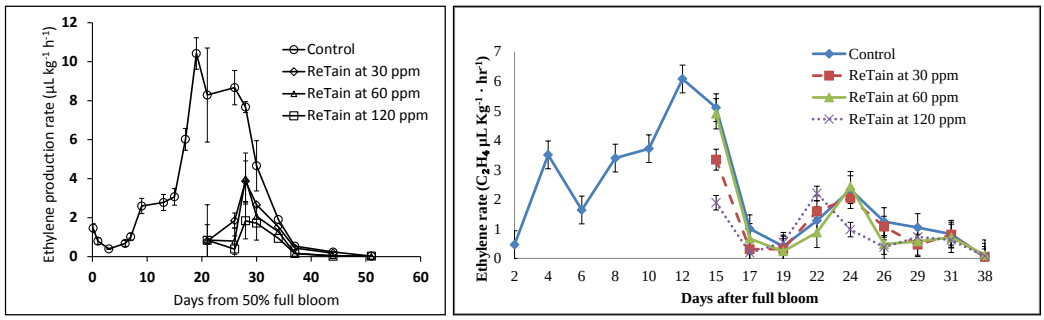

Figure 1. Ethylene production rate of flowers and fruitlets from 2013 (left) and 2014 (right).

The mode of action of ReTain on fruit set is via disruption of ethylene synthesis in fruit tissues. Ethylene is a natural plant hormone responsible, in part, for senescence processes. Hence, the objective of using ReTain to improve fruit set is to limit ethylene production in fruitlets that might otherwise induce abscission. Despite the label recommendation of bloom-time applications, ReTain **was not** effective when evaluated during the bloom period, with the exception of one experiment; however, applications at ~2 weeks after bloom had significantly greater efficacy in that trial. In fact, in all trials where a positive effect on fruit set was observed, the timing was ~14 days after bloom at a rate of 1 pouch per acre (~133 ppm).

Ethylene production rate of untreated 'd'Anjou' and Comice flowers steadily increased from bloom, peaking around 14 days after bloom and then declining to ~0 by 30 d after bloom. ReTain markedly reduced, but did not completely inhibit, ethylene production of flowers and fruitlets (greater effect in 2013). Differences in the absolute rate of production were observed in 2013 and 2014 (Fig 1). In 2014, we were anticipating higher levels of ethylene production, and as a consequence, missed the ideal application timing; prior to the peak (Fig 1). In fact, there may be a threshold level of ethylene necessary for fruitlet abscission (>5 µl/kg/hr). In this case, 2014 treatments would not have been

expected to improved fruit set. The fairly rapid metabolism of ReTain, in combination with low threshold ethylene rates, provide further support for the lack of effect from bloom-time applications.

Exp 1. 2012- 'd'Anjou'	Yield per tree		Projected Yield	Fruit size	Seeds
	(lb)	(fruit no.)	(0.55 ton bins per ha)	(no. per 20 kg box)	(no. per fruit)
Untreated Control	88 b	172 c	59	90 a	4.9 a
40 ppm ReTain® (80% FB)	57 c	118 cd	40	90 a	3.5 ab
80 ppm ReTain® (80% FB)	52 c	111 cd	35	90 a	3.2 b
40 ppm ReTain® (2 WAFB)	160 a	409 b	109	110 b	4.0 ab
80 ppm ReTain® (2 WAFB)	198 a	558 a	136	120 c	4.0 ab

Exp 2. 2012- 'Comice'	Yield per tree		Projected Yield	Fruit size	Seed count
	(lb)	(fruit no.)	(0.55 ton bins per ha)	(no. per 20 kg box)	(no. per fruit)
Untreated Control	77 c	138 c	52	80 a	5.4 a
40 ppm ReTain® (80% FB)	95 b	189 bc	64	90 bc	4.5 ab
80 ppm ReTain® (80% FB)	106 b	235 ab	72	100 c	3.9 b
40 ppm ReTain® (2 WAFB)	100 b	215 b	69	100 c	5.6 a
80 ppm ReTain® (2 WAFB)	127 a	269 a	86	100 c	5.8 a

Exp 3. 2013- 'Comice'	Yield per tree		Projected Yield	Fruit size	
	(lb)	(fruit no.)	(0.55 ton bins per ha)	(no. per 20 kg box)	g
Untreated Control	181 b	405 b	50	100	206 a
30 ppm ReTain® (50% FB)	150 c	348 c	41	100	197 a
60 ppm ReTain® (50% FB)	148 c	362 c	41	110	185 ab
120 ppm ReTain® (50% FB)	149c	365 c	41	110	184 ab
30 ppm ReTain® (2 WAFB)	161 bc	391 bc	44	100	191 ab
60 ppm ReTain® (2 WAFB)	195 ab	445 b	54	100	199 a
120 ppm ReTain® (2 WAFB)	219 a	569 a	60	110	176 b

Exp 4. 2013- 'd'Anjou'	Yield per tree		Projected Yield	Fruit size	
	(lb)	(fruit no.)	(0.55 ton bins per ha)	(no. per 20 kg box)	g
Untreated Control	150 a	95 a	45	70	289
30 ppm ReTain® (50% FB)	134 ab	75 b	36	80	264
60 ppm ReTain® (50% FB)	122 b	74 b	35	70	276
120 ppm ReTain® (50% FB)	168 a	98 ab	46	70	268
30 ppm ReTain® (1 WAFB)	119 b	79 b	37	70	299
60 ppm ReTain® (1 WAFB)	133 ab	76 b	36	80	262
120 ppm ReTain® (1 WAFB)	151 a	91 a	43	70	272
30 ppm ReTain® (2 WAFB)	111 b	69 b	33	70	281
60 ppm ReTain® (2 WAFB)	161 a	97 a	46	80	264
120 ppm ReTain® (2 WAFB)	175 a	101 a	48	70	268

Exp 5. 2013- 'd'Anjou' Trials	Fruit set (%)		
	7th leaf	30-year-old	4th leaf
Untreated Control	11 b	22	5 b
60 ppm ReTain® (10 dafb)	18 ab	21	10 ab
120 ppm ReTain® (10 dafb)	22 a	31	14 a

Exp 6. 2013- 'd'Anjou' RP	Yield per tree		Projected Yield	Fruit size	
	(lb)	(fruit no.)	(0.55 ton bins per ha)	(no. per 20 kg box)	g
Untreated Control	16 b	11 b	13	80	265 a
1-side Root Pruning NO ReTain	13 b	12 b	11	90	228 b
2-side Root Pruning NO ReTain	18 b	11 b	15	90	234 b
ReTain 120 ppm 10 dafb	27 a	24 a	23	100	202 bc
1-side Root Pruning + ReTain	25 a	22 a	21	100	222 b
2-side Root Pruning + ReTain	22 ab	26 a	18	100	198 bc

Exp 7. 2014- 'd'Anjou'	Yield per tree		Projected Yield	Fruit size	
	(lb)	(fruit no.)	(0.55 ton bins per ha)	(no. per 20 kg box)	g
Untreated Control	213	420 ab	59	100	201
30 ppm ReTain® (2 WAFB)	194	378 b	53	100	191
60 ppm ReTain® (2 WAFB)	227	454 ab	63	90	228
120 ppm ReTain® (2 WAFB)	214	471 a	59	90	212

Exp 8. 2014- 'Comice'	Yield per tree		Projected Yield	Fruit size	
	(lb)	(fruit no.)	(0.55 ton bins per ha)	(no. per 20 kg box)	g
Untreated Control	112	275	31	100	209
30 ppm ReTain® (2 WAFB)	95	217	26	90	217
60 ppm ReTain® (2 WAFB)	118	277	32	100	203
120 ppm ReTain® (2 WAFB)	96	252	26	100	199

The fact that fruits from ReTain treatments showed no difference in seed number relative to control fruit (Table 1) suggests that a lack of fertilization was not the critical factor limiting fruit set potential.

Ethephon and NAA: Three trials were established to evaluate ethephon or NAA applications on flowering and production of 'd'Anjou' in the season subsequent to application. In 2012, 300 ppm ethephon was applied at either 20 dafb or 50 dafb; the latter timing was meant to coincide with the flower imitation period of 'd'Anjou'. Ethephon significantly increased yield with the greatest response occurring at the later application timing (Fig 2). In 2014, the experiment was repeated using 150, 300 or 450 ppm Ethephon. Return bloom, fruit set and yield need to be evaluated in 2015. In addition, NAA was also trialed to improve return bloom and productivity. Applications were made at 45 dafb using low concentrations (5 ppm) and repeated weekly for 3 weeks (5 ppm at each timing). Return bloom, fruit set and yield need to be evaluated in 2015.

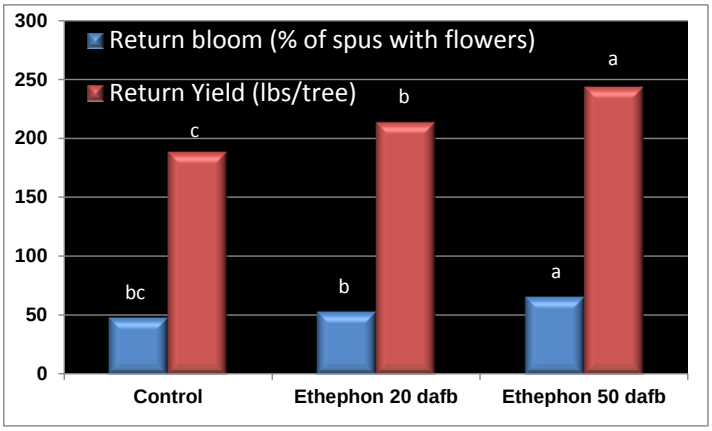

Figure 2. Effect of ethephon applications on 'd'Anjou' return bloom and yield the year after application. Ethephon was applied at 300 ppm to runoff. Data are means of 6 replicate trees.

Objective 2 (Root Pruning):
Root pruning was performed in commercial orchards prior to bloom when ~10% of the flowers were open. The implement (fabricated by Mr. Herbie Annala, Hood River producer) was tractor mounted and pulled in low gear ~1.5 ft. from tree trunks down either one or two sides of the tree row. Root pruning treatments were compared to untreated control trees in randomized complete block designs, replicated four times throughout the orchard. Whole rows were treated in experiment 1; in experiment 2, replicates comprised 8 contiguous trees. The depth of the steel shank was 1.5 ft. and the angle was

5 degrees off from the vertical (angle facing into the tree row). All other cultural practices were performed according to commercial standards.

Experiment 1: 2012 6th leaf 'd'Anjou' Trial- Double-sided root pruning reduced shoot growth of 6th leaf 'd'Anjou' trees by 40% in year one (Fig 3). Single-sided root pruning also significantly reduced shoot growth, compared to controls. Trunk size (TCA) was slightly reduced in 2012; however, yield, fruit size and vegetative growth were all significantly reduced compared to untreated control trees (in a rate responsive manner; Table 2). Typically, root pruning does not negatively affect production the year of application. We can speculate that too much root volume was removed; hence, we did not impose root pruning in this orchard in subsequent years. In 2013 (year 2), return bloom and fruit set were significantly increased by double sided root pruning (Table 2).

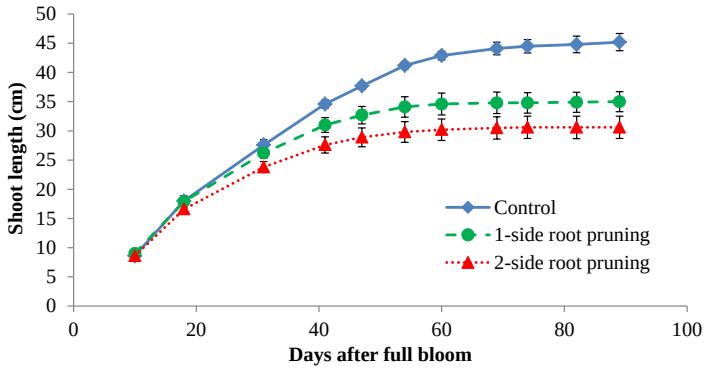

Figure 3. Shoot growth of 6th leaf 'd'Anjou' trees root pruned in 2012 to two levels (1 side of tree row or both sides of tree row).

Table 2. Effects of root pruning (one or both sides of the tree row) on 6th, 7th, and 8th leaf Anjou production and vegetative growth compared to an untreated control. Due to the severe root pruning effects on growth and production in 2012, trees were not root pruned in subsequent years.

Expt./Treatment	PAR Light Interception	TCA	Avg. Annual Shoot Length	Fruit Set	Avg. Fruit Size	Avg. Total Yield	Avg. Fruit Count	Avg. Bloom/ Limb	Yield Efficiency
	(%)	(cm^2)	(cm)	(%)	(g)	(lbs/tree)	(Fruit #/tree)	(Cluster #)	(%)
				2012					
Untreated control	-	96.4 a*	47.2 a	10 a	230 c	75 c	148 a	80	0.353
One-sided root prune	-	94.1 ab	36.3 b	10 a	205 b	55 b	122 b	71	0.266
Two-sided root prune	-	89.8 b	30.7 c	7 b	191 a	46 a	106 c	70	0.234
Pr(>F)		0.017**	<.001	<.001	<.001	<.001	<.001	0.3938	0.1606
				2013					
Untreated control	41.1 a	124.4 a	50.4 a	8 a	265	62	104.5	62.3 a	0.225
One-sided root prune	39.6 b	118.7 a	45.7 b	10 ab	255	62	110.5	79.9 b	0.236
Two-sided root prune	37.2 b	110.5 b	41.1 c	14 b	245	73	132.6	77.8 b	0.299
Pr(>F)	<.05	<.001	<.001	0.0294	0.1654	0.0853	0.1216	<.05	0.244
				2014					
Untreated control	42.2 a	140.6 a	49.9	24 b	251 b	64	114	49.9 b	0.206
One-sided root prune	41.9 a	132.6 b	48.3	21 ab	231 a	62	120	59.7 ab	0.211
Two-sided root prune	39.6 b	123.2 c	47.7	16 a	226 a	66	132	61.9 a	0.244
Pr(>F)	<.001	<.001	0.2342	0.0483	<.001	0.0678	0.0518	<.05	0.688

*letters signify significant difference with LSD test, all values are means of 4 replicates, n=25

**analysis of variance pr(>f).05

Positive effects of root pruning on year 2 bloom and fruit set have been previously documented. Generally, the effect is associated with altered partitioning of carbohydrates, since shoot growth is typically reduced. Subsequently, light interception is often improved, which, in turn, strengthens fruit bud development. The higher bloom and fruit set in 2013 led to increased yield, though not significant at 0.05 (Table 2). Combined yield over the two years, however, did not compensate for the reductions in year-one production. Strong carryover effects were also observed on vegetative growth (trunks and shoots were significantly reduced by root pruning). In the third year, reproductive

parameters responded similarly to 2013; double-sided root pruned trees had higher bloom and fruit set (% and number of fruit at harvest) compared to other treatments (Table 2). Interestingly, fruit size remained significantly smaller than control fruit, precluding a yield advantage. Tree size remained smaller, but annual shoot growth recovered. Ultimately, root pruning limited carbon pools (both storage and annual) necessary to support the increased fruiting response.

Experiment 2: 2013 4th leaf 'd'Anjou' Trial- The second experiment comprised a trellised block of 4th leaf Anjou/OH×F 87 (4 x 12 ft; 908 trees/acre) trained to a V. Trees had completely filled their space. Equivalent root pruning treatments were performed in year 1 as described above for Exp 1.

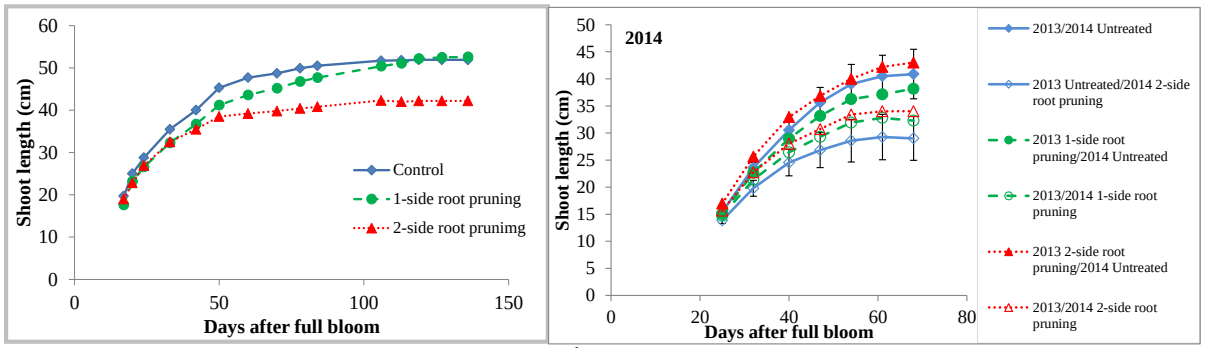

Figure 4. Shoot growth as affected by root pruning 4th leaf 'd'Anjou' trees in 2013 (left panel) and/or in 2014 (right panel).

Shoot growth was reduced relative to the severity of root pruning (Fig 4 left); however, the overall reduction in growth was ~half that observed in experiment 1- somewhat intuitive, since trees were nearly half the age and size. Interestingly, one-sided shoots appeared to recover during the season, plausibly due to the limited stress and regeneration of roots.

Table 3. Effects of root pruning (one or two sides of the tree row) on 4th leaf and 5th leaf 'd'Anjou' tree growth and production. In 2014 (year 2), treatments were either reapplied to the same trees (i.e., at the same level as in 2013) or not administered (i.e., trees left untreated). In addition, untreated trees were root pruned (2-sided only) for the first time in their 5th leaf.

Expt./Treatment by year		TCA	Avg. Annual Shoot Length	Avg. Bloom/ Limb	Fruit Set	Total Yield	Avg. Fruit Count	Projected Prod.	Avg. Fruit Size (g)	Yield Efficiency
		(cm^2)	(cm)	(# clusters)	(%)	(lbs/tree)	(no. fruits/tree)	(bins/acre)	(g)	(%)
2013 treatment		2013								
Untreated control		40.8*	53.2 a	30	11	16.1	28	13	265.3	0.179
One-sided root pruning		27.5	51.3 ab	26	13	13.5	27	11	228.3	0.221
Two-sided root pruning		30.8	42.2 b	33	13	18.3	34	15	233.7	0.269
Pr(>F)		0.0773	<.05	0.5621	0.7376	0.5608	0.4941		0.1648	0.0773
2013 treatment	2014 treatment	2014								
Untreated control	Untreated control	57.8 a	40.1	34.3 b	70.0	29.3 b	53	24	220 d*	0.23 b
Untreated control	Two-sided root pruning	48.2 b	32.1	34.3 b	73.6	31.6 b	62	26	221 d	0.297 b
One-sided root pruning	Untreated	37.7 c	40.6	40.1 b	69.8	28.9 b	67	24	233 cd	0.34 b
One-sided root pruning	One-sided root pruning	48.6 b	33.1	40.1 b	58.8	28.7 b	57	24	236 c	0.267 b
Two-sided root pruning	Untreated	41.6 c	43.4	54.7 a	85.8	45.1 a	93	37	253 b	0.49 a
Two-sided root pruning	Two-sided root pruning	50.1 b	32.3	54.7 a	85.4	49.5 a	85	41	273 a	0.447 a
Pr(>F)		<.001	0.0585	<.001	0.0616	0.0314	0.0513		<.001	<.001

*letters signify significant difference with LSD test, all values are means of 4 replicates, n=25

**analysis of variance pr(>f).05

As might be expected from the moderate vegetative stress induced by root pruning, first-year (2013) fruit set and yield were not negatively affected, irrespective of the level of root pruning (Table 3). Fruit size of root pruned treatments, however, was smaller; possibly due to a shortage of carbon (as presumed to be the case in experiment 1). In year 2 (2014), we split the replicates in half (4 contiguous trees per rep); one half was re-root pruned to the same level while the other half was left untreated. In addition, double-sided root pruning was applied to trees previously untreated in year 1.

Shoot growth of trees untreated in year 2, recovered fully in the second year (Fig 4, right). Trees pruned to the same level in consecutive years had a similar 20% reduction in shoot growth. Trees root pruned for the first time in their 5th leaf showed less overall shoot growth than other treatments (Fig 4, right). Bloom, fruit set and yield were all markedly increased by double-sided root pruning, but only when applied the previous year (Table 3). Projected per-acre production was increased by ~70% for these treatments relative to controls (from 24 bins to 41 bins). Fruit size or quality (data not shown) were unaffected by root pruning in year 2. These data support application of root pruning to young plantings to improve early production. We continue to evaluate the benefits of annual root pruning.

Objective 3:

2012 Planting: The 2012 planting to compare bi-axe and single axe training systems using multiple in-row tree spacing (2, 4 and 6 ft. for Bartlett, and 4 and 8 ft. for 'd'Anjou x 12 ft. alley spacing) needed to be restarted in 2013 due to excessively poor vigor and tree health. 2014 tree growth responded well to pruning, but production was delayed. Trunk size was only slightly smaller for bi-axe trees (compared to single axe) probably because bi-axe trees were developed by heading; a technique which generated vigorous axes. Bartlett trees were slightly smaller at the 2 ft. spacing compared to wider spacings. 'd'Anjou' tree size was not influenced by spacing. This is likely due to the wider spacings of 'd'Anjou', compared to Bartlett, and the poor growth in the formative years (i.e., no root competition yet). Bartlett trees have just now filled their space at the 2 ft. spacing. Minimum yield was recorded for Bartlett trees (~10 fruits per tree) equating to ~ 2.4, 1.5 and 1 bins per acre for the 2, 4 and 6 ft. spacing, respectively (data not shown).

2013 Planting: The performance of 'd'Anjou' trees is being evaluated on OH×F 87, OH×F 69 and Pyro 2-33 at three levels of training (V, single axe, bi-axe) and three levels of intra-row spacing (3, 4.5, and 6 ft.) at Hood River, OR. Alley spacing is 12 ft. resulting in a range of tree densities per acre of 605, 807, and 1,210. Tree growth and development (branching) was excellent after 2 years. Much of the uniformity among reps (see photos) of the bi-axe trees can be attributed to the nursery practice of chip budding scion buds opposite one another, as opposed to establishing the bi-axe by heading in the field. All trees reached the top wire (8 ft.) by August, 2014. Tree height will be managed at 10 ft. Trunk cross-sectional area of bi-axe trees was 40% smaller than single axe trees (whether planted to a V or vertical; Table 3). These data show the vigor control achieved by dividing vigor over two axes. Trees on Pyro 2-33 remained smaller than either of the OH×F clones (i.e., trees on Pyro 2-33 were significantly smaller at planting). Effects of inter-row spacing on trunk size were not significant, but 'd'Anjou' trees planted at 3' spacing were numerically smaller than those at 4.5 or 6 ft. Trees planted at 3 ft. have just filled their space. Bi-axe trees also resulted in significantly higher flower clusters per tree than either of the single axe training systems. While flower density was quite low, the results suggest a potential improvement in precocity of bi-axe trees.

Training. Individual axes of bi-axe trees were tied to bamboo (fastened to wires). Initially, bi-axe trees planted at 6 ft. in-row spacing were spread at ~45 degree angles until reaching the second wire (~5 ft.) then trained vertical. At 3 ft. spacing, axes were not spread as wide and were tied to a vertical position after the first wire (~2.5 ft.). The objective of changing the angle of the axes was to fill space. Trees in the V training system (each pole tipped 10 degrees from vertical) were attached to the wires in spring of 2014. Adjacent trees were tipped opposite one another. For trees in the 6' spacing, 3 leaders (palmette) per tree were trained to fill space (i.e., 12 ft. between trees on the same plane). V-trained trees at 3 and 4.5 ft. spacing were maintained as central leaders. The single axe trees were trained as spindles. All systems were pruned in the dormant period. For all systems, shoots in the top 25% of the tree were snapped at roughly 1/3rd of their length (a technique of S. Musacchi). All limbs that exceeded ~50% of the trunk diameter were removed with a bevel cut (i.e., Dutch cut). Shortening (heading) was only performed on primary scaffolds that were deemed too vigorous. The primary criterion for heading was the presence of weak or blind nodes within the basal foot of the

scaffold. The objective was to invigorate these nodes by heading in order to maintain fruiting potential at the base of limbs. Otherwise no other limbs were headed. All new shoots that developed in the spring were either tied, tooth-picked or clothes-pinned to establish wide-branched angles (30 to 45 degrees from horizontal). Throughout the season, wide branch angles were encouraged by bending, tying to wires, spreading and/or hop clipping (for those limbs oriented into the alley).

Table 3. Effect of rootstock, training system and in-row spacing on 2nd leaf 'd'Anjou' tree growth & flowering.

Treatment Effects	Trunk size		Flower clusters
	Above graft union (cm^2)	Below graft union (cm^2)	no./tree
Rootstock			
OH×F 69	11.6	24 a	2.2
OH×F 87	11	21.2 ab	1.6
Pyro 2-33	9	20.1 b	1.6
Training system			
Bi-axe	7.2 b	21.9	3.5 a
Single-axe	12.6 a	22.5	1.1 b
V	11.8 a	21	0.8 b
In-row spacing			
3 ft.	9.9	20.5	1.6
4.5 ft.	10.9	22.4	2
6 ft.	10.8	22.5	1.6

Executive Summary:

PGR Experiments

Eight trials were conducted to evaluate ReTain on pear fruit set and production; 60% of the trials resulted in significantly greater fruit set or yield, 40% did not. When effective, yield improvements ranged from a three-fold increase to only modest, numerical gains. For 'd'Anjou', ReTain was only effective when applications were made ~10 to 14 days after bloom, at a rate of 1 pouch per acre. Applications near full bloom were ineffective. In fact, bloom applications only improved fruit set for 'Comice' in one of three trials. 'Comice' is purported to have a short ovule longevity period. In that trial, however, applications made 2 weeks after bloom had a significantly greater response.

The rate of ethylene production of untreated 'd'Anjou' and 'Comice' flowers steadily increased from bloom (near 0), peaking around 14 days after bloom, and returning to ~0 by 30 d after bloom. ReTain markedly reduced, but did not completely inhibit, ethylene production of flowers and fruitlets. Differences in the absolute rate of ethylene were observed between 2013 and 2014. Plausibly, a critical level of ethylene contributes to fruit abscission, and to be effective, ReTain applications should be applied just prior to this peak. Further work is required to better understand the relationship. Interestingly, fruits harvested from ReTain treatments had equivalent seed counts as untreated fruits.

Ethephon applications (300 ppm) ~45 d after full bloom significantly improved return bloom, fruit set and yield of 'd'Anjou' trees the year after application.

Root Pruning Experiments

Root pruning was applied to orchards between 2012 (6th leaf d'Anjou') and 2014 (4^{th} and 5^{th} leaf 'd'Anjou'). In all trials, root pruning reduced vegetative growth (between 20% and 40%). Reduction in shoot length and trunk growth was positively related to tree age and the severity of pruning (two sides elicited a stronger response than one).

Root pruning was too severe on 6^{th} leaf trees, resulting in reduced yield and fruit size the year of application. Root pruning was not re-applied to this orchard, but vegetative growth and fruit size remained restricted through 2014. In contrast, shoot length of trees pruned in their 4^{th} leaf recovered fully the year subsequent to application (when not root pruned again), but trunks remained significantly smaller.

In all trials, double-sided root pruning consistently improved return bloom, fruit set, yield, and yield efficiency the year after application. In 2014, trees root-pruned in their 4^{th} leaf (2013) had ~65% greater yield than controls (i.e., 41 bins per acre vs. 24 bins per acre) with no negative effect on fruit size or quality.

Root pruning in consecutive years reduced shoot length by 20% and increased yields by ~70% compared to untreated trees.

Training Systems

In 2013, a rootstock trial was planted to evaluate the performance of 'd'Anjou' on OH x F 87, OH x F 69 or Pyro 2-33 at three different training systems (V, bi-axe, single-axe) and three different in-row spacings (3, 4.5 and 6 ft.). After the 2nd leaf, trees on Pyro 2-33 were significantly smaller than OH x F 87 or OH x F 69. Individual axes of bi-axe trained trees were 40% smaller than single axe trees, irrespective of rootstock.

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

Project Title: ABA chemical fruit thinning of Bartlett Pears

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

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

Other funding sources: None presently.

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

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

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

Objectives:

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

Significant Findings:

- A total of 5 trials were administered to evaluate the thinning efficacy of ABA (ProTone®, Valent Biosciences Corp.) on Bartlett pear trees. Thinning was rate-responsive but inconsistent from year-to-year: Strong thinning was observed in 60% of the trials and insignificant thinning in 40%. When ABA thinned, the most effective rate was ~100-150 ppm; higher concentrations removed too many fruits and lower concentrations not enough. Concentrations above 400 ppm were phytotoxic to leaves resulting in necrotic leaf spots and partial defoliation.
- We showed that ABA reduced photosynthesis, indirectly, by reducing stomatal conductance (partial closure of stomates). Photosynthesis responded to ABA rate. This action persisted in the plant for a relatively short period of time. Photosynthesis was inhibited 1 HR after application. Four days after application, 100 ppm ABA leaves were photosynthesizing at~ 80% of control levels and nearly 100% by day 12.
- ABA, on its own, did not appear to produce enough carbon stress to elicit sufficient thinning when sunny conditions prevailed the week after treatment. However, when combined with cloudy, overcast weather, thinning efficacy was high. Daily solar radiation (light) data supported this observation.
- In 2014, we conducted a shade x ABA trial to test the relationship between light level and ABA on thinning. Shade houses were constructed out of 30% or 60% shade cloth and placed over whole canopies for 15 d. ABA (125 ppm) was applied to trees in the presence or absence of shade. Under natural light (no shade), ABA-treated trees retained ~35% fewer fruits compared to unshaded controls. Fruit set of canopies under 30% shade had less fruit set than full-sun trees, but ABA + 30% shade did not improve the effect. Trees receiving 60% shade had ~ half the fruits of control trees; at this level of shade, ABA only slightly increased the thinning response.
- For the shade study, 125 ppm ABA reduced photosynthesis by 95% on day 1, but a recovery to 75-80% of control photosynthesis had occurred by day 3. Photosynthesis remained ~20% reduced until day 11. Leaf photosynthesis of trees shaded 30% were only slightly reduced from unshaded levels, indicating that sufficient light was available to saturate the response; however, leaf photosynthesis of 60% shaded trees was markedly reduced. Under this level of shade, interestingly, ABA did not augment the effect.
- In addition to solar radiation, tree age or, more importantly, carbon stores, may have played a key role in the inconsistent results. For the 3 trials where thinning was effective, trees were relatively small (between 7-10 years-old); however, the 2 trials in which relatively no thinning was observed were performed on 18 and 19-year-old trees with markedly larger canopies and, presumably, root systems.

Methods:

ABA (ProTone, Valent BioSciences Corp.) plus 0.1% surfactant was sprayed to drip using a pressurized handgun. Rates of ABA varied depending on the trial. In all 5 experiments, whole canopies of Bartlett pear trees were treated. At all sites, each treatment was replicated 4 times.

Timing of application occurred ~10mm fruit size, though one trial examined earlier applications at petal-fall. For each replicate tree, a minimum of 200 flower clusters were counted and tagged on scaffold limbs. Just prior to hand thinning timing (~35 days after bloom), fruit were counted on tagged sections of scaffolds and fruit set was determined. Scaffolds were roughly chest-height and adequately represented the condition of the lower and mid-canopy, but fruit set, in general, was greater in the top portion of the canopy (probably due to less spray coverage in the tops).

Photosynthesis measurements were intended to begin one day prior to each of the two treatment timings and continue every couple of days until the effect disappeared; however, our photosynthesis chamber malfunctioned, necessitating shipment to the (MA) for repairs in 2013. We received the instrument 5 days after the 10 mm treatments were applied and measurements began the next day, rendering only a partial data set. We did, however, repeat the trial in 2013 in Parkdale. Complete photosynthesis data sets were recorded for 2 of the 5 trials.

All fruits were counted at harvest and weighed. Fruit quality at harvest and PH was assessed in 2014.

Results and Discussion:

The 2013 Parkdale site was a good example of ABA's potential as a 'Bartlett' thinning compound (Figure 1). Fruit set, in general, at this site was high. Absolute fruit set and the number of fruit removed by hand-thinning, were significantly reduced by ABA; the effect was more pronounced with increasing rate. Rates of 200 and 400 ppm were excessive, resulting in too much drop for acceptable commercial harvest levels. In fact, rates of 400 ppm resulted in a high degree of leaf phyto-toxicity (necrotic spots and defoliation of ~25% of the canopy). Hence, the combined effects of 400 ppm ABA on photosynthesis and defoliation led to the drastic thinning response observed. The 100 ppm rate, however, reduced hand thinning by ~half.

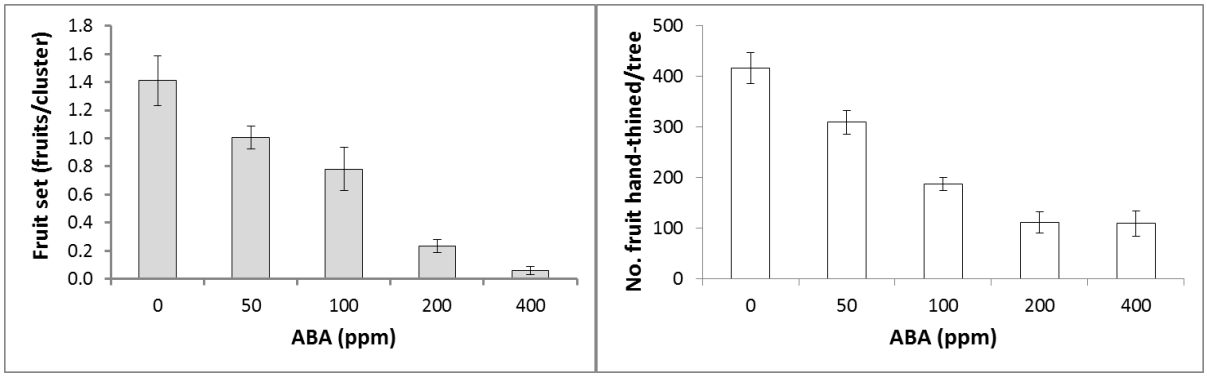


Figure 1. Fruit set (left) and hand thinned fruit (right) as affected by ABA rate. Both data sets are from the 2013 Parkdale trial.

In addition to reducing overall fruit set, ABA had a positive effect on the fruit density of individual spurs (Figure 2). An increase in the number of blank spurs (i.e., spurs failing to set any fruits) was observed with increasing ABA rate. For control trees, ~50% of the spurs were void of any fruit following natural 'June' drop, compared to 94% in trees sprayed with 400 ppm ABA. Prior to hand thinning, a higher percentage of the total fruit on ABA-treated trees resided singly on spurs. In general, fewer fruit were set on multiple-fruit spurs of ABA-treated trees compared to controls. However, for the concentration range best-suited for commercial use (~100 ppm), ABA still produced a small percentage of spurs possessing 3 or 4 fruits/spur.

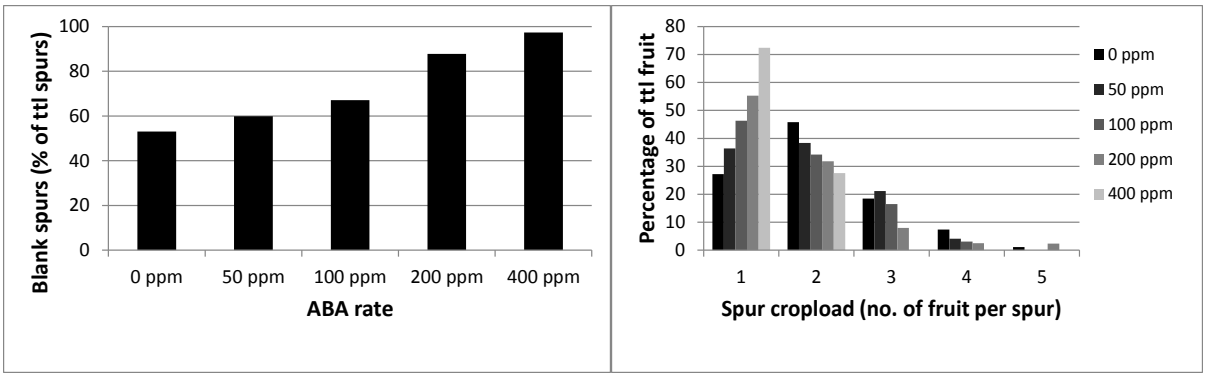


Figure 2. Percentage of total spurs on selected scaffold limbs that did not set fruit (i.e., blank) following applications of ABA at 10 mm timing (left). The right panel shows the distribution of spurs bearing 1 to 5 fruits as affected by ABA rate. Frequency distributions were done prior to hand-thinning, but after natural (and ABA induced) fruit drop occurred. Data were collected from pre-selected scaffolds and are means of 4 replicates.

After 3 years of trials, however, thinning was not consistent and good thinning efficacy of ABA was only observed in 3 of the 5 trials (Table 1). The lack of consistent thinning by ABA, as observed in the 2013 and 2014 (19-year-old trees) Hood River trials (Table 1), suggests that other factors contributed to the thinning efficacy of ABA. Recently, Middelberg et al. (2014) demonstrated that the uptake of ABA at the leaf cuticle was unresponsive to fluctuations in temperature or humidity. Hence, poor ABA uptake as a function of differing application temperatures from year-to-year or site-to-site can likely be ruled out as a limiting factor to thinning efficacy.

Summary of 5 ABA thinning trials (site and tree age provided). Data are percentage fruit set before hand thinning relative to controls (i.e., treatment fruit set as a percentage of control fruit set).

ABA (ppm)	2012 Hood River 8-year-old	2013 Hood River 18-year-old	2013 Parkdale 9-year-old	2014 Hood River 19-year-old	2014 Hood River 9-year-old
0	100	100	100	100	100
50		100	71	95	
100		100	57	86	
125	59				65
150		96			
200		78	18	79	
250	12				
400			7	58	
500	2				

ABA applied ~10 mm fruit diameter.

In fact, photosynthesis data support that ABA was taken up in the 2013 Hood River trial, despite our instrument issues described above. In that trial, photosynthesis of 200 ppm ABA leaves, 6 days after the 10 mm application timing, was 72% of control levels (Figure 3). In comparison, photosynthesis

of leaves at Parkdale treated with 200 ppm ABA was 60% of control levels on day 4 and 73% of control levels by day 8 from treatment application (Figure 3). The effects are harder to see for the lower rates of ABA (50 and 100 ppm) because they had largely disappeared by day 6 in Hood River (~10% reduced from control levels) and were undetectable by day 8 in Parkdale (Figure 3). In fact, at Parkdale photosynthesis of leaves treated with 100 ppm ABA recovered to ~82% of control levels by day 4 from application.

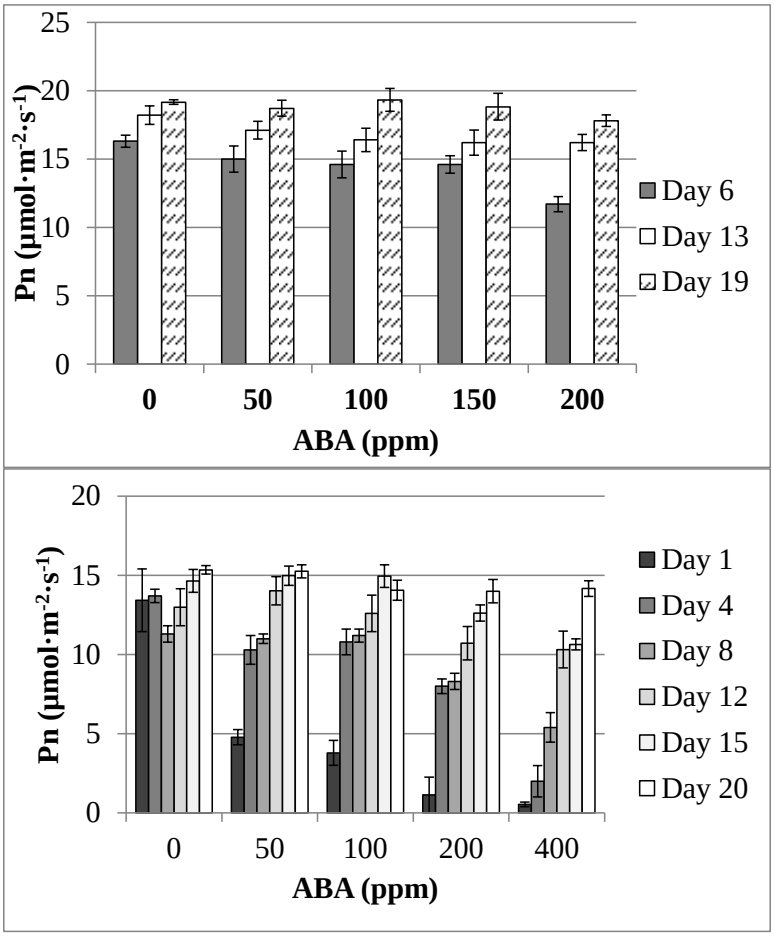


Figure 3. Photosynthesis of pear leaves 6, 13 and 19 days after applications of ABA at Hood River 2013 (above), and more frequently at Parkdale 2013 (below). Equipment malfunction precluded comprehensive measurements in Hood River. Data are means of 4 replicates (n=4).

The most efficacious rate of ABA for commercial thinning would likely fall between 100 and 125 ppm (as seen for trials whose columns are highlighted in grey in Table 1). Clearly, at these rates the effect of ABA on gas exchange (photosynthesis) is quite transient. Presumably, ABA thins by two possible modes of action. The first may be attributed to hormonal effects on fruits which likely involves ethylene (ABA stimulates ethylene synthesis) and possibly direct effects of ABA, though other hormonal interactions cannot be ignored. The second is an indirect effect, via carbon reduction associated with limited photosynthesis. Many chemical thinners work, in part, on this principle and thinning is the outcome of fruit competition for limited available carbohydrates (i.e., demand exceeds supply and weak fruit are at a competitive disadvantage). However, pear trees are quite large and likely have sufficient reserve carbohydrates (in wood and root tissues) to supplement such a short interruption in photosynthate production (i.e., only a day or two of severe reductions and up to 3 to 5

days at 70% to 80% of optimal levels). In fact, average tree age at trials where good thinning was observed was 9-years, while those that appeared unresponsive to ABA were double in age (Table 1). This observation implies that the thinning activity of ABA may be better related to the compound's effect on carbon balance and gas exchange than hormone balance, per se.

In 2012 and 2013, we plotted daily solar radiation (light) values for each of the three trials (Figure 4). Interestingly, a general relationship was apparent between thinning and light intensity for the 10-day period after ABA application. Sites where thinning was strong (Hood River 2012 and Parkdale 2013) were also plagued by low light levels (typical cloudy spring conditions in OR); the poor thinning observed in 2013 at the Hood River site experienced full sun conditions for the entire 10-day period after ABA application (Figure 4). These data, though only correlative, provide additional support that ABA thinning is dependent upon the carbohydrate status in the plant. Incidentally, these data (carbohydrate status leading up to thinning in combination with forecasted conditions) largely form the basis for carbon driven (deficit : surplus) thinning models (i.e., Malusim).

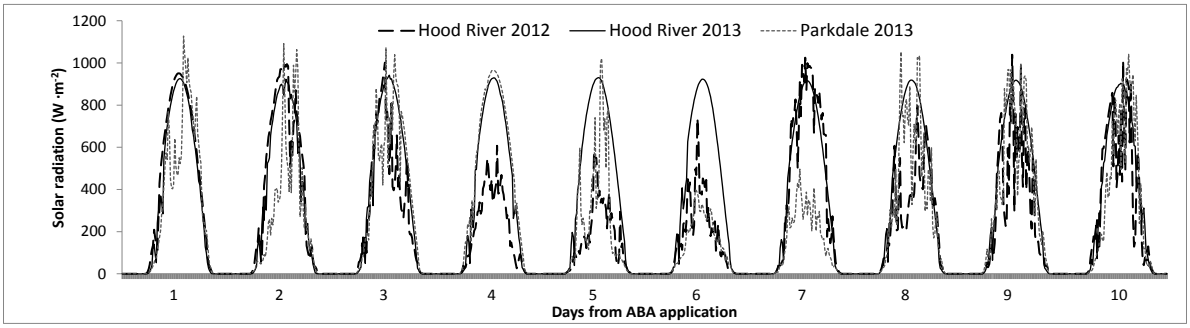


Figure 4. Daily solar radiation levels throughout the 10-day period immediately succeeding ABA applications at the 10 mm timing for 2012 and 2013 trials. Cloudy, low-light conditions in Hood River (2012) and Parkdale (2013) contrast the sunny period immediately following the Hood River 2013 experiment. Data were calculated from meteorological stations in Hood River and Parkdale.

Based on this assumption, we hypothesized that a lack of thinning (2013 Hood River and later observed in 2014) was attributed to the combination of a short-lived reduction in photosynthesis elicited by ABA and high light (sunny) conditions following treatment. In contrast, the high thinning achieved by ABA in 2012 and 2013 Parkdale were commensurate with several weeks of cloudy, overcast weather. In 2014 we designed a factorial trial to test this hypothesis.

Shade houses were constructed out of shade cloth (either 30% or 60% shade) and pvc tubing. Steel fence posts were inserted into the orchard and pre-assembled shade houses were fitted over entire trees and secured to fence posts immediately after ABA (125 ppm) application on 29-April. Control trees were left uncovered. There were a total of 6 treatments: 1) Uncovered control; 2) uncovered control + 125 ppm ABA; 3) 30% shade; 4) 30% shade +125 ppm ABA; 5) 60% Shade; and, 6) 60% shade +125 ppm ABA. Treatments were randomized in rows (blocks) and replicated 4 times.

Photos. Overview of shade experiment plot (top) and close-up of one replicate 60% shade house (left) and 30% shade house (right).

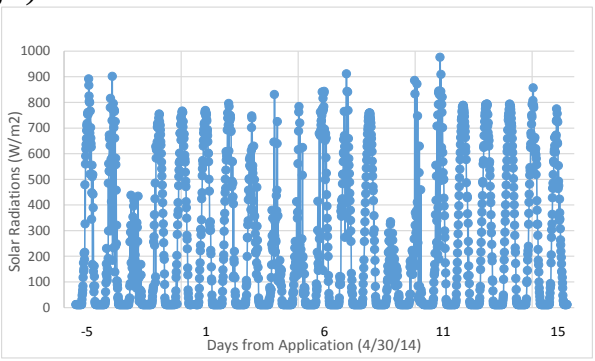


Figure 5. Daily solar radiation -5 days to +15 days after ABA and shade application.

Using forecasted weather, we targeted a cloud-free week between petal fall and 10 mm fruit size to conduct the experiment. Despite our best efforts, two partial-sun/cloud days occurred during the first week of the experimental period (Figure 5). Fruit set of ABA 125 ppm trees was ~35% reduced relative to untreated trees (Table 1 and Figure 6). Thirty percent shade alone reduced fruit set, compared to unshaded controls, but ABA 125 ppm applied to these trees did not improve the response. The highest level of shade resulted in more than 50% reduction in control fruit set. The addition of ABA did not, however, drastically alter thinning. These data indicate that both shade and ABA have the potential to thin, but when combined, the effect was not additive.

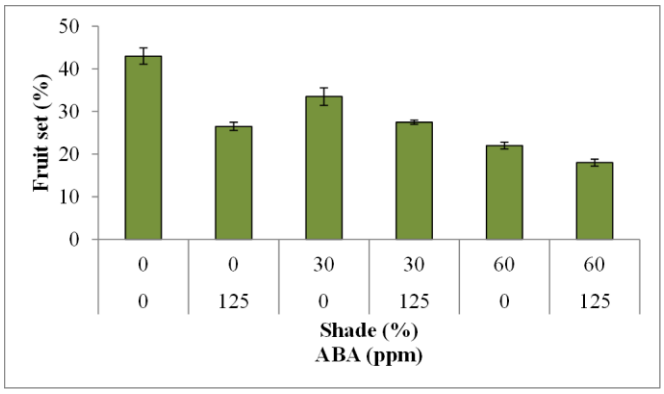
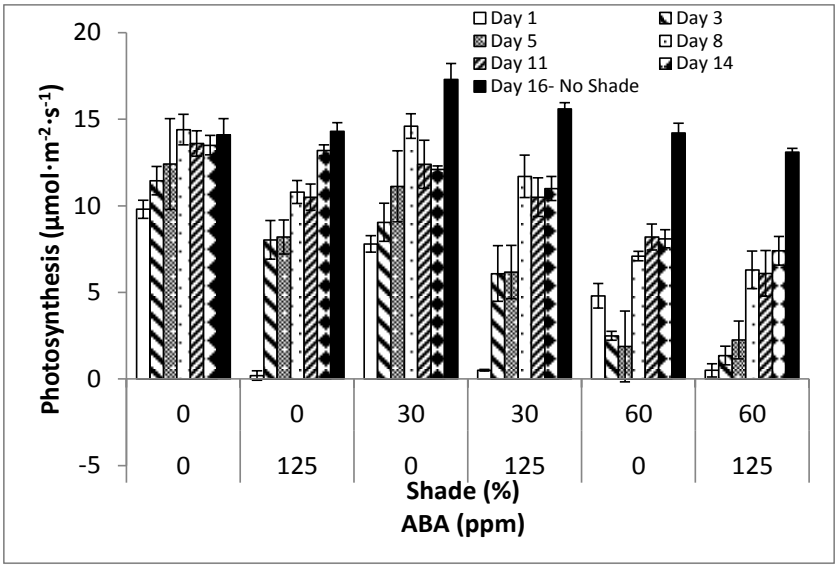
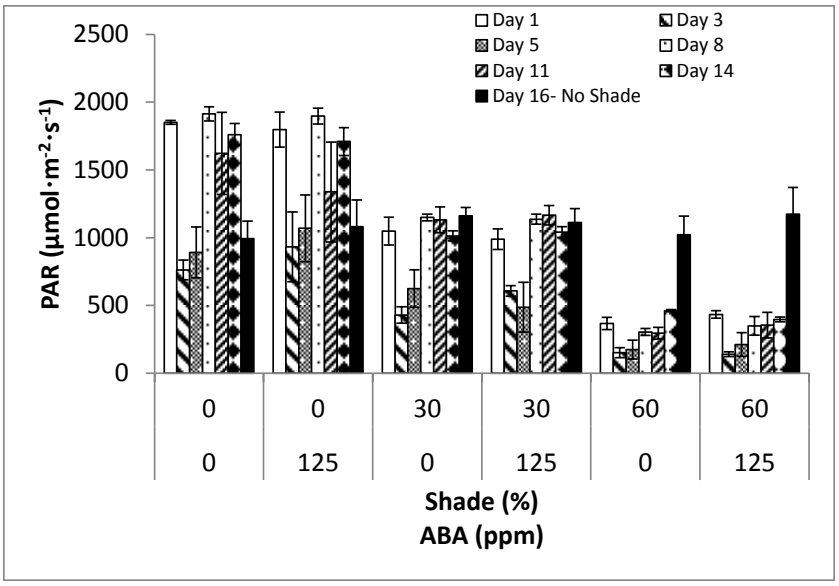


Figure 6. Fruit set as affected by ABA 125 ppm and/or shade (30% or 60%). Trees were 9-year-old Bartlett. Data are from 4 reps.

125 ppm ABA markedly reduced photosynthesis of control trees by 95% on day 1, but a recovery to 75-80% of control levels had occurred by day 3 (Figure 7 top panel). Photosynthesis remained ~20% reduced until day 11. Leaf photosynthesis of trees shaded 30% were reduced to levels similar to those induced by ABA on its own (i.e., without shade), with the exception of day 1 when photosynthesis was nearly 0 for ABA-treated trees. Only minor reductions in photosynthesis were observed for 30% shade leaves relative to unshaded trees, indicating that light was non-limiting for maximum photosynthesis. In fact, PAR (light levels) measured at the leaf surface of 30% shade trees was above saturating conditions (~750 to 1,000 units PAR) for leaf photosynthesis on most days (Figure 7 bottom panel). Despite the fact that these trees were small, this would not likely be the case for the whole canopy given that internal canopy leaves would experience more shade. The slightly lower photosynthetic rates of the 30% shade + ABA leaves during the first 5 days of the experiment had only minor effects on thinning relative to 30% shade alone (Figures 6 and 7 top panel). For trees shaded 60%, leaf photosynthesis was markedly reduced, as were light levels (Figure 7). Under this level of shade, interestingly, ABA did not augment the depression in photosynthesis. These data do not support the additive effects of ABA and shade on fruit set.





ABA did not reduce fruit size in the shade x ABA experiment (Table 2) as was the case for all other experiments (data not shown). In fact, when measured, fruit growth rates were unaffected by ABA rate (data not shown). Fruit quality (firmness, soluble solids and titratable acidity) was also unaffected at harvest and following ripening (data not shown).

Effect of ABA and Shade on Hand thinnning and harvest parameters.

Treatment		Fruits hand thinned	Harvest		
ABA	Shade	no./tree	Fruits/tree	lbs/tree	Fruit sz (g)
0 ppm	0%	18 a	225	21.9	193 d
ABA 125 ppm	0%	3 b	142	15	211 bc
0 ppm	30%	11 ab	165	15.1	197 cd
ABA 125 ppm	30%	3 b	144	16.8	215 b
0 ppm	60%	3 b	166	14.6	195 d
ABA 125 ppm	60%	0 bc	142	16.6	252 a
Pr (>F)		0.01	0.1	0.23	0.012

In summary, these results present somewhat of a challenge for moving forward with additional work on ABA. Ample carbon supplies of older trees might preclude the thinning activity of ABA for pear. When sunny, high-light conditions occurred during the first few days from application, ABA did not induce fruit drop. However, we demonstrated that ABA thinning was not drastically altered by shade when provided at a relatively high level (i.e., 60%). The effects of ABA and shade do not appear to be additive. Increasing ABA rate is not a solution due to phytotoxicity at high rates.

Executive Summary

Between 2012 and 2014, a total of 5 trials were administered to evaluate the thinning efficacy of ABA (ProTone®, Valent Biosciences Corp.) on Bartlett pear trees. Thinning was rate-responsive but inconsistent from year-to-year: Strong thinning was observed in 60% of the trials and insignificant thinning in 40%. When ABA thinned, the most effective rate was ~100-150 ppm; higher concentrations removed too many fruits and lower concentrations not enough. When effective, ABA significantly increased the proportion of blank spurs and spurs with a fruit density of 1. The frequency of multiple-fruited spurs was reduced as ABA rate increased.

ABA reduced photosynthesis, indirectly, by reducing stomatal conductance (partial closure of stomates). The rate of photosynthesis was inversely related to the rate of ABA. One hour after ABA application, photosynthesis was completely inhibited. Four days later, 100 ppm ABA leaves were photosynthesizing at~ 80% of control levels and nearly 100% by day 12. High rates of ABA (400 ppm) reduced control photosynthesis by roughly half for a period of 8 days beyond application. Photosynthesis only recovered to 75% of control levels by 15 days after application. Moreover, 400 ppm ABA was phytotoxic to leaves resulting in necrotic leaf spots and partial defoliation.

ABA, on its own, did not appear to produce enough carbon stress to elicit sufficient thinning when sunny conditions prevailed the week after treatment. However, when combined with cloudy, overcast weather, thinning efficacy was high. Daily solar radiation (light) data supported this observation.

Therefore, in 2014 we conducted a shade x ABA trial to test the relationship of these factors on thinning. Pre-assembled shade houses, constructed from PVC and 30% or 60% shade cloth, were placed over whole canopies immediately after ABA application and were maintained for 15 d. There were a total of 6 treatments: 1) Uncovered control [0% shade]; 2) uncovered control [0% shade] + 125 ppm ABA; 3) 30% shade; 4) 30% shade +125 ppm ABA; 5) 60% shade; and, 6) 60% shade +125 ppm ABA. Trees treated with ABA alone retained ~35% fewer fruits compared to uncovered controls. 30% shade produced fruit drop similar to ABA on its own, but the addition of 125 ppm ABA to 30% shaded trees did not improve the effect. Trees provided 60% shade had roughly half the fruit retention of control trees; at this level of shade, ABA only slightly increased the thinning response.

Application of 125 ppm ABA to unshaded trees reduced photosynthesis by 95% on day 1, but a recovery to 75-80% of control photosynthesis occurred by day 3 and remained ~20% reduced until day 11. Leaf photosynthesis of trees shaded 30% was only slightly reduced from unshaded levels for the first few days, indicating that light was nonlimiting for photosynthesis. PAR data at the leaf surface supported this conclusion. The addition of ABA to trees provided 30% shade only slightly lowered leaf photosynthesis relative to 30% shade alone. At 60% shade, photosynthesis was markedly reduced. Under this level of shade, interestingly, ABA did not augment the effect.

Tree age or, more importantly, carbon stores, may have played a key role in the inconsistent results. For the 3 trials where thinning was effective, trees were relatively small (between 7-9 years-old); however, the 2 trials in which no thinning was observed comprised 18 and 19-year-old trees, possessing markedly larger canopies and, presumably, root systems.

When sunny, high-light conditions occurred during the first few days from application, ABA did not induce fruit drop. However, we demonstrated that ABA thinning was not drastically altered by shade when provided at a relatively high level (i.e., 60%). The effects of ABA and shade do not appear to be additive. Improving thinning by increasing ABA rate is not a viable solution due to the phytotoxicity observed at high rates.

FINAL PROJECT REPORT

Project Title: Pear scion trials in the Pacific Northwest

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Other funding sources: None

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

Budget History:

Budget 1 – Kate Evans (WSU)

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

Budget 2 – Todd Einhorn (OSU_MCAREC)

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

Budget 3 – Tom Auvil (WTFRC)

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

Budget 4 – Grower reimbursement (WTFRC)

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

Budget 5 – Richard Bell (USDA-ARS)

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

OBJECTIVES

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

SIGNIFICANT FINDINGS

- Randomized replicated plantings of the USDA-ARS pear scion selections were established in two sites in WA (Wenatchee Valley and Wapato) and one site in OR (MCAREC, Hood River). Trees are growing well and no mortality occurred at any of the sites.
- The two new Coregeo Australian scions ('Deliza' [ANP-0131] and 'Lanya' [ANP-0118]) have been planted at MCAREC, Hood River. Uniform, good growth accrued in the first season.
- 'Lanya' (ANP-0118) has been planted at the two Washington sites and additional trees of 'Deliza' (ANP-0131) have been budded for planting in 2016.

RESULTS & DISCUSSION

Objective 1:

The five new USDA-ARS scion selections were planted in two sites in Washington and one site in Oregon. Trees were planted in Wapato (Chuck Peters), in the Wenatchee Valley (Josh Koempel) and at MCAREC, Hood River. All trees are propagated on OH×F87 rootstock.

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

In Hood River, Oregon, trees were established 5 ft in-row and 12 ft between rows in a randomized complete block design. Trees were left un-headed at planting. The few pre-existing limbs that exceeded half the size of the trunk were removed at planting with a 'Dutch' cut to encourage a new, flatter shoot and improve the uniformity of shoots. All remaining limbs were spread to widen branch angels (between 30-45 degrees). Trees were tied to the wire in spring of 2014 and trained to a 10° V whereby each tree was tipped in the opposite direction. Tree architecture is palmette, with each tree possessing a central leader and two primary scaffolds (originating from a similar point and opposite one another). Primary scaffolds were trained to the trellis wires at an angle of ~45 degrees from the horizontal to fill space. Throughout the season, large limbs were removed with a thinning cut (leaving a stub to generate a flatter, weaker limb) and all vertical limbs were pulled down to ~30-45 degree angles. Irrigation was provided at a frequency of two to three times per week for 4 hours per set. Urea was applied (side-dressed) at a rate of 10 lbs per acre every 10-14 days beginning June 1. There was no mortality and all trees of a rep grew uniformly; however, scion selections varied in their vigor and were significantly smaller than 'd'Anjou' and 'Bartlett' (Table 1).

Trunk diameters were recorded fall/winter 2014 and cross-sectional areas calculated (Table 1).

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

Scions	*Trunk cross-sectional area (cm^2)*		
	Wapato	Wenatchee Valley	MCAREC
US-69426-038	5.3	7.7ab	8.1 dc
US-71655-014	6.4	6.8ab	n/a
US-84907-069	5.1	4.5b	6.3 d
US-84907-078	6.4	8.6a	9.2 c
US-84907-166	4.7	6.3ab	9.8 bc
Anjou	1.6	5.8ab	11.6 ab
Bartlett	8.5	8.5a	12.3 a

Note: Each site mean analyzed independently;

Some of the selections started to fruit in 2014 but the overall volume of fruit was insufficient for more than basic observation (Table 2). No fruit was harvested in Hood River.

Table 2. Total and mean fruit weight (harvest 2014) from the Washington plantings.

Wapato

	total fruit weight (lb)	total fruit number	mean fruit weight (lb)
US-69426-038	0	0	0
US-71655-014	0.76	2	0.38
US-84907-069	1.49	4	0.37
US-84907-078	0	0	0
US-84907-166	0	0	0
Anjou	0	0	0
Bartlett	0	0	0

Wenatchee Valley

	total fruit weight (lb)	total fruit number	mean fruit weight (lb)
US-69426-038	0.88	3	0.29
US-71655-014	0	0	0
US-84907-069	0.72	1	0.72
US-84907-078	0	0	0
US-84907-166	11.03	27	0.41
Anjou	0	0	0
Bartlett	1.16	2	0.58

Objective 2:

The tree count from the nursery of the two new Coregeo Australian scions was lower than estimated: 'Deliza' [ANP-0131] 40 trees, and 'Lanya' [ANP-0118] 257 trees. The decision was taken that there were too few trees of ANP-0131 to distribute to all three sites so all 40 trees were planted at MCAREC, Hood River. A further order for the extra trees for the trial was placed with C&O nursery; trees should be available for planting in 2016. All trees are on OH×F87.

For 'Lanya' (ANP-0118), trees were distributed as follows: 100 trees to MCAREC, 78 trees to Josh Koempel's planting at Dryden and 79 trees to Chuck Peters at Wapato.

In Hood River, Oregon, trees were established 5 ft in-row and 12 ft between rows in a randomized complete block design. Trees were delayed-dormant headed when ~ 6 inches of new shoot growth had accumulated. Heading was performed to improve vigor given the weak, spindly starting material received from the nursery. The few, pre-existing limbs that exceeded half the size of the trunk were removed at heading with a 'Dutch' cut to encourage a new shoot and improve the uniformity of shoots. All remaining limbs were spread to widen branch angels (between 30-45 degrees). Trees were tied to wires in a 10° V as described above under Objective 1 in Hood River. Throughout the season limbs were trained to wires and overly-vigorous shoots were removed leaving a stub. Irrigation was provided at a frequency of three times per week for 3 hours per set. Urea was applied (side-dressed) at a rate of 10 lbs per acre every 10-14 days beginning June 1. Excellent, uniform tree growth was realized and cultivars did not significantly differ in trunk size after year one (Table 3).

All trees were planted in the same spacings and orchard systems as described for the USDA-ARS selections in Objective 1.

Table 3. Trunk size of two new Coregeo Australian scions: 'Deliza' [ANP-0131] and 'Lanya' [ANP-0118] measured fall of 2014 (year 1 in the orchard) at MCAREC.

	Trunk cross-sectional area (cm^2)
ANP-0118 ('Lanya')	14.6
ANP-0131 ('Deliza')	13.1
P>F	0.376

EXECUTIVE SUMMARY

Five new scion selections from the USDA-ARS program are now established in replicated trial plantings at two sites in Washington (Blewitt and Wapato) and at the MCAREC, Hood River, Oregon. These trees will enter their third leaf in 2015 and should begin producing sufficient fruit for evaluation.

Two new Coregeo Australian scions (‘Deliza’ [ANP-0131] and ‘Lanya’ [ANP-0118]) were also established, although ANP-0131 is only present in Oregon until additional trees can be propagated. All trees are on OH×F87 rootstock.

The next stage of this work will assess the precocity (bloom and fruit set), production, and fruit quality to determine the viability of these cultivars in the Pacific Northwest. We now propose to transition the leadership of the follow-on project to Dr. Todd Einhorn with collaboration with Dr. Yan Wang for post-harvest evaluation of the fruit.

FINAL PROJECT REPORT

Project Title: Genotype work for pear

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

Total Project Funding: **Year 1:** $25,000

Budget History:

Item	**Year 1:**
Miscellaneous[1]	$25,000
Total	$25,000

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

OBJECTIVES

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

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

SIGNIFICANT FINDINGS

- New potential pear rootstock germplasm was imported into the US from the University of Bologna, Italy.

RESULTS & DISCUSSION

Initial delays in this project were primarily due to the complex issues surrounding the importation of germplasm into the US through quarantine and the negotiation of Material Transfer Agreements (MTA).

Several different international sources of possible new germplasm were identified (EMR UK, INRA France, IRTA Spain and the University of Bologna Italy) and MTAs were drafted and submitted. MTAs were approved by EMR and the University of Bologna but so far no signed MTA from France or Italy has been achieved.

With the appointment of Dr. Stefano Musacchi to WSU and, therefore, the potential for complications with further collaboration with the University of Bologna program, the decision was taken to focus on the Musacchi germplasm within this project. Dr. Musacchi identified eight selections that he considered would have the most value for the PNW as new rootstocks. The population structure analysis (Dhingra PR13-109) was also taken into account to by Dr. Musacchi when selecting the germplasm to ensure a diverse set.

This germplasm has been imported into the US by two different routes, to better ensure its success through the quarantine process. Propagating wood was sent directly to APHIS Beltsville in January 2015 where it will go through the routine testing required for all imported wood (which can take an indefinite amount of time). In addition, further wood was sent to the Clean Plant Center, Prosser in January 2015 where testing will be expedited thus allowing the preliminary release of wood in less than two years if no problems are detected.

The germplasm accessed is described in Table 1.

The contacts with other programs and access to other germplasm are being exploited by the population structure analysis in the Dhingra lab.

Table 1: Pear accessions imported from University of Bologna, Italy.

	cross	Features	nursery at UNIBO grafted with
P2	US309 × Nijisseiki	expanded habit, medium vigor, short internodes, early bloom, many fruit	
P5	Abbé Fétel × California	up-right habit , vigorous, abundant bloom	Abbé Fétel Anjou Lucy Sweet
P6	Passa Crassana × Decana del Comizio	small and compact tree, no fruit	Abbé Fétel Anjou
P7	Passa Crassana × Decana del Comizio	medium vigor, low bloom, some big fruit	
P8	Passa Crassana × Decana del Comizio	small and compact trees, abundant bloom, some fruit	Abbé Fétel Anjou Lucy Sweet
P9	Abbé Fétel × California	expanded habit; but low vigor	
P14	Abbé Fétel × sel.79504074	expanded habit, very compact and small tree, high bloom, medium-big fruit size, green leaf	
P16	Abbé Fétel × sel.79504074	medium low vigor, basitone habit with close shoots, short internodes, abundant bloom, big fruit	

EXECUTIVE SUMMARY

Eight new possible pear rootstock accessions have been imported into the US and are currently in quarantine. The selections were chosen as the most promising by Dr. Stefano Musacchi from the University of Bologna breeding program that he managed prior to his move to WSU.

Once through the quarantine process, we expect that these rootstocks will be established in tissue culture and micropropagated to provide replicate stocks for a full rootstock trial with one or more standard scions. All these rootstocks have previously proved to be suitable for micropropagation; unfortunately we were not able to access this tissue for importation. This is, however, beyond the scope of this project.

Relationships will be maintained with the other breeding programs mentioned with a view to possibly importing more accessions in the future.

FINAL PROJECT REPORT **YEAR**: 3 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:** $13,197

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	1,667
Benefits	2,168	2,233	1,150
Wages			
Benefits			
Equipment			
Supplies			
Travel[2]	500	1,300	650
Miscellaneous			
Total	$5,810	$6,769	$3,467

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

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

Item	2012	2013	2014
Salaries[1]	3,000	3,500	3,600
Benefits[1]	1,050	1,225	1,260
Wages[1]	2,675	2,800	2,900
Benefits	800	840	870
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'. OH×F 87 will be used as the standard.

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

Significant Findings:

Cumulative

- After 6 years, OH×F 87 consistently ranked highest for yield and yield efficiency compared to Horner 10 and Horner 4 at four separate trials. In some cases, numerical differences were not significant. For a given tree density, the relatively high yield efficiency of OH×F 87 suggests that it is more effective allocating carbon to fruits relative to vegetative growth.
- Horner 4 yields were either similar to or slightly less than OH×F 87, with the exception of one Bartlett site where Horner 4 produced markedly lower yields than OH×F 87. Yield efficiency of Horner 4, however, was typically lower than OH×F 87, due to its higher vigor. Interestingly, precocity was not delayed by the invigorating effect of Horner 4. Generally, annual variations in fruit size obscured rootstock effects; however, Horner 4 typically had the largest fruit size.
- A general trend in tree size was Horner 4 ≥ OH×F 87 ≥ Horner 10. The largest differences were observed for 'd'Anjou', where trees were ~40% larger on Horner 4 than either OH×F 87 or Horner 10.
- In three of four trials, Horner 10 produced the lowest yields, poorest yield efficiency and smallest fruit size. For 'Bosc', yield efficiency on Horner 10 was intermediate, primarily due to the rootstock's significant reduction of Bosc tree size.
- Tree mortality varied across sites (for a range of reasons) but was unrelated to rootstock genotype.

2014

- 'Bartlett' trees at Wapato produced excellent yields in the sixth leaf for all rootstocks (projected per acre yields between 55-59 bins). Horner 10 yields (44 bins) were significantly less than OH×F 87. Tree size was only slightly larger on Horner 4. Fruit size was similar among rootstocks but on the smaller end (average box size between 100s and 110s).
- 'Bartlett' yields at Methow were nearly double for OH×F 87 trees (47 bins/acre) compared to the Horner rootstocks (~25 bins/acre). Fruit size was good (size 80s and 90s) on all rootstocks.
- 'GR Bosc' yields were ~ 20% to 30% lower than the previous year, presumably as a result of biennial bearing. Comparatively, rootstocks performed similarly in both years; OH×F 87 had higher yields than the Horner rootstocks. Trees on Horner 10 were ~25% smaller compared to other rootstocks.
- Sixth leaf 'd'Anjou' yields were excellent for trees on OH×F 87 and Horner 4 (~42 bins/acre). Although Horner 10 produced an equivalent number of fruit per tree as OH×F 87 and Horner 4, fruit size was markedly smaller (box size 135, compared to 100s), resulting in 30% less total yield (29 bins/acre)… of box size 135 fruit. Trees on Horner 4 were 55% larger than the other rootstocks.

Results and Discussion:

1. Sites

Wapato (Bartlett and Bosc) had roughly 40% of the planting affected by fire blight. Rootstock genotype did not relate to fire blight susceptibility (i.e., trees on all rootstocks were similarly affected). As indicated in the 2013 report, the Parkdale, OR 'd'Anjou' site was removed after the 2013 season based on a high incidence of fire blight and Pseudomonas infection. Despite severe fires surrounding the Methow (Bartlett) site, trees were largely unaffected. Averaged across sites, tree mortality remained at 2013 levels of 33%, 25% and 29% for OH×F 87, Horner 4 and Horner 10, respectively.

Details pertaining to the existing trial sites are provided below:

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

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

For these trials, cultivar is confounded with site; therefore, overall performance comparison among rootstocks (i.e., averaged across sites) carries little value. While general trends in the measured parameters can be observed, unique habits of cultivars and/or cultivar:rootstock combinations can disproportionately alter the data. Therefore, rootstocks will only be compared 'within' individual cultivars.

2. Rootstock effects on Cultivars

D'Anjou'

Yields of 42 bins per acre in the 6th leaf are considered excellent for 'd'Anjou' (Table 1). These yields were achieved on OH×F 87 and Horner 4. Horner 10, in contrast, produced 30% less yield despite setting a similar number of fruits per tree (~250 fruits per tree). The yield reduction was attributed to fruit size; 'D'Anjou' pears on Horner 10 peaked on box size 135. Tree size on Horner 10 was no different than tree size on OH×F 87 underscoring the direct rootstock effect on fruit size. In Hood River, trees on Horner 4 were 55% larger than trees on Horner 10 or OH×F 87. This was an

effect observed in preliminary trials with Horner 4 (all performed with 'd'Anjou' as the cultivar) and can be seen in the mother block (Horner 4 is in the upper 5% for tree size in a population of over 450 individuals). Interestingly, the magnitude of vigor induced by Horner 4 compared to other rootstocks varies with the scion and, to a lesser degree, site. Horner 4 may offer distinct advantages over alternative rootstocks of comparable vigor in low-fertility soils, lower density configurations, or as replacement trees in established blocks. An evaluation of Horner 4 in replant sites might also prove useful. Trees on Horner 4 have filled their in-row space of 6 ft., but given their high bearing potential (fruit buds look good for 2015) the grower is comfortable managing the trees. In contrast to this planting, Horner 4 did not perform so well in a recently concluded 10-year evaluation of 'd'Anjou' performance on ten promising pear rootstocks. Horner 4 ranked 5th in yield, 9th in yield efficiency and 1st in trunk size. OH×F 87 was the best performing rootstock in that trial for yield and yield efficiency, despite ranking 7th in tree size.

Table 1. Horner Rootstock On-Farm Trials. 2014 and cumulative (through 6th leaf) production at four sites in the PNW: 'd'Anjou', Hood River, OR; Bartlett, Methow, WA; Bartlett; Wapato, WA; Bosc, Wapato, WA.

	2014 #				Cumulative		
d'Anjou	TCA (cm^2)	Yield (lbs/tree)	Fruit wt. (g)	Proj. production (bins/a)	Yield (lbs/tree)	Yield effic. (kg/cm^2 TCA)	Fruit wt. (g)
OH×F87	57.5 b	107.2 a	185 a	41.5 a	139.2 a	1.1 a	214 ab
Horner 4	87.2 a	110.5 a	204 a	42.4 a	139 a	0.72 b	225 a
Horner 10	55.4 b	78.7 b	150 b	28.7 b	99.7 b	0.79 b	200 b

#6th leaf production; 427 trees/acre

	2014 #				Cumulative		
Bartlett	TCA (cm^2)	Yield (lbs/tree)	Fruit wt. (g)	Proj. production (bins/a)	Yield (lbs/tree)	Yield effic. (kg/cm^2 TCA)	Fruit wt. (g)
OH×F87	32.7	56.8 a	232	46.6 a	114.3 a	1.62 a	202 b
Horner 4	33.4	28.3 b	253	23.3 b	79.8 b	1.1 b	228 a
Horner 10	29.6	32.9 b	228	27.3 b	77.6 b	1.17 b	197 b

#6th leaf production; 908 trees/acre

	2014 #				Cumulative		
Bartlett	TCA (cm^2)	Yield (lbs/tree)	Fruit wt. (g)	Proj. production (bins/a)	Yield (lbs/tree)	Yield effic. (kg/cm^2 TCA)	Fruit wt. (g)
OH×F87	30.2 ab	58.1	194	57.3	150.9 a	2.26 a	191
Horner 4	33.1 a	59.9	190	59.1	140.3 ab	1.93 ab	195
Horner 10	28.2 b	55.3	195	54.4	116.5 b	1.78 b	191

#6th leaf production; 1,089 trees/acre

	2014 #				Cumulative		
Bosc	TCA (cm^2)	Yield (lbs/tree)	Fruit wt. (g)	Proj. production (bins/a)	Yield (lbs/tree)	Yield effic. (kg/cm^2 TCA)	Fruit wt. (g)
OH×F87	42 a	30.9	247	30.4	70.9	1.01 a	245
Horner 4	46.3 a	22.3	246	22	67.4	0.66 b	257
Horner 10	33.1 b	23.9	230	23.6	57.2	0.84 ab	242

#6th leaf production; 1,089 trees/acre

Data- 6 reps for 'd'Anjou. All other sites 5 reps (5 contiguous trees/rep).

‘Golden Russet Bosc’

‘Bosc’ trees attained good 5th leaf yields in 2013 with excellent fruit size (70s). Yields were reduced in 2014, however, by ~20%. This reduction in yield was attributed to both biennial bearing and corrective pruning of fire-blight infected wood (reducing bearing volume). Rootstocks did not affect this biennial swing in bearing. OH×F 87 produced the highest yields in 2013 (significantly) and 2014 (nonsignificantly). In 2014, Horner 4 and Horner 10 produced similar tree yields, but fruits were larger on Horner 4 (Table 1). Fruit size was excellent, however, for all rootstocks. Tree size was positively influenced by Horner 10; producing trees ~25% smaller than the other rootstocks. In fact, this was the only trial where Horner 10 produced significantly smaller trees than OH×F 87. Despite the dwarfing conferred by Horner 10, yield efficiency, over the entire project, was highest for OH×F 87 (Table 1).

‘Bartlett’.

Two sites were trialed with fairly similar outcomes with respect to yield and yield efficiency (Table 1). OH×F 87 performed best, but Horner 4 was similar at one trial (Wapato). In Wapato, ‘Bartlett’ production exceeded 50 bins per acre for the third consecutive year (~58 bins per acre for Horner 4 and OH×F 87 in 2014). Fruit size was on the low end of box size 100. Tree size was largest on Horner 4 but differences among rootstocks were not large (~15% difference between the largest and smallest trees). The cumulative yield efficiency of OH×F 87 through the 6th leaf (2.26 kg/cm^2 TCA) is roughly two-fold that achieved in comparable rootstock evaluation trials in the US (Elkins et al., 2011). Consistent high production was associated with good fertigation and irrigation practices. Horner 10 yields were high in 2014 despite being consistently low in the previous years. Overall, however, Horner 10 had significantly lower yields and yield efficiency compared to OH×F 87 despite similar tree size (Table 1).

At Methow, OH×F 87 produced significantly higher Bartlett yields than either of the Horner rootstocks (Table 1). Fruit size on Horner 4 was usually a box size larger than OH×F 87 each year, but at a cost of 30% less yield over the duration of the project (Table 1). Despite having lower yields and yield efficiency, Horner 10 had the smallest fruit size.

Executive Summary
Five trial sites were established in 2009 to test effects of Horner 4 and Horner 10 rootstocks on performance of 'GR-Bosc', 'd'Anjou', and 'Bartlett'. OHxF 87 was included as the control at each site. Trial sites were established as high-density plantings in commercial orchards. Cultivar selection, planting design and training system varied from site to site. Trees were well-managed at all sites.

After 6 years, OH×F 87 consistently ranked highest for yield and yield efficiency compared to Horner 10 and Horner 4 at four separate trials. In some cases, numerical differences were not significant. For a given tree density, the relatively high yield efficiency of OH×F 87 suggests that it is more effective allocating carbon to fruits relative to vegetative growth.

Horner 4 yields were either similar to or slightly less than OH×F 87, with the exception of one Bartlett site where Horner 4 produced markedly lower yields than OH×F 87. Yield efficiency of Horner 4, however, was typically lower than OH×F 87, due to its relatively higher vigor. Interestingly, precocity was not delayed by the invigorating effect of Horner 4. Generally, annual variations in fruit size obscured rootstock effects; however, Horner 4 typically had the largest fruit size.

A general trend in tree size was Horner 4 ≥ OH×F 87 ≥ Horner 10. The largest differences were observed for 'd'Anjou'; trees were ~40% larger on Horner 4 than either OH×F 87 or Horner 10. Given Horner 4's effect on tree size, we would not recommend the combination 'd'Anjou'/Horner 4 in high-density plantings. However, on difficult, weak sites, lower density configurations, or as replacement trees in established blocks, Horner 4 may have distinct advantages over comparably vigorous rootstocks.

In three of four trials, Horner 10 produced the lowest yields, poorest yield efficiency and smallest fruit size. We do not recommend continued trialing of Horner 10.

Given the high productivity of all sites, trees appear well balanced and manageable in their high-density configurations through 6th leaf production.

FINAL PROJECT REPORT **YEAR**: **3 of 3**

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

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Co-PI: Richard Bell
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Cooperators: Stefano Musacchi

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

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

Other funding sources: None

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Three Year Project Objectives:

1) Determine effective propagation methods for quince with commercial nursery partners.

2) Test graft compatibility of cold hardy quince rootstocks and commercial pear cultivars.

3) Determine fire-blight resistance/sensitivity of cold-hardy quince rootstocks.

4) Deliver 10-12 rootstock clones grafted to Bartlett and Anjou for field trials in Wenatchee and Hood River.

Significant Findings:

1. **Cutting Propagation/Stoolbed Establishment –** Rooting of cuttings (hardwood and softwood) ranged from 0% to 62%. In general, propagation by softwood cuttings was more successful than hardwood. Hormone dips improved rooting. Many of the quince accessions were observed to root more efficiently than OH x F clones (success rates between 0% and 7%). Rooted hardwood cuttings of 15 of the 22 cold-hardy accessions were successfully stablished in a stool bed at Helios Nursery.

2. **In vitro multiplication-**
    a. 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. A multiplication rate of 6 is considered good. Only 8 of the accessions had low multiplication rates.
    b. After a thrips outbreak in 2012, 70% of the cold-hardy quince clones were successfully cultured *in vitro* at N.A.Plants in 2013. All accessions were transplanted to media and successfully rooted. In most cases, the numbers of transplants per accession exceeded the number required for field trials.
    c. The 7 remaining clones are presently being cultured at North American Plants for multiplication.
    d. Cultures have been maintained for all accessions.

3. **Production of quince trees for fire blight field trial** – 15 of the 20 accessions were evaluated for fire blight susceptibility in 2013 and 2014 field trials. The level of susceptibility was much less than anticipated. Few infections spread into older wood, and no trees of any of the quince clones were lost to fire blight. Many of the accessions could apparently have a level of resistance sufficient for commercial use as rootstocks. It is recommended that these clones be inoculated in the coming year to verify the level of resistance.

4. **Liner production for field trials** - In 2014, a sufficient number of rootstock liners of ~15 accessions, received by Helios nursery as explants from N. A. Plants, were successfully grown to budding/grafting size. Half of each accession was budded/grafted in late summer 2014 with 'Comice' (selected as the interstem). The other half was left un-grafted. Plants will be grown out for the 2015 season. In late summer of 2015, the total number of plants per accession (50% with interstems and 50% without) will be divided into equal groups–one group will be budded to 'd'Anjou', the other to 'Bartlett'. Trees will either be established in field plots in spring 2016 as sleeping eyes or spring 2017 as 1-year-old scions. Initial compatibility between 'Comice' and quince accessions will be evaluated spring of 2015; compatibility between quince and 'Barteltt' and 'd'Anjou' will be evaluated fall of 2015.

Results and Discussion:

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

Propagation- *In vitro* initiation was successful on newly developed pear medium (Reed, USDA-ARS NCGR). Eight quince clones had multiplication rates >10 and 21 had multiplication rates ≥ 6 during in vitro establishment (Table 3). A multiplication rate of 6 or higher is considered good. Despite being delayed from our initial timeframe due to loss of these cultures to a thrips infestation (October, 2012), N.A. Plants rapidly multiplied a sufficient number of transplants for liner production. Fifteen of the 22 clones were cultured, transplanted, and rooted in 2013 at N. A. Plants (Table 1 and photos). Slight alterations to tissue culture media were made to optimize in-vitro production of the different genotypes. In 2014, plants were delivered to Helios Nursery and transplant to a liner bed. Acceptable growth accrued during 2014 and half the plants of each accession were budded to 'Comice' (selected as the interstem). Plants will be grown out in 2014 (half with interstems and half without). In late summer all plants will be T-budded to the scions ('d'Anjou' and 'Bartlett'). All clones are maintained in culture permitting rapid production of additional plants should the need arise.

In general, propagation by softwood cuttings was more successful than hardwood cuttings (Table 2), though some clones had superior rooting from softwood and others from hardwood cuttings. 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 2). 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). Only one clone (the hardiest of the population) did not root easily from either soft or hardwood cuttings.

Fire blight- 1). 2013 Planting. Fifteen of the 20 cold-hardy quince clones were evaluated for fire blight susceptibility (Table 3). Several additional quince accessions were evaluated; the cold hardy clones in Table 3 are highlighted in grey. For the entire population evaluated, mean shoot length varied from 60 mm for V-7 o. p. seedling3 to 301 mm for 'Avia from Gebeseud'. Mean lesion length varied from 0 mm for OHF 87 to 255 mm for IV-36 o. p. Lesion lengths and percent lesion lengths for the first inoculation were generally more severe. The mean percent lesion length varied from 0 for OHF 87 to 149 for V-46 o. p., indicating spread of infection into 1-year-old wood. Due to considerable variability among trees, there were many overlapping significance classes for all traits, but especially for mean percent lesion length. Based on lesion length and percent lesion length, the most resistant quince rootstocks were V-46 o.p. seedling 3, V-7 o. p. seedling 3, and ×Pyronia vetchii IRP 82-1. However, these values were only based on 2, 1 and 1 replicate trees. There were six additional clones which had percent lesion lengths of 20 mm or less. There was a great difference between V-46 o.p. and it's offspring V-46 o. p. seedling 3, exhibiting the most susceptible and most resistant disease reactions.

2). 2014 Planting. The 2014 planting included 10 of the 20 cold hardy quince accessions. As in the 2013 planting, additional quince were evaluated for fire blight susceptibility. Of the entire population evaluated, mean shoot length varied from 121 mm for W-4 to 300 for 'Avia' from Gebeseud. The mean lesion length varied from 0.1 mm for OHF 97 to 162 mm for 'Bartlett' seedling. The mean percent lesion length varied from 0.1 for OHF 97, indicating only one infected leaf, to 116 for 'Bartlett' seedling, indicating infection into 1-year old wood. The results for these resistant and susceptible standards are similar to previous results. Based on the percent lesion length, the most resistant quince clone was 'Avia' from Gebeseud, the second most hardy of the 20 hardy clones. However, there was

only one replicate tree of this clone. Quince C7/1, 'Bereczki', 'Megri' and Pigwa S-1 were the next least susceptible clones.

Overall, the level of susceptibility was much lower than expected, especially given the high inoculum concentration. However, the level of susceptibility of 'Bartlett' seedling rootstock was similar to many previous studies. The Spearman's correlations between plantings for shoot length, lesion length and percent lesion length were all non-significant.

Objectives not yet completed:
An evaluation of initial graft incompatibility (with the interstem, 'Comice') will take place this spring. An evaluation of T-budding success with the scions 'Bartlett' and 'd'Anjou' will be performed in fall of 2015.

Methods:
Fire Blight-

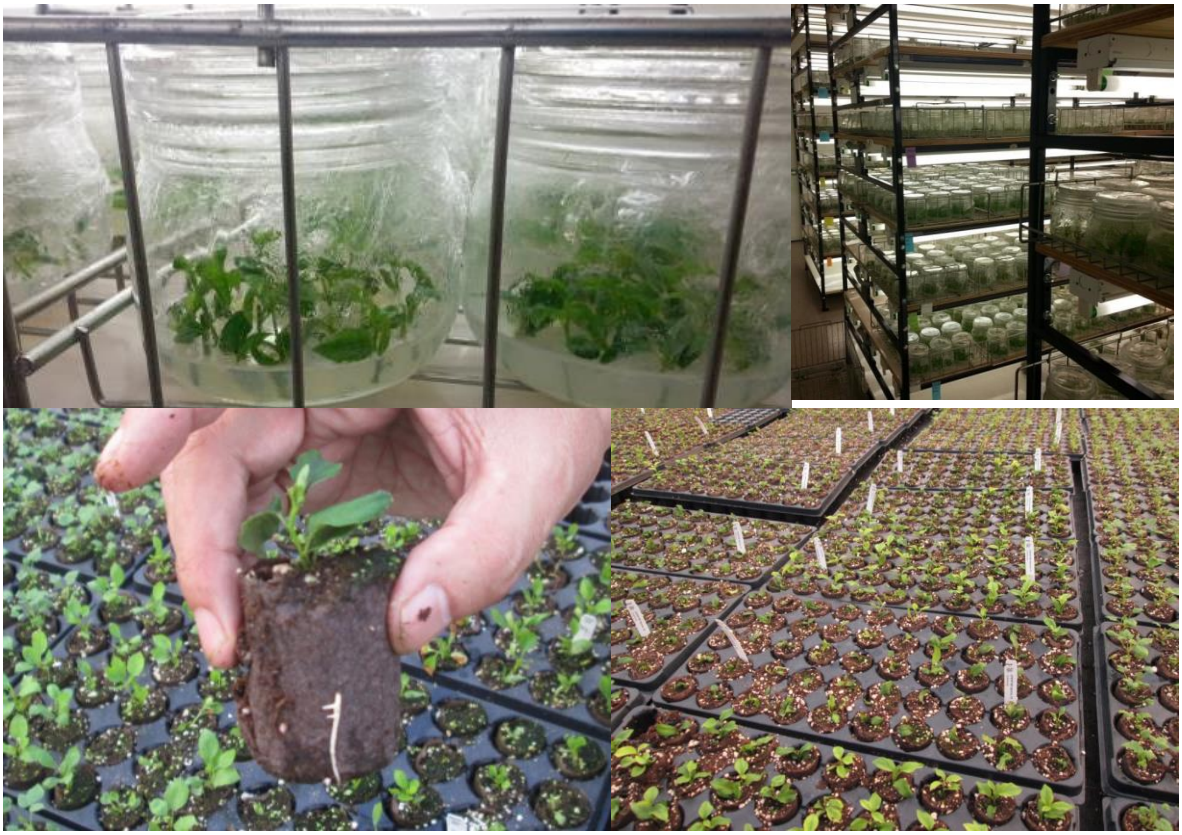
Thirty-five quince clones propagated and sent from the National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon were planted in the spring of 2013 in a field plot at the Appalachian Fruit Research Station in Kearneysville, WV. The plot design was a randomized incomplete block design. The number of replicate trees of each clone varied primarily from one to 14. Because many trees were small and the trees varied considerably in size and number of shoots available for inoculation, the trees were grown for one year (2013) before fire blight assays were commenced in the spring of 2014. Trees of Quince A were considered the susceptible control and 10 trees of 'Old Home × Farmingdale 87' were the resistance controls.

In 2014 a second set of 13 quince clones was received from NCGR, and were planted in a separate plot, again in a single-row randomized incomplete block design with 10 blocks. Bartlett seedling rootstocks were planted as the susceptible controls and trees of 'Old Home × Farmingdale 97' were planted as the resistant controls. The number of replicate trees for each clone varied from 1 to 10.

A suspension of fire blight inoculum was prepared using the isolate Ea273, with the concentration adjusted to 1 × 109 cfu•ml-1. One inoculation was performed on 23 May 2014 in which one shoot per tree was inoculated, but because the characteristic necrotic symptoms were slow to develop after 4 days, a second set of inoculations was performed on 30 May 2014. On this date one to three shoots on each plant were inoculated, where available. Shoot growth was vigorous on both inoculation dates, but the current year's extension growth was relatively short, compared with that usually seen on established pear seedlings. Inoculations were performed by dipping a pair of scissors in the inoculum suspension and cutting the top two expanding leaves on a selected shoot of each clone. The length of current season's growth of each shoot was measured. Inoculated shoots which only exhibited necrosis in the leaf midrib, with no infection in the shoot were scored as "1 mm" to indicate infection. For the first inoculation, the trees were inspected for infection 10 days post infection, but no data was taken, due to limited symptom development. Data on the length of the resulting necrotic lesions for both inoculations were measured beginning on day 17 post infection, and repeated on days 24, 31, and 38, when progression of the lesions ceased for most inoculated shoots. End of season data were collected at 122 days postinfection.

Table 1. Fifteen of the of twenty-two clones previously determined to possess adequate cold hardiness for the PNW have been successfully tissue cultured by Dr. Yongjian Chang at N.A. Plants. Explants were planted in three stages (October, 2013; January, 2014; and, February, 2014).

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



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

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

			% rooting w/ hormone			
Hardiness Rank	**Accession**	**Browning Score (1-6)**	**hardwood cutting 01,11/2012**	**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**	**33.3**	**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**	**23.8**	**-**	**-**	**-**
Standard	**OHxF 97**	**3.86**	**7.1**	**0.0**	**-**	**-**
Standard	**OHxF 87**	**3.83**	**-**	**4.2**	**-**	**-**

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

Table 3. Fire blight infection in 2013 planting of prospective quince rootstock germplasm (2014 data, 122 days post-infection). Accessions highlighted in grey represent cold hardy clones; the number preceding the name represents the cold hardy rank

Clone	No. trees	Mean shoot length (mm)	Mean lesion length (mm)	Mean percent lesion length
V-46 o.p.	2	149 cd	112 bcd	149 a
IV-36 o.p.	1	295 abcd	255 a	89 abcde
9) Seghani o.p.	6	139 bcd	99 bcd	85 abcde
4) Tashkent AR-232 Sdlg 4	4	280 ab	231 a	82 ab
17) Bereczki	2	274 abcd	168 abcd	68 abcde
VI-7 o.p. Sdlg 3	2	179 abcd	81 bcd	68 abcde
10) Tashkent AR-232 Sdlg 2	12	213 bcd	129 b	66 abc
14) W-4	5	205 bcd	74 bcd	65 abcd
13) Krukovskaya o.p. Sdlg 2	4	265 abc	161 ab	58 abcde
8) Megri	1	164 abcd	86 bcd	53 abcde
IV-40 o.p. Sdlg 3	2	188 abcd	113 bcd	48 abcde
Akhtubinskaya o.p. Sdlg 1	2	173 abcd	80 bcd	44 abcde
18) Kashenko No. 8	3	174 bcd	48 cd	39 bcde
7) Quince W	10	152 cd	50 cd	36 bcde
Texas Scarlett	5	239 abcd	81 bc	33 bcde
19) Quince C7/1	9	222 abced	71 cd	33 bcde
Pillnitz 2	3	238 abcd	65 bcd	32 abcde
I-83 o.p. Sdlg 1	3	199 abcd	76 bcd	32 bcde
Pigwa S-1	14	173 d	53 cd	29 cde
2) Avia from Gebesud	5	301 a	71 bcd	25 bcde
1) Arakseni	5	129 bcd	29 cd	24 bcde
Pillnitz 3	11	166 de	39 cd	24 bcde
Pillnitz 5	18	145 d	31 cd	24 cde
Quince A	10	233 abcd	54 cd	23 cde
Pillnitz 1	6	185 bcd	42 cd	22 bcde

5) Skorospelka o.p Sdlg	3	190 abcd	36 cd	20 bcde
BA-29C	10	199 bcd	40 cd	20 de
Quince E	6	179 bcd	34 cd	19 de
Babaneuri	5	239 abcd	47 cd	19 cde
3) Akhtubinskaya o.p. Sdlg 4	11	246 abc	45 cd	17 de
Pigwa S-2	6	195 bcd	30 cd	16 cde
20) xPyronia vetchii IPR 82-1	2	140 bcd	11 d	11 de
V-7 o.p. Sdlg 3	1	60 e	1 d	2 e
V-46 o.p. Sdlg 3	1	65 e	1 d	2 e
OHF 87	9	242 abcd	0 d	0 e
Significance of effects				
Clone		<0.0001	<0.0001	<0.0001
Inoculation		<0.0001	0.0007	0.0002
Clone x Inoculation		0.6424	0.0909	0.0004

Table 4. Fire blight infection in 2014 planting of prospective quince rootstock germplasm (2014 data, 122 days post-infection). Accessions highlighted in grey represent cold hardy clones; the number preceding the name represents the cold hardy rank.

Clone	No. trees	Mean shoot length (mm)	Mean lesion length (mm)	Mean percent lesion length
Bartlett seedling	7	140 c	162 a	116 a
18) Kashenko No. 8	7	153 c	68 b	78 b
Babaneuri	4	183 bc	77 b	48 c
9) Seghani	1	175 bc	75 b	43 c
4) Tashkent AR-232 Sdlg 4	7	172 bc	66 b	40 c
14) W-4	6	121 d	31 c	39 c
Pigwa S-2	10	159 c	59 b	36 c
5) Skoropelka o.p. Sdlg	8	149 c	51 b	35 c
16) WF-17	4	173 bc	44 b	30 c
19) Quince C7/1	7	177 bc	40 bc	26 cd
17) Bereczki	2	173 bc	20 c	17 cd
8) Megri	5	167 c	26 c	15 d
Pigwa S-1	3	228 b	12 cd	7 d
2) Avia from Gebeseud	1	300 a	1 d	0.3 e
OHF 97	9	162 c	0.1 d	0.1 e
Significance of effects				
Clone		0.1218	<0.0001	0.0016
Inoculation		0.8733	0.7075	0.8433
Clone x Inoculation		0.1197	0.7866	0.9364

Executive Summary:

In the project's initial phase (2009-2011) we characterized 22 quince accessions as cold hardy; defined by visual observation of ≤ 50% oxidative browning of vascular tissues following exposure to -22 °F (predetermined critical temperature). These accessions had equal or greater cold hardiness than the commonly utilized commercial Pyrus rootstocks (OH × F 87, OH × F 97). Our main objectives for the second phase of the project were to develop propagation knowledge/protocols for these accessions and to produce an adequate volume of trees for field evaluations.

In general, propagation by softwood cuttings was more successful than hardwood cuttings. Rooting hormone improved levels of rooting. Eight accessions had ≥ 19% rooting success from hardwood cuttings; 12 accessions had > 25% rooting from softwood cuttings. Although the proportion of cuttings that rooted was relatively low, many of the quince accessions were observed to root more efficiently than OH x F clones (rooting success between 0% and 4%). Fifteen of the 22 cold-hardy accessions were successfully stablished in a stool bed at Helios Nursery (from hardwood cuttings).

In vitro initiation was successful on newly developed pear medium (Reed, USDA-ARS NCGR). Eight quince clones had multiplication rates >10 and 21 had multiplication rates ≥ 6 during in vitro establishment. A multiplication rate of 6 or higher is considered good. Despite being delayed from our initial timeframe due to loss of these cultures to a thrips infestation (October, 2012), N.A. Plants rapidly multiplied a sufficient number of transplants for liner production. Seventy percent of the cold-hardy quince clones were successfully cultured *in vitro* at N.A.Plants. All accessions were transplanted to media and successfully rooted. In most cases, the numbers of transplants per accession exceeded the number required for field trials. Cultures have been maintained for all accessions.

In 2014, sufficient numbers of rootstock liners of ~15 accessions, received by Helios nursery as explants from N. A. Plants, were successfully grown to budding/grafting size. Half of each accession was budded/grafted in late summer 2014 with 'Comice' (selected as the interstem). The other half was left un-grafted. Plants will be grown for the 2015 season. In late summer of 2015, the total number of plants per accession (half with interstems and half without) will be divided into equal groups–one group will be budded to 'd'Anjou', the other to 'Bartlett'. An evaluation of initial graft compatibility between the quince accessions and pear scions will be performed in the fall of 2015.

Fifteen of the 20 accessions were evaluated for fire blight susceptibility in 2013 and 2014 field trials. The level of susceptibility was much less than anticipated. Few infections spread into older wood, and no trees of any of the quince clones were lost to fire blight. Many of the accessions could apparently have a level of resistance sufficient for commercial use as rootstocks. It is recommended that these clones be inoculated in the coming year to verify the level of resistance.

FINAL PROJECT REPORT

Project Title: Improve electronic data collection/public access to USDA pear genebank

PI: Joseph Postman
Organization: USDA Agricultural Research Service
National Clonal Germplasm Repository (NCGR)
Telephone: 541-738-4220
Email: Joseph.Postman@ars.usda.gov:
Address: 33447 Peoria Road
City/State/Zip: Corvallis, Oregon 97333

Total Project Request: $2500

Other funding sources:
Agency Name: North American Raspberry & Blackberry Association (NARBA)
Amount awarded: $1000
Notes: Funding received for supplies to expand QR code labeling to include USDA Rubus collection.

Budget History:

Item	2014
Wages	$1100
Equipment	$ 900
Supplies	$ 500
Total	$2500

Objectives:

- Add the capacity at NCGR to generate weather-proof labels using a thermal-transfer printer, and to collect field data using a tablet computer and barcode reader.
- Improve the durability and information content on Pyrus field labels at the USDA germplasm collection in Corvallis, Oregon.
- Provide field access to online germplasm data by adding QR (quick response) codes to field tags.

Procedure:

1. High density polyethylene sheets (1/8" Polymax) were purchased, and we manufactured 4" x 6" HDPE tree tags with an expected 25 year lifespan.
2. Tree labels were generated on a laser printer using Avery 5524 weatherproof address labels.
3. Labels were affixed to HDPE tags and fastened to orchard pear trees with 2" stainless steel screws and 1" nylon spacers.
4. We worked with Westmark Industries, Inc. (Lake Oswego, OR) to obtain 1.25" square, weather-proof labels, and adapt barcode labeling software (BarTender, Seagull Scientific) to be used with a recently purchased thermal-transfer printer to generate quick response (QR) codes for each pear tree accession.
5. QR codes were designed to encode a custom URL for each tree accession that links to the specific accession record in the USDA Germplasm database (GRIN).
6. A tablet computer and barcode reader were purchased for field use.

Introduction

The USDA National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon houses a globally diverse living collection of pear cultivars and *Pyrus* wild relatives as 13 acres of orchard trees with a

single tree per accession. The collection includes about 2200 clonal accessions representing 36 Pyrus taxa from 56 countries. Included in the collection are:

913 European Cultivars	171 Rootstock Selections
177 Asian Cultivars	25 Perry (cider) Cultivars
115 Hybrid Cultivars	946 Pear Wild Relative Trees

Pear accessions are evaluated for phenotypic and genotypic traits which are documented in the national GRIN germplasm database, and scions are freely distributed to researchers worldwide.

Long-lasting, high quality tags are commercially available for forestry, botanic garden, and museum applications with costs ranging from $3.00 to more than $10.00 each. These costs are prohibitive for labeling the thousands of long-lived woody tree and shrub accessions conserved in the USDA field genebank collections. We have developed a durable, attractive and functional high density polyethylene (HDPE) tag that can be easily manufactured for direct mounting onto a tree trunk for a total cost of less than $0.70 each (including 4" x 6" HDPE tag, stainless steel fastener, nylon spacer, printed 3 ⅓" x 4" weather-proof label). For small trees a 3 ft. mounting stake is used until trees are large enough to relocate tags directly onto lower trunk.

Significant Findings, Results & Discussion

A developmentally disabled young adult had been helping with labeling tasks at NCGR through a school district training program. We were able to use part of the tree fruit industry funds hire him for the summer to manufacture and install HDPE tags on more than 2000 pear tree accessions. After custom QR coded labels were developed, they were printed using a thermal transfer printer and affixed to each field tag.

Thanks in part to Tree Fruit Research Commission funding, the entire clonal pear germplasm collection is now labeled with durable, long-life tags. The weather-proof labels on each tree include cultivar, taxonomy and origin information. Barcoded inventory numbers incorporated in the labels allow electronic data collection. Custom QR codes added to each tag allows public access to each plant's database information directly from the field using any smart phone or tablet computer equipped with a free QR reader app. The QR code generates a URL using the germplasm accession number and links to the USDA GRIN germplasm database. The link displays all public information in GRIN for the accession including plant origin, pedigree, source history, as well as voucher images and evaluation results.

Executive Summary

1. More than 2000 pear germplasm accessions were labeled with durable, attractive and functional high density polyethylene (HDPE) tags.
2. The weather-proof labels on each tree tag include cultivar, taxonomy and origin information.
3. Barcoded inventory numbers incorporated in the labels allow electronic data collection to expedite germplasm evaluation efforts.
4. Custom QR codes added to each tag provides public access to plant origin, pedigree, taxonomy, source history, voucher images and evaluation results directly from the field using a smart phone.

HDPE Tags with Tree and QR Labels

John attaches tag to pear tree.

Tree Tag without QR Label

Namrata adds QR Labels

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: PR-14-101

Project Title: Tests of a sprayable pheromone formulation against winterform psylla

PI: David Horton
Organization: USDA-ARS
Telephone: 509-454-5639
Email: david.horton@ars.usda.gov
Address: 5230 Konnowac Pass Rd.
City/State/Zip: Wapato, WA 98951

Total Project Request: **Year 1:** $16,000 **Year 2:** $**3,000 (revised)**

Other funding sources

Agency Name: Western Integrated Pest Management Center
Amt. awarded: $23,844
Notes: WIPMC funding is to be the primary source of funding for the second year of this project, leading to a lowering of funds requested here (to $3,000)

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510) 559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2014	2015
Salaries	$11,250	$1,500
Benefits	$ 3,750	$ 500
Plot Fees[1]	$ 1,000	$1,000
Total[2]	$16,000	**$3,000**

Footnotes:
[1] Pruning costs
[2] Request for 2015 lowered from the original ($16,000) due to new funding obtained from WIPMC

OBJECTIVES
Goal is to determine whether a sprayable formulation of the pear psylla pheromone (1% oil + pheromone) interferes with male success at finding and mating females

Objective 1: Confirm attractiveness of pheromone in oil using paired control and treatment traps

Objective 2: Determine effects of oil + pheromone on mating success in cage studies

Objective 3: Compare the following between control and pheromone plots under field conditions:

1. Adult densities (are males settling preferentially in pheromone plots?)
2. Mating success (do females in pheromone plots have fewer matings?)
3. Egg fertility (does egg fertility drop in pheromone plots because of poorer mating success)?
4. Densities of spring nymphal populations (are nymphal densities lower in pheromone plots)?

SIGNIFICANT FINDINGS

- Demonstrated weak but statistically significant attraction of males to oil + pheromone treated shoots under field conditions (result confirms earlier studies)
- In small and large cage studies, showed that drenching of potted trees or clippings of prunings with oil + pheromone product led to ***increases*** in rates of mating, in contrast to what we had hoped to obtain
- Large field trials also indicated ***higher*** rates of mating in oil + pheromone plots than oil plots, again in contrast to what we had hoped to obtain. No effects of oil + pheromone were seen on adult densities, egg fertility, or densities of the first generation immatures

METHODS
Sprayable. Pheromone was mixed in 1% oil (IAP Horticultural Oil) in water at rates described below for each objective. Control solutions (oil alone) were mixed at 1% oil in water.

Objective 1: Trapping study to confirm activity of sprayable. Paired limbs (each 2-3 foot in length) were drenched with oil + pheromone solution or oil solution, and then enclosed in a clear sticky mesh (to trap psylla). Solutions were applied to drip using a salad spritzer. Overspray was collected by holding a towel behind sprayed limbs. I estimate each oil + pheromone limb to have received about 50 female equivalents of spray (some of which was lost to overspray). Traps were collected after 3 days in the field. The trial was done on 6 dates during the winterform generation.

Objective 2: Cage studies to examine mating rates. *Small cage study (winterforms).* Approximately 2 dozen pear shoots (delayed dormant stage) cut from trees at the Moxee farm were placed into each of four ventilated dome cages (2 x 2 x 2 foot); cages were then placed in a greenhouse. Shoots were first cut to about 1½ foot in length, and cut ends were placed in water. In two of the cages, shoots were treated with oil + pheromone, applied using a salad spritzer to produce approximately 50 female equivalents per cage. Shoots in the other two cages were misted with 1% oil. Thirty female winterforms (unmated) were added to each cage and allowed to settle. Twenty-four hours later, thirty male winterforms were added. After 48 hours, all females were collected from each cage and dissected to determine number of matings.

Large cage study (summerforms). The winterform study was repeated out-of-doors using lab-reared (virgin) summerforms, in two large (6 x 6 x 6) ventilated cages. A fully leaved potted tree (approximately 4 foot in height) was set in the center of each cage and misted with either the pheromone solution (to produce 200 female equivalents) or with oil. To both cages were added 100 females, followed (24 hours later) with 100 males. After 48 hours, 50 females from both cages were collected, and then dissected to determine number of matings.

Objective 3: Large field trial. Twelve plots (each16 trees in size) were established at the Moxee research farm in late February; six plots were designated oil + pheromone, and six were control (oil alone) plots (see Figure 1 for design). Psylla were collected at approximately weekly intervals beginning in early February and dissected to determine onset of ovarian development. Once the first mature eggs were seen in dissected females and before mating had begun, plots were sprayed with the two solutions (March 13). Each plot received approximately 4 gallons of solution applied through a 25 gallon weed sprayer (Scorpion Sprayer) attached to a 4 wheeler. The application rates that were used would produce a signal of approximately 6000 females per tree (not adjusted for loss to overspray).

Tray samples were taken 1 week following application to determine adult densities. At 5 and 7 days following application, 20 females were collected from each plot and dissected to determine number of matings. On April 3, 5 to 10 spurs (enough to provide data on at least 100 eggs) were collected from each plot for monitoring egg fertility. Eggs were counted on each spur, cut ends of spurs were placed in water, and spurs were re-examined 1 week later to determine hatch rates. Finally, 10 spurs were collected from each plot on 30 April to determine numbers of eggs, small nymphs, and medium/large nymphs per spur.

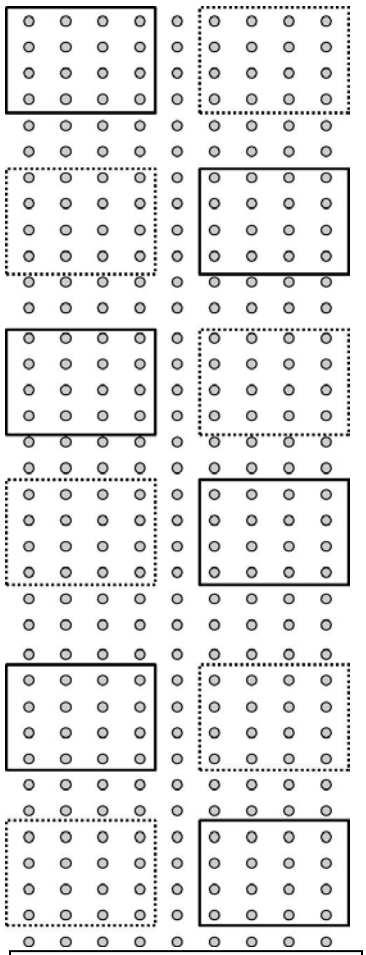


Figure 1. Solid boxes: control plots; dotted boxes: pheromone plots.

RESULTS AND DISCUSSION

Objective 1: Trapping study to confirm activity of sprayable. As shown also in 2013, I confirmed that the sprayable has weak but statistically significant attractiveness to male winterform psylla (Figure 2). Trap catch was approximately 1.5-fold higher for the oil + pheromone limbs than the oil alone limbs (averaged over sampling dates).

Objective 2: Cage studies to examine mating rates. *Small cage study (winterforms)*. Results indicated that probability of a winterform female being mated (top panel Figure 3) as well as number of matings per female (bottom panel Figure 3) were higher in the Oil + pheromone treatment than in the oil alone treatment. These results are opposite of what would occur if the pheromone had disrupted mating.

Large cage study (summerforms). In the large cage study, probability of a female summerform being mated was approximately 1.5-fold higher in the oil + pheromone cage than in the oil alone cage (left panel Figure 4); number of matings per female was almost twice as high in the oil + pheromone treatment than the oil treatment (right panel Figure 4). These results are opposite of what would occur if the pheromone had disrupted mating.

Objective 3: Large field trial. The proportion of females that had been mated was higher in the oil + pheromone plots than in the oil plots (Figure 5 upper panel; $P = 0.024$), results which are consistent with the cage studies. There was also a suggestion ($P = 0.08$) that mating rates (spermatophores per female) were higher in the oil + pheromone plots (Figure 5 lower panel). Again, these results are opposite of what would occur if the pheromone had been disruptive. At this time, it is unclear what would lead to results in both field and cage studies that would suggest that the pheromone enhanced rather than disrupted mating of pear psylla. There was no indication that sex ratios in pheromone vs

oil plots were different, as shown by tray counts (Figure 6), thus the higher mating rates in the pheromone plots do not appear to have been due to a male biased sex ratio in the pheromone plots.

Unsurprisingly, given that most females in both treatments had been mated by 20 March (Figure 5), egg hatch rates were high in all plots and were similar between treatments (Figure 7); between 17 and 22% of eggs failed to hatch in the two treatments, which is a typical rate for the earliest eggs deposited by winterform psylla under Central Washington conditions (Horton unpublished). Densities of eggs and nymphs in late April were similar between treatments, although with a (non-significant) suggestion of possibly higher counts for small nymphs in the control (oil) plots (Figure 8).

Figure 2.

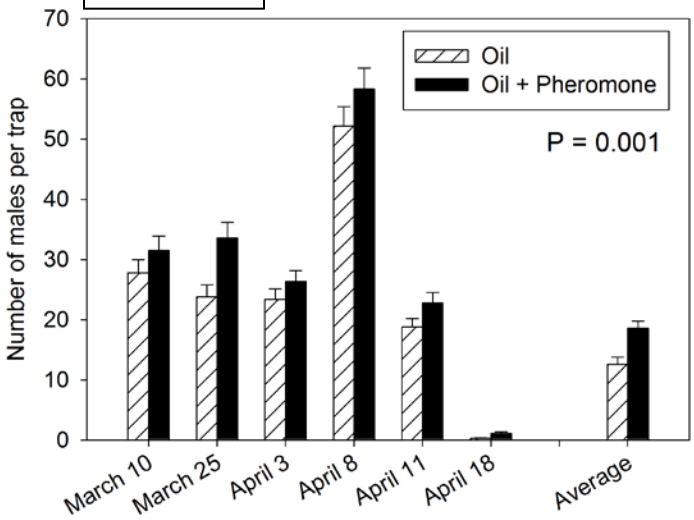
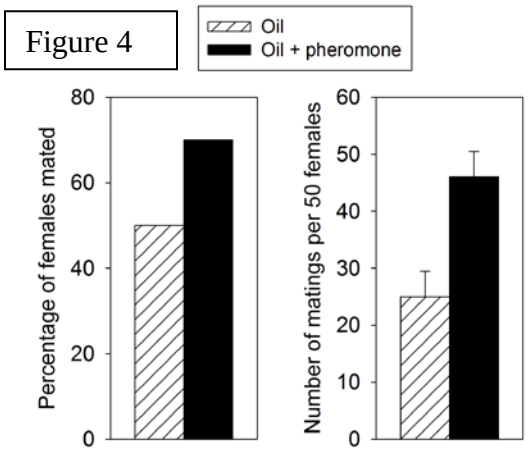


Figure 4



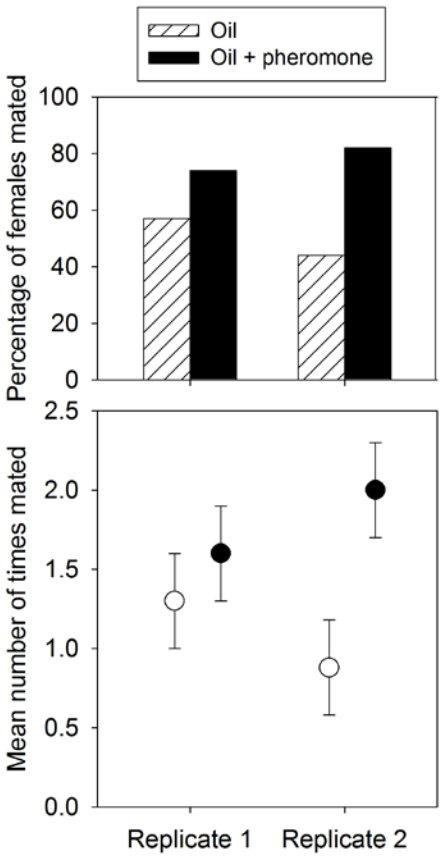


Figure 3

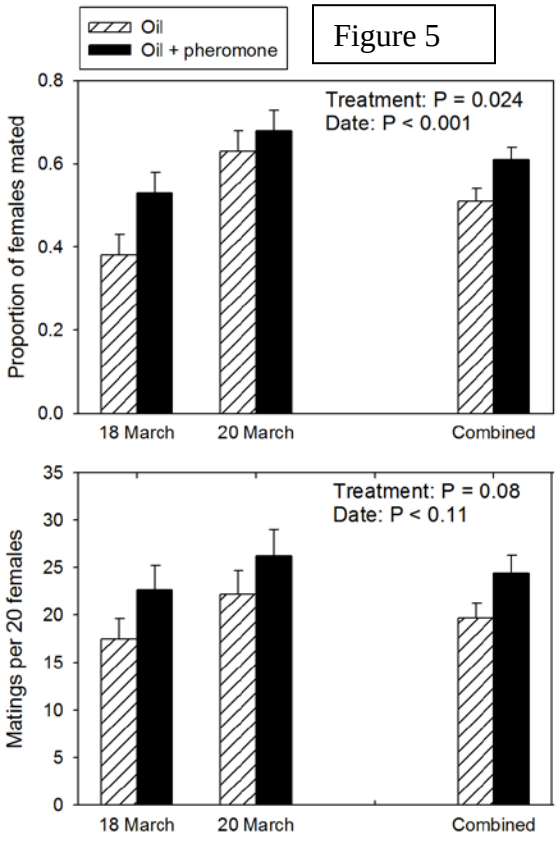


Figure 5

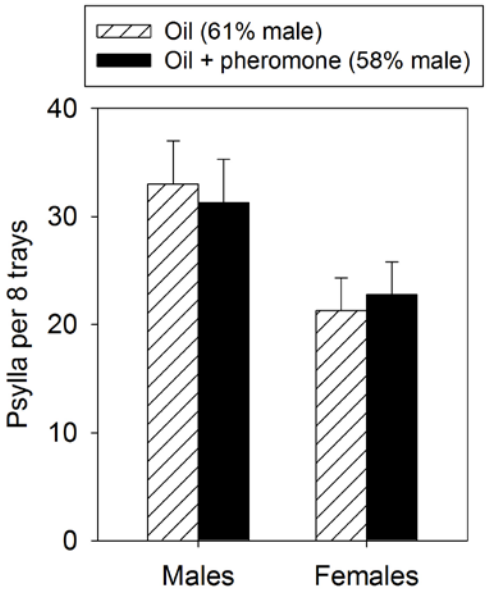


Figure 6

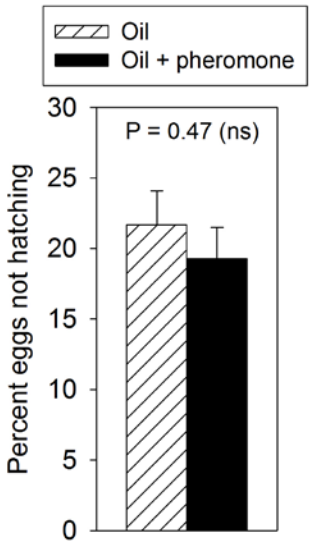
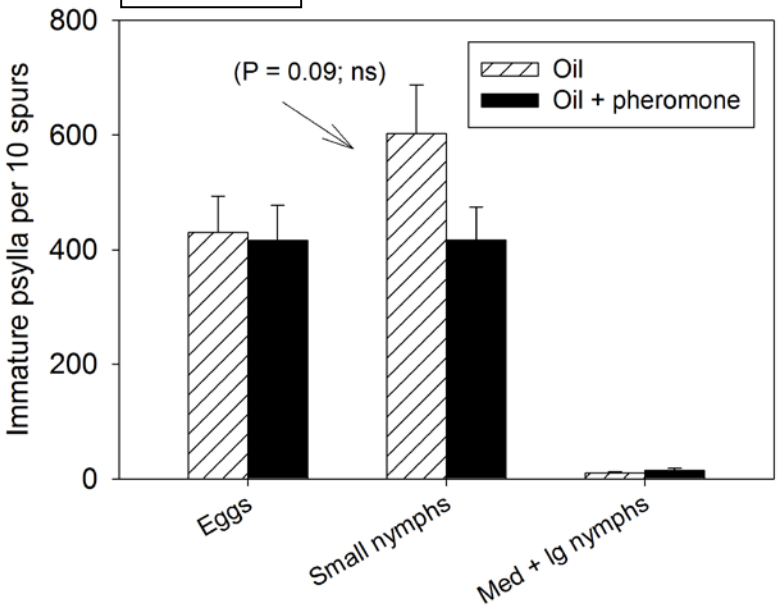


Figure 7

Figure 8



CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number:

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

PI: W. Rodney Cooper
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City/State/Zip: Wapato, WA 98951

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

Total Project Request: **Year 1:** $25,000 **Year 2: $25,000** **Year 3:** $25,000

Other funding sources: None

Budget 1
Organization Name: USDA-ARS-YARL **Contract Administrator: Chuck Myers**
Telephone: 510/559-5769 **Email address: Chuck.Myers@ars.usda.gov**

Item	2014	2015	2016
Salaries	$16,000	$16,000	$16,000
Benefits	$1000	$1000	$1000
Wages			
Benefits			
Equipment			
Supplies	$5000	$5000	$5000
Travel			
Plot Fees	$3000	$3000	$3000
Miscellaneous			
Total	$25,000	**$25,000**	$25,000

Footnotes:
[1] Temporary employee to help with field studies
[2] Defense elicitors, field supplies, greenhouse supplies, trees, PCR primers, probes, and reagents, FISH probes and reagents.

OBJECTIVES

1) Test the effects of commercially available elicitors of host defenses on pear psylla population growth in pear orchards.
2) Test the effects of defense elicitors on recruitment of natural enemies.
3) Test the combined effects of defense elicitors and potassium or magnesium fertilization on pear psylla performance.
4) Test the effects of defense elicitors on obligate bacterial symbionts of pear psylla.

SIGNFICANT FINDINGS

1) We observed fewer pear psylla nymphs on trees treated with defense elicitors than on untreated trees.
2) We developed methods using qPCR and fluorescence in situ hybridization (FISH) to compare *Carsonella* densities in pear psylla. Preliminary results suggest that *Carsonella* is reduced in pear psylla collected from trees treated with Acitgard compared with psylla collected from untreated trees, but Employ and ODC did not appear to influence *Carsonella* densities

METHODS

Objectives 1. Test the effects of commercially available elicitors of host defenses on pear psylla population growth in pear orchards.

Experiments will be conducted in a Bartlett pear orchard located at the USDA-ARS experimental farm near Moxee, WA. The orchard was planted in 2001 with 16 × 16-ft spacing. The orchard will be divided into six main plots each with four subplots (one for each treatment). Each subplot will be randomly assigned a foliar treatment: Employ, Actigard, ODC, or untreated control. Foliar treatments will be applied according to the product labels every four weeks beginning mid-March and ending mid-August.

Populations of pear psylla will be monitored weekly beginning one week before the first application of defense elicitors and ending at fruit harvest. Populations of adult pear psylla will be estimated using beat tray samples from each of the four cardinal directions around each tree. Populations of pear psylla nymphs will be estimated by counting the numbers of nymphs on the ten most terminal leaves from ten shoots per tree. Fruit will be harvested from each tree in the fall to assess fruit downgrading due to honeydew as described by Pfeiffer and Burts (1983). The experiment will be repeated each year for three years. Data will be used to estimate the effects of each defense elicitor on populations of pear psylla and fruit damage caused by honeydew production.

Objective 2. Test the effects of defense elicitors on recruitment of natural enemies.

Experiments will be conducted using the same trees used for objective 1. Populations of common orchard predators (Coccinellids, Anthocorids, *Deraeocoris*, and lacewings), and the common parasitoid of pear psylla (*Trechnites* spp.) will be estimated based on samples obtained using a standard beat-tray. Nymphal stages of predators and parasitized pear psylla nymphs (mummies) will be sampled by observing the ten most terminal leaves of ten shoots per tree.

Objective 3. Test the combined effects of potassium and magnesium fertilization on induced defenses against pear psylla.

Experiments will be conducted within a screened pavilion located at the USDA-ARS experimental farm. Bartlett saplings grafted to OHXF-87 rootstock will be planted in 30 liter plastic pots. Potted plants will be arranged in 6 blocks each with 8 trees. Each tree within a block will be randomly assigned a fertilizer by defense elicitor combination. Three blocks will be used to assess the interaction between potassium availability and application of defense elicitors on pear psylla. The remaining three blocks will be used to assess the interaction between foliar application of magnesium sulfate and defense elicitors on pear psylla. To assess the effects of potassium availability on induced defenses, each tree will be fertilized prior to bud break with 24 g of nitrogen (nitrate of soda), 18 g of

phosphate (super phosphate), and either 30 g of potassium (potash) or no potassium (native levels present in the soil) (Neilsen 1994). A second application of fertilizers will be applied in mid-season. To test the effects of magnesium on induced defenses, each tree will be fertilized prior to bud break with 24 g of nitrogen, 18 g of phosphate, and 30 g of potassium. Magnesium sulfate (20 g/4 l water) will be applied the foliage of half of the trees in each block one week before treating the trees with defense elicitors.

Twenty-four hours after treating trees with defense elicitors, 10 field-collected adults will be confined to a shoot of each tree for thirty days. The combined effects of fertilization and defense elicitors will be estimated by counting the numbers of nymphs and adults on trees thirty days after insect releases. The thirty-day period will be used because eggs oviposited on the day of insect releases should be fifth instars, but not adults (McMullen and Jong 1977). The experiment will be conducted each year for three years. Data will be used to determine whether increasing potassium or magnesium availability increases the efficacy of defense elicitors against pear psylla.

Objective 4. Test the effects of defense elicitors on the obligate bacterial symbiont of pear psylla.

Bartlett seedlings will be grown in a greenhouse and treated with each defense elicitor (5 trees per treatment). Twenty-four hours after foliar treatments, six fourth instar pear psylla will be confined to a single leaf of each tree using a sleeve cage. The insects will be collected after 36 hours. The obligate symbiont of psyllids, *Carsonella ruddi*, will be quantified in three insects from each tree using qPCR and FISH. Data will be used to assess whether plant defenses activated by Employ, Actigard, or ODC reduce symbiont populations in pear psylla nymphs.

RESULTS AND DISCUSSION

Objectives 1. Test the effects of commercially available elicitors of host defenses on pear psylla population growth in pear orchards.

Peak populations of pear psylla nymphs were observed on weeks 5-6 (1-7-May) and on weeks 11 and 12 (18-25 June) of our study (Figure 1A). Analyses indicated a significant treatment by week interaction, indicating that the effects of treatment were not consistent among weeks (Figure 1A). Untreated trees supported significantly more nymphs than did trees treated with Actigard, Employ, or ODC from weeks 11-15 (11-June to 9-July), and on the final sampling day (6-Aug). Averaged over all weeks, there were numerically more nymphs present on untreated trees (mean ± SE nymphs per shoot, 33.1 ± 1.97) than on trees treated with Actigard (23.9 ± 1.97), Employ (25.6 ± 1.97) or ODC (26.8 ± 1.97). Although the observed effects of defense elicitors on pear psylla nymphs were consistent with our previous study, the effects of elicitors were not as great as previously observed in the laboratory, where Actigard, Employ, and ODC reduced populations by nearly 50% compared with untreated trees. Because only single trees were treated with elicitors, it is possible that adult movement from adjacent untreated trees reduced the treatment effects on trees treated with elicitors. It is also possible that the plant responses to elicitors are more variable in larger trees than in the potted trees used in our laboratory study.

We did not observe a significant treatment effect on pear psylla adults, or a significant treatment by week interaction (Figure 1B). Results were consistent with our laboratory studies, which also suggested that adults are not affected by plant responses to applications of defense elicitors. However, the numerically (but non-significant) greater number of adults observed on control trees compared with trees treated with elicitors on week 15 (9-July) and weeks 17-19 (23-July-6-Aug) suggest that more nymphs reached adulthood on untreated trees (Figure 1B). As expected, adult populations varied by week (Figure 1B). Peak adult populations occurred on week 8 (21-May) and weeks 14-15 (2-9-July), which was about 3-4 weeks later than peak nymph populations (Figure 1).

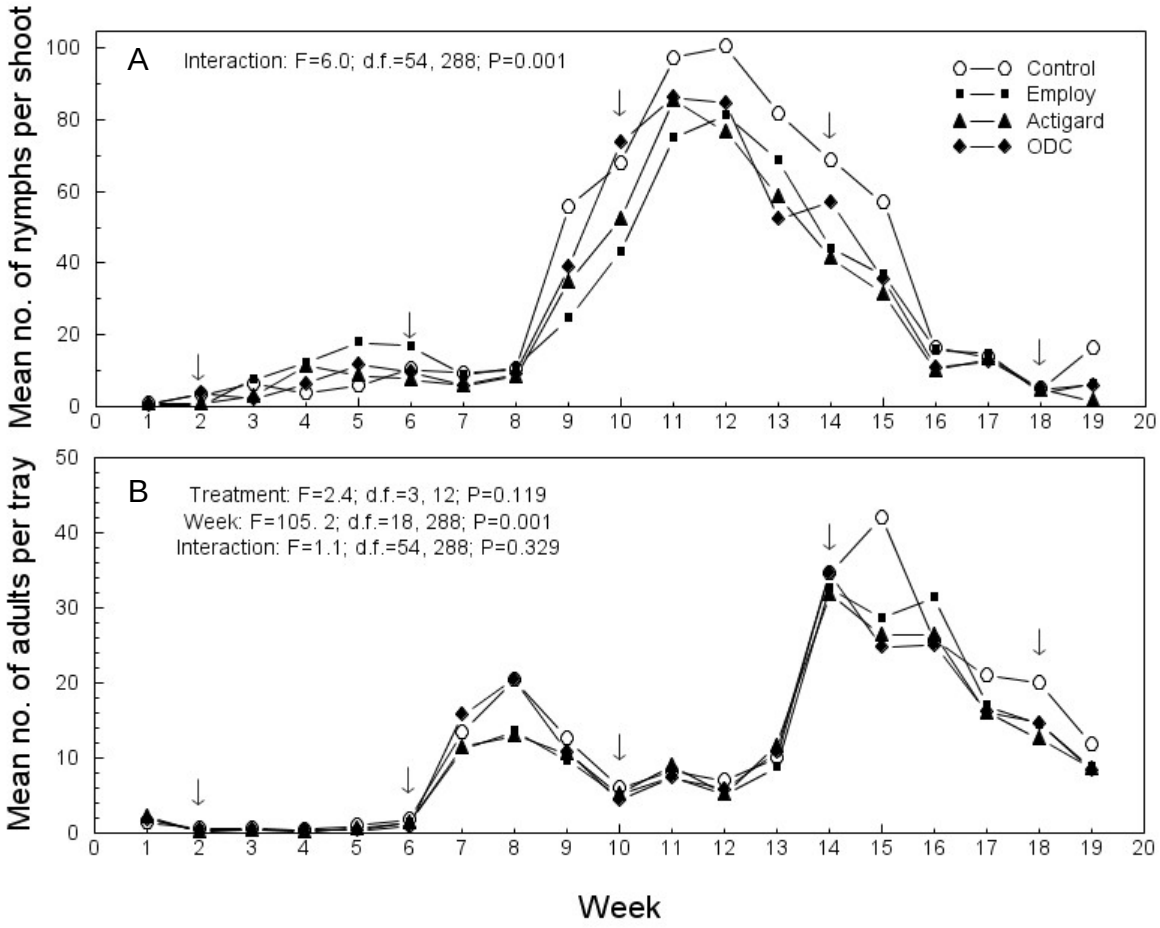

Figure 1. Effects of defense elicitors on pear psylla nymphs (A) and adults (B). Arrows denote treatment application dates (9-April, 7-May, 4-June, 2-July, and 30-July).

A rainstorm several days before harvest washed honeydew from fruit, so we were not able to compare honeydew accumulation on fruit among treatments. Severity of fruit russetting was ranked based on the percentage of each fruit (10 per tree) marked by russetting where 1=0-25%, 2=26-50%, 3=51-75%, and 4=76-100%. We did not observe significant differences in damage among treatments (Figure 2; χ^2=1.56; df=3; *P*=667). Although not significant, a greater percentage of fruit from trees treated with any of the three elicitors were ranked with the lowest damage rating than fruit from untreated trees (Figure 2). Data from multiple years may exhibit significant trends.

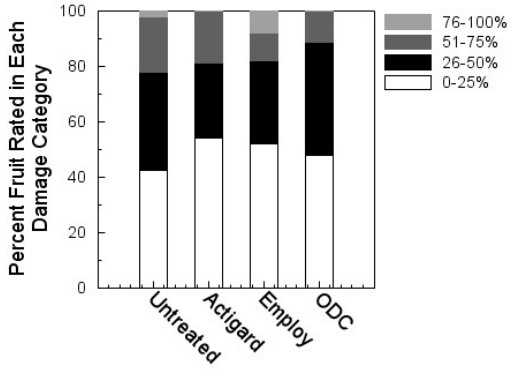

Figure 2. Fruit damage caused by fruit russetting.

Objective 2. Test the effects of defense elicitors on recruitment of natural enemies.

Populations of natural enemies were generally low throughout the year, and most varied by week (Table 1). We did not observe significant differences in natural enemy populations among treatments, but a significant treatment by week interaction for lacewing adults indicated that the

effects of treatment were not consistent among weeks (Table 1). On week 8 of our study (21-May), which conincided with the first psylla adult population peak (Figure 1B), there were more lacewing adults captured on trees treated with Actigard or Employ than on untreated trees or trees treated with ODC. A similar trend was observed on week 11 (11-June) when more lacewing adults collected from trees treated with Employ than on other trees. Previous studies have shown that activation of aquired defenses, including salicylic acid-dependent defenses, can lead to increased recruitment of natural enemies (Thaler et al., 2001; Zhu and Park, 2005; van Driesche et al., 2009). However, our results do not provide substantial evidence that natural enemy populations increase on pear trees treated with defense elicitors. It is possible that pear trees with induced defenses do not release volatiles that are attractive to natural enemies, or that volatile signals from single pear trees are not strong enough to attract adequate numbers of natural enemies. It is also possible that natural enemy populations were too small at the Moxee farm in 2014 to reliably compare their populations among treatments.

Table 1. Statistical analyses comparing natural enemy populations on trees treated with Actigard, Employ, ODC, or water.

Natural Enemy	Foliar Treatment	Week	Treatment × Week
Coccinellids			
Nymphs	F=0.7; df= 3, 300; P=0.574	F=1.4; df=18, 300; P=0.151	F=0.8; df=54, 300; P=0.777
Adults	F=0.26; df= 3, 284; P=0.581	F=4.8; df=17, 284; P<0.001	F=0.49; df=51, 284; P=0.999
Anthocorids			
Nymphs	F=1.2; df= 3, 300; P=0.297	F=1.6; df=18, 300; P=0.062	F=1.4; df=54, 300; P=0.056
Adults	F=0.3; df= 3, 284; P=0.820	F=3.2; df=17, 284; P<0.001	F=0.5; df=51, 284; P=0.999
Deraeocoris			
Nymphs	F=0.3; df= 3, 300; P=0.839	F=2.4; df=18, 300; P=0.001	F=0.6; df=54, 300; P=0.981
Adults	F=1.5; df= 3, 284; P=0.215	F=10; df=17, 284; P<0.001	F=0.5; df=51, 284; P=0.999
Lacewings			
Nymphs	F=1.2; df= 3, 300; P=0.300	F=0.9; df=18, 300; P=0.588	F=1.0; df=54, 300; P=0.496
Adults	F=1.8; df= 3, 284; P=0.149	F=4.2; df=17, 284; P<0.001	**F=2.8; df=51, 284; P<0.001**
Trechnites			
Nymphs	F=0.1; df= 3, 300; P=0.956	F=3.7; df=18, 300; P<0.001	F=0.4; df=54, 300; P=0.999
Adults	F=0.2; df= 3, 284; P=0.928	F=4.8; df=17, 284; P<0.001	F=0.4; df=51, 284; P=0.999

Objective 3. Test the combined effects of potassium and magnesium fertilization on induced defenses against pear psylla.

Experiments were setup in the screenhouse located at the experimental farm in Moxee, and each potted tree was inoculated with psyllids. However, all of the trees were infected with fire blight, and many of the trees died before the study was completed 31 days after foliar treatments. Averaged over all treatments, trees treated with foliar applications of magnesium sulfate had 220 pear psylla compared with 570 psylla on untreated trees, but these values were not significantly different. We plan to repeat these studies in 2015.

Objective 4. Test the effects of defense elicitors on the obligate bacterial symbiont of pear psylla.

We first developed methods to compare populations of the obligate symbiont of pear psylla, *Carsonella*, among different insects. One method uses fluorescence *in situ* hybridization (FISH) to visually detect *Carsonella* in bacteriocytes, specialized insect cells which harbor the bacteria. This method was largely based on our FISH assay to detect *Liberibacter* in specific tissues of potato psyllid (Cooper et al., 2014). Using FISH, we labeled *Carsonella* with a fluorescent probe and measured the intensity of fluorescence to estimate relative bacteria densities in individual bacteriocytes (Figure 3A inset). Our second method relies on quantitative real time PCR (qPCR) to estimate bacteria densities in whole insects. Using these methods, we compared *Carsonella* between male and female pear psylla. Results of FISH indicated that bacteriocytes of females harbored greater numbers of *Carsonella* than do those of males (F=19.2; d.f.=1, 22; P<0.001), but densities did not significantly change with increasing adult age (F=2.4; d.f.=3, 22; P=0.095) (Figure 3A). The lack of a significant sex×week interaction (F=1.1; d.f.=3, 22; P=0.391) indicated that the differences between sexes in fluorescence intensity were consistent among the different insect ages. Results of qPCR showed similar trends (Figure 3B).

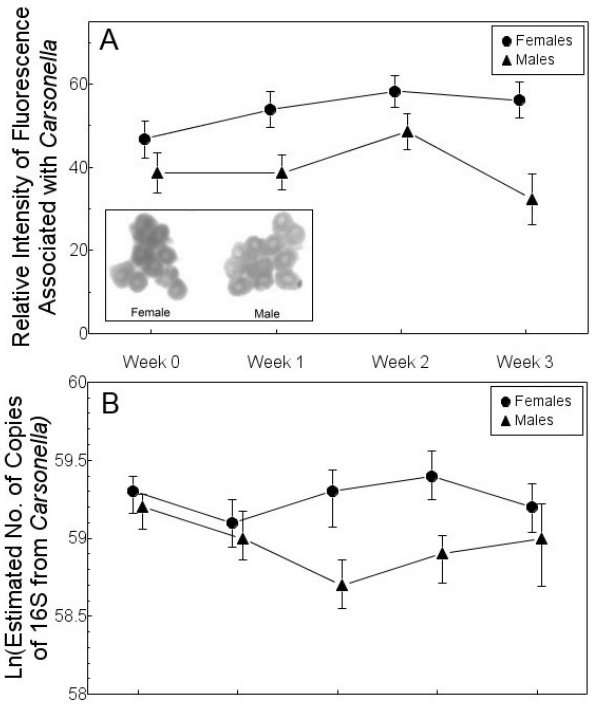

Figure 3. Comparison of *Carsonella* densities among females and males of different ages using FISH (A) and qPCR (B). Inset shows samples of bacteriocytes containing *Carsonella* labeled with a fluorescent probe; the darker cells corresponds with a greater density of *Carsonella*.

Carsonella was more abundant in females than in males (Fig. 3; F=8.4; d.f.=1, 22; P=0.009), but we did not observe a significant effect for age (F=0.9; d.f.=4, 22; P=0.513) or a significant sex×age interaction (F=1.2; d.f.=4, 22; P=0.362). These results confirm that our methods are suitable for comparing *Carsonella* among pear psylla. These findings also indicate that insect sex should be controlled in our future studies, which is consistent with our previous study that showed that bacteriomes of female psylla are larger than those of males (Cooper and Horton 2014).

Groups of nymphs collected from a previous study and storied in -20°C were used to compare *Carsonella* populations using qPCR in psylla collected from trees treated with Actigard, Employ, ODC, or water. *Carsonella* densities were significantly lower in pear psylla collected from trees treated with Actigard compared with untreatedd trees (F=3.8; df=3, 12; P=0.041), but *Carsonella* populations did not differ in psylla untreated trees and trees treated with Employ or ODC (Table 2). These preliminary results suggest that the acquired defense responses in pear elicited by Actigard lead to reduced populations of the obligate endosymbiont of pear psylla. Studies to confirm these preliminary results are currently underway.

Treatment	*Carsonella* titers
Untreated	54.03 ± 0.462 a
Actigard	52.20 ± 0.446 b
Employ	53.46 ± 0.457 ab
ODC	54.10 ± 0.463 a

Table 2. Natural log of the estimated number of copies of 16S from *Carsonella* (±S.E.) in pear psylla nymphs collected from trees treated with Actigard, Employ, ODC, or water (untreated).

REFERENCES

Cooper WR, and Horton DR (2014) Gender- and species-specific characteristics of bacteriomes from three psyllid species (Hemiptera: Psylloidea). Journal of Entomological Science.

Cooper WR, Sengoda VG, and Munyaneza JE (2014) Localization of 'Candidatus Liberibacter solanacearum' (Rhizobiales: Rhizobiaceae) in *Bactericera cockerelli* (Hemiptera: Triozidae). Annals of the Entomological Society of America. 107: 204-210.

McMullen RD, and Jong C (1977) Effect of temperature on developmental rate and fecundity of the pear psylla, *Psylla pyricola* (Homoptera: Psyllidae). Canadian Entomologist 109: 165-169.

Thaler JS, Stout MJ, Karban R, and Duffey SS (2001) Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology 26: 213-324.

van Driesche R., Hoddle M, and Center T (2009) Control of Pests and Weeds by Natural Enemies. John Wiley and Sons, Hoboken, NJ.

Zhu JW & Park KC (2005) Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*. Journal of Chemical Ecology 31: 1733-1796.

CONTINUING PROJECT REPORT **NO COST EXTENSION**
WTFRC Project Number: PR-14-103 (A, B&C)

Project Title: Pesticide resistance in pear psylla and discovery of resistance genes

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Co-PI: Joanna Chiu
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Total Project Request: $48,700 **Year 1**: $48,700

Budget 1 (Unruh)
Organization Name: USDA-ARS **Contract Administrator:** Charles W. Myers
Telephone: 510-559-5769 **Email address:** Chuck.myers@ars.usda.gov

Item	2014	NA	NA
Salaries			
Benefits			
Wages	$13800		
Benefits	$ 1200		
Equipment			
Supplies[1]	$ 1000		
Travel			
Miscellaneous			
Plot Fees[2]	$ 1000		
Total	$17,000		

Footnotes:[1] Insecticides, collection materials, computer program for DNA analysis. [2]Moxee farm pears-fertilizer

Budget 2 (Shearer and Hilton)
Organization Name: OSU MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2014	NA	NA
Salaries[1]	$5,215		
Benefits[1]	$3,454		
Wages[2]	$5,787		
Benefits[2]	$1,739		
Equipment	$0		
Supplies[3]	$346		
Travel[4]	$459		
Plot Fees	--		
Miscellaneous	--		
Total	$17,000		

Footnotes: Footnotes: [1]Salary and Benefits: Faculty Research Assistant 0.75 mo. Bioscience Research Technician 0.75 mo. [2]Wages and Benefits: Summer Technician(s), 10 weeks [3]Supplies: Lab supplies for assay and rearing [4]Travel to field. 0.556/mi.

Budget 3 (Chiu)
Organization Name: University of California Dav**is** **Contract Administrator:** Guyla Yoak
Telephone: (530) 752-3794 **Email address:** gfyoak@ucdavis.edu

Item	2014	NA	NA
Salaries[1]	$5,896		
Benefits[1]	$2,252		
Wages	--		
Benefits	--		
Equipment	--		
Supplies[2]	$3,552		
Travel	--		
Miscellaneous[3]	$3,000		
Plot Fees	--		
Total	$14,700		

Footnotes: [1]Salary and Benefits: Technician (2 months of full time); [2]Supplies: Lab supplies for generating transcriptome sequencing libraries and library quality control including NEB Next Ultra RNA library Prep Kit for Illumina, NEB Next Multiplex Oligos for Illumina, NEB Next Poly(A) mRNA Magnetic Isolation Module, Biorad Experion Nucleic Acid Analysis Kit, and consumables such as pipet tips and microcentrifuge tubes
[3]Miscellaneous: Transcriptome sequencing costs at the UC Davis Genome Sequencing Center

OBJECTIVES:

1. **Conduct resistance survey of winter form pear psylla in 16 orchards, 8 in WA and 8 in OR**

We will measure resistance status using the slide dip bio-assay well suited to for pear psylla adults We will attempt to assay Warrior and Pounce (pyrethroids), Delegate (spinosyn), Admire (provado, a neonicotinoid), Nexter (METI interferes with mitochondrial energy cascade), sulfur (inorganic), Manzate (bis-dithiocarbamate), Agri-Mek (avermectin), and one or two more compounds to be determined. ($34,000)

2. **Produce and analyze transcriptomes from 6 populations of pear psylla to identify genetic variations that confer insecticide resistance**

We (Dr. Chiu) will identify inter-population variation at genes related to insecticide resistance using transcriptomes of expressed genes from pear psylla population samples collected from two isolated populations that have a history of minimal chemical control and evidence of low resistance from bioassays. We will compare these to four distinct populations chosen because they have shown significant resistance in bioassays and are from orchards with a history of significant pesticide use. The transcriptomes will be shared with colleagues to support other applications of this valuable genetic information. ($14,700)

Deviations from the Objectives

1. Collaborators in Northern Oregon were unable to collect adequate numbers of pear psylla to conduct studies in autumn of 2014 due to uncommonly low psylla abundance, conflicting travel restrictions and a significant and disruptive freeze in autumn. No Pear -WTFRC funds were spent in OR and a reduced number of collections and assays were conducted in Medford. Plans are to complete studies in autumn of 2015 and extending existing funding .
2. Dr. Chui has just received preserved pear psylla for transcriptome work, which has delayed transcriptome work by a month or two.
3. Sulfur and manzate are not suitable for slide dip assays and we will not proceed with those important materials unless we can put together a better assay.

SIGNIFICANT FINDINGS

- Six of the eight pesticides were successfully assayed against psylla at multiple sites, including: Admire (9 sites), Agrimec (9 sites), Delegate (12 sites), Nexter (8 sites), Pounce (12 sites) and Warrior (7 sites).
- Sites included: West Valley, Lombard Loop, Tieton, Sunnyside, Sawyer, Cashmere, Mesa, Omak, Orondo, Yakima in WA and six sites were in or near Jackson Co. OR.
- **Good News:** Nexter and Delegate show little evidence of resistance or tolerance
- **Mediocre News:** Agrimec and Admire are showing moderate efficacy but some sites appear to have some level or resistance to these two materials.
- **Bad news:** Psyllas at all sites show extreme resistance to Pounce and Warrior
- Pear psylla is ridiculously difficult to collect in numbers in autumn in organically managed sites (if you know of organic orchards that have psylla post-harvest please contact TRU.)
- John Dunley and Bruce Greenfield have provided psylla bioassay data from the Wenatchee region for 2000 and 2006 which will give a historical context to the data we are analyzing.

MATERIALS and METHODS

Objective 1

We will continue to evaluate resistance status of adult winter form psylla from several orchards in WA collected in autumn prior to leaf fall. The standard slide dip bio-assay will be used. Up to eight products will be tested for each of the 16 populations: Warrior and/or Ambush/Pounce (pyrethroids), Delegate (spinosyn), Assail and/or Provado (neonicotinoids), Nexter (METI), Agri-Mek (avermectin), and one or two more compounds may be tested. *Development of an assay to evaluate sulfur and manzate are being considered but may not work for this proposal.*

Adult winter form psylla will be collected from the field from mid-September through leaf fall using beat trays and an aspirator or a collection funnel (Figure 1). Collected psylla will be returned to the laboratory and kept cool (40^{O}F) and in short day length (10:14, L:D); leaf bearing shoots will be placed with the adults to provide water.

Figure 1. Psylla adult collection funnel; 4 liter plastic vials screws to bottom

The psylla will be assayed using a slide-dip method. For a single test, 30-35 adults will be aspirated into a vial with tissue paper and anesthetized with CO2 for 30 s; anesthetized adults will be placed on their back on a sticky tape affixed to a glass microscope slide (Figure 2). Slides with psylla are dipped into the pesticide solution for 5 sec, then incubated for 48 hours and survival assessed for each individual. The assay will be repeated three times (~90-100 psylla per concentration). Five to six insecticide concentrations plus a water control will be used. Several populations will be assayed using the complete dose response curve as described. Additional populations will be assayed with two to three discriminating doses as developed from the dose response observed in the full dose-response analyses.

Figure 2. Psylla affixed to slide for testing

Objective 2

We will identify inter-population variation at genes related to insecticide resistance utilizing deep sequencing of transcriptomes. Specifically we will compare DNA sequence differences seen in the transcriptomes in psylla collected from 2-3 sites that are determined to be susceptible to 3-4 sites determined to be resistant. Transcriptome libraries will be sequenced using 100bp paired-end Illumina HiSeq at the UC Davis Genome Center Sequencing facility.

Our bioinformatic analyses will yield transcriptomes for the different psylla populations will uncover genetic variations that confer insecticide resistance or susceptibility among test populations. We will perform comparative sequence analysis against all available genomes in the public database to identify pear psylla genes that have similarity to genes from other insects known to be associated with insecticide target site or metabolic resistance Psyllidae and the Hemiptera.

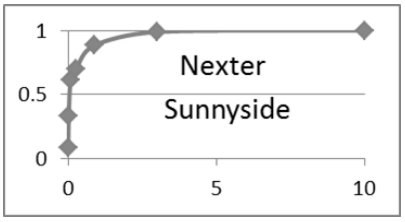
We will examine both metabolic resistance genes among psylla populations to determine if there is differential expression (upregulation) and if they show genetic substitutions that may increase detoxification efficiency. Similarly we will look for target site substitutions that would render specific pesticides ineffective because they are tolerated as opposed to being degraded. Results from gene expression should correlate with results of bioassays and will provide new basic understanding

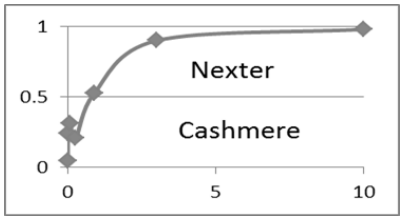
of resistance in this insect group. In addition, the psylla transcriptome produced will be shared with the scientific community to facilitate basic and applied research using the myriad of other genes of pear psylla captured by a transcriptome, providing molecular for other trait variations.

RESULTS & DISCUSSION

Our initial results provide insights on potential resistant levels based on our pesticide bioassays. Here we focus on the findings from studies that began in early October and finished in early December. We are still analyzing the bioassay data and will be collecting more for future analysis and will show a brief summary supporting our good, mediocre and bad news announced above. To do so we provide summary of efficacy of each insecticide across populations based on mortality produced by field rates. The good news first.

Mortality for **Nexter** is best characterized by the dose mortality curve pictured. The vertical axis measures proportion of mortality to 1 (100%) mortality. The horizontal axis describes the dose rate of the insecticide in field rate used from zero to ten times field rate. In this graph 0.9 field rate is just below 100% mortality (third diamond from the right). But a couple of sites show modest tolerance to Nexter as evident in graph for a site in Cashmere where 0.9 field rate is near to 0.5 mortality. In Table 1, 1x and 10x field rates are summarized and it is clear that some sites show hints of tolerance Omak and Sunnyside.





The pattern for **Delegate** at Cashmere WA shows no hint of resistance but other sites do show such some hints of tolerance. These are summarized in Table 2. One should note from the results in Table 2 that the 1x rate for Delegate is low at some sites, notably at Omak, Mesa and Sawyer. It is clear that Delegate is less effective than Nexter for control of autumn winter form psylla adults. To the extent this represents a development of tolerance to Delegate or the nature of uptake of Delegate by the psylla remains an open question.

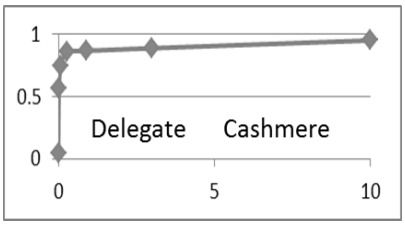


Table 1. Prop. Mortality Nexter		
Site\rate	1x	10x
Cashmer	0.5	0.98
Mesa	0.5	xxxx
Omak	0.04	1
Orondo	0.5	1
Sawyer	0.97	xxxx
Sunnyside	0.37	0.98
Medforn	0.54	xxxx

Table 2. Prop. Mortality Delegate		
Site\rate	1x	10x
West Vally	0.5	0.83
Mesa	0.4	0.87
Omak	0.06	0.67
Orondo	0.45	0.94
Sawyer	0.27	0.85
Sunnyside	0.89	1
Medforn	0.33	0.98

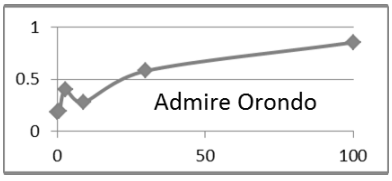
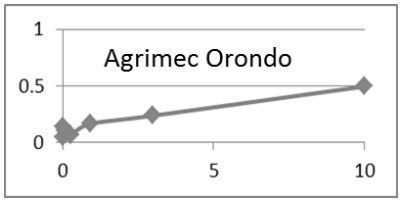


Table 3. Prop. Mortality Agrimec		
Site\rate	1x	10x
West Vally	0	0.1
Mesa	0.11	0.31
Omak	0.09	0.88
Orondo	0.16	0.49
Sawyer	0.31	0.49
Sunnyside	0.15	0.34
Cashmere	0.08	0.26

The patterns seen with Admire and Agrimec are more worrisome. The figure for Admire at Orondo shows that even at 100x field rate mortality is less than 90%. The figure for Agrimec at Orondo show similar problems attaining 50% mortality at 10x field rate. Agrimec seldom goes above 50% mortality at 10x as pictured in Table 3. Admire shows a more variable pattern among sites as evident in Table 4.



Finally, the ugly situation for the pyrethroids is made evident in tables 5 and 6 below and two exemplar figures. Pounce shows little capacity to kill psylla as evident from the mortality observed at Sawyer ep to 50x field rate. Warrior shows similar high level of resistance.

The knowledge that we had pyrethroid resistance in psylla dates back to late 1970's. Growers continue to use these products because they are inexpensive. But that is a false economy because the literature is replete with evidence that these pyrethroids kill most beneficial natural enemies that would contribute to psylla (and mite) biological control. Similar arguments can be made about Admire and Agrimec with one caveat. The latter two products are systemic and their reduced activity on psylla may be partially due to that mode of action. IN previous studies by TRU, Agrimec was shown to be acutely lethal to the parasitoid *Trechnites* , and psylla predators *Anthocoris* and *Deraeocoris* at rates of 25% of field rates. There is ample evidence to suggest these products do much more harm than good.

Table 4. Prop. Mortality Admire		
Site\rate	1x	10x
West Vally	0.02	0.15
Mesa	0.11	0.27
Omak	0.07	0.3
Orondo	0.28	0.28
Sawyer	0.27	0.85
Sunnyside	0.89	1
Medforn	0.5	0.7

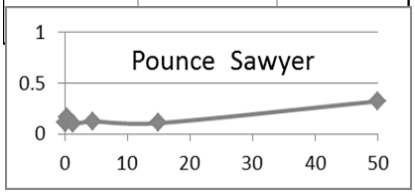


Table5. Prop. Mortality Pounce		
Site\rate	10x	50x
Tieton	0.07	xxxx
Mesa	0.01	xxxx
Omak	0.21	xxxx
Orondo	0.39	0.83
Sawyer	0.11	0.32
Cashmere	0.33	xxxx
Medford	0.98	xxxx

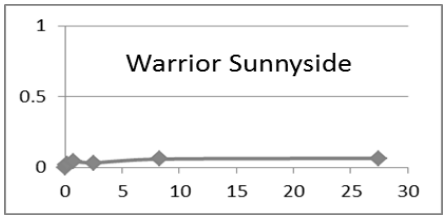


Table 6. Prop. Mortality Warrior		
Site\rate	1x	10x
West Valle	0.03	0.066
Mesa	0.13	0.18
Omak	0.08	0.16
Orondo	0.2	0.44
Sawyer	0.2	0.43
Cashmere	0.09	0.34

CONTINUING PROJECT REPORT **NO COST EXTENSION**

Project Title: Miticide resistance in spider mite pests of pears (PR-13-106)

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Cooperators: None

Other funding sources: None

Budget History:

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

Objectives

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

Significant Findings

- The adulticides (Agri-Mek, Acramite, and FujiMite) were affected by resistance in the populations tested, in decreasing order of strength of effect.
- Agri-Mek and Acramite are predicted to provide little control in the field (with the exception of Acramite for the Yakima population, where efficacy was much higher).
- FujiMite shows only incipient resistance, but field performance may still be retained.
- The ovicides (Onager, Zeal and Envidor) were less affected by resistance than the adulticides, with resistance to Onager and Zeal currently only found in one region. Where resistance occurred, it occurred at an extremely high level. No evidence of resistance to Envidor was found in any population.
- Onager (MOA 10A) resistance and Zeal (MOA 10B) resistance appear to be related, although Zeal is a much newer product.

Methods - Obj. 1 - Survey

The eight commercial orchard populations were collected over two growing seasons (four per season), representing pear orchards in the Chelan, Douglas, Okanogan and Yakima Counties. Initiating a colony from the field was made by transferring individual mites with a fine-tipped paintbrush, taking care to avoid transferring other arthropods. The populations were reared on bean plants, *Phaseolus vulgaris* L., at a constant temperature of ca. 75°F, and 16:8 light:dark photoperiod. Colonies were kept isolated in different rooms, and supplied with fresh bean plants every ≈2 weeks.

An additional susceptible reference colony was obtained from Cornell's Geneva Laboratory in New York. The latter has been reared in the laboratory for >15 years without exposure to pesticides.

Table 1. Acaricides tested against populations of twospotted spider mites from pear

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

The bioassays were performed using commercial formulations of six acaricides (Table 1), including three adulticides and three ovicides. The acaricides chosen represent six different modes of action (MOAs), however, Onager and Zeal (10A and 10B, respectively) are be considered closely related MOAsEach bioassay consisted of four to six concentrations of the acaricide and a distilled water check. All bioassays were conducted on bean leaf disks (3 cm/1.18 inch diam) with the lower surface facing up in a 3.25 oz plastic cup with cotton and water. Acaricide concentrations were mixed by serial dilution of a 1 liter stock solution, and sprayed in a Potter Spray Tower with 2 ml (0.06766 fl oz) of mixture at 6.5 psi.

Adulticide bioassays used 20 adult female mites/disk and were evaluated after 24, 48, and 72 h (the 72 h data are shown throughout this report). For ovicidal bioassays, 10 adult females were

transferred to the disks and allowed to lay eggs for 24 h. Eggs were counted, and their positions marked with a felt-tip pen, and the females removed. The initial number of eggs was standardized to 20/disk by removing excess eggs. Eggs were treated and then held at 25°C (77°F) in a growth room for 10 days, when they were evaluated for treatment mortality (unhatched eggs). These methods are essentially the same as have been used historically in collecting information on mites from Washington tree fruits, allowing for comparisons across time.

The dose-response curves were calculated with POLO-Plus (LeOra software), which provided LC_{50}s (the concentration needed to kill 50% of mites) and associated 95% confidence intervals.

An additional calculation was made using the probit regression parameters (slope, intercept, natural response). Using the maximum label field rate, the predicted percentage mortality of the various populations was estimated. It should be noted that these are relative indicators of activity because of the differences between laboratory studies and field conditions. However, they provide an index of predicted activity in the context of actual use rates, which is difficult to ascertain from the degree of change in the LC_{50}.

Rate ranges for the bioassays were chosen based initially on historical data, and adjusted if mortality was too high or too low to produce an LC_{50} using probit analysis. Because of the variable (and much higher than anticipated) levels of resistance, many of the bioassays failed probit analysis, and were re-run. Only those bioassays with six concentrations, an acceptable level of check mortality (<20%) and valid estimates of the LD10, 50, 90 and 99 with 95% confidence intervals were retained. Resistance ratios were (LC_{50}/baseline) calculated from the LC_{50} of the New York susceptible colony as the baseline; historical data are shown for reference. Resistance ratios are useful metrics in assessing the degree of resistance and likelihood for it to spread in the field. Values < 3 indicate no resistance, values 3-10 represent low levels of resistance that may spread in the field, values between 10-100 represent statistically significant resistance that may or may not cause field failure, and values > 100 indicate high levels of resistance that are likely to lead to field failure of the acaricide.

Obj. 2. Dominance of Resistance

Making crosses. Crosses were made on whole bean plants by adding at least 80 female *T. urticae* deutonymphs in teleochrysalises from the resistant mite colony and 40 males from the susceptible colony to the plants. Mites were taken from the same colonies used in Objective 1. For adulticide tests, crosses were observed for 1-2 wk until F_1 larvae began hatching. At this point, all adults were removed from the cross. This was done by removing a leaf from the plant, removing all adults from the leaf, then attaching that leaf to a new plant using a paper clip. The juveniles from the leaf would move to the new plant as the old leaf desiccated. This was done until the entire original plant was harvested. These juveniles were observed for ~1 week until all had matured and adult females were available for use in bioassays. Although only adulticide crosses have been tested to date, a similar technique can be used to test ovicides. Here, adults can be left to mate on the whole plant for about one week, and then adult females can be transferred onto bioassay arenas (described below) to lay eggs to be used in the assay.

Bioassays of crosses. Disks (3.5 cm diam) were cut from clean beans and placed with the lower surface facing up in a plastic cup (30 ml) filled with cotton and water Twenty F1 *T. urticae* females were placed on each disk. For an ovicide, mated females will be left on disks for 24 h to lay eggs. After this period, egg numbers will be adjusted to 20 per arena.

There were five replications per concentration tested, with a total of 5-7 concentrations (including the check). The number of concentrations was dependent on the number of F1 individuals available. The treatments were applied by contact to females on the disks. The concentrations range used was set so that it included values that approximated the LC_{95} of the resistant colony, LC_{25} of the resistant colony, LC_{75} of the susceptible colony, LC_5 of the susceptible colony, and some intermediate values. The LC values were determined in Objective 1. The solutions were made by mixing the appropriate amount of the formulated pesticide in 1 liter of water. Pesticides were applied with a

Potter Spray Tower (Burkard Mfg, Rickmansworth, England) set at 44.8 kPa using the intermediate nozzle.

For adulticides, bioassays were held in a growth room at 22°C and evaluated every 24 h for three days after treatment. Mites were counted as live, dead, runoff, or moribund. For ovicides, bioassays will be held for 10 d and then eggs will be counted as hatched or unhatched. All juveniles from hatched eggs will be moved onto fresh arenas and observed until mature so the number of males and females for each replication can be recorded.

Calculating *h*. Dominance of resistance (*h*) is defined as: $h = (W_h - W_s)/(W_r - W_s)$, where W_s, W_r, and W_h are the survival of susceptible, resistant, and hybrid (the cross) females, respectively (Liu and Tabashnik 1997). When $W_s \leq W_h \leq W_r$, h=0 indicates completely recessive resistance and h=1 indicates completely dominant resistance, with calculated values falling in between these two extremes. Resistance is expected to evolve slower when h is close to 0; resistance evolves more quickly as h approaches 1.

This value (h) was calculated for all doses of each pesticide assayed against a specific cross at 3 DAT. Values of W_h for these doses were obtained directly from the assays. Because the doses used in the cross bioassays were different from those used to assay resistant and susceptible colonies (Objective 1), survival at the doses used in the cross bioassay for the resistant and susceptible colonies was estimated using the probit curve for each colony.

Results and Discussion - Obj. 1

Agri-Mek. Resistance ratios (RR) for this material were extremely high for all populations tested (Table 2), ranging from ca. 9,391 to 125,760- fold increase in the LC_{50}. Of the mite populations examined, the lowest RR was from Yakima county population; all those from Chelan County (in this case, the Wenatchee River Valley), were uniformly high. This high level of resistance is the probable cause for field failure as a miticide for spider mites. However, it may still be useful for rust mites and pear psylla. The elevated resistance levels reflect its continued and frequent use since the late 1980s in Washington's pear industry. The predicted percentage mortality at the maximum label rate of Agri-Mek varied from 1 to 43% (Table 3).

Acramite. The RRs for Acramite were considerably lower than those for Agri-Mek (4.63-947) (Table 2). This material has been used for a much shorter period of time. However, with the exception of the Y1-2013 colony from Yakima, RRs were still very high, indicating a major shift in the LC_{50}s. The predicted percentage mortality at the maximum label rate of Acramite varied from 13 to 94% (Table 3).

FujiMite. RRs were lower for FujiMite than the other two adulticides (1.04-16.14); the Yakima colony showed no increase in resistance, and the other three colonies a moderate increase (Table 2). The predicted percentage mortality at the maximum label rate of FujiMite was 99-100% for all populations (Table 3).

Onager. RRs were quite variable for this material (Table 2). Two of the populations (both from the Wenatchee River Valley, and essentially contiguous, although under different management) were very high (8,450 and 12,751). All other populations had very low RRs, well within the range of variation for bioassays. The predicted percentage mortality at the maximum label rate of Onager was 100% for all populations except the two resistant ones, where the predicted mortality was zero (Table 3).

Zeal. RRs for Zeal all indicated that a moderate level of resistance has occurred, including the Yakima population (Table 2). One of the Wenatchee River Valley populations (C3-2013) apparently had extreme levels of resistance, such that no significant mortality was measured at 200,000 ppm AI, making the RR > 3.2 million. The population with this high level of resistance is the same one with high (but measurable) levels of resistance to Onager, the other IRAC group 10 material. The predicted percentage mortality with Onager at the maximum label rate (Fig. 2e) is 100%, with the exception of the highly resistant population (0% predicted mortality). Although it has not yet been tested, the 2014 population that was highly resistant to Onager will likely show a high level of resistance to Zeal.

Envidor. None of the populations tested showed any measureable resistance to Envidor; all RRs were <2 (Table 2). Envidor is one of the more recent materials to be used on pear. It is classed as IRAC MOA group 23, the same MOA as Ultor, which is routinely used on pears for psylla, and also has mite activity. All populations tested had a predicted mortality of 100% based on probit regression (Table 3).

Obj. 2. Dominance of Resistance

To date, two crosses have been performed; crosses of the FS and KK mites with the resistant colony were both assayed with FujiMite (Tables 4 and 5). Summary results of the crosses are reported in Tables 3 and 4. Calculations of *h* are reported in Tables 5 and 6. Except for the two doses on the extreme ends of the range, all values of *h* for the KK cross were <0.5, indicating recessive inheritance. At the extreme doses, survival of the crosses was lower than that of the susceptible individuals, resulting in negative values. In these cases resistance is assumed to be completely recessive and results are due to variation in survival

Table 2. LC_{50}s and resistance ratios (LC_{50} of tested field-derived colony divided by LC_{50} of susceptible laboratory colony) of six acaricides tested against populations of twospotted spider mites collected from commercial pear orchards in eastern Washington.

				95% CI		
Acaricide	TSM population	Baseline LC_{50}[x]	Calc LC_{50}	lower	upper	Resistance Ratio
Agri-Mek	C1-2013	0.004	271.20	142.38	409.74	**67,801**
Agri-Mek	C2-2013	0.004	503.04	413.28	604.14	**125,760**
Agri-Mek	C3-2013	0.004	389.33	277.54	508.77	**97,332**
Agri-Mek	Y1-2013	0.004	37.56	24.13	51.14	**9,391**
Agri-Mek	C2-2014	0.004	116.02	64.10	170.53	**29,012**
Acramite	C1-2013	2.29	1213.51	982.09	1476.08	**531**
Acramite	C2-2013	2.29	2165.29	1730.02	2626.31	**947**
Acramite	C3-2013	2.29	687.14	599.95	789.71	**300**
Acramite	Y1-2013	2.29	10.59	0.00	53.65	**4.63**
FujiMite	C1-2013	1.29	8.94	7.95	10.02	**6.93**
FujiMite	C2-2013	1.29	11.68	8.87	14.39	**9.05**
FujiMite	C3-2013	1.29	20.82	13.77	26.29	**16.14**
FujiMite	Y1-2013	1.29	1.35	0.13	3.37	**1.04**
Onager[y]	C1-2013	0.14	0.51	0.37	0.74	**3.61**
Onager	C2-2013	0.14	0.39	0.34	0.45	**2.79**
Onager	C3-2013	0.14	1785.18	1573.97	1995.69	**12,751**
Onager	Y1-2013	0.14	0.42	0.29	0.51	**3.01**
Onager	C2-2014	0.14	1182.94	1019.41	1367.30	**8,450**
Onager	D1-2014	0.14	0.15	0.11	0.18	**1.04**
Zeal	C1-2013	0.062	5.02	2.81	7.25	**81.02**
Zeal	C2-2013	0.062	5.77	5.00	6.47	**93.06**

Zeal	C3-2013	0.062	[z]			
Zeal	Y1-2013	0.062	1.57	1.29	1.83	**25.39**

Envidor	C1-2013	5.96	9.76	5.57	13.32	**1.64**
Envidor	C2-2013	5.96	11.41	9.20	14.15	**1.91**
Envidor	C3-2013	5.96	8.22	6.24	10.09	**1.38**
Envidor	Y1-2013	5.96	9.70	6.08	12.94	**1.63**
Envidor	C2-2014	5.96	6.43	5.66	7.21	**1.08**
Envidor	O1-2014	5.96	11.08	6.24	14.70	**1.86**

[x]Baseline LC_{50} is from contemporary bioassays on the susceptible New York colony. [y]Provisional baseline for NY colony; historical baseline 0.102 ppm AI. [z]Unable to obtain significant mortality at 200,000 ppm AI (near limits of solubility).

Table 3. Predicted percentage mortality at the field rate of various acaricides

Pesticide	Colony	Field rate	field rate (ppm ai)	intercept	natural	slope	Predicted % mortality
Agrimek	C1-2013	4.25 fl oz	27.85	-3.165	0.056	1.301	10.95
Agrimek	C2-2013		27.85	-4.746	0	1.757	1.36
Agrimek	C3-2013		27.85	-4.746	0.055	1.832	2.05
Agrimek	Y1-2013		27.85	-2.366	0.019	1.503	43.04
Agrimek	C2-2014		27.85	-2.622	0.02	1.27	22.15
Acramite	C1-2013	1 lb	599.20	-7.213	0.057	2.339	25.49
Acramite	C2-2013		599.20	-7.078	0.037	2.122	12.57
Acramite	C3-2013		599.20	-9.94	0.056	3.503	43.87
Acramite	Y1-2013		599.20	-0.907	0.02	0.885	94.19
FujiMite	C1-2013	2 pt	119.84	-3.867	0	4.065	100.00
FujiMite	C2-2013		119.84	-2.596	0.041	2.432	99.38
FujiMite	C3-2013		119.84	-3.892	0.02	2.952	98.82
FujiMite	Y1-2013		119.84	-0.149	0.03	1.157	98.89
Onager	C1-2013	24 fl oz	224.70	0.781	0	2.641	100.00
Onager	C2-2013		224.70	2.294	0.062	5.665	100.00
Onager	C3-2013		224.70	-22.689	0.042	6.977	0.00
Onager	Y1-2013		224.70	2.94	0.214	7.835	100.00
Onager	C2-2014		224.70	-13.138	0	4.275	0.10
Onager	D1-2014		224.70	2.216	0.201	2.645	100.00
Zeal	C1-2013	3 oz	161.78	-1.739	0.03	2.48	99.99
Zeal	C2-2013		161.78	-5.055	0.089	6.641	100.00
Zeal	C3-2013		161.78				
Zeal	Y1-2013		161.78	-0.77	0.065	3.906	100.00

Envidor	C1-2013	18 fl oz	337.05	-2.451	0.01	2.477	99.99
Envidor	C2-2013		337.05	-3.288	0	3.11	100.00
Envidor	C3-2013		337.05	-3.578	0.044	3.912	100.00
Envidor	Y1-2013		337.05	-2.251	0.05	2.281	99.98
Envidor	C2-2014		337.05	-3.23	0.023	3.996	100.00
Envidor	O1-2014		337.05	-3.727	0.171	3.568	100.00

Table 4. Percentage mortality (± SE) for the offspring of KK ♀ crossed with NY♂ treated with FujiMite (*, values rounded up to zero).

			KK ♀ x NY ♂cross			
Conc (mg AI/liter)	% Mortality (3 DAT)	Conc (mg AI/liter)	W_r	W_s	W_h	h
75	100.00 ± 0.00	75	0.05	0.01	0	0.00*
12	88.89 ± 5.09	12	0.75	0.09	0.11	0.03
9	85.74 ± 6.24	9	0.85	0.12	0.14	0.03
7	74.89 ± 7.84	7	0.92	0.15	0.25	0.13
4.55	45.00 ± 6.89	4.55	0.97	0.21	0.55	0.44
0.06	17.00 ± 4.06	0.06	--	--	--	--
0	2.22 ± 2.22		--	--	--	--

Table 5. Percentage mortality (± SE) for the offspring of FS ♀ crossed with NY♂ treated with FujiMite (*, values rounded up to zero).

			FS ♀ x NY ♂cross			
Conc (mg AI/liter)	% Mortality (3 DAT)	Conc (mg AI/liter)	W_r	W_s	W_h	h
23	100.00 ± 0.00	75	*0.05*	*0.05*	*0*	--
6	98 ± 1.22	12	--	--	--	--
0.6	41.53 ± 3.69	9	--	--	--	--
0.06	11.56 ± 4.38	7	0.76	0.17	0.02	0.00*
0	5.22 ± 2.30	4.55	1	0.61	0.58	0.00*
		0.06	1	0.94	0.88	0.00*

CONTINUING PROJECT REPORT **YEAR: 1 of 3**
Project Number: PR14-104

Project Title: Fall and summer pruning to control vigor and psylla in Anjou pear

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Cooperators: Sara Serra (WSU/TFREC)

Total Project Request: **Year 1**: $72,707 **Year 2: $71,589** **Year 3**: $71,170

Other funding sources:

Agency Name: USDA/ARS
Amt. awarded: Harvest and postharvest quality analyses conducted by Jim Mattheis to be supported with base USDA, ARS funds.

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

Item	2014	2015	2016
Salaries[1]	36,480	37,939	39,456
Wages[2]	11,440	11,898	12,374
Benefit[3]	14,130	14,695	15,283
Travel[4]	757	757	757
Goods and Services[5]	9,900	6,300	3,300
Total	72,707	**71,589**	71,170

Footnotes:
[1] Salary for a new hire Research Intern (Musacchi), a Research Intern (Beers).
[2] One non-Student temporary for 13 wks: 40/wk at $11/hr (Musacchi) and one non-Student temporary for 13 wks: 40/wk at $11/hr (Beers).
[3] Benefits at 9.7% (Musacchi and Beers).
[4] 676 miles/year for domestic travel to go to the orchard (Musacchi) and 676 miles/year for domestic travel to go to the orchard (Beers).
[5] Fruit mineral analyses, data loggers, light bar, laboratory supplies for fruit quality analyses (Musacchi).

OBJECTIVES

1. *Control vigor through pruning practices in a mature Anjou orchard while maintaining yield and quality, and reduce psylla densities throughout the tree.*

SIGNIFICANT FINDINGS

Vigor control

- Similar amounts of branch and leaf material were removed from winter pruned and summer/fall pruned trees during 2014.
- Summer/fall pruned trees had greater light interception during the growing season due to higher canopy density prior to final fall pruning.
- Although light measures did not differ significantly between winter and summer/fall pruning treatments, at the end of the 2014 growing season, summer/fall pruned trees appeared to have greater light incident on branches.
- Rootstocks (OHF97, OHF69, and OHF87) did not differ in pruning weight removed by pruning treatment both in summer/fall and winter.
- The quantity of leaves, one-year old shoots, old wood and good small fruit did not differ among rootstocks for summer pruning in summer/fall pruning treatments.

Yield and quality

- Yield was higher on winter pruned trees, but yield efficiency (kg/cm^2 trunk cross sectional area) was not different between summer/fall and winter pruning, nor among rootstocks.
- Fruit were significantly smaller in summer/fall pruning compared to winter pruning, but fruit from summer/fall pruned trees were of commercial size (80 fruit/box).
- Pruning timing had no significant effect on fruit calcium, nitrogen, or cork spot.
- Fruit sunburn incidence was higher in summer/fall pruned trees.

Psylla and Mite Densities

- With the exception of the pre-spray delayed dormant count, psylla densities were low throughout the season.
- OHF 69 had significantly higher levels of older nymphs than the other two rootstocks.
- Mite densities were generally high, peaking in mid-July and late September; however, there were no significant treatment or rootstock differences.
- Fruit damage from insects was very low, but significantly higher psylla russet occurred in the winter-pruned treatment.

METHODS

The trial was carried out in an Anjou orchard planted in 1998 on three different rootstocks: Old Home x Farmingdale (OHF) 97, 69, and 87. OHF 97 is considered a vigorous rootstock in comparison with the other two (semi-vigorous). The three combinations of Anjou on different rootstocks are fully randomized inside the orchard.

Vigor

Half of the rows were winter pruned (19 Mar 2014). The other half of the rows were summer pruned (12 Jun 2014) to remove vigorous suckers, with the intent of reducing nutrient competition between shoots and fruit and to reduce psylla presence. The summer pruned rows were pruned again in the fall after harvest (24 Oct 2014). These pruning treatments will be repeated every year of the trial (from 2014 to 2016) to assess the potential for improvement of fruit quality and distribution in the canopy. The weight of the pruned branches and leaves from a subset of 18 trees were determined at

each pruning. Vigor was also assessed using light penetration data collected beneath tree canopies via a photosynthetically active radiation (PAR) portable measurement systems. Fresh and dry weight were also calculated for the branches, leaves, and fruit removed during summer pruning.

Yield and Quality

Pre-harvest assessment of fruit maturity was carried out one week before harvest on one tree per rootstock per each pruning treatment (total 6 trees) to determine differences between them. Postharvest fruit quantity and quality measurements were carried out to define yield and quality effects of winter compared to summer/fall pruning:

1. At harvest time (2-3 Sep 2014), yield (fruit number and weight) per tree was determined for each treatment combination (two pruning times and three rootstocks). A sample of fruit per each treatment was divided according to their I_{AD} index to optimize the samples for the storability. Fruit quality and maturity, including skin color parameters (L, a, b), weight, firmness, soluble solids content, acidity, and pH were assessed at harvest.
2. After two and four months of storage at -1°C, fruit quality and maturity was assessed and samples were analyzed for calcium, nitrogen and other nutrient content by enzymatic digestion (Best Test Analytics, Moses Lake, WA). Cork spot incidence was assessed on ~900 fruit on Nov 3 and 4, 2014. The fourth month analysis (January 2015) were not entirely completed when this report was written.

Psylla and Mite Sampling

Psylla adults. Adult psylla were sampled with a beating tray (10 taps/subplot, or 20 per treatment x rootstock x replicate combination) every 2-3 weeks from mid-March through the end of September. The number of adult psylla falling on the tray was recorded, and the average of the 20 taps was used for analyses.

Psylla eggs and nymphs. Pear psylla eggs and nymphs were counted from mid-April through the end of September. Pre-bloom counts were taken from spur samples, and post-bloom counts were taken from leaf samples. On 14 and 28 Apr, 10 fruit spurs per subplot were collected, brought back to the lab and examined. From that point forward (13 May), leaf samples were used to assess psylla densities. Four leaves per each tree in the subplot (40 leaves total) were collected and kept cool during transportation and storage. Leaves were brushed with a leaf-brushing machine (Leedom Mfg, Mi-Wuk Village, CA) and collected on a revolving glass plate coated with undiluted dishwashing liquid. Psylla nymphs were recorded as either young (1st, 2nd or 3rd instar) or as old (4th or 5th instar). Psylla eggs and nymphs on spur and leaf samples were counted using a stereoscopic microscope.

Mites. The most common orchard mite species were also counted on the same leaf samples used for pear psylla starting on 13 May. All stages and species of phytophagous and predatory mites were recorded, including the eggs and motile stages of European red mite (ERM), *Panonychus ulmi* (Koch); twospotted spider mite (TSM), Tetranychus urticae Koch; McDaniel spider mite (MCD), *Tetranychus mcdanieli* McGregor [the eggs of TSM and MCD could not be distinguished, and were recorded as a group]; western predatory mite, *Typhlodromus* (=*Galendromus*) *occidentalis* (Nesbitt); and motile stages of pear rust mite (PRM), *Epitremerus pyri* (Nalepa). For both psylla and mites, the composite sample on the plate was counted using a stereoscopic microscope, and divided by the number of leaves to obtain a per-leaf estimate

Fruit damage. Following harvest on 2 Sep, fruit damage was assessed on 100 fruit per subplot (200 fruit/replicate). Each fruit was rated for russet and the source of the russet (pear psylla, grape mealybug or pear rust mite) was noted. The russet rating was based on a severity scale of 0 = no

russet, 1 = 1 to 10% of the fruit surface with russet, 2 = 11 to 20% russet, and 3 = 21 to 30% russet. In addition, the absence or presence of grape mealybug in the calyx of each fruit was noted.

RESULTS AND DISCUSSION

Vigor

In early summer (11 Jun 2014), winter pruned trees had greater light penetration (61%), implying lower canopy density than summer/fall pruned trees (50% light penetration) (Table 1). Immediately following summer pruning (12 Jun 2014), summer/fall pruned trees had similar light penetration (61%, 18 Jun 2014) to winter pruned trees (61%), implying similar canopy density (Figure 1). The quantity of pruned material removed from either pruning treatment did not differ according to rootstock. Fresh and dry weights for the branches, leaves, and fruit removed during summer pruning did not differ significantly among the rootstocks (Figure 2). Leaf area removed did not differ significantly among the rootstocks (data not shown).

Table 1. Average material removed per tree at each pruning date and overall light penetration in the canopy by pruning treatment, 2014.

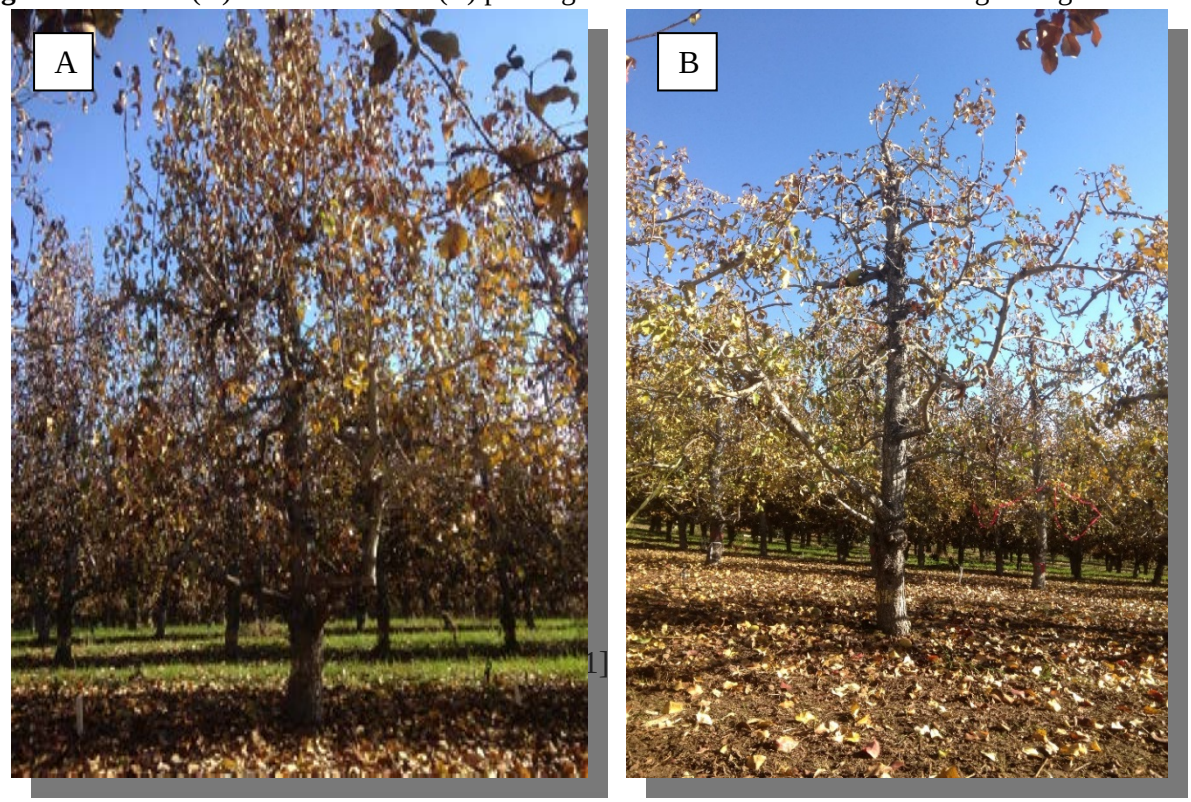
Date	**19 Mar**	**11 Jun**	**12 Jun**	**18 Jun**	**29 Oct**	
Pruning treatment	winter prune (lb/tree)	**%LP*†**	summer prune (lb/tree)	**%LP***	fall prune (lb/tree)	**Total pruned** (lb/tree)
Winter	34.4	61.0% a	--		--	34.4
Summer/fall	--	49.6% b	21.7	61.0%	12.2	33.9
Significance†‡	--	*	--	--	--	*ns*

† For light penetration, ANOVA, *post hoc* Tukey test.

‡ For pruned material, proc GLM in SAS ANOVA; type III sums of squares significance: *, p <0.05, **, p < 0.01; ***, p <0.001.

*LP = Light penetration

Figure 1. Winter (A) and summer/fall (B) pruning treatments at the end of the 2014 growing season.



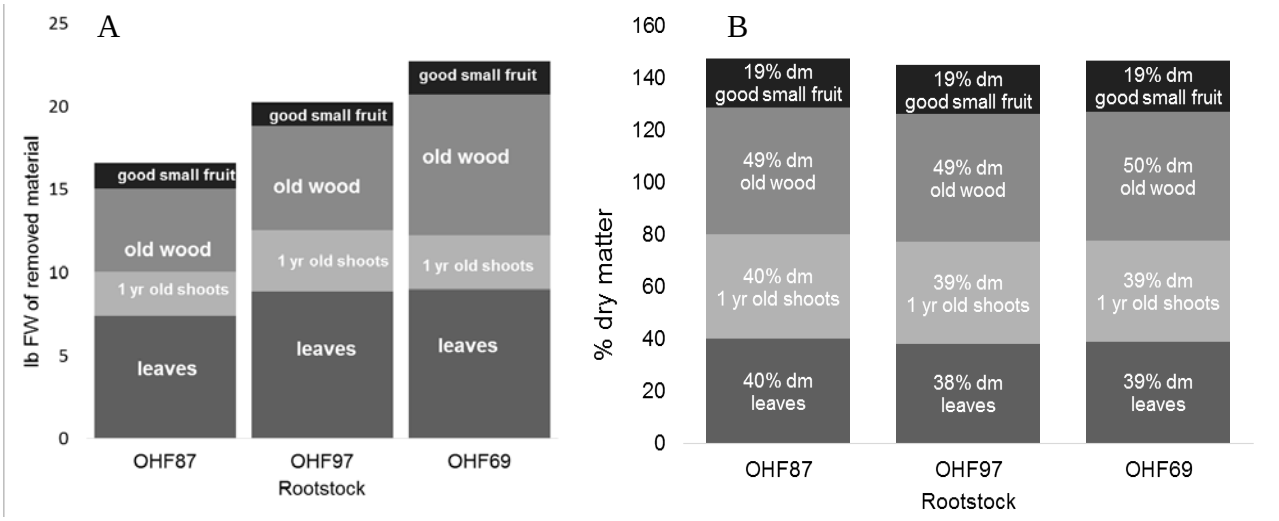


Figure 2. Fresh weight (A) and percent dry matter (B) of pruned materials did not differ significantly among the rootstocks for summer pruning in summer/fall pruned trees.

Yield and Quality

Pre-harvest exploration of fruit maturity distribution with the I_{AD} (DA) meter indicated that winter pruned trees had a higher percentage of fruit belonging to the more ripe I_{AD} classes (Figure 3). This survey helped determine when to start picking the winter pruning treatment.

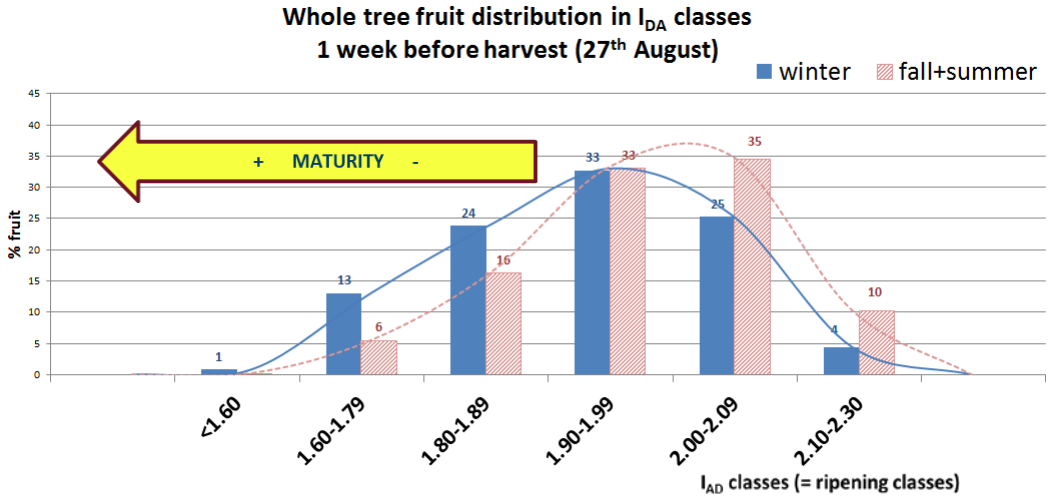


Figure 3: Fruit maturity distribution of the whole canopy of central leader winter pruned and summer/fall pruned trees at pre-harvest (27 August 2014).

Yield was higher for winter pruned trees (Table 2), but neither pruning nor rootstock significantly affected yield or yield efficiency (yield per tree/ trunk cross-sectional area). Average fruit weight was lower for summer/fall pruned trees compared to winter-pruned trees (Table 2).

Table 2. Anjou yield by pruning treatment, 2014.

Pruning	Average fruit weight (g)	Number of fruit per tree	Yield (lb) per tree	Yield efficiency (lb/in^2 TSCA)
Summer/fall	208.4 b	377	171.4 b	4.7
Winter	236.3 a	370	191.3 a	4.4
Significance‡	***	*ns*	*	*ns*

‡ proc GLM in SAS; type III sums of squares significance: *, p <0.05, **, p < 0.01; ***, p <0.001.

Calcium, nitrogen, and cork spot were not statistically significant in summer/fall pruned fruit compared to winter pruned fruit (Table 3). Cork spot incidence overall was low. Sunburn incidence was slightly higher in summer/fall pruned trees compared to winter pruned trees.

Table 3. Calcium, nitrogen, and cork spot incidence by pruning treatment, 2014**.**

Pruning	Fruit Calcium (%)	Cork spot incidence (%)#	Fruit Nitrogen (%)	Sunburn (%)
Summer/fall	0.054	2.9	0.297	1.10 a
Winter	0.045	5.2	0.325	0.42 b
Significance	*ns*‡	*ns*†	*ns*‡	*‡

‡ proc GLM in SAS; type III sums of squares significance: *, p <0.05, **, p < 0.01; ***, p <0.001.

†Chi-square test of proportions, ns = not significant; *, p <0.05; **, p < 0.01; ***, p < 0.001.

At harvest, summer/fall pruned fruit had a higher I_{AD} reading, indicating the fruit were less ripe (Table 4). Correspondingly, summer/fall pruned fruit had lower soluble solids content. Summer/fall pruned fruit were lighter with a slightly more yellow hue angle. Chroma was not significantly different between the pruning treatments (data not shown). Summer/fall pruned tree also had a lower titratable acidity than winter pruned trees.

Table 4. Fruit quality measures at harvest.

Pruning	I_{AD}	Firmness	Soluble solids (degrees Brix)	Lightness (L)	Hue angle	Titratable acidity (% malic acid)
Summer/Fall	1.92 a	13.9	12.2 b	59.8 a	114.1 b	0.258 b
Winter	1.89 b	13.9	12.4 a	59.0 b	114.7 a	0.285 a
Significance‡	***	*ns*	***	*	***	***

‡ proc GLM in SAS; type III sums of squares significance: *, p <0.05, **, p < 0.01; ***, p <0.001.

Psylla and Mite Densities

Overwintering psylla adult densities were moderate (19-20/tap) on 18 Mar before the first insecticide applications were made. They remained low throughout the season, rising slightly in the fall after harvest. Few treatment or rootstock differences occurred on any of the count dates, nor was the treatment x rootstock interaction significant.

Psylla eggs and nymphs were also high on the first spur count, but reduced by the insecticide application. No treatment or rootstock differences in mean psylla numbers occurred. Early and late instar nymphs remained at low densities throughout the rest of the growing season, never approaching

the action threshold of 0.3 nymphs/leaf. While the densities were low, the OHF 69 rootstock had higher levels of older nymphs than the other two rootstocks.

Tetranychid mites peaked in mid-July at ca. 6 mites/leaf. A second peak occurred late September (9-12 mites/leaf) although no treatment or rootstock mean differences occurred at any time. The high mite densities in the study orchard reflected higher mite densities throughout the Wenatchee River Valley in the 2014 growing season.

Fruit damage from psylla, mealybug and rust mite were low, with an average damage rating in all cases of <1 (1-10% of the fruit surface russetted). However, the damaged from psylla in the winter-pruned treatment was significantly higher than that in the summer/fall pruned treatments.

CONTINUING PROJECT REPORT **YEAR: 1 of 3**
Project Number: PR-14-108A

Project Title: Improving quality and maturity consistency of 'D'Anjou'

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City/State/Zip: Wenatchee/WA/98801

Cooperators: Sara Serra, JingJin Zhang, Rachel Leisso (WSU/TFREC)

Total Project Request: **Year 1**: $ 65,992 **Year 2**: **$67,272** **Year 3**: $ 68,602

Other funding sources: None

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

Item	2014	2015	2016
Salaries[1]	24,000	24,960	25,958
Benefit[1]	7,992	8,312	8,644
Travel[2]	500	500	500
Goods and Services[3]	3,000	3,000	3,000
Total	35,492	**36,772**	38,102

Footnotes:
[1]Salaries and benefits for 50% Ag. Research Assistant (Musacchi).
[2]Travel to different orchards and farm where the different trials will be conducted (Musacchi).
[3] Consumable lab ware and mineral analyses.

Budget 2
Organization Name: USDA, ARS **Contract Administrator: Chuck Myers**
Telephone: 510-559-5769 **Email address: Chuck.Myers@ARS.USDA.GOV**

Item	2014	2015[2]	2016[2]
Wages[1]	15,000	15,000	15,000
Goods and Services[2]	15,500	15,500	15,500
Total	30,500	**30,500**	30,500

Footnotes:
[1] $12,500 for 25% annual instrument service contracts. $3,000 for consumables
[2]Add proposed same amount for year 1 if work is to be performed in years 2 or 3.

OBJECTIVES:

1) *Determine maturity and quality variation as impacted by tree and orchard management regimes.*
2) *Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.*

SIGNIFICANT FINDINGS

1) *Determine maturity and quality variation as impacted by tree and orchard management regimes.*

- Considerable variability in fruit maturity exists within the large canopy of an open vase tree.
- The DA meter values (I_{AD}) for internal and external canopy fruit were different at harvest. External fruit tend to have lower I_{AD} values compared to internal fruit.
- At harvest, external fruit were significantly heavier, larger, and had higher titratable acidity and soluble solids compared to internal fruit. Internal fruit had greener peel color.

2) *Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.*

- Peel chemistry during the postharvest period is dependent on fruit on-tree position at harvest.
- Postharvest degreening occurs at a different rate depending upon fruit on-tree position.
- Many of the peel phytochemicals are important for pear quality and ripening and were different based on fruit on-tree position.

METHODS

1) Determine maturity and quality variation as impacted by tree and orchard management regimes.

Pre-harvest exploration

A mature D'Anjou orchard with trees trained as open vase (20 ft x 20 ft, 109 trees/acre) was used for this trial. Trees in this system have a large canopy volume with high maturity variability among fruit. At the end of August, we picked a representative tree from the orchard for an estimate of the total number of fruit (1971 good fruit and 135 <60 mm size and/or with defects), yield (344 kg/tree), and fruit maturity variability in the whole canopy. Fruit were assessed for their ripening stage using the DA meter.

PAR measurement portable system

A portable spectrometer was used to quantify light intensity in different horizontal layers within tree canopies. This system consisted of an implemented light-bar (Figure 1(a)) with total measuring length of 2.4 m, resulting in 24 readings (0.1 m/reading). A data acquisition box was developed and connected to a laptop to monitor and record data.

We identified 15 similar trees in the same orchard and evaluated them for light penetration. Branches were categorized by two height levels, approximate 2.0 m and 3.5 m. Therefore, midday light measures were conducted from above each height. Light measurements were performed in two passes, one on each side of the canopy. The pass length was 6.0 m (3.0 m across the row on both sides of each trunk). During each pass, light-bars were held perpendicular to each row and data were collected at 0.3 m intervals creating a grid of 21 readings × 24 readings (across row × with row). Midday light measures were mainly conducted on the lower level (approximate 2.0 m). Internal fruit were harvested based on measurements taken from the lower level. External fruit were sampled from the outer layer of the canopies on the upper level when there was nothing above the fruit.

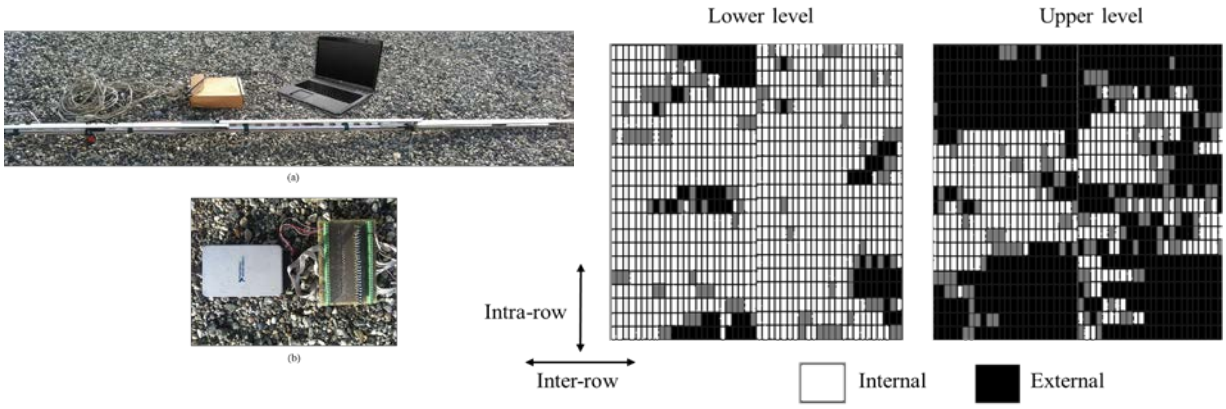

Figure 2: PAR measurement portable system: light bar (a) and data acquisition box (b). On the right the light penetration map of one tree canopy build up with this measurement. White color represents area with light penetration lower than 30%, classified as internal portion, and black color represents area with light penetration greater than 70%, classified as external portion.

2014 harvest and fruit sorting

Fruit from each of the two light penetration levels were harvested on 9/10. Fruit from the lowest zone (internal; <30% light) and highest (external; 70-100% light) canopy regions were separately picked. Fruit from each light condition were collected into two bins and immediately moved to 40°F where fruit maturity distribution was assessed on 1013 external fruit and 934 internal pears.

Fruit were sorted into additional groups by fruit weight. Within each group (external and internal), fruit were also classified according to I_{AD} reading (Table 1).

Fruit belonging to each class were randomized and subdivided in 4 groups: T0 (= after harvest, no controlled atmosphere (CA) storage) and three pull outs from CA storage (T1, T2, and T3).
Fruit to be stored were moved to a research CA room (31°F, 2% O_2 and 0.8% CO_2).
The first pull out has been analyzed at 3 months (T1) and will be again at 6 and 8 months (T2 and T3).

Table 1: I_{AD} classifications (DA meter readings) for sorting fruit within two canopy positions.

I_{AD} range	**ripening class**	**canopy position**	
1.40-1.59	A	.	EXTERNAL
1.60-1.79	*B*	INTERNAL	EXTERNAL
1.80-1.89	*C*	INTERNAL	EXTERNAL
1.90-1.99	*D*	INTERNAL	EXTERNAL
2.00-2.19	E	INTERNAL	.

Fruit Quality Analysis

At harvest (T0), for each I_{AD} class within each canopy position we estimated/measured the percentage of blush over-color surface, the background color (Minolta colorimeter), the exogenous ethylene (continuous flow method), I_{DA} index (DA meter), and weight as non-destructive parameters, followed by assessing firmness, fruit diameter, cork spot incidence, soluble solid content, acidity and pH.

After 3 months of CA storage (T1), we subdivided again all fruit in two batches in order to evaluate fruit immediately after CA room exit ("unripe stage") and after 7 days at room temperature ("ripe stage") assessing the fruit behavior of the different ripening classes within each position in the canopy.

2) Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.
A preliminary study aimed at determining differences in peel and cortex chemistry from upper and lower portions of the fruit associated with canopy position was performed on fruit harvested from external and internal positions. Fruit were evaluated using the DA meter at harvest and then stored at room temperature for 24 days after harvest to provide an initial assessment of the impact of tree position on postharvest maturation. At harvest and following storage, peel and cortex tissue were separated and frozen prior to analysis of 400 non-polar chemicals simultaneously using LC-MS. Differences in peel chemistry associated with tree position were determined by interrogating chemical levels using multivariate statistics.

In 2014, peel and cortex of fruit grown and harvested from different canopy positions were sampled (5 rep/I_{AD} class/canopy position=80 samples) immediately following harvest (T0, no CA storage) and at T1 for metabolomics analysis.

RESULTS AND DISCUSSION

1) Determine maturity and quality variation as impacted by tree and orchard management regimes.

Fruit maturity as related to canopy position
By rating I_{AD} before harvest, we confirmed the variability of maturity within a tree: 52% of fruit were classified in the least mature I_{AD} classes (over 2.00 I_{AD}) and only a small percentage of fruit were classified in the more ripe classes (below 1.80 I_{AD}, Fig. 2).

Fruit maturity distribution within I_{AD} classes at harvest was different. Twenty percent of internal fruit were still in the least ripe class, while only the 2.7% of external one belonged to that class (Fig. 2). Almost 28% of external fruit were classified in the most ripe categories (<1.60 I_{AD}), while only 0.7% of the internal ones resided in those classes.

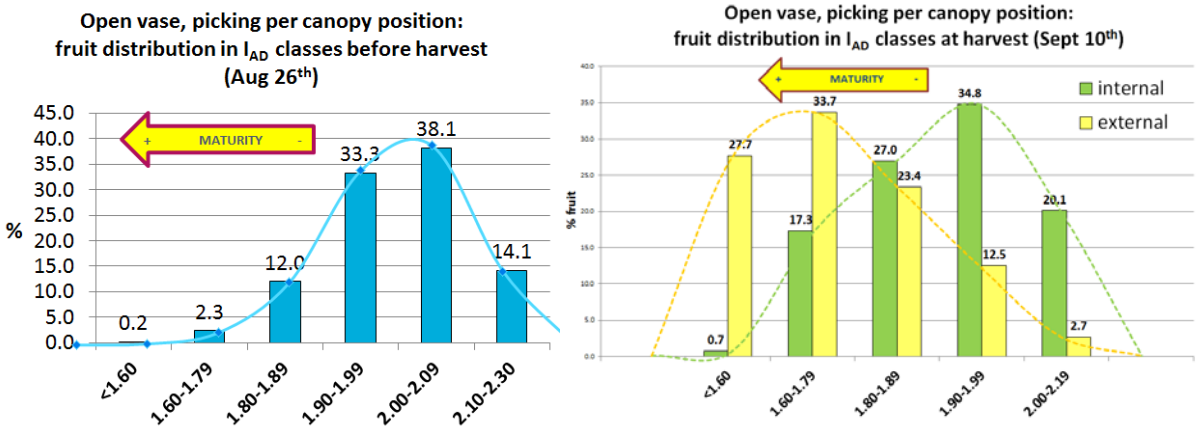

Figure 3: Fruit maturity distribution of the whole canopy of an open vase tree at pre-harvest (left) and distribution of all the fruit picked divided by position in the canopy: external and internal (right).

Fruit Quality Analysis

At T0 no ethylene production was detected. External fruit were significantly larger (higher average fruit weight and diameter) and had higher SSC and titratable acidity than internal ones (Table 2). Peel of internal fruit was significantly greener than external ones. A percentage of blushed over-color was observed only in external fruit with an estimated covered percentage ranging from 5 to 9%. There was no difference in cork spot or firmness (Table 2).

When all eight combinations were compared (four ripening classes x two canopy positions), significant differences were found in fruit weight, firmness and soluble solid contents. Class E internal (the least ripe of the classes) were significantly smaller than external (all External from A to D) fruit. The firmest fruit were from classes External D, C and Internal D, while the softest ones were in class Internal B. Soluble solids content was clearly different among the eight combinations, all four classes of internal fruit had comparable values statistically lower from those in the four classes of external fruit (Fig. 3).

Table 2: Fruit quality analysis at T0 (harvest): comparison between external and internal fruit of an open vase tree.

Canopy position	Weight (g) T0	I_{DA} index T0	Hue	Chroma	Diameter (in)	Firmness (lb)	SSC (Brix)	pH	TA (% malic acid)
External	241 a	1.73 b	112.4 b	41.5	2.7 a	13.80	13.89 a	3.95 b	0.38 a
Internal	213 b	1.89 a	114.8 a	40.5	2.6 b	13.65	12.07 b	4.04 a	0.36 b
significance	***	***	***	ns	***	ns	***	**	*

*p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant for Type III sums of squares model significance.*
Arithmetic means are presented; post hoc tests were done with LSMEANS option and the Bonferonni adjustment provided letter.

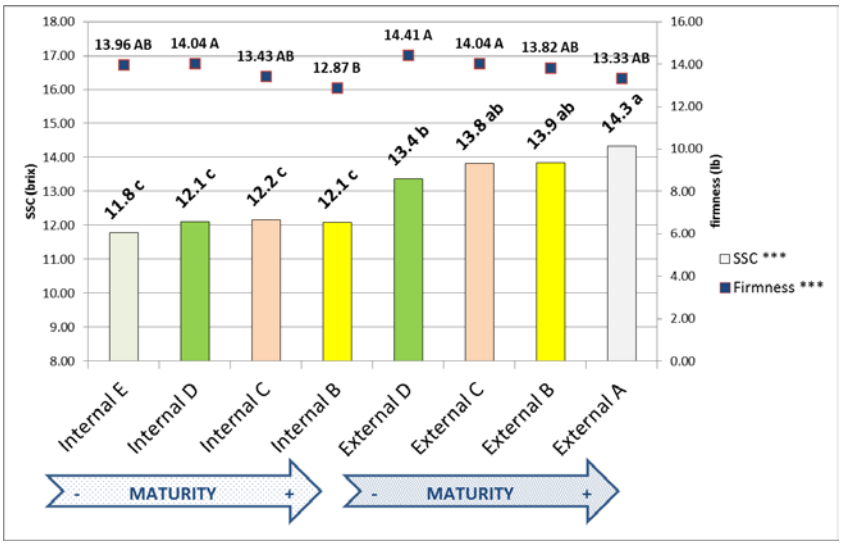

Figure 3: All eight combinations (4 ripening classes x 2 canopy positions) under comparison for soluble solid contents and firmness at T0.

2) Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.

In our preliminary study (2013), fruit harvested from the internal canopy were greener than the external ones and differences in peel color, water retention (shriveling), and, ostensibly, fruit maturity (measured by DA meter) were observed. Differences in natural chemical levels (Fig. 4) of stem-end (top) and calyx-end (bottom) Anjou pears harvested from internal (int) and external (ext) portions of the tree canopy sampled after 24 days at room temperature were reported. DA meter readings were different at harvest (internal fruit were less ripe than the external ones according to DA meter readings) and those differences increased during the shelf-life period (the decrease of this index was more pronounced in external fruit than in the internal ones suggesting a difference in their ripening kinetics and chemistry). Differences in chlorophyll levels of tops and bottoms of Anjou pears harvested from internal and external portions of the tree canopy and sampled after 24 days at room temperature indicates degreening occurs at a different rate depending upon on-tree position (Fig. 5). This summary of differences in 400 pear peel phytochemicals shows that tree position and peel location impacts changes in natural peel chemicals associated with many important quality and ripening processes.

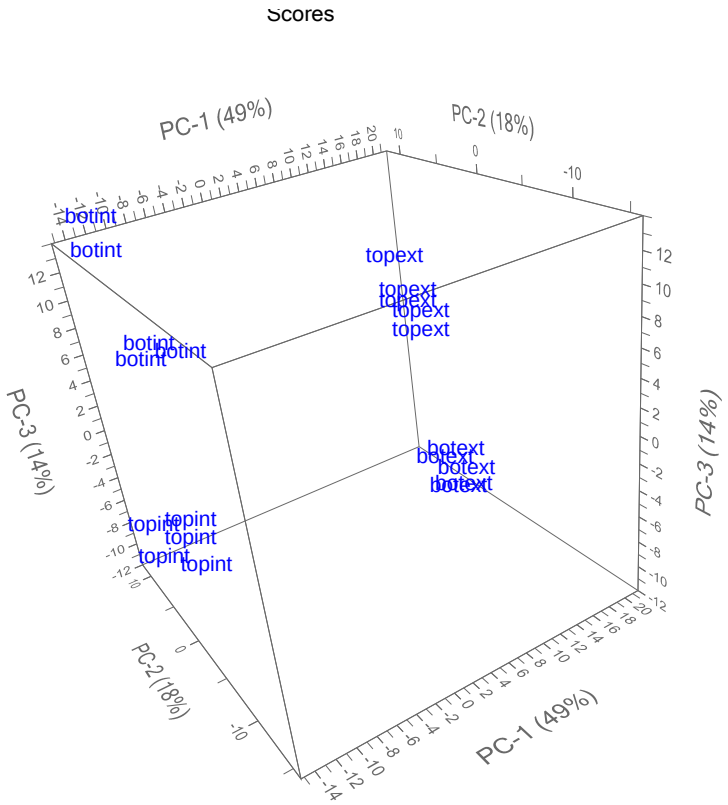

Figure 4: Differences in natural chemical levels of stem-end (top) and calyx-end (bottom) Anjou pears harvested from internal (int) and external (ext) portions of the tree canopy sampled after 24 days at room temperature.

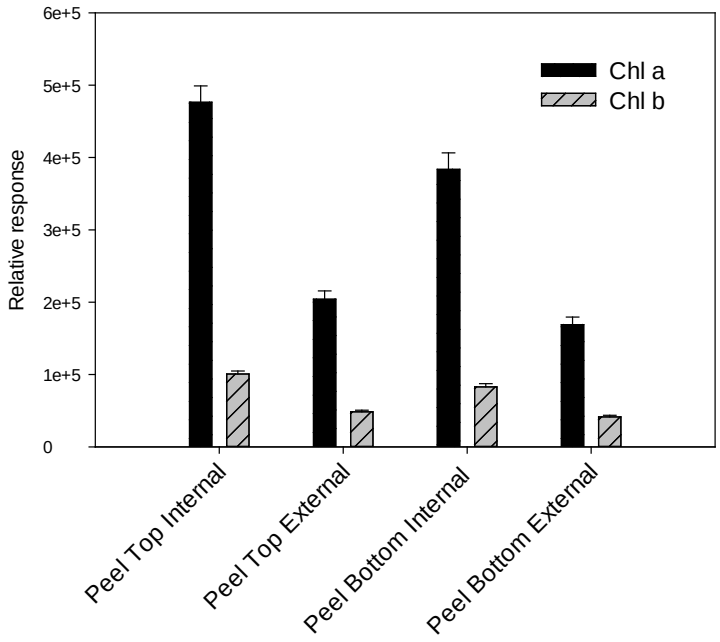

Figure 5: Differences in chlorophyll levels of tops and bottoms Anjou pears harvested from internal and external portions of the tree canopy sampled after 24 days at room temperature.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-14-107

Project Title: Establishing NW-acclimated pyrus rootstock breeding material

PI: Amit Dhingra
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Co-PI: Kate Evans
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Total Project Request: **Year 1**: 21,409 **Year 2**: **22,185** **Year 3**: 22,992

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

Item	2014	2015	2016
Salaries			
Benefits			
Wages[a]	13832	14385	14960
Benefits	5577	5800	6032
Equipment			
Supplies[b]	1000	1000	1000
Travel			
Miscellaneous			
Plot Fees[c]	1000	1000	1000
Total	21,409	**22,185**	22,992

Footnotes: a. Technical support for plant handling in greenhouse
b. Greenhouse supplies, pots, soil etc.
c. Greenhouse space fees

OBJECTIVES

The objectives of the project are:
1. Screen seedlings germinated in 2012 for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions (2013-2015)

> Protocols and approaches developed for accelerating plant growth for apples continues to be used as a model for adaptation to accelerating pear seedling growth in the greenhouse.

2015-2016: The plants have been taken through two cycles of growth since the project was funded. The activities for the next year would be continue the maintenance of the trees and push the growth of the plants to complete 2-3 additional growth cycles. This will necessitate greenhouse growth and incubation in cold chambers to provide 1000 hours of chilling. The plants are currently in the juvenile state as evident from the presence of thorns. Once this phase is over, trees will be phenotyped for node number, internode length, height and architecture.

2. Germinate and subsequent phenotypic screening of 618 seeds derived from irradiated pollen in 2013 (2013-2016)

> Over 60% of the seeds obtained with irradiated pollen will require embryo rescue since they are deformed without proper formation of cotyledons. In this technique, the embryo is excised from the seeds and developed using tissue culture procedures. Remaining 40% of the seeds will be germinated conventionally and characterized for desirable phenotypes.

2015-2016: Seeds derived from irradiated pollen demonstrated high rate of mortality. A total of 51 seeds germinated and have gone through one cycle of rapid growth already. The embryo rescue with several deformed seeds has remained unsuccessful. The radiation levels may have been too high and could have resulted in embryo lethal phenotype. This part of the work will be continued to screen all deformed seeds.

An overall schedule of timelines and milestones follows:

Timeline and Milestones

Objective	Timeline	Expected Results
1. Screen 2012 seedlings for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions (2014-2016)	Ongoing - 2017	Continue maintenance of seedlings from 2011 population. Identification of dwarf and semi dwarf genotypes Obtain additional seeds with crosses in 2014
	Feb 2015 and 2016	Progress updates
2. Germinate and phenotypically screen 618 seeds derived from irradiated pollen in 2013 (2014-2016)	July 2014 onwards	Germination of 2013 seeds and embryo rescue and development of deformed aborted seeds
	February 2017	Final report

Additional Information on labor requirements in the green house: Based on previous calculations, total labor time for greenhouse and embryo rescue work was estimated to be approximately 22 hours per week. With the trees getting larger, the requirement has gone up by an average of 6 additional hours per week.

Greenhouse and Embryo Rescue Work:

1.) There are a total of 168 pear plants in 2 gallon pots; ~600 seedlings are expected to be added in 2014.

2.) Labor: Ongoing care labor is estimated to be ~16 hrs / week

Watering/fertilizing of trees	12 hrs/week
Transferring trees from greenhouse to cold room and back	~40 hrs (one time)
Watering/fertilizing of trees in 2 gal pots	10 hrs/week
Securing trees to stakes with flag-tape, adjusting tape	~5 hrs (one time) 1hr/week
Monitoring for pests and pest control requests	3 hrs/week
Pest control	(done by PGF staff)
Application of slow-release fertilizer (Osmocote), top dressing	~3hrs (every three months)

3.) Labor is needed at about 6 hrs/week for the following activities

Media preparation	2hrs/week
Supply prep (magenta box washing and sterilizing)	2hrs/week
Transfers, observations	2hrs/week

Embryo rescue procedure – one time effort at a total of 12 hours

SIGNIFICANT FINDINGS

- Several of the trees demonstrate juvenile phenotype as evident from the presence of thorns.
- Compared to apple, the time to move out of juvenile phase is longer in pears.
- A total of 51 seeds out of 618 seeds that were derived using irradiated pollen were viable.
- Seedlings derived from irradiate pollen demonstrate a large variation in size and architecture

METHODS

Greenhouse growth of seedlings and maintenance of plants: Seeds obtained from crosses made in the 2013 season were stratified and were germinated in 12 inch pots filled with potting soil. Once the seedlings were 6 inches tall, they were moved to larger pots. Previously germinated plants continue to be maintained in 2 gallon pots. Irrigation and fertilization is being performed on an ongoing schedule standardized for greenhouse plants. Seedlings are periodically moved to the cold room to provide 1000 hours of chilling (ecodormancy) at the first sign of phenotypic markers of shoot growth. Plants are completely defoliated prior to being moved back to ambient growth conditions to initiate vigorous growth.

Embryo rescue: Standard tissue culture protocols have been used for embryo rescue experiments. Briefly, the deformed seeds were surface sterilized in a laminar flow hood using 50% bleach solution. After 10 minutes of treatment, seeds were washed with autoclaved water 5 times. Using a scalpel, the seed coat was excised and the cotyledons were exposed. The embryo area was carefully excised and placed on Murashige and Skoog media for embryo growth.

RESULTS & DISCUSSION

Growth characteristics: Most of the trees are exhibiting juvenile phase of growth. This phase is characterized by presence of thorns (fig 1C). There are a total of approximately 200 pear trees in the greenhouse. Due to the limitation of space in the cold chambers, trees are provided ecodormancy (chilling) in separate batches. Figure 1 shows a set of trees that has just been moved to the greenhouse from the cold chamber.

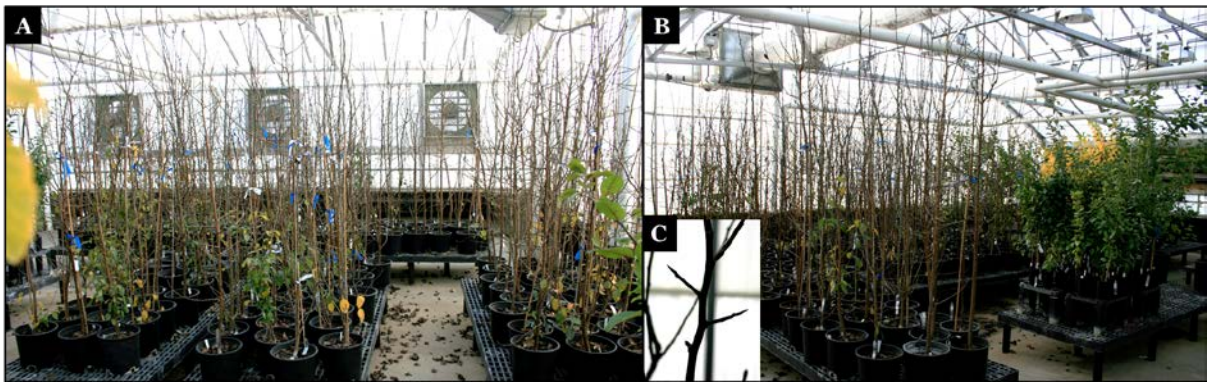

Figure 1: A and B. Pear trees from 2013 and 2014. B. Note the actively growing pears in the right panel. That group of trees is being prepared to undergo ecodormancy in February 2015. C. Thorns that are characteristic of juvenile phase are clearly evident on most trees

Seedlings from irradiated pollen: Approximately 51 trees derived from irradiated pollen continue to grow in the greenhouse (Figure 2). This set of plants has been through one set of rapid cycling. The trees demonstrate a large degree of variation is size and growth characteristics. These trees will continue to be pushed for growth and taken through 2-3 rapid growth cycles. Once these plants go through a chilling or dormancy cycle, they will be phenotyped for number of nodes, internode length, overall size and growth traits.

Figure 2: Seedlings derived from irradiated pollen. Note the variation in height and growth characteristics

The PNW pear industry needs a locally suited rootstock. Since Bartlett, d'Anjou and Comice have been cultivated for a century in this area these varieties are good parental material for the pear breeding program. Seedlings derived from these populations can directly serve as potential rootstocks having a large economic impact on the US pear industry.

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

Project Title: Optimizing use of Actigard for post-infection fire blight control

PI: Ken Johnson
Organization: Dept. Botany and Plant Pathology, Oregon State University, Corvallis
Telephone/email: 541-737-5249 johnsonk@science.oregonstate.edu

Cooperators: Rachel Elkins UC-ANR, Lake County
Tim Smith WSU, Wenatchee
Steve Castognoli OSU, Hood River

Budget: **Year 1:** $21,400 **Year 2: $22,042**

Other funding sources

Agency Name: Syngenta Crop Protection ($5K)

WTFRC Collaborative expenses: None

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

Item	**2014-15**	**2015-16**	
Salaries Faculty Res. Assist.	12,000	12360	
Benefits OPE 58%	6,960	7168.8	
Wages undergrads	500	515	
Benefits OPE 12%	60	61.8	
Equipment			
Supplies	880	906.4	
Local Travel	500	515	
Miscellaneous			
Plot Fees	500	515	
Total	$21,400	**$22,042**	

Footnotes: Annually**:** FRA 3 mo plus fringe, 50 hr undergrad labor, 0.9K M&S, 1K local travel & plot fee, 3% inflation

OBJECTIVES

Obj. 1: In the field, evaluate the timing of Actigard paints to prevent running fire blight cankers and to suppress canker re-ignition.

Obj. 2: In the greenhouse, re-evaluate the concentration of Actigard in paints applied to slow fire blight canker expansion in pear.

Obj. 3: Evaluate alternative SAR inducers and surfactants.

SIGNIFICANT FINDINGS

- For a 4th season, a paint of concentrated acibenzolar-S-methyl (ASM, Actigard®) used in combination with cutting reduced the severity of 're-ignited' fire blight cankers in Bosc pear.
- In one field experiment, an early-May timing of ASM (at symptoms first observed) resulted in greater suppression of 're-ignited' cankers compared to a timing of this treatment later in May.
- For a 4th season, the addition of ASM to antibiotic sprays enhanced fire blight control over antibiotics alone.
- For a 2nd season, ASM sprays reduced shoot infection on artificially-inoculated Concorde pear.

Materials and Methods

Objective 1: In the field, evaluate the timing of Actigard paints to prevent running fire blight cankers and to suppress canker re-ignition**.**

This objective was addressed in two Bosc pear blocks (6-yr-old and a 4-yr-old) located at the Oregon State University Botany and Plant Pathology Field Laboratory near Corvallis, OR. The experiments were arranged in a randomized complete block design with 18 to 22 replications. Flowers on trees were mist inoculated with the pathogen on 7 and 9 April. (Experimental details are in **Table 1** on the next page.)

After running cankers were established in the trees, ASM treatments were timed to occur 'at first symptoms' (early May), 'most symptoms appeared' (mid-May), and at 'traditional cutting time' (late May); all primary cuts were made at the 'traditional cutting time': 23 May for 6-yr-old Bosc and 28 May for 4-yr-old Bosc. ASM treatments associated with the primary cut were applied to the central leader with a small Solo sprayer (Fig. 1); the length of leader treated was approximately one meter and was located within the branching zone. ASM treatments associated with the secondary cut (2 July) were applied to 25-30 cm of healthy branch below immediately below each removed canker.

Fig.1 ASM treatments were 'painted' onto to central leaders of Bosc pear trees with a 1-liter Solo pump sprayer.

Table 1. Experimental details of 2014 ASM post-infection treatments applied to 6-yr-old and 4-yr-old Bosc pear in orchards location near Corvallis, OR

Cultivar & year	Tree age (years)	Pathogen inoculation type and date	Treatments	Rate of ASM (a .i.)	Amount of ASM applied	Number of replicate trees	Cankers per tree (± s.e.) at 1^0 cut	Date(s) cankers removed 1^0, 2^0 and 3^0 cuts	Cut distance below edge of canker	Date(s) ASM painted
					On central leader:			Twice		Twice
Bosc	6	Flowers 1×10^6	Cut only	-	-	18	26.4 (4.0)	23-May, 2-Jul, 15-Sep	15-20 cm	-
2014		CFU/ml	Cut & Paint	15 g /L	~750 mg in 50 ml	18	24.9 (3.5)	23-May, 2-Jul, 15-Sep	15-20 cm	2-May, 2-Jul[#]
		on 7-Apr	(sprayer)	1% Pentrabark						
			Cut & Paint	15 g /L	~750 mg in 50 ml	19	26.0 (3.6)	23-May, 2-Jul, 15-Sep	15-20 cm	16-May, 2-Jul[#]
			(sprayer)	1% Pentrabark						
			Cut & Paint	7.5 g /L	~325 mg in 50 ml	19	37.8 (5.1)	23-May, 2-Jul, 15-Sep	15-20 cm	16-May, 2-Jul[#]
			(sprayer)	1% Pentrabark						
					On central leader:			Twice		Twice
Bosc	4	Flowers 1×10^8	Cut only	-	-	23	20.2 (2.8)	28-May, 2-Jul, 15-Sep	15-20 cm	-
2014		CFU/ml	Cut & Paint	15 g /L	~750 mg in 50 ml	22	20.1 (2.8)	28-May, 2-Jul, 15-Sep	15-20 cm	2-May, 2-Jul[#]
		on 9-Apr	(sprayer)	1% Pentrabark						
			Cut & Paint	15 g /L	~750 mg in 50 ml	22	21.5 (2.7)	28-May, 2-Jul, 15-Sep	15-20 cm	16-May, 2-Jul[#]
			(sprayer)	1% Pentrabark						
			Cut & Paint	15 g /L	~750 mg in 50 ml	22	19.6 (2.6)	28-May, 2-Jul, 15-Sep	15-20 cm	28-May, 2-Jul[#]
			(sprayer)	1% Pentrabark						

[#]Painted branch below 2^0 cuts

Obj. 2: In the greenhouse, re-evaluate the concentration of Actigard in paints applied to slow fire blight canker expansion in pear.
Obj. 3: Evaluate alternative SAR inducers and surfactants.

Greenhouse experiments in 2014 failed because the 200 Bosc pear trees we purchased to address these objectives apparently had been frozen after digging in the nursery. After potting into new growth medium (Rexius Forest Products, Eugene, OR) in late March, headed trees developed leaves, but did not initiate shoot growth. After re-heading in May, remaining leaves on all trees dried up. At disposal in early July, inspection of the root zone showed no new root development on any of the trees. New trees have been ordered for 2015 and materials (alternative SAR-inducers and surfactants) required to conducted the proposed experiments are all in hand.

RESULTS

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

6-yr-old Bosc pear. An average of 30 fire blight cankers developed on each tree as a result of the pathogen inoculation at full bloom (**Table 1**). ASM treatments were made on 2 and 16 May; the primary cut occurred on 23 May. After cutting, running cankers re-ignited in 75% of the trees. The secondary cut was made on 2 July, with the final evaluation (cuts) of re-ignited cankers made on 15 September. Compared to cut only, the early (2 May) ASM paint treatment significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers but the 16 May treatments (full and half rate) did not (**Fig. 2**).

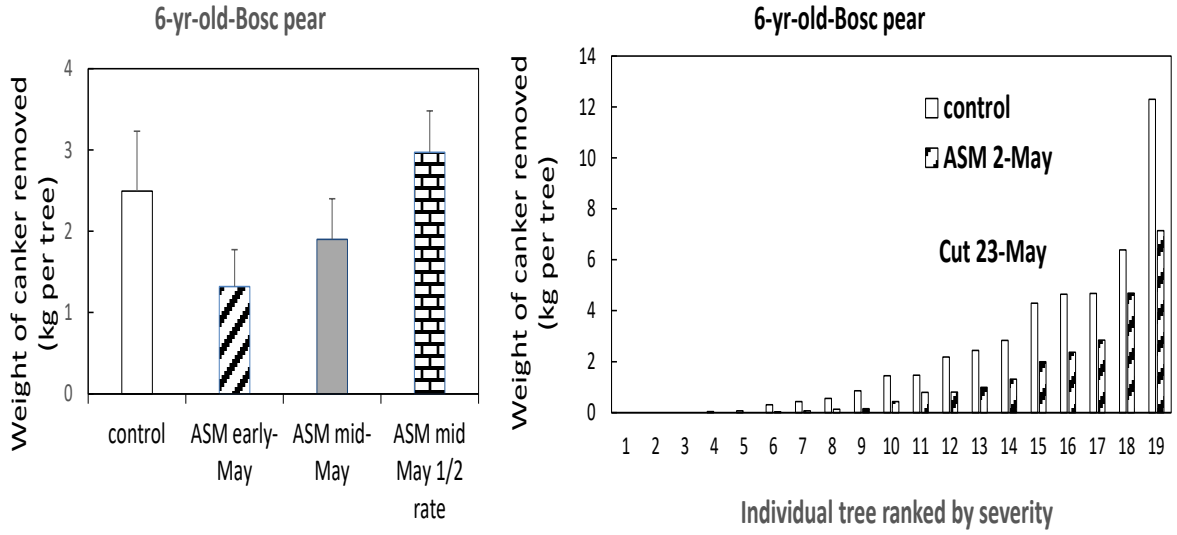

Fig. 2. Effect of the SAR-inducer, ASM, on re-ignited fire blight cankers in 6-yr-old 'Bosc' pear. Trees were inoculated with the fire blight pathogen on 7 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 23 May and 2 July. ASM was applied by 'paint' to the central leader (Actigard 30g/L in 1% Pentrabark, or half rate, Actigard 15g/L in 1% Pentrabark). Paints were applied to 1 m of central leader in the branch zone. Weight of cankered branches removed was assessed on July 2 and September 15. A) Each bar is the mean and standard error of 19 trees. B) Ranked comparison of the disease severity on individual 'ASM early-May' trees compared to individual 'cut only' trees.

4-yr-old Bosc pear. An average of 20 fire blight cankers developed on each tree as a result of the pathogen inoculation at full bloom (**Table 1**). ASM treatments were made on 2, 16 and 28 May, with the primary cut occurring on 28 May. After cutting, running cankers re-ignited in 64% of the trees. The secondary cut was made on 2 July, with the final tertiary cut of re-ignited cankers made on 15 September. Compared to cut only, all of the ASM paint treatment timings (early-, mid-, and late-May significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers (**Fig. 3**).

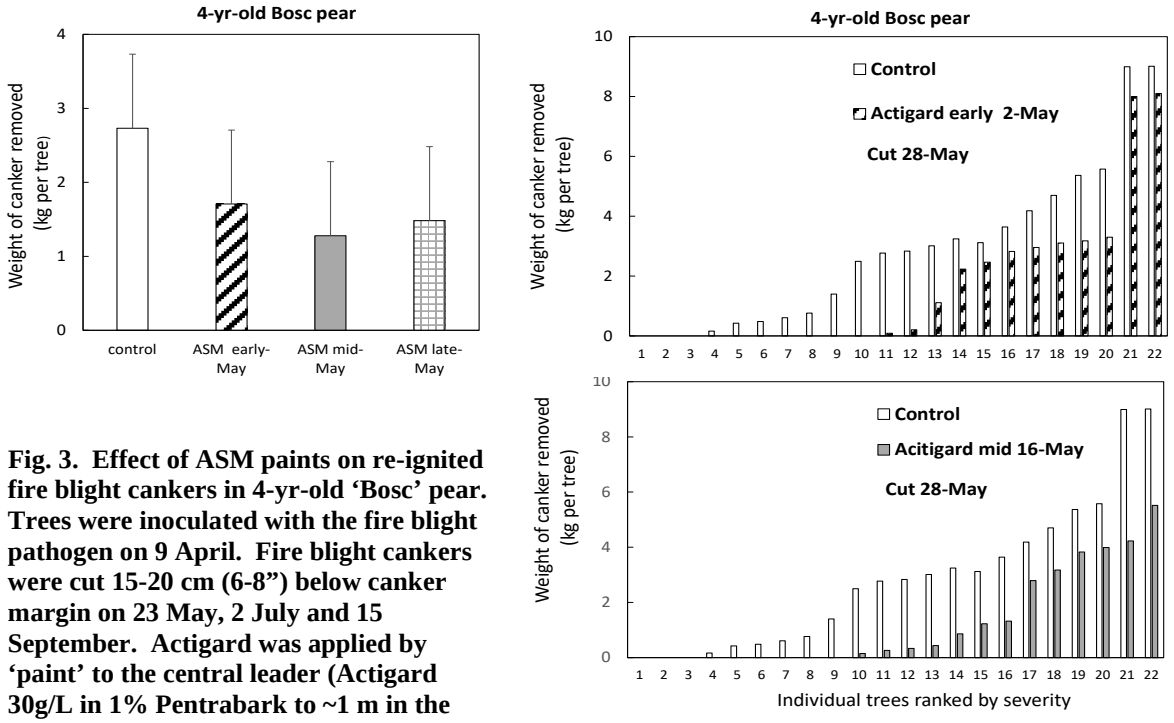

Fig. 3. Effect of ASM paints on re-ignited fire blight cankers in 4-yr-old 'Bosc' pear. Trees were inoculated with the fire blight pathogen on 9 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 23 May, 2 July and 15 September. Actigard was applied by 'paint' to the central leader (Actigard 30g/L in 1% Pentrabark to ~1 m in the branch zone). Weight of re-ignited cankers was assessed after cutting on 2 July and 15 September. Left panel: Each bar is the mean and standard error of 22 trees. Right panels: Ranked comparison of disease severity on individual 'ASM early-May' and 'ASM mid-May' trees compared to individual 'cut only' trees.

Discussion. The experiments above were harsh in that a) the number of fire blight strikes per tree was higher than typically observed in commercial orchards, and b) we applied the SAR treatment at various times in May, but did not cut any cankers until late May, which gave the pathogen additional time to move into the healthy portions of the trees. In spite of this, the central leader 'paint' of ASM (applied by small sprayer) slowed fire canker re-ignition and expansion in diseased trees at levels comparable to ASM treatments made to the 12-18 inches of healthy branch immediately below each cut canker (see previous WTFRC reports). Treatment of the central leader requires much less time to implement than painting of specific diseased branches. Future experiments will utilize this treatment approach exclusively. We hypothesized that ASM treatment in early May would be superior to treatment later in May, and the results from the 6-yr-old Bosc pear supported this hypothesis. In contrast, in the 4-yr-old Bosc pear, all the treatment timings resulted in less diseased wood after canker re-ignition. Interestingly, for both experiments, the proportions of re-ignited cankers removed from the cut-only treatment were 55-60% and 40-45%, respectively, for the secondary (2-Jul) and tertiary (15-Sep) cut timings. Conversely, for ASM-treated trees, these proportions were reversed: 36-44% of re-ignited cankers removed at the secondary cut and 56-64% removed at the tertiary cut. That more disease occurred in the latter half of summer on ASM-treated

trees suggests that the effect of the treatment declines over time. Potentially, trees recovering from fire blight could benefit from an additional ASM treatment to the central leader near the time of the secondary cut. This treatment will be evaluated in 2105.

Supplemental Results: Other research with ASM in 2014.

In 2014, Actigard (acetyl-S-methyl) was evaluated for fire blight suppression in a 14-yr-old Bartlett pear orchard located near Corvallis, OR. The design was an RCB with 4 replications. Trees were inoculated with the pathogen at full bloom. Relative to water-treated control, oxytetracycline alone significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree (**Fig. 4**). The addition of two ASM treatments (Actigard 50W, 2 oz./100 gal) in combination with oxytetracycline significantly improved ($P \leq 0.05$) control of fire blight compared to oxytetracycline by itself. This result is consistent with previous trials (see previous WTFRC reports) in pear and apple conducted from 2011- 2013.

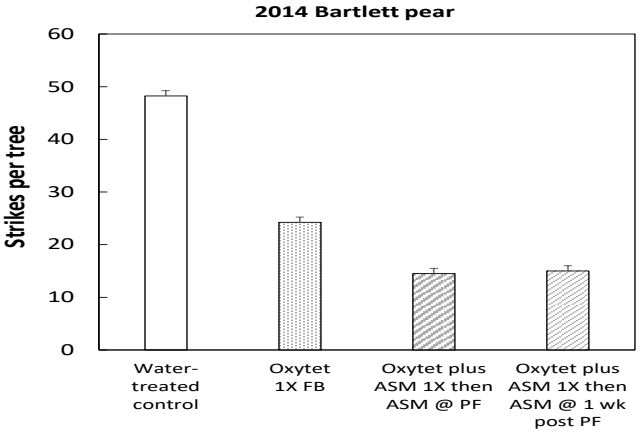

Fig. 4. Fire blight strikes per tree as affected by treatment with oxytetracycline once or in a program with 2 additional Actigard treatments. The antibiotic was applied at full bloom; Actigard treatments occurred at full bloom and again near petal fall.

Also in 2014, we evaluated ASM for prevention of shoot blight. Fresh wounds at the tips of expanding shoots (24 per treatment) on potted 2-yr-old Concorde pear trees were inoculated on 30 April with a high dose (10^7 cfu/ml) or a low dose (10^4 cfu/ml) of the fire blight pathogen. Prior to inoculation, the trees were sprayed once (15 days before inoculation) or twice (15 days before and 2 days after inoculation) with ASM (Actigard 50W, 2 oz./100 gal. As we observed in 2013, the incidence of shoot infection on trees treated twice with ASM was reduced by 40-45% relative to the water-treated control (**Fig. 5**).

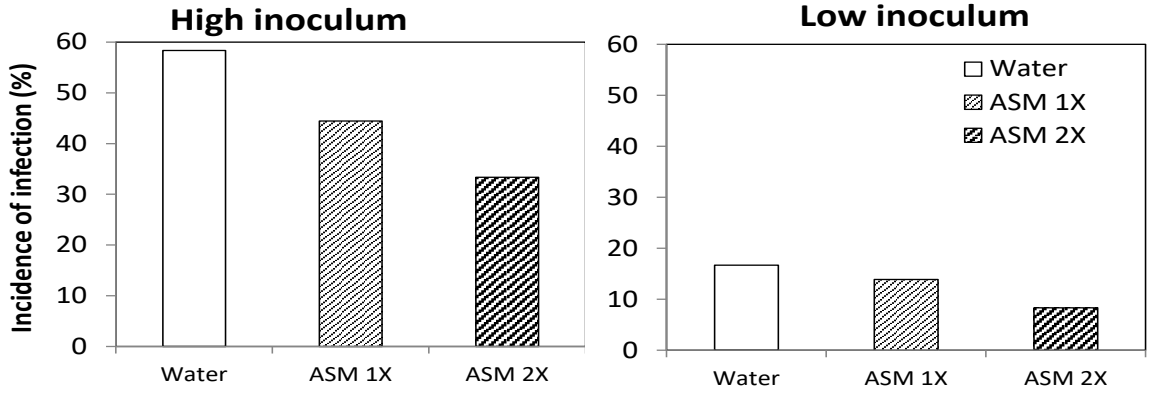

Fig. 5. Incidence of shoot blight on potted Concorde pear inoculated with the fire blight pathogen after 1 or 2 spray treatments of Actigard 50W (24 shoots inoculated per dose per treatment).

Discussion. ASM continues to show value as program partner with antibiotics during bloom, which could prove to be cost ineffective in high risk/high value orchards. We speculate that suppression is due to a longer residual time (7-15 days) compared to antibiotics (~3 days). This property may extend its usefulness to suppression of rattail and shoot infection, and of trauma blight

(infection from storm-induced wounds), which is difficult to suppress with antibiotics. The registration package for Actigard 50W for use on pome fruit has been submitted by Syngenta to the EPA with a section 3 label expected in late 2015; first commercial use is expected in 2016. Each method of application discussed in this report has been included on the proposed label.

CONTINUING PROJECT REPORT **YEAR**: 2015
WTFRC Project Number: PR-14-111

Project Title: Development of marker-based breeding technologies for pear improvement

PI: David Neale
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Cooperators: Amit Dhingra, Washington State University (WSU) Department of Horticulture, Richard Bell, USDA/ARS Kearneysville, WVa; Joseph Postman and Nahla Basil, USDA/ARS National Clonal Germplasm Repository, Corvallis, OR; and Katherine Evans, Washington State University Department of Horticulture, WSU

Total Project Request: **Year 1:** $50,000 **Year 2:** $50,000

Other funding sources
None

WTFRC Collaborative expenses: None

Item	2014	2015
Salaries		
Benefits		
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	50,000	50,000
Travel		
Plot Fees		
Miscellaneous		
Total	50,000	**50,000**

Footnotes:

ABSTRACT

Traditional pear breeding, like most woody perennial crops, takes a long time due to the breeding cycle time and time to trait evaluation and can be quite expensive due to large land and labor requirements. Marker-based breeding technologies, as are routinely used in nearly all agricultural systems now, can potentially increase pear breeding efficiency. A new project to obtain a genome-wide genetic variation data from the entire *Pyrus* germplasm collection maintained by the ARS in Corvallis, OR, was funded.

PROCEDURES, RESULTS AND DISCUSION

A leaf tissue sample (around 15 leaves) for each one of the accessions in the entire *Pyrus* germplasm collection, maintained by the ARS in Corvallis, OR, was collected in June 2014. A subset of samples, covering a wide range, from a breeding and genetic diversity perspective, of genetic diversity was selected (46 samples) (Table 1). This set of samples will be sequenced using a whole-genome shotgun sequencing strategy. The experience gained for our group (UC Davis) in sequencing and re-sequencing large and complex genomes will make the re-sequencing pear strategy relatively quick and inexpensive. The Pear Genomics group is collaborative group of researchers, all working on some aspect of pear genetics and genomics. The purpose of the group is to facilitate information exchange and foster creativity and innovation toward pear genetic improvement. The complete list of participants is shown below.

UC Davis
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CONCLUSIONS

A total set of 46 samples was selected. The whole genome sequence of the selected samples is in progress.

TABLE 1.

	Clade	Name	Cpyr	Species	Comment
1	Xviii	P. betulifolia shaanxi	2291.001	betulifolia	
2	Xxv	P. calleryana 96107	2588.001	Calleryana	
3	Vi	Beurre d'Anjou	63.001	Communis	
4	Vii	Coscia tardive	159.002	Communis	
5	X	Seckel	519.001	Communis	Fire blight resistant
6	Xii	Gin	246.001	Communis	
7	Xxiii	Zao su li	2594.001	Pyrifolia	
8		P. salicifolia GE-2004-141	2849.001	Salicifolia	
9	Xix	Xiang shui li	2640.002	Ussuriensis	
10		Xuehua li	2681.002	X bretschneideri	Q27647
11	Xxiv	P. ussuriensis Korea	1202.001	Ussuriensis	
12	Xvi	P. syriaca Armenia	920.001	Syriaca	
13	Xiv	Para de zahar de bihor	1663.001	Communis	
14	Xv	Mednik	1549.001	Communis	Psylla resistant
15		Roi Charles de Wurtemburg	489.002	Communis	Fire blight resistant
16		US 309		Communis	Fire blight resistant, dwarf
17	Xvii	Erabasma	1524.002	Communis hybrid	Psylla resistant
18	Xvi	P. communis ssp caucasica	680.001	Communis ssp caucasica	
19		P. communis ssp pyraster alb-2011-024	2965.001	Communis ssp pyraster	
20	V	NY 10353	1660.001	Communis x ussuriensis	Psylla resistant
21		NJ B9 R1 T117		Communis x ussuriensis	Psylla resistant
22	Xv	P. cordata Turkey	1589.001	Cordata	
23	Xviii	P. cossonii	828.001	Cossonii	
24	Xviii	P. elaeagrifolia	765.001	Elaeagrifolia	
25		P. fauriei	772.004	fauriei	
26	Xvi	P. gharbiana 1	787.001	Gharbiana	
27		P. glabra	1205.001	Glabra	

28	Xxi	P. hondoensis Japan	2117.001	Hondoensis	
29	Xx	P. koehnei	825.001	Koehnei	
30	Xx	P. mamorensis	835.001	Mamorensis	
31	Xvi	P. nivalis	256.002	Nivalis	
32	Xx	Naspati	411.001	Pashia	
33		P. pseudopashia	875.001	Pseudopashia	
34	I	Beurre bosc	1165.001	Communis	
35	Ix	Takisha	1675.003	Communis	
36		Dan bae	2623	Pyrifolia	
37		Nijisseiki	413.001	Pyrifolia	
38	Xviii	P. regelii	890.001	Regelii	
39		P. sachokiana GE-2006-115	2882.001	Sachokiana	
40	Vi	Ho mon	2723.001	Sinkiangensis	
41	Xix	P. amygdaliformis Turkey	634.001	Spinosa	
42	Xviii	P. cordata pure	745.001	Cordata	
43		Illinois 76		Ussuriensis (x pyrifolia?)	Fire blight resistant
44		Ya li	1678.001	X bretschneideri	
45		Old Home	431.001	Communis	delete if PFR data available
46		Bartlett	38.001	Communis	delete if PFR data available

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

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

PI: Yan Wang
Organization: OSU MCAREC
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Email: yan.wang@oregonstate.edu
Address: 3005 Experiment Station
City/State/Zip: Hood River/OR/97031

Cooperators: Todd Einhorn, David Sugar, Xingbin Xie, Wade Root (Duckwall-Pooley Fruit)

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

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

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

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

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

OBJECTIVES

1. Improving storability and reducing senescent disorders of summer pears

- Ensure a consistent 1-MCP efficacy on maintaining storage and export quality of 'Bartlett'.
- Improve storage quality of 'Bartlett' pears by pre-harvest ReTain® application.
- Extend storage life of 'Starkrimson' by ReTain® and 1-MCP.
- Optimize MAP conditions for storage and export of 'Bartlett' and 'Starkrimson'.

2. Reducing scuffing of 'Bartlett', 'Comice', and 'Anjou' pears

- Develop cultivar-specific wax coating application protocol to reduce friction forces on epidermal cells to reduce scuffing by lubricating fruit surface.
- Reduce enzymatic discoloration by antioxidant-ethoxyquin.

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

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

SIGNIFICANT FINDINGS: year-2

Objective 1. Improving storability and reducing senescent disorders of summer pears

1. 1-MCP efficacy on inhibiting senescence of 'Bartlett' is inconsistent at commercial application in PNW. To ensure a consistent 1-MCP efficacy:
    a. Harvest fruit at 19-18lb, especially for fruit from higher production elevations.
    b. Treat fruit within 10-12 days after harvest. Eliminate field heat quickly and store fruit at 30°F during the treatment delay.
    c. Vent out exogenous ethylene (if > 300ppb) before 1-MCP treatment.

2. Pre-harvest ReTain® spray efficacy in improving storability of 'Bartlett' pears is affected by application rate, timing, and fruit harvest maturity.
    a. AVG at 60ppm applied 1 week before H1=19lb (WBH1) suppressed ethylene production and respiration rate, retarded fruit firmness and green color losses, and reduced senescence disorders for H1 and H2 (12d after H1) fruit. H3 (17d after H1) fruit did not response to the AVG treatments regarding ethylene production and storage quality. Compared to 60ppm, AVG at 120ppm applied 1WBH1 did not improve storage quality but delayed ripening capacity by one month. Compared to control, AVG at 30ppm applied 1 WBH1 did not improve 'Bartlett' storability.
    b. AVG at 30-120ppm applied 2 WBH1 had little effect on any of the storage responses measured.
    c. AVG at 60-120ppm applied 1 or 2 WBH1 did not affect the initial harvest maturity (H1), but delayed fruit maturation on the tree about 5d for H2 and H3 fruit.

3. 'Starkrimson' produces a higher amount of ethylene and has a higher respiration rate and therefore a shorter storage life compared to other PNW pear cultivars. Pre-harvest ReTain® or postharvest 1-MCP treatments inhibit ethylene production and extend storage life of 'Starkrimson'.
    a. ReTain® at 60-120ppm applied 1 week before harvest extended 'Starkrimson' storage life without significant effect on ripening capacity.
    b. 1-MCP at 300ppb extended 'Starkrimson' storage life to 4 months at 30°F. However, it took 2 weeks to ripen at 68°F following 4 months of cold storage.

Objective 2. Reducing scuffing of 'Bartlett', 'Comice', and 'Anjou' pears (see year-1 report)

Objective 3. Controlling storage decay of pears by introducing new postharvest fungicides (see year-1 report)

METHODS

Objective 1. Improving storability and controlling senescent disorders of summer pears.

1) 'Bartlett' and 1-MCP. (see year-1 report)

2) 'Bartlett' and ReTain®. The effects of pre-harvest AVG spray rate (30, 60, and 120ppm) and timing [1 and 2 WBH1 (weeks before H1)] on storability of 'Bartlett' fruit at three harvest maturities [H1: when control fruit firmness (CFF) ≈ 83.6 N; H2: 12 d after H1 when CFF ≈ 74.8 N; and H3: 17 d after H1 when CFF ≈ 72.6 N] were measured with respect to ethylene production, storage quality and ripening capacity during 5 months of storage at 30°F.

3) 'Starkrimson'. AVG at 30, 60, and 120 ppm was sprayed 1 week before commercial harvest. Fruit quality and ripening capacity were determined after 1, 2, 3, and 4 months of cold storage at 30°F. Postharvest treatment with 1-MCP at 300 ppb was as the same as described for 'Bartlett'.

RESULTS AND DISCUSSION

1. Ensure a consistent 1-MCP efficacy on improving storability of 'Bartlett' pears

1-MCP efficacy on inhibiting senescence of 'Bartlett' pears has been reported being inconsistent from year to year and from lot to lot in the PNW. The effects of harvest maturity, orchard elevations, delayed treatment after harvest, holding temperature during treatment delay, and exogenous ethylene concentration in treating room on 1-MCP efficacy were studied repeatedly in 2013. Since similar trends with that of 2012, the results of 2013 are not presented in this report. Please see year-1 report.

2. Pre-harvest ReTain® spray efficacy in improving storability of 'Bartlett' pears is affected by application rate, timing, and fruit harvest maturity

2.1. Ethylene production

In H1, the control fruit started to produce significant amount of ethylene after 2 months of cold storage (Fig. 1A). AVG at 60 and 120ppm applied 1 WBH1 reduced ethylene production rate significantly during 2-5 months of storage. Compared to 60ppm, AVG at 120ppm 1 WBH1 further reduced ethylene production rate numerically but not at statistically significant level ($p = 0.05$) during the experimental period. In contrast, AVG at 30ppm applied 1 WBH1 and AVG at 120ppm applied 2 WBH1 did not inhibit ethylene production compared to the control. For H2 fruit following 4 months of storage, similar to H1 fruit, AVG at 60 and 120ppm applied 1 WBH1 reduced ethylene production, but AVG at 30 mg L^{-1} applied 1 WBH1 and 120 mg L^{-1} applied 2 WBH1 did not affect ethylene production (Fig. 1B). Ethylene production in H3 fruit following 4 months of storage was not affected by the AVG treatments (Fig. 1B).

2.2. Fruit storage quality

In H1 fruit, FF, I_{AD}, and TA decreased gradually in all the treatments. Their losses were not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1, but slowed down significantly by AVG at 60 and 120ppm applied 1 WBH1 during 5 months of storage (Fig. 2). In H2 fruit following 4 months of storage, AVG at 60 and 120ppm applied 1 WBH1 maintained higher FF, I_{AD}, and TA, but AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1 did not affect the losses of FF, I_{AD}, and TA. H3 fruit did not response to the AVG treatments in terms of the losses of FF, I_{AD}, and TA (data not shown). SSC increased in a small magnitude in each of the three maturities during storage, but it was not affected by the AVG treatments.

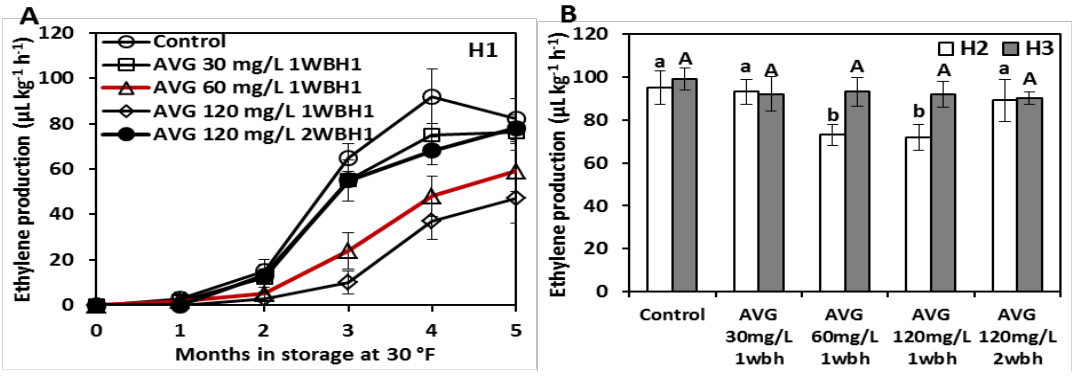

Fig. 1. Effects of pre-harvest AVG sprays on ethylene production of 'Bartlett' pears with three harvest maturities (H1, H2, and H3) on day 1 at 68°F following cold storage at 30°F for 1-5 months in H1 and 4 months in H2 and H3.

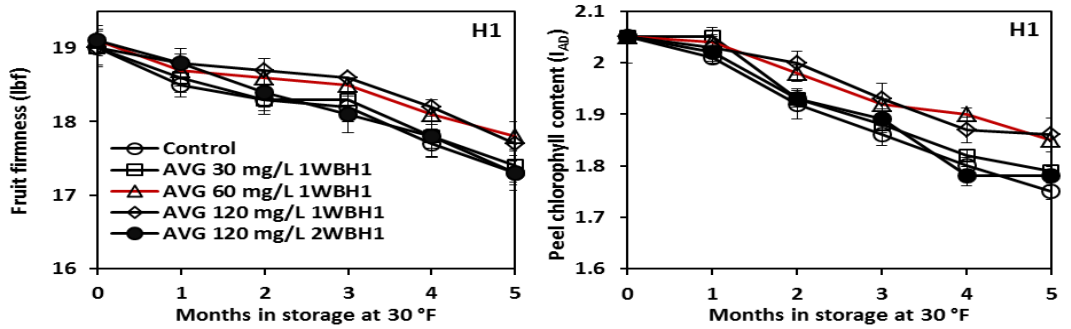

Fig. 2. Effects of pre-harvest AVG sprays on fruit flesh firmness and peel chlorophyll content (I_{AD}) of 'Bartlett' pears on day 1 at 68°F following cold storage at 30°F for 1-5 months in H1.

2.3. Senescence disorders

In the control, H1 and H2 fruit developed senescence disorders of 30.3% and 35.0% after 5 and 4 months of storage, respectively (Fig. 3). AVG at 60ppm applied 1 WBH1 reduced senescence disorders to 5.6% and 16.1% in H1 and H2 fruit after storing for 5 and 4 months, respectively. Senescence disorders in H1 and H2 fruit were not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1. Compared to AVG at 60ppm, AVG at 120ppm did not improve its efficacy on reducing senescence disorders. H3 control fruit developed 49.5% senescence disorders following 4 months of storage and the AVG treatments did not affect the senescence disorders significantly.

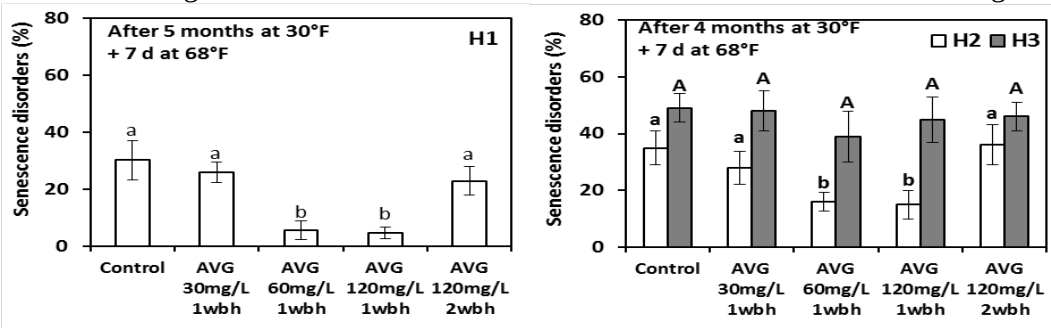

Fig. 3. Effects of pre-harvest AVG sprays on senescence disorders of 'Bartlett' pears with three harvest maturities (H1, H2, and H3) on day 7 at 68°F following cold storage at 30°F.

2.4. Ripening capacity

In H1, the control fruit developed ripening capacity following 1-4 months, but developed mealy texture with increased EJ > 650 mL kg^{-1} within 7d at 68°F following 5 months of cold storage (Fig. 6A&C). Following 1 month of cold storage, fruit treated with AVG at 60ppm applied 1 WBH1 could ripen to FF = 5.5lb with EJ = 649 mL kg^{-1} in 7 d at 68°F, however, fruit treated with AVG at 120ppm

applied 1 WBH1 did not develop ripening capacity. Fruit treated with AVG at 60 and 120ppm applied 1 WBH1 developed ripening capacity with FF < 5lb and EJ < 650 mL kg^{-1} after 7 d at 68°F following 2-5 months of cold storage. Compared to the control, the ripening capacity was not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1.

Following 4 months of storage, while H2 fruit in all the treatments developed ripening capacity, the fruit treated with AVG at 60 and 120ppm applied 1 WBH1 had less EJ after 7d at 68°F compared to the control and fruit treated with AVG at 30 mg L^{-1} applied 1 WBH1 and 120 mg L^{-1} applied 2 WBH1 (Fig. 6D). In contrast, H3 fruit, regardless of the control and AVG treatments, developed mealy texture with EJ > 650 mL kg^{-1} after 7d at 68°F following 4 months of cold storage (Fig. 6D).

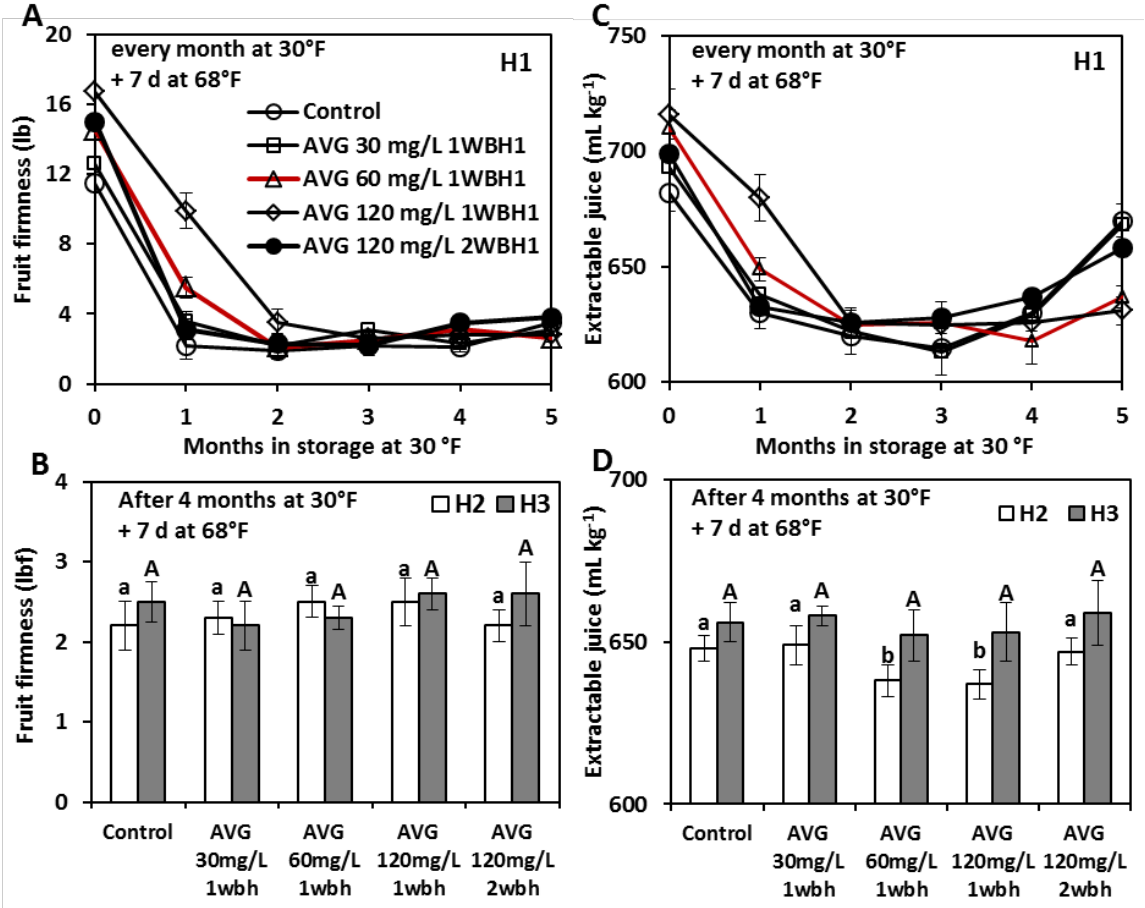

Fig. 4. Effects of pre-harvest AVG sprays on fruit ripening capacity expressed as flesh firmness and extractable juice on day 7 at 68°F following cold storage at 30°F for 1-5 months in H1 and 4 months in H2 and H3.

3. Extending storage life of 'Starkrimson' by pre-harvest ReTain® or postharvest 1-MCP applications

3.1. Ethylene production and respiration rate

Control fruit started accumulating IEC at about 0.6ppm after 4 weeks. Thereafter, IEC increased gradually and reached the highest amount of 4.8ppm at 16 weeks of storage at 30°F (Fig. 5). Fruit treated with AVG at 30, 60 and 120ppm started accumulating IEC at 1.7, 0.9, and 0.6ppm, respectively, after 8 weeks and IEC peaked at 4.7, 3.3, and 3.2ppm, respectively, after 16 weeks of storage. There was no difference between AVG at 60 and 120ppm on IEC accumulation. Compared to the AVG treatment, 1-MCP was more effective in inhibiting the IEC during storage. 1-MCP treated fruit started accumulating IEC at 0.1ppm after 8 weeks and had IEC lower than 1.0ppm for the 16

weeks of storage. Ethylene production rate (EPR) in control fruit increased significantly after 4 weeks, increased thereafter and reached a maximum value after 16 weeks. Fruit EPR was not affected by AVG at 30ppm but decreased significantly by AVG at 60 and 120ppm during 4-16 weeks. 1-MCP prevented EPR during 16 weeks of storage period (Fig. 5). The respiration rate (RR) of control fruit increased during 16 weeks of storage and was generally higher than that of AVG at 60 and 120ppm. AVG at 30ppm did not affect RR compared to control. 1-MCP treated fruit maintained the lowest RR which decreased in the first 4 weeks and then increased during 4–16 weeks of storage (Fig. 5).

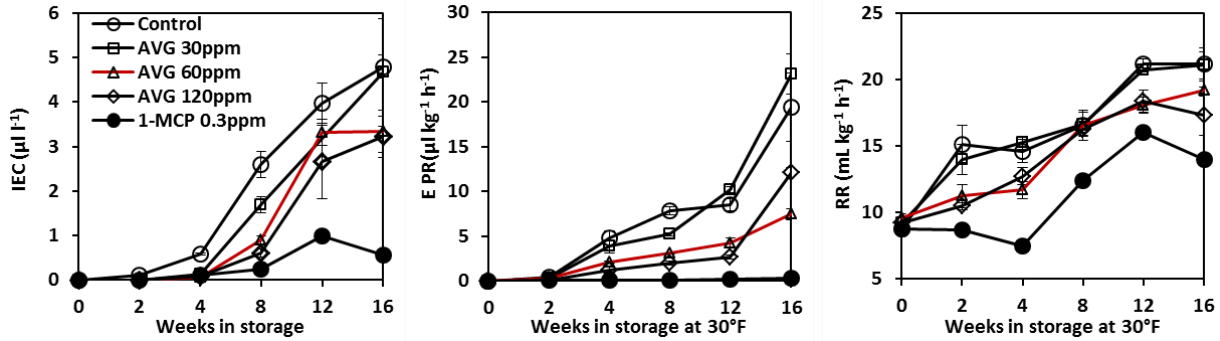

Fig. 5. Effects of AVG and 1-MCP on internal ethylene concentration (IEC), ethylene production rate (EPR) and respiration rate (RR) of 'Starkrimson' pears during 16 weeks of storage at 30°F.

3.2. Fruit storage quality

AVG sprayed one week before harvest at 60, but not 30 and 120ppm slowed down the FF reduction of fruit on the trees compared to control. At nearly the commercial harvest date, FF of fruit treated with AVG at 0, 30, 60, and 120ppm were 58.7, 58.0, 62.6, and 59.9 N, respectively (Fig. 6). Control fruit decreased FF from 58.7 to 53.1 N and maintained SSC at about 11.5% for 12 weeks of storage at -1.1 °C (Fig. 6). AVG and 1-MCP applications did not affect FF and SSC. TA decreased gradually and lost 40% in control fruit after 16 weeks of storage (Fig. 6). AVG and 1-MCP treatments inhibited TA reduction (Fig. 6). For example, AVG at 30, 60, and 120 µL L^{-1} and 1-MCP reduced TA loss from 40% to 28, 20, 28, and 23%, respectively, after 16 weeks of storage.

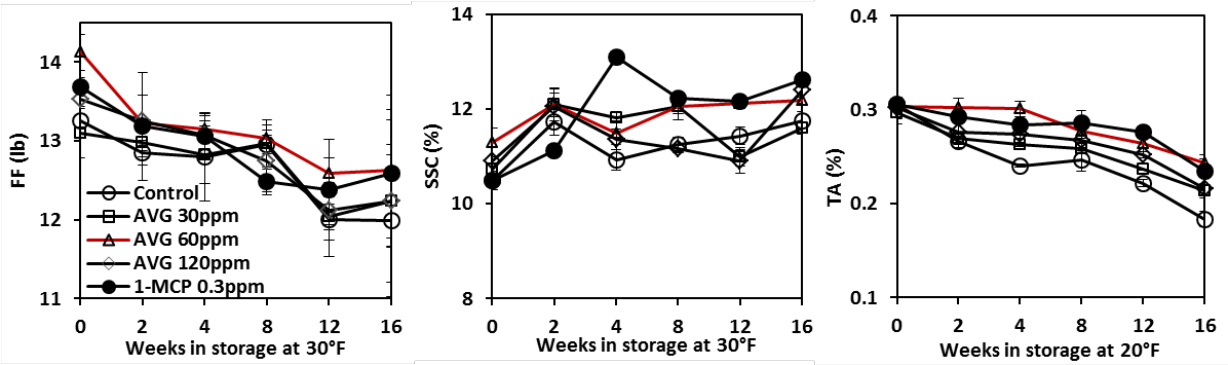

Fig. 6. Effects of AVG and 1-MCP on FF, SSC, and TA of 'Starkrimson' pears during 16 weeks of storage at 30°F.

3.3. Senescence disorders

After 16 weeks of cold storage, control fruit developed internal breakdown (IB) and decay at 12.3 and 7.1%, respectively. AVG at 30, 60, and 120 µL L^{-1} and 1-MCP at 0.3 µL L^{-1} reduced IB to 10.5, 2.5, 3.3, and 0%, and decay to 6.6, 1.2, 3.3, and 1.1%, respectively (Fig. 7).

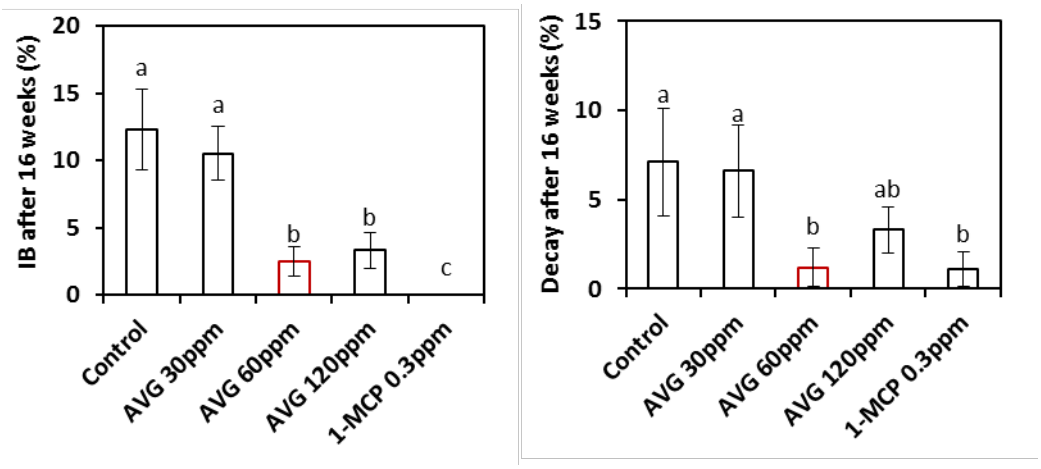

Fig. 7. Effects of AVG and 1-MCP on internal breakdown (IB) and decay of 'Starkrimson' pears after 16 weeks of storage at 30°F.

3.4. Ripening capacity

The control fruit could not ripen immediately after harvest, but developed ripening capacity within 5d at 68°F after 2 weeks of storage at 30°F. Ripening capacity was not affected by AVG at 30 and 60ppm. Fruit treated with AVG at 120ppm developed ripening capacity after 4 weeks of cold storage. Both control and fruit treated with AVG at 30ppm maintained low EJ (i.e., < 600 mL kg^{-1} FW) and high eating quality (i.e., > 7) between 2–8 weeks of storage and increased EJ and lost eating quality thereafter. Fruit treated with AVG at 60 and 120ppm maintained low EJ and high eating quality between 2–16 and 4–16 weeks of cold storage, respectively. 1-MCP treated fruit could not develop ripening capacity within 5d at 68°F for 16 weeks of storage, but could ripen in 15d at 68°F with high eating quality following 4–16 weeks of cold storage (Fig. 8).

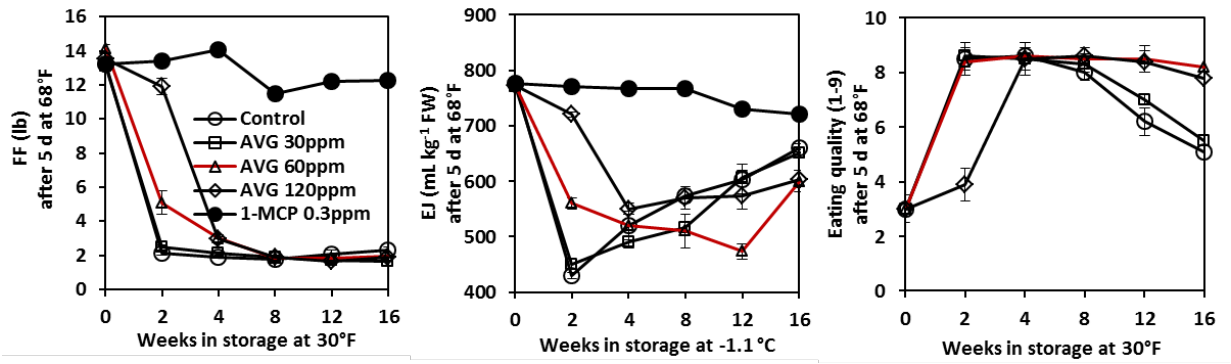

Fig. 8. Effects of AVG and 1-MCP on FF, extractable juice (EJ), and eating quality of 'Starkrimson' pears after 5 d at 68 F following 16 weeks of storage at 30°F.

4. Optimize MAP conditions for storage and export of 'Bartlett' and 'Starkrimson' (See year-1 report)

OBJECTIVE 2 & 3 (see year-1 report)

CONTINUING PROJECT REPORT **YEAR**: Year 1 of 2
WTFRC Project Number: PR-14-100

Project Title: Health role of pear for metabolic syndrome

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Co-PI: Sarah A. Johnson, PhD, RD, CSO
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Email: sba07@my.fsu.edu
Address: 428 Sandels Building, 120 Convocation Way, Tallahassee, Florida 32306-1493

Cooperators: Pear Bureau Northwest

Total Project Request: Year 1: $32,185 **Year 2: $29,871**

Other funding sources

Agency Name: Pear Bureau Northwest
Amt. requested: Year 1: $32,185 **Year 2**: $29,871
Notes: Pear Bureau Northwest will match the amount funded by the Pear Marketing Order 927 to bring the total funded amount to $64,370 for Year 1 and $59,742 for Year 2.

Budget 1
Organization Name: Florida State University
Contract Administrator: Gina Wells, Grants Compliance Analyst
Telephone: (850) 644-3658 **Email address:** glwells@fsu.edu

Item	2014	2015
Salaries	$16,457.50	$16,951
Benefits	$2,688	$2,853
Wages	$0	$0
Benefits	$0	$0
Equipment	$0	$0
Supplies	$12,539.50	$9,067
Travel	$0	$0
Miscellaneous	$500	$1,000
Plot Fees	$0	$0
Total	$32,185	**$29,871**

Footnotes:

A. OBJECTIVES

The ***central hypothesis*** of the proposed study is that the daily consumption of 2 pears (medium sized Green Bartlett and Green Anjou pears weighing ~166 g each) for twelve weeks will improve blood pressure, lipid profiles, glycemic control and insulin resistance, inflammatory and oxidative status in men and women with MetS. Because pears are high in pectin, a soluble and fermentable dietary fiber, we propose two ***ancillary hypotheses*** as follows: **1)** regular intake of pears will promote gastrointestinal health (GI); and **2)** will improve measures of body composition. The hypotheses of the study will be tested in a randomized, crossover design study using 2 pears or 50 g isocaloric control drink powder with 50 men and women between the ages of 45 and 65 years with three of the five features of MetS using the following four ***specific aims***:

Specific Aim 1: To investigate the extent to which daily pear consumption reduces blood pressure and improves lipid profiles by measuring total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A1 and apolipoprotein B100 levels will be measured. Atherogenic risk ratios (TC/HDL-C, LDL-C/HDL-C, HDL-C/LDL-C) will also be assessed.

***Specific Aim 2*:** To determine the degree to which daily pear consumption will improve biochemical markers of **a)** inflammation [C-reactive protein (CRP), leptin, and adiponectin]; **b)** antioxidant defense [total antioxidant capacity (TAC)]; **c)** oxidative stress [oxidized low-density lipoprotein (LDL) and 8-hydroxy-2'-deoxyguanosine (8-OHdG)]; and **d)** insulin sensitivity [(fasting glucose, insulin, the homeostatic model assessment-insulin resistance (HOMA-IR), and hemoglobin A1c (Hgb A1c)].

Specific Aim 3: To investigate the ability of pear consumption to improve GI health using a validated Seven-Day Bowel Movement Questionnaire and serum levels of short-chain fatty acids.

***Specific Aim 4*:** To examine whether pear consumption has positive effects on body weight and composition including lean body mass (LBM), fat mass (FM) and percent body fat (%BF) using dual-energy x-ray absorptiometry (DXA).

The **goals** for the next year are as follows (Please see **Study Timeline** below for anticipated dates):

1. To finish subject recruitment and data collection.
2. To analyze blood and urine samples for the abovementioned specific aims.
3. To statistically analyze all data collected for the abovementioned specific aims.
4. To prepare abstracts and manuscripts for presentation at national conferences and publication in peer-reviewed journals (after approval from the Washington Tree Fruit Research Commission, Pear Marketing Order 927, and the Pear Bureau Northwest).

B. SIGNIFICANT FINDINGS

We are very pleased with the progress we have made with the recruitment, subject retention, and compliance. We do not anticipate any obstacles in carrying out the remainder of the study.

- We have 39 CURRENTLY ENROLLED PARTICIPANTS
  - 2 participants dropped from the study due to health and personal reasons
- 24 participants have completed the first 12 weeks of the study
- 4 participants will complete the first 12 weeks of the study in January 2015
- 6 participants will complete the first 12 weeks of the study in February 2015
- 6 participants will complete the first 12 weeks of the study in March 2015
- 1 participant will complete the first 12 weeks of the study in April 2015

*Please see **Flowchart of Enrollment** below for more details.
**We are continuing to recruit and enroll participants into the study. We project that we will be finished with recruitment by the end of February 2015 as we originally projected (please see Study Timeline below) and hence expect that data collection and analysis, as well as dissemination of study findings will also follow this timeline.

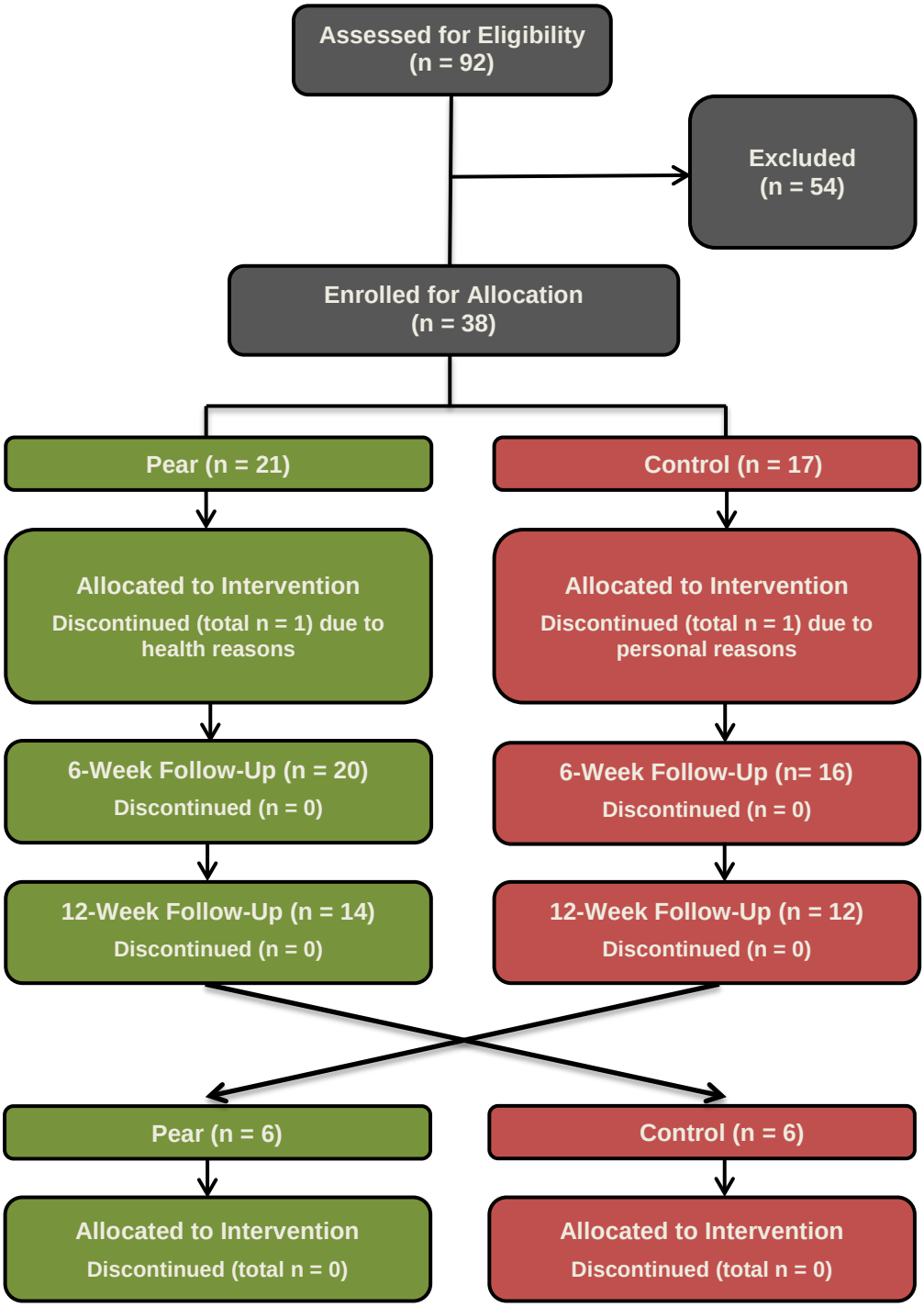
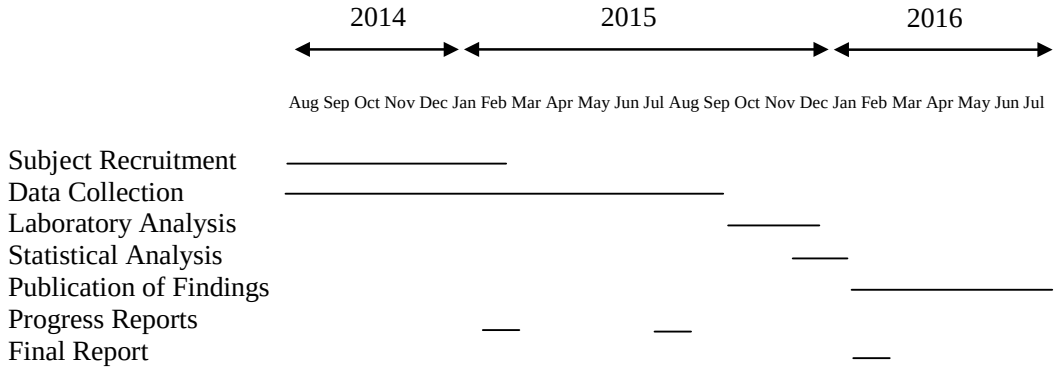


Figure 1. Flowchart of Enrollment.

Figure 2. Study Timeline.



C. OUTLINE OF METHODS

A total of 50 men and women between the ages of 45 and 65 years who have three of the five features of MetS as defined by the ATP III will be included in the study (see Subjects Inclusion Criteria below). After a two-week run-in phase, eligible men and women will be randomly assigned to receive one of two treatments daily for twelve weeks: 1) Two medium-sized pears or 2) 50 g isocaloric maltodextrin-based pear-flavored control drink powder. After an initial ***telephone screening***, all participants will be requested to report to the study site for their first visit. On the **first visit (screening)**, the Study Coordinator, Sarah A. Johnson, PhD, RD, CSO, will provide the potential subjects with verbal and written explanation of the project and will answer any questions regarding the study. Then the individual will be asked to sign an informed consent form, followed by measuring waist circumference, resting brachial blood pressure, fasting serum triglycerides, HDL cholesterol, and glucose levels using the Cholestech LDX® System (Waltham, MA) to confirm MetS. Baseline assessments will be performed for medical history, medications use, dietary intake, and physical activity. If volunteers meet the study criteria they will be scheduled for their second visit two weeks later (actual baseline data collection) and randomly assigned to their treatment group. They will be given a three-day food record to take home and bring back on the second visit. Additionally, subjects will be asked to collect 25-50 ml their first void on the morning of the **second (baseline) visit (2-weeks)** and bring this with them to the clinical research facility. During this visit between the hours of 7-10 A.M., blood pressure will be measured followed by blood draw (20 ml venous blood). Subjects' anthropometrics including height, weight, and waist and hip circumferences will be measured. Participants will be asked to complete Physical Activity and Bowel Movement Questionnaires. Next participants will undergo a DXA scan for body composition measurements. They will be provided with their assigned treatment and will receive standard instructions on how to fill out daily diaries for their treatment, and for food records. Urine collection, blood pressure, blood draw, and anthropometric, body composition, diet, physical activity, and bowel movement assessments will be repeated at **6- (third visit) and 12-week (final visit)** intervals. Participants will be provided with light breakfast items before leaving the clinical research facility. After completing the assigned 12-week intervention, subjects will undergo a 4-week washout period before crossing over to the other intervention and all respective procedures will be followed at baseline, 6- and 12-week visits. See Figure 2 below.

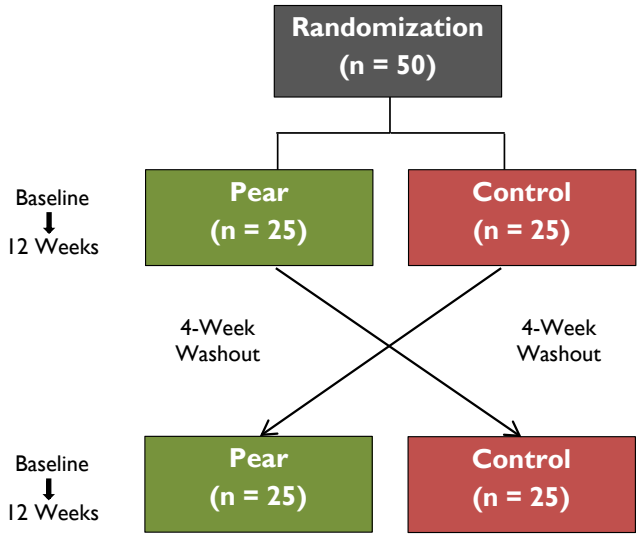


Figure 3. Study Design.

Study Procedures	**Screening**	**Baseline**	**6-Weeks**	**12-Weeks**
Eligibility Criteria and Informed Consent	X			
Medical History	X			
Three-Day Food Record	X	X	X	
Physical Activity Questionnaire		X	X	X
Seven-Day Bowel Movement Questionnaire		X	X	X
Anthropometrics	X	X	X	X
DXA		X		X
Blood Draws	X	X	X	X
Urine Collection		X	X	X
Blood Pressure	X	X	X	X
Assess Compliance	Assessment of compliance will be ongoing throughout the study.			

Table 1. Study Flowchart.

Data Analyses and Management:

Sample Size and Power Calculation: An initial sample size of 50 participants, with attrition rate of 20% will produce a sample size of approximately 20 participants per group in a crossover design with greater than 80% power of more than 0.85 at an $\alpha = 0.05$ to detect a significant difference ($P < 0.05$).

Statistical Analysis: Statistical analysis will be performed using SAS Version 9.3 (SAS Institute, Cary, NC). Descriptive statistics will be calculated for all variables and will include means, standard deviations, medians, minima and maxima. Distributions of outcome variables will be examined graphically for asymmetry and for outliers. If a lack of symmetry is noted, the variable will be transformed before analysis. Baseline characteristics for the study groups will be compared and if differences occur in variables that could influence the results, subsequent analyses will adjust for the effects of these variables. Baseline values of blood pressure, serum, plasma, and urine biomarkers, anthropometric variables, body composition, and questionnaires for the two experimental groups will be compared using two-sample t-tests. The effects of dietary treatments on primary outcomes of interest (blood pressure and serum markers of lipids, insulin sensitivity, inflammation, oxidative and antioxidative status), and secondary outcome variables (body composition and gastrointestinal function) will be evaluated by 2 (group) x 3 (time) repeated measures ANOVA applied to changes in these measurements during the treatment periods. The effectiveness of the washout phase will be tested by comparing baseline values to the values at the end of the washout phase, and also by evaluating the change in the response during the washout phase. Appropriate multiple comparisons will be employed to investigate main or interaction effects. Some covariates such as age, initial BMI, and baseline characteristics identified in the preliminary analysis will be included. Other factors that might affect the results, such as physical activity and dietary intakes will also be examined.

D. RESULTS AND DISCUSSION

At this point we are continuing with subject recruitment and data collection and do not have any results to present at this time (please see **Study Timeline** above).