Northwest Pear Research Review

Hood River Inn, Oregon

Thursday, 2/16/2017

Time	Page	Presenter	Title	Yrs
8:00		Gix	Welcome, introduction	
8:10		Willett	WTFRC, ethoxyquin, TFREC entomology position updates	
8:20		Godwin	WSU Endowment Advisory Committee	
8:25		Schmidt	Housekeeping	
			Final Project Reports	
8:30	1	Neale	Development of marker-based breeding technologies	14-15
8:45	9	Dhingra	Establishing NW-acclimated Pyrus rootstock breeding material	14-16
9:00	17	Moffitt	Health role of pear for Metabolic Syndrome	14-16
9:15	27	Einhorn	Advancing Metamitron as a chemical thinner for Bartlett pear	16
9:30	35	Musacchi	Improving quality and maturity consistency of 'd'Anjou'	14-16
9:45	47	Cooper	Suppression of pear psylla using elicitors of host-defenses	14-16
10:00	55	Beers	Pesticide resistance in pear psylla	14
10:15	64	Beers	Miticide resistance in spider mite pests of pears	13-14
10:30			*Break*	
			Continuing Projects 10:50 - 12:00	
1	75	Wang	Delivering quality pear fruit to consumers	15-17
1	81	Einhorn	Evaluation of potential new pear cultivars for the PNW	15-17
1	87	Evans	Pear rootstock breeding*	15-17
1	94	Musacchi	Fall and summer pruning to control vigor and psylla in d'Anjou pear: *No-cost extension*	14-16
1	101	Serra	Dry matter assessment in pear and consumer perception	16-17
2	108	Cooper	Gut content analysis to pinpoint where psylla overwinter: *No-cost extension*	16
2	114	Johnson	Improved late - and post-bloom sanitation of fire blight pathogen	16-17
2	121	Amiri	Enhancement of postharvest decay management in pear	16-18
2	126	Beers	Integrated fruit production for pears	16-18

FINAL PROJECT REPORT

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

PI:	David Neale	**Co-PI (2):**	Amit Dhingra
Organization:	UC Davis	**Organization**:	Washington State University
Telephone:	530-754-8431	**Telephone**:	509-335-3625
Email:	dbneale@ucdavis.edu	**Email**:	adhingra@wsu.edu
Address:	One Shields Avenue	**Address**:	Dept. of Horticulture
Address 2:	262 Robbins Hall	**Address 2**:	155 Johnson Hall
City/State/Zip:	Davis	**City/State/Zip**:	Pullman
State/Zip:	CA 95616	**State/Zip**:	WA 99164

Cooperators: Richard Bell (USDA/ARS Kearneysville, WV), Joseph Postman and Nahla Bassil (USDA/ARS Corvallis, OR), Kate Evans (Washington State University), Sara Montanari (UC Davis), Rachel Elkins (UC Cooperative Extension)

Other funding sources: none
Agency Name: UC Davis
Amount awarded: $100,000

Total Project Funding: $100,000

Budget History:

Item	2014:	2015	2016
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$50,000	$50,000	$0, NCE
Travel			
Plot Fees			
Miscellaneous			
Total	50,000	50,000	0

OBJECTIVES

Pear production can be increased by developing new varieties with improved agronomic characteristics, such as disease/insect resistances and dwarfing stature, which can be combined with high fruit quality and many other traits. In traditional breeding the selection of such elite cultivars is based on the visual evaluation of phenotype, and in woody perennial crops, including pear, this process is time consuming and expensive, because of the trees' long juvenile phase, laborious trait assessment, and large land requirement. Marker-assisted selection (MAS) technologies are currently routinely and successfully applied for several plant crops, and they can potentially increase pear breeding efficacy. The objective of this project was to develop a high number of molecular markers to be used to screen ~2000 different pear cultivars collected from the National Clonal Germplasm Repository (USDA/ARS NCGR) in Corvallis, OR. These genotypic data will be useful to find strong marker-trait associations to be applied for MAS in pear, information which is currently lacking for most of the traits of interest in this crop.

All U.S. pear genetics researchers have teamed up under the new Pear Genomics Research Network, and collaborations with other foreign pear scientists have also been set up, with the objective of working together towards a common goal.

Activities:

1. Design a re-sequencing project and a SNP genotyping assay (accomplished).
2. Collect leaf samples from *Pyrus* spp. accessions from the National Clonal Germplasm Repository (NGCR) in Corvallis, OR (accomplished).
3. Conduct bioinformatics analysis of the re-sequencing data and design a SNP array (accomplished).
4. Genotype all the collected samples (in progress).
5. Submit the re-sequencing and genotypic data to the Genome Database of Rosaceae (https://www.rosaceae.org/).

SIGNIFICANT FINDINGS

1. We selected 55 accessions to represent the SNP discovery panel and we extracted high quality DNA from them.
2. We collected leaf samples from ~2000 *Pyrus* spp. accessions from the National Clonal Germplasm Repository (NGCR) in Corvallis, OR.
3. We processed the 55 selected accessions for whole-genome, low-coverage sequencing (re-sequencing).
4. We performed bioinformatics analysis of the re-sequencing data, SNP calling, filtering and we designed a high-density SNP array.
5. We extracted high-quantity DNA from a subset of the collected samples for genotyping.

METHODS

Design a re-sequencing project and a SNP genotyping assay for pear

Researchers working on pear breeding and genomics in the U.S., their extension collaborators, and the pear marketing boards created the Pear Genomics Research Network (PGRN), with the aim of bringing together their efforts for the enhancement of the pear-growing industry in the U.S. Within this collaboration, we started a re-sequencing project for the evaluation of Pyrus genetic diversity. We selected 55 pear accessions, representing founding cultivars and a total of 29 species and hybrids, within the NCGR in Corvallis, OR, and the Appalachian Fruit Research Station (AFRS) in Kearneysville, WV, to constitute the polymorphism discovery panel in this project (Table 1). These accessions were processed for whole-genome, low-coverage sequencing.

Sample collections and DNA extraction

During the summer 2014 we collected leaves from 1870 different Pyrus spp. cultivars and hybrids maintained at NGCR and AFRS. For the 55 samples included in the discovery panel, we extracted DNA from freeze-dried leaves using the DNeasy Plant Mini Kit (Qiagen®). For each sample, paired-end libraries were constructed using the Nextera DNA Sample Preparation kit (Illumina®) at the UC Davis Dept. of Evolution and Ecology. Libraries were sent to the Institute for Genomic Medicine at UC San Diego for sequencing on an Illumina® HiSeq2500 in high output mode with v4 chemistry and 2x100 bps runs.

Bioinformatics analyses of re-sequencing data and SNPs calling and filtering

Sequences of the 55 different pear accessions were evaluated, and the low quality bases (usually at the boarders of the sequences) were trimmed off. Sequences from similar accessions were divided into 6 groups, as in Table 1: i) Group Communis, including all P. communis cultivars, P. communis subsp. caucasica and P. communis subsp. pyraster; ii) Group 1, including wild relatives of P. communis; iii) Group 2, including Middle East/Central Asia arid adapted species; iv) Group 3, including East Asian "pea" pears; v) Group 4, including East Asia large fruited cultivars and wild relatives; and vi) Group Hybrids, including all interspecific hybrids. The objective was to group together accessions with expected similar genomes and apply ad hoc parameters for both the sequence alignment and the SNP calling. The trimmed sequences were aligned to the 'Bartlett' v1.0 reference genome, applying more stringent parameters for the Group Communis. The aligned sequences within each group were pooled and searched for polymorphisms against the reference genome. The polymorphic sites (variants) were then subjected to a Quality filter (Fig. 1), with parameters calculated for each of the 6 groups. Afterwards, all the detected variants from each group were combined into a unique file and subjected to the Affymetrix filter (Fig. 1), aimed at discarding possible false SNPs.

SNP selection

The most informative set of SNPs was selected basing on their predicted effect on genes (according to the software SnpEff), their position on the genome (according to a Focal Point strategy), and the level of diversity across the 55 re-sequenced accessions (Fig. 1). Two different files were submitted to Affymetrix for the array design: a high priority file and a low priority file. SnpEff is a software that predict how a certain SNP, if it falls inside a coding region, might modify the protein, and it classifies the SNPs according to the impact of such a change. SNPs inside coding regions (those classified with HIGH, MODERATE and LOW effect by SnpEff), and SNPs close to coding regions (those with MODIFIER effect and not categorized as "intergenic") were given high priority for inclusion in the array. Also SNPs developed with other technologies and validated in mapping population were given high priority. These are Illumina Infinium II SNPs (Montanari et al, 2013) and SNPs developed by Genotyping-by-sequencing (GBS) at PFR.

The remaining SNPs (those with MODIFIER effect and intergenic) were given low priority for the array design, and a sorted list was submitted to Affymetrix. We divided the genome in windows of constant size, called Focal Points (FP). Of all the SNPs inside each FP, we removed those with the same genotypes (redundant information). Then we chose one SNP for each FP, the SNP with the higher number of heterozygous genotypes, and we put them at the top of the list; these were followed by the second SNPs with the higher number of heterozygous genotypes from each FP, and so on. This way, we selected SNPs that were evenly spread across the genome and more informative.

At Affymetrix, SNPs from the high priority file were tiled on the array first, then the SNP from the low priority file were selected starting from the top of the list and going down, until completion of the array.

Genotyping of the collected samples

DNA was first extracted from a subset of 284 highly diverse pear accessions (the "screening panel"). The SNPs and the DNA were sent to Affymetrix for the construction of a draft genotyping array, according to the Axiom myDesign™ protocol, and for genotyping. Basing on the results of this first round of genotyping, we will discard all non-functioning markers and the less informative SNPs. SNPs passing the "screening" step will be again sent to Affymetrix, along with the DNA of the remaining samples, for designing a final, highly-efficient SNP array and for genotyping.

RESULTS & DISCUSSION

The Pear Genomics Research Network

The University of California (UC) Davis, UC Cooperative Extension, the NGCR in Corvallis, OR, the AFRS in Kearneysville, WV, Washington State University (WSU) and Oregon State University (OSU), have teamed up under the new Pear Genomics Research Network (PGRN), which also involves the industry organizations California Pear Advisory Board (CPAB), Pear Pest Management Research Fund (PPMRF), Pear Bureau Northwest (USA Pears), and Washington Tree Fruit Research Commission (WTFRC). A website for the PGRN (http://ucanr.edu/sites/peargenomics/) was developed in March 2015.

Re-sequencing, SNP calling and selection of SNPs for first draft genotyping array

Sequencing of the 55 accessions included in the discovery panel resulted in a total of 731.2 Million read pairs, with a per sample coverage of 3.3x to 5.4x. Variants were called from each of the 6 groups and i) 3,809,750 were discovered in Group Communis; ii) 5,484,730 in Group 1; iii) 5,957,246 in Group 2; iv) 7,004,301 in Group 3; v) 7,339,331 in Group 4; and vi) 5,732,197 in Group Hybrids. After the Quality filter and combination of the variants from all the groups into a single file, a total number of 9,662,991 unique variants were left and were submitted to Affymetrix for scoring. After the Affymetrix filter, 1,195,301 SNPs were left and were analyzed with SnpEff. 85,152 SNPs (643 tri-allelic and 84,509 bi-allelic) with HIGH or MODERATE effects were all kept; SNPs with LOW effect were subjected to further filtering (Fig. 1) and 93,302 were left (461 tri-allelic and 92,841 bi-allelic); SNPs with MODIFIER effect were subjected to further filtering (Fig. 1) and 552,485 were left (6138 tri-allelic and 546,347 bi-allelic, of which 98,557 intergenic). Also validated SNPs were scored by Affymetrix and filtered (Fig. 1): 1139 Illumina Infinium II SNPs (Montanari et al., 2013), filtered down to 558, and 9151 SNPs developed by GBS at PFR, reduced to 2452. In total, 733,949 were submitted to Affymetrix and 659,183 were successfully tiled on the first draft array.

Screening panel

The 284 samples constituting the screening panel were chosen to be representative of the entire diversity held at NCGR. A total of 35 different species and interspecific hybrid were included in the screening panel. Some cultivars with known pedigree information and their two parents ("trios") were also included, for a total of 21 trios, whose genotypic information will be useful to validate the SNP markers. Moreover, three samples were replicated, in order to double check, the reliability of the genotyping and identify possible causes of errors: P. communis 'Bartlett' was replicated three times, double haploid 'Bartlett' twice and P. pyrifolia 'Dan Bae' twice.

56,700 SNPs will be chosen for the final array.

Discussion

The number of SNPs we discovered is the highest ever found for pear. By performing the screening step, we will guarantee the design of a highly-efficient SNP array, with a success rate close to 100%, which is fundamental for the evaluation of a large genetic diversity. With this genotypic data we will be able to characterize the pear germplasm collection. From these studies, we will gain information about unknown genotypes identity and pedigrees, which is fundamental for their

employment in breeding. We will also be able to elucidate the degree of relatedness among different species, and the comparison of wild species with cultivars might also help us identifying regions linked to domestication patterns, which are assumed to be associated with important agronomic features.

Moreover, we will use this genotypic information to do associations with phenotypes and identify markers to be used in MAS. First of all, historic phenotypic data collected at NCGR will be used, although they are not expected to provide highly reliable information. Secondly, appropriate phenotypic experiments will be designed for the collection of new data and the identification of robust marker-trait associations.

REFERENCES

Chagné, D., Crowhurst, R. N., Pindo, M., Thrimawithana, A., Deng, C., Ireland, H., … Velasco, R. (2014). The draft genome sequence of European pear (Pyrus communis L. "Bartlett"). *PLOS ONE*, *9*(4), 1–12. http://doi.org/10.1371/journal.pone.0092644

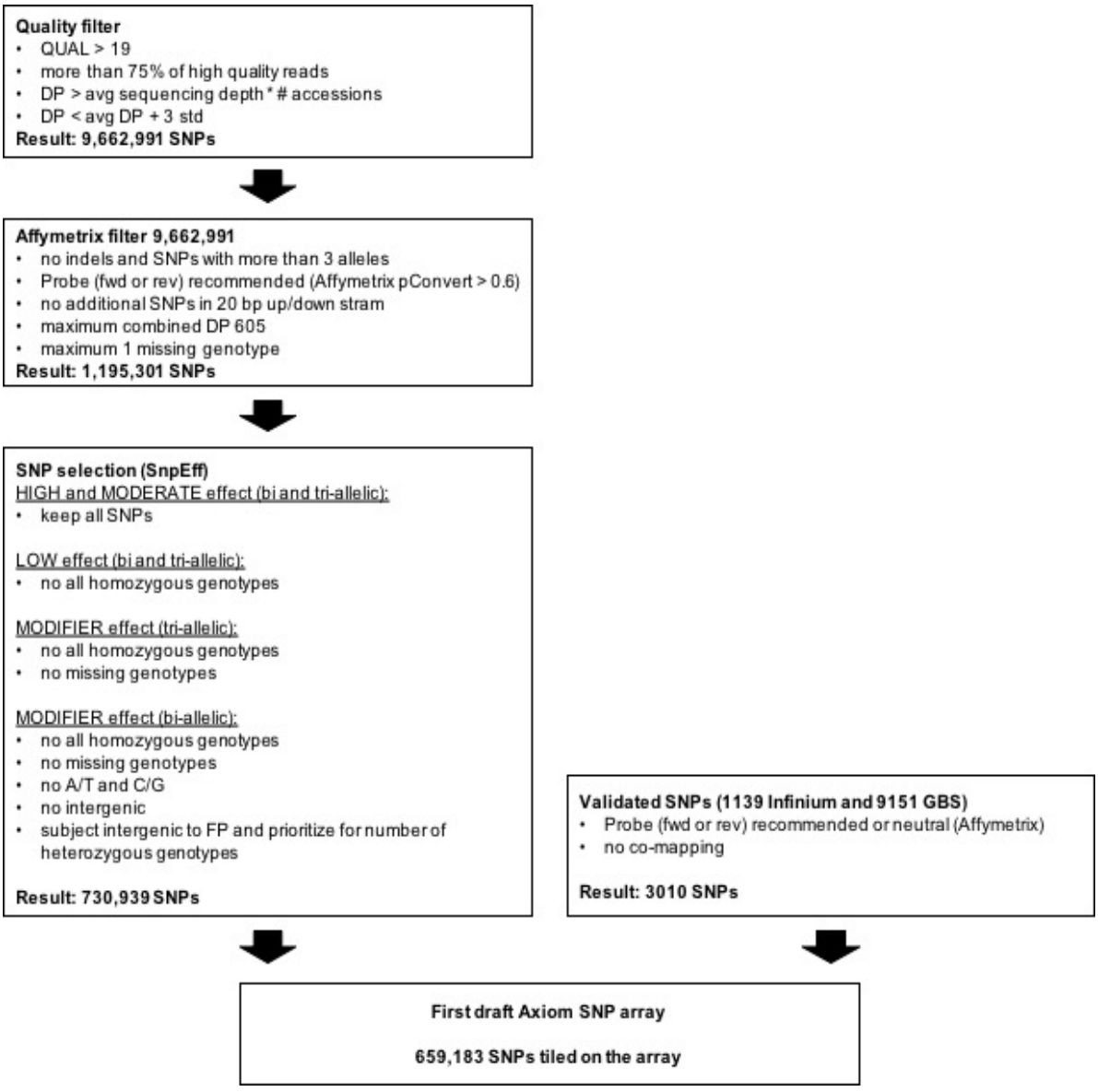
Montanari, S., Saeed, M., Knäbel, M., Kim, Y., Troggio, M., Malnoy, M., … Chagné, D. (2013). Identification of Pyrus Single Nucleotide Polymorphisms (SNPs) and evaluation for genetic mapping in European pear and interspecific Pyrus hybrids. *PLOS ONE*, *8*(10), 1–11. http://doi.org/10.1371/journal.pone.0077022

Table 1: List of 55 re-sequenced pear accessions, with subdivision into 6 groups of genetic similarity.

Pear accession	Group	Pear accession	Group
P. communis 'Anjou'	Communis	*P. elaeagrifolia* MSU6768	Group 2
P. communis 'Bartlett'	Communis	*P. glabra*	Group 2
P. communis 'Bosc'	Communis	*P. regelii*	Group 2
P. communis 'Coscia'	Communis	*P. sachokiana* GE-2006-115	Group 2
P. communis 'Gem'	Communis	*P. salicifolia* GE-2004-141	Group 2
P. communis 'Gin'	Communis	*P. spinosa* (*amygdaliformis*)	Group 2
P. communis 'Harrow Delight'	Communis	*P. syriaca*	Group 2
P. communis 'Harrow Sweet'	Communis	*P. betulifolia*	Group 3
P. communis 'Old Home'	Communis	*P. betulifolia*	Group 3
P. communis 'Para de Zahar de Bihor'	Communis	*P. fauriei*	Group 3
P. communis 'Roi Charles de Würtemburg'	Communis	*P. koehnei*	Group 3
P. communis 'Seckel'	Communis	*P.* × *bretschneider*i 'Ta Shian Sui Li'	Group 4
P. communis subsp. *caucasica*	Communis	*P.* × *bretschneideri* 'Xuehuali' (Snowflake)	Group 4
P. communis subsp. pyraster 'Erabasma'	Communis	*P.* × *bretschneideri* 'Ya Li'	Group 4
P. communis subsp. *pyraster* 'Mednik'	Communis	*P.* × *sinkiangensis* 'Ho Mon'	Group 4
P. communis subsp. *pyraster* ALB-2011-024	Communis	*P. hondoensis*	Group 4
P. communis US 309	Communis	*P. pashia* 'Naspati'	Group 4
P. communis US76128-009	Communis	*P. pseudopashia*	Group 4
P. communis US82720-002	Communis	*P. pyrifolia* 'Dan Bae' (Olympic)	Group 4
P. cordata (Turkey)	Group 1	*P. pyrifolia* 'Nijisseiki'	Group 4
P. cordata pure	Group 1	*P. pyrifolia* 'Zao Su'	Group 4
P. cossonii (Russia)	Group 1	*P. ussuriensis* 'Pai Li' (Beijing White Pear)	Group 4
P. gharbiana No. 1	Group 1	*P. ussuriensis* No. 2 (Korea)	Group 4
P. mamorensis	Group 1	*P. ussuriensis* x *P. pyrifolia* Illinois 76	Group 4
P. nivalis	Group 1		
(*P. ussuriensis* x *P. pyrifolia*) x *P. communis* NJ487601193	Hybrids	*P. communis* x *P. ussuriensis* NJB9R1T117	Hybrids
(*P. ussuriensis* x *P. pyrifolia*) x *P. communis* NJA2R59T69	Hybrids	*P. communis* x *P. ussuriensis* NY 10262	Hybrids
P. communis x *P. ussuriensis* 'Takisha'	Hybrids	*P. communis* x *P. ussuriensis* NY 10353	Hybrids

Communis = *Pyrus communis*; Group 1 = *P. communis* wild relatives; Group 2 = Middle East/Central Asia arid adapted species; Group 3 = East Asian "pea" pears; Group 4 = East Asia large fruited wild relatives; Hybrids = interspecific hybrids

Figure 1: SNP filtering pipeline.



EXECUTIVE SUMMARY

1. We designed a large number of SNP markers for pear and we included them in an array for high-throughput genotyping.
2. The genotypic data developed with this tool will be used to characterize the pear germplasm collection, evaluate *Pyrus* genetic diversity and build linkage maps for breeding populations.
3. Such studies will provide information that can be used for breeding in several ways: localize genomic regions associated with traits of interest; identify degrees of relationship among cultivars, in order to optimize their use for breeding; elucidate *Pyrus* domestication patterns, which are assumed to be associated with important agronomic features.
4. Available phenotypic data collected for the genotyped accessions will be used directly for association studies, and new phenotypic experiments will be designed for the confirmation of such associations and the study of new, important characters.
5. The final objective is to implement MAS in pear, for a faster development of new, high-performance cultivars.

FINAL PROJECT REPORT

Project Title: Establishing NW-acclimated Pyrus rootstock breeding material

PI:	Amit Dhingra	**Co-PI**:	Kate Evans
Organization:	Washington State University	**Organization**:	Washington State University
Telephone:	509 335 3625	**Telephone**:	509-663-8181
Email:	adhingra@wsu.edu	**Email**:	kate_evans@wsu.edu

Other funding sources

Agency Name: PNW Pear Bureau
Amt. awarded: $273,253 (2015-2018)
Notes: "Pear Rootstock Breeding" PI Evans, Co- PI Dhingra. Synergistic project to advance the selected pear rootstock seedlings via phenotyping and propagation.

Agency Name: WSU CAHNRS Ignite Program
Amt. awarded: $2500
Notes: Support for an undergraduate student to perform phenotyping and tissue culture of selected seedlings and embryo rescue.

Agency Name: Washington State University Graduate school
Amt. awarded: $34,000 (2016)
Notes: Support for Danielle Guzman, Graduate student – she will perform additional crosses with irradiated pollen in 2016.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

Total Project Request: **Year 1**: 22,000 **Year 2**: 22,185 **Year 3**: 22,992

Budget History:

Item	**2014**	**2015**	**2016**
Wages[a]	13832	14385	14960
Benefits	5577	5800	6032
Supplies[b]	1000	1000	1000
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

RECAP OF THE ORIGINAL OBJECTIVES

1. Screen seedlings germinated in 2012 for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions
2. Germinate and subsequent phenotypic screening of seeds derived from irradiated pollen

This project addresses the long-term need for NW acclimated pear rootstocks in the US and is complementary to larger efforts in this direction. In particular, this project focuses on rapid growth of 149 seedlings derived from crosses between 'Bartlett', 'd'Anjou' and 'Comice' and 49 seedlings derived from crosses using gamma irradiate pollen between 'Bartlett', 'd'Anjou', 'Comice' and 'Abate Fetel' in the greenhouse to perform phenotypic screening.

SIGNIFICANT FINDINGS

- Seedlings from crosses made between 'Bartlett', 'd'Anjou' and 'Comice' are in the fifth dormancy cycle. Most seedlings have gone beyond the juvenile phenotype of exhibiting thorns. Two of the "Bartlett" × "d'Anjou" seedlings flowered in spring 2016 in just 4 years from seed germination.
- The technique of rapid cycling through generations to overcome juvenility works in pears and can be utilized for future breeding experiments.
- A selected dwarf subset of the seedling populations have been propagated for small scale replicated trials and will be planted in Wenatchee spring 2017.
- A total of 58 dwarf seedlings have been established from crosses made with gamma irradiated pollen and are ready for evaluation via grafting to see if the dwarfing is transmitted to the scions. A new project has been submitted in 2017 to enable this next step in identifying a dwarf pear rootstock.
- The ratio of number of nodes to height in the irradiated pollen ranges from 0.53 in a 'Bartlett' × 'Abate Fetel' (irradiated) cross to 1.4 in a 'Bartlett' × 'Comice' (irradiated) cross indicating a great degree of spread between vigor and dwarfing.

RESULS AND DISCUSSION

Objective 1: Screen seedlings for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions

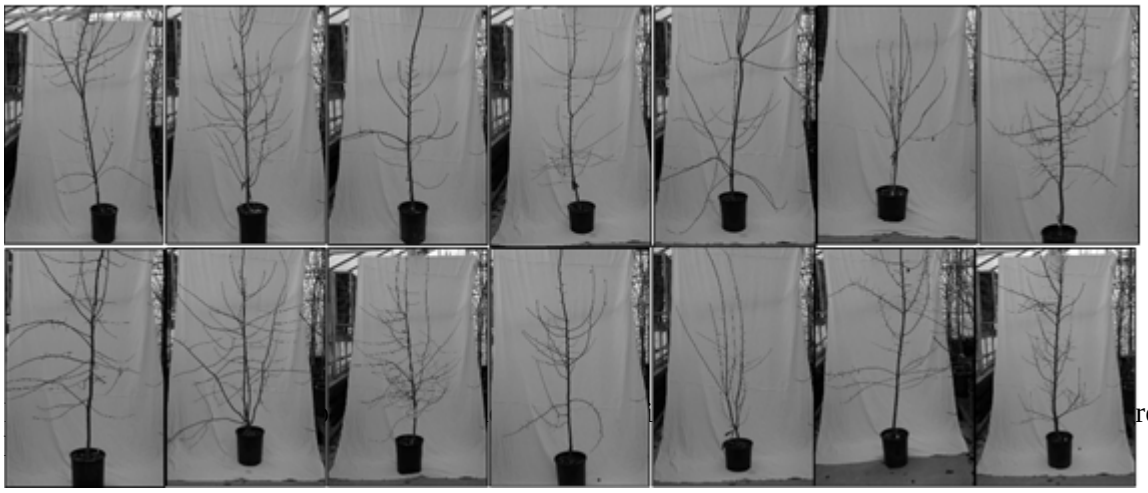
A total of 149 potted trees representing seedlings obtained from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' have been established. The seeds germinated in 2012 are undergoing fifth dormancy cycle and are being maintained in the greenhouse in Pullman. These potted seedlings were scored for node count and height in May 2015. Based on the ratio of number of nodes to height and growth habit, preliminary plant selections were made for desirable seedlings for a complementary project being led by Co-PI Evans. Considerable phenotypic variation was observed in plant habit and wide distribution of ratio of number of nodes to height was recorded. Figure 1 illustrates the extent of variation in habit. A subset of 13 individuals was selected for propagation from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' and three trees of each will be planted in a randomized complete block design at the Columbia View orchard, Wenatchee, in spring 2017. The trees will be budded with a standard scion in August 2017. Vigor data will be taken in 2018 and 2019. Currently these plants are in a dormant state (Figure 2) and will be moved to Wenatchee in early spring. A summary of the selected individuals is presented in Table 1.

Table 1 Propagated seedlings for planting in Wenatchee

Cross	Number of plants
B × A 12-13	4
B × A 12-26	4
B × A 12-6	9
B × A 12-21	6
B × A 12-32	3
B × A 12-60	6
B × A 12-9	3
B × C 12-10	4
B × C 12-79	5
B × C 12-69	4
B × C 12-71	2
B × C 12-42	2
B × C 12-37	2

Protocols and approaches developed previously for apples, to accelerate plant growth and cycling through dormancy, continues to be used as a guide for accelerating pear seedling growth in the greenhouse. The plants derived from seeds germinated in 2012 have been taken through five cycles of dormancy since the project was funded. Interestingly, two of the seedlings, B×A 12-44 and B×A 12-19 obtained from "Bartlett" × "d'Anjou" cross produced flowers in 2016 spring (Figure 3). Remaining plants are expected to flower in 2017 spring. This is an exciting outcome as seedlings produced flowers within 4 years of the seeds being germinated.

Seeds obtained from crosses made in the 2013 season were also 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. Irrigation and fertilization was performed on a regular schedule standardized for greenhouse plants. For dormancy cycling, these seedlings were moved to the cold room to provide 1000 hours of chilling (ecodormancy) at the first sign of phenotypic markers of shoot growth. Plants were completely defoliated prior to being moved back to ambient growth conditions to initiate vigorous growth. Of the 149 seedlings, only 5 were lost and 144 seedlings continue to be maintained in large pots at the Tukey Orchard, Pullman.



rowth

Figure 3: Flowering observed in two seedlings 12-44 and 12-19 derived from “Bartlett” x “d’Anjou” crosses in 2016.

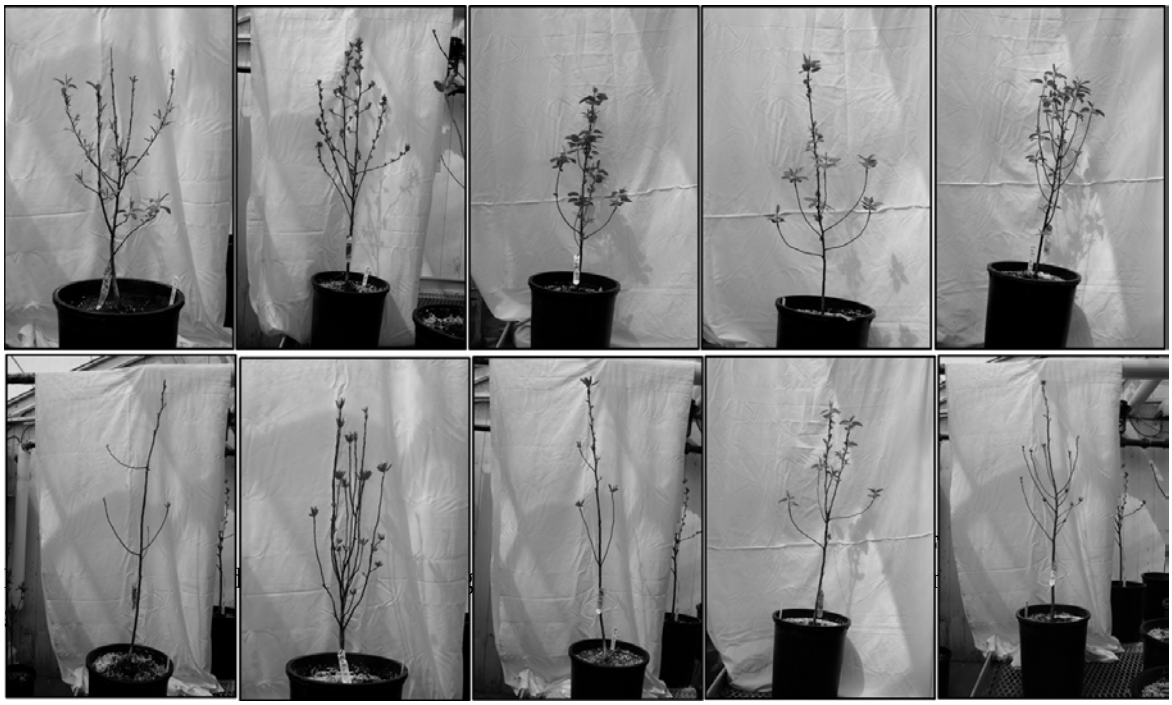
In summary, this objective has yielded two important and tangible outputs.

1. Identification of 13 seedlings exhibiting the desirable dwarf habit representing plant material that is naturally acclimatized to the PNW region. These seedlings will be planted in Wenatchee for a replicated trial with scions grafted atop these selections. The experiment will be done to evaluate the transmission of dwarfing trait to scions.

2. Identification of two precocious seedlings for which further phenotyping will be performed in the future.

Objective 2. Germinate and subsequent phenotypic screening of seeds derived from irradiated pollen

A total of 49 seedlings derived from 2013 crosses made with irradiated pollen were established. Irradiated pollen was used to generate the foundational trait of dwarfing in pears. This set of plants were cycled through 3 sets of dormancy. The seedlings demonstrate a large degree of variation is size and growth characteristics. The plants were phenotyped for height and number of nodes and the ratio between the two parameters was calculated. It is interesting to note that only two crosses yielded a ratio greater than 1. However there were several seedlings where the ratio was closer to 1. Please refer to Table3. Figure 4 illustrates the diversity in growth habit. These plants are now ready to be grafted and screened for transmission of the dwarfing habit to the scion. A new proposal to evaluate this aspect has been submitted for consideration in 2017.



4: in

Table 3: Ratio of number of nodes/height for seedlings derived from irradiated pollen.

‘Bartlett’ × ‘Bartlett’(irradiated)			
ID	Height(cm)	mber of no	Ratio # nodes/ height
13-6	**71**	**70**	**0.99**
13-4	68	58	0.85
13-1	69	53	0.77
13-2	113	83	0.73
13-5	86	60	0.70
13-7	62	43	0.69
13-3	107	74	0.69

‘Bartlett’ × ‘Comice’ (irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-4	**30**	**42**	**1.40**
13-1	56	42	0.75
13-3	95	71	0.75
13-2	100	67	0.67

‘Comice’ × ‘Comice’(irradiated)			
ID	Height(cm)	Number of nodes	Ratio # nodes/ height
13-5	**61**	**60**	**0.98**
13-6	68	61	0.90
13-1	78	60	0.77
13-7	74	52	0.70
13-2	112	77	0.69
13-4	65	43	0.66

‘Abate Fetel’ × ‘Comice’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-1	88	76	0.86
13-2	83	69	0.83
13-4	81	59	0.73
13-1	81	57	0.70
13-3	96	60	0.63

‘Bartlett’ × ‘d’Anjou’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-4	**41**	**45**	**1.10**
13-1	87	80	0.92
13-8	81	73	0.90
13-9	111	97	0.87
13-6	71	62	0.87
13-7	66	57	0.86
13-3	99	68	0.69
13-2	153	103	0.67
13-5	77	51	0.66

‘Bartlett’ × ‘Abate Fetel’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-2	92	79	0.86
13-3	77	66	0.86
13-9	62	53	0.85
13-14	84	70	0.83
13-8	61	50	0.82
13-11	76	61	0.80
13-5	96	75	0.78
13-13	80	62	0.78
13-6	90	68	0.76
13-15	141	105	0.74
13-10	129	89	0.69
13-4	82	56	0.68
13-1	97	65	0.67
13-16	87	58	0.67
13-7	88	58	0.66
13-17	127	70	0.55
13-12	116	61	0.53

‘Comice’ × ‘d’Anjou’(irradiated)			
ID	Height(cm)	Number of nodes	Ratio # nodes/ height
13-1	55	37	0.67

In summary, this objective has yielded two important and tangible outputs.

1. Generation of several dwarf seedlings derived from material that is naturally acclimatized to the PNW region.

2. Potential rootstocks or rootstock parental material that can be utilized in subsequent rootstock or variety development.

OUTREACH

- Good Fruit Grower article focused on the pear rootstock breeding program was published in September 2015.
- Amit Dhingra hosted the Washington AgForestry leadership group at WSU Pullman; pear rootstock breeding was discussed during a visit to the greenhouses to look at the germplasm.
- Amit Dhingra hosted Doug Hemly (CA pear grower); advances in pear rootstocks was the primary discussion point.
- Kate Evans presented the outline of the breeding program at the Washington State Tree Fruit Association meeting in Yakima December, 2015 in a talk entitled 'Developing and implementing new technologies for and from the WSU pome fruit breeding program'.
- Amit Dhingra presented efforts on developing material for pear rootstocks at the Washington State Tree Fruit Association meeting in Yakima December, 2015 in a talk titled, "Smart Plants".
- Kate Evans presented the outline of the breeding program at the Washington State Horticultural Association Show, Wenatchee, December 2016, in a talk entitled 'Update on pear rootstock breeding'.
- Amit Dhingra's article 'The pear industry has unlimited potential and is ripe for a revolution' was published in the Good Fruit Grower, September 2016 (http://www.goodfruit.com/the-age-of-the-pear/ September 14, 2016) and his research on pears was featured in an article in The Atlantic, June 2016 (The push to make pears the new apples). The Atlantic. http://www.theatlantic.com/science/archive/2016/06/battle-of-the-pomes/488687/ June 27, 2016.
- Amit Dhingra presented the role of rootstocks in managing fruit quality in a talk entitled, "Improving Fruit Quality in Pears" at the GS Long Annual Growers Meeting in Chelan, WA on December 15, 2016.

EXECUTIVE SUMMARY

Background: Dwarfing rootstocks are the key to transforming the pear industry. There are 3 primary strategies being pursued to identify a set of dwarf pear rootstocks. 1. Import dwarf rootstocks developed outside of the USA. 2. Make new crosses with imported and domestic parental material and 3. Utilize regionally acclimatized material for traditional and mutation breeding. This project represents the number 3 approach which was initiated by Dhingra and Evans programs prior to obtaining funding for this project in recognition of the urgent need of the USA pear industry.

Outcomes and significant findings: The project resulted in the establishment of 144 F1 seedling population derived from regionally acclimatized parental material. In 2016, 13 seedlings from this group where identified that exhibited the desirable dwarf habit and these seedlings will be planted in Wenatchee for a replicated trial with scions grafted atop these selections. The experiment will be done to evaluate the transmission of dwarfing trait to scions. Two of the 144 seedlings produced flowers and these individuals will be phenotyped in the future. As an outcome of the mutation breeding, several dwarf seedlings were generated which can directly serve as potential rootstocks or parental material that can be utilized in subsequent rootstock or variety development.

Future directions: The seedlings with dwarf habit derived from crosses made with both traditional and irradiated pollen need to be evaluated if they will transmit the dwarf habit to the scion. While a replicated trial has been planned in a complementary project with 13 seedlings from the traditional crosses, additional trials will need to be conducted at multiple locations. One of the exciting outcomes is the establishment of a range of dwarfing habit in seedlings derived from irradiated pollen. It is imperative to evaluate if these seedlings will transmit the dwarf habit to the scions. An economic and rapid way to evaluate that will be to perform the grafting and screening in the greenhouse to select a few desirable candidates.

FINAL PROJECT REPORT

Project Title: Health role of pear for Metabolic Syndrome

PI: Bahram H. Arjmandi, PhD, RD
Organization: Department of Nutrition, Food and Exercise Sciences, Florida State University
Telephone: (850) 645-1517
Email: barjmandi@fsu.edu
Address: 412 Sandels Building, 120 Convocation Way, Tallahassee, Florida 32306-1493

Co-PI: Sarah A. Johnson, PhD, RDN
Organization: Department of Food Science and Human Nutrition, Colorado State University
Telephone: (970) 491-3807
Email: sarah.johnson@colostate.edu
Address: 206 Gifford Building, 502 W. Lake St., Fort Collins, Colorado 80523-1571

Cooperators: Pear Bureau Northwest

Total Project Request: **Year 1:** $32,185 **Year 2:** $29,871 **Year 3:** $18,000

Other funding sources

Agency Name: Pear Bureau Northwest
Amount awarded: Year 1: $32,185 **Year 2**: $29,871 **Year 3:** $N/A
Notes: Pear Bureau Northwest matched 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.

Total Project Funding: $80,056

Budget History

Item	2014	2015	2016
Salaries	$16,457.50	$16,951	$0
Benefits	$2,688	$2,853	$0
Wages	$0	$0	$0
Benefits	$0	$0	$0
Equipment	$0	$0	$0
Supplies	$12,539.50	$9,067	$18,000
Travel	$0	$0	$0
Miscellaneous	$500	$1,000	$0
Plot Fees	$0	$0	$0
Total	$32,185	$29,871	$18,000

A. OBJECTIVES

The ***central hypothesis*** of this study was that daily consumption of 2 pears (medium sized Green Bartlett and/or Green Anjou pears weighing ~166 g each) for twelve weeks would 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 were 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)].

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).

B. SIGNIFICANT FINDINGS

- Subject recruitment and overall subject retention was excellent (**Fig. 1**) with only 7 participants dropping from the study (14% attrition).
- Systolic blood pressure was reduced by 3.7% ($p = 0.01$) and pulse pressure (difference between systolic and diastolic blood pressure) was significantly ($p < 0.05$) reduced by 7.4% at 12 weeks in the Pear group but not in the Control group. There were no differences between groups so a treatment effect cannot be confirmed; however, this is suggestive of blood pressure reducing effects of pears.
- Triglyceride levels were significantly ($p < 0.05$) reduced by 3.5% and HDL-C levels were ($p < 0.1$) increased by 6.8% in the Pear group but not in the Control group. There were no significant differences between groups so a treatment effect cannot be confirmed; however, this is suggestive of improvements in lipid parameters due to pear consumption.
- Total cholesterol and LDL-C were increased at 6 and 12 weeks in both groups. The changes over time were in both groups so cannot be attributed to pears, but rather a time effect.
- Waist circumference was significantly ($p < 0.05$) reduced by 0.56% at 12 weeks and waist-to-hip ratio was significantly ($p < 0.05$) reduced by 0.54% at 6 and 12 weeks, respectively. There were no differences between groups so a treatment effect cannot be confirmed; however, a significant increase in waist circumference was noted at 6 weeks in the Control group and was sustained at 12 weeks, while percent android (abdominal) fat was increased at 6 and 12 weeks compared to baseline in the control group. Android-to-gynoid ratio (abdominal fat to hip fat) was increased in the control group at 12 weeks. Additionally, leptin was significantly ($p < 0.05$) reduced at 12 weeks by 4.3% and levels were significantly ($p < 0.05$) lower than the Control group. *This suggests a possible shift in fat distribution favoring less leptin production due to a reduction in leptin resistance.* Importantly, the control drink

(addition of calories in the form of carbohydrates) had moderate but detrimental effects on body composition while the pear intervention improved parameters of body composition. Although a treatment effect was not noted (with the exception of leptin), these results suggest that pear consumption may have favorable effects on body composition.

C. OUTLINE OF METHODS

A total of 50 men and women between the ages of 45 and 65 years with three of the five features of MetS were included in the study. After a two-week run-in phase, eligible men and women were 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 were requested to report to the study site for their first visit. On the **first visit (screening)**, potential subjects were provided with verbal and written explanation of the project and individuals were then asked to sign an informed consent form, followed by measuring waist circumference, resting brachial blood pressure, fasting serum triglycerides, HDL-C, and glucose levels using the Cholestech LDX® System (Waltham, MA) to confirm MetS. Baseline assessments were performed for medical history, medication use, dietary intake, and physical activity. Volunteers who met the study criteria were scheduled for their second visit two weeks later (actual baseline data collection) and randomly assigned to their treatment group. They were given a three-day food record to take home and bring back on the second visit. During the **second (baseline) visit (2-weeks)** this visit between the hours of 7-10 A.M., urine was collected, blood pressure was measured followed by blood draw. Subjects' anthropometrics including height, weight, and waist and hip circumferences were measured. Participants were asked to complete Physical Activity and Bowel Movement Questionnaires. Next participants underwent a DXA scan for body composition measurements. They were then 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 were repeated at **6- (third visit) and 12-week (final visit)** intervals. Participants were provided with light breakfast items before leaving the clinical research facility. After completing the assigned 12-week intervention, subjects underwent a 4-week washout period before crossing over to the other intervention and all respective procedures were followed at baseline, 6- and 12-week visits.

Study Procedures	**Screening**	**Baseline**	**6 Weeks**	**12 Weeks**
Informed Consent	X			
Medical History	X			
Three-Day Food Record	X	X	X	
Physical Activity Questionnaire		X	X	X
7-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	Ongoing throughout the study.			

Table 1. Study Procedures.

<u>Data Analyses and Management:</u>

An initial sample size of 50 subjects, with a projected attrition rate of 20% was projected to produce a sample size of approximately 40 participants in a crossover design with greater than 80% power of more than 0.85 at an $\alpha = 0.05$ to detect a significant difference ($p < 0.05$). SAS v9.4 (SAS Institute Inc., Cary, NC) was used for all statistical analyses. A linear regression analysis was used to evaluate the difference between the groups as well as the difference between different time points taking into account the clustering effect of each subject. If the outcome data was not normally distributed, log conversion was performed. A *p*-value of 0.05 was used to evaluate statistical significance.

D. RESULTS AND DISCUSSION

Results:

Subject Enrollment and Attrition

As mentioned in the Significant Findings section, subject recruitment and overall subject retention was excellent with only 7 participants dropping from the study (14% attrition) (**Fig. 1**). Reasons for dropping from the study included personal reasons such as lack of time or moving, not wanting to take the placebo powder, and not wanting to give blood. Tolerance to daily pear consumption was generally reported as good; however, there were reports of taste fatigue towards the end of the 12-week pear interventions.

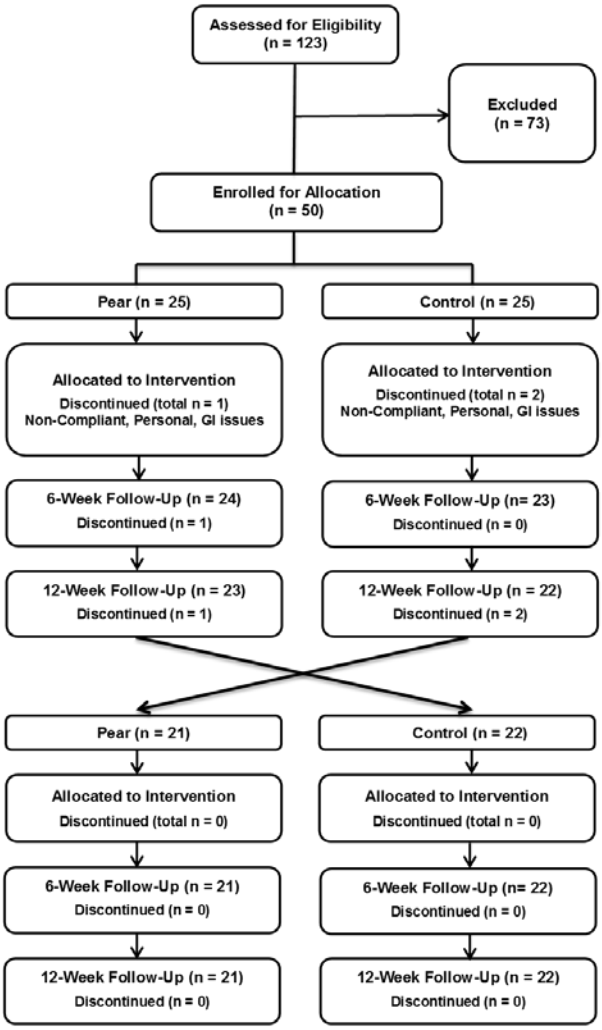


Figure 1. Flowchart of Enrollment

Anthropometrics, Physical Activity Expenditure, and Energy Intake

No differences were observed over time or between groups for weight, BMI, or energy intake. Self-reported physical activity expenditure increased (172 Kcal) from baseline to 6 weeks in the Control group but not in the Pear group. Waist circumference and waist-to-hip ratio were improved at 12 weeks and at 6 and 12 weeks, respectively. There were no differences between groups so a treatment effect cannot be confirmed; however, a significant increase in waist circumference was noted at 6 weeks in the control group and was sustained at 12 weeks.

Table 1. Anthropometric measurements, physical activity expenditure, and energy intake.

	Pear			Control		
Measures	**Baseline**	**6 weeks**	**12 weeks**	**Baseline**	**6 weeks**	**12 weeks**
Weight, kg	92 ± 2	92 ± 2	92 ± 3	92 ± 2	92 ± 2	92 ± 3
BMI, kg/m²	33 ± 1	33 ± 1	33 ± 1	33 ± 1	33 ± 1	34 ± 1
WC, cm	108.1 ± 1.9	107.8 ± 1.9	107.5 ± 2.0*	107.9 ± 2.0	108.4 ± 1.9*	108.1 ± 1.9
Waist/Hip	0.930	0.926$^{\pm}$	0.925$^{\pm}$	0.936	0.923	0.932
PA, Kcal	3256 ± 94	3345 ± 114	3439 ± 150	3222.5 ± 100	3394 ± 124*	3356 ± 135
EI, Kcal	1777 ± 128	1984 ± 113	2012 ± 146	2033 ± 124	1960 ± 165	2167 ± 164

Values reported as mean ± SEM. *Significantly ($p < 0.05$) different compared to baseline. $^{\pm}$Significantly ($p < 0.05$) different compared to Control. **Abbreviations:** BMI, body mass index; EI, energy intake; PA, physical activity; WC, waist circumference.

Blood Pressure

Blood pressure parameters are presented in **Table 2**. Systolic blood pressure (-5 mmHg) and pulse pressure (-4) were significantly lower at 12 weeks compared to baseline in the Pear group while no changes were noted in the control group. Heart rate was significantly greater (+2 beats/min) at 12 weeks compared to baseline in the Pear group but not in the Control group. No significant differences were noted between groups at any time point and therefore a treatment effect cannot be confirmed; however, this is suggestive of blood pressure lowering effects due to pear consumption.

Table 2. Blood pressure parameters.

	Pear			Control		
Measures	**Baseline**	**6 weeks**	**12 weeks**	**Baseline**	**6 weeks**	**12 weeks**
SBP, mmHg	135 ± 2.0	133 ± 2	130 ± 2†	133 ± 2	134 ± 2	131 ± 2
DBP, mmHg	80 ± 1	80 ± 1	80 ± 1	81 ± 1	81 ± 1	80 ± 1
PP	54 ± 1	54 ± 1	50 ± 1*	52 ± 2	53 ± 2	51 ± 2
MAP, mmHg	98 ± 1	98 ± 1	97 ± 1	98 ± 1	98 ± 1	98 ± 1
HR, beats/min	69 ± 1	70 ± 1	71 ± 1*	71 ± 2	71 ± 1	71 ± 2

Values are mean ± SEM. *Significantly different compared to baseline. **Abbreviations:** SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure.

Blood and Urinary Biomarkers

Blood and urinary biomarker results are presented in **Table 3**. Triglyceride levels were reduced (-5.11 mg/dL) and HDL-C tended to increase (3.34 mg/dL) in the Pear group but not in the Control group. There were no differences between groups so a treatment effect cannot be confirmed; however, this is suggestive of improvements in lipid parameters due to pear consumption. Total cholesterol and LDL-C were increased at 6 and 12 weeks in both groups. The changes over time were in both groups so cannot be attributed to pears, but rather a time effect. Leptin was reduced at 12 weeks and levels were significantly lower than the control group at this time point suggesting a treatment effect due to pear consumption.

Table 3. Blood and urinary biomarkers.

	Pear			Control		
Measures	**Baseline**	**6 weeks**	**12 weeks**	**Baseline**	**6 weeks**	**12 weeks**
Fasting Glucose, mg/dL	107.58 ± 2.17	109.52 ± 2.44	109.91 ± 2.50	106.40 ± 2.25	108.63 ± 2.45	106.98 ± 2.23
Insulin (pmol/L)	131.96 (117.58, 177.66)	131.96 (120.65, 172.33)	128.49 (123.13, 173.52)	125.01 (119.39, 169.72)	125.01 (120.80, 195.77)	118.07 (117.76, 203.01)
HOMA-IR	2.51 (2.26, 3.31)	2.59 (2.32, 3.23)	2.46 (2.36, 3.27)	2.38 (2.25, 3.29)	2.39 (2.32, 3.61)	2.30 (2.29, 3.68)
HOMA-B%	131.79 ± 7.08	129.86 ± 7.15	130.54 ± 7.28	135.50 ± 6.54	134.21 ± 8.44	140.21 ± 9.73
QUICKI	2.78 ± 0.26	2.78 ± 0.26	2.78 ± 0.26	2.78 ± 0.26	2.78 ± 0.26	2.78 ± 0.26
TG, mg/dL	145.28 ± 12.41	156.44 ± 12.0	140.17 ± 12.19*	143.07 ± 9.65	145.05 ± 10.42	149.56 ± 11.07
TC, mg/dL	195.61 ± 5.91$^{\#}$	201.21 ± 6.73*$^{\#}$	200.02 ± 5.97	202.05 ± 5.66	208.72 ± 6.58*	203.81 ± 5.96
LDL-C, mg/dL	94.14 ± 4.99	99.07 ± 5.34*	100.69 ± 4.92*	95.53 ± 4.62	103.67 ± 5.07*	103.07 ± 4.69*
HDL-C, mg/dL	49.16 ± 1.46	49.14 ± 1.63	52.50 ± 3.41†	50.26 ± 1.76	51.21 ± 1.82	50.19 ± 1.61
Apo B	101.98 ± 3.58	103.28 ± 3.97	100.61 ± 3.56	103.86 ± 3.54	106.74 ±3.99	101.35 ± 3.73
Apo A	2.30 ± 0.09	2.37 ± 0.10	2.38 ± 0.09	2.35 ± 0.12	2.33 ± 0.07	2.45 ± 0.10
Leptin	52.72 (46.78, 66.40)	46.67 (36.74, 108.47)	50.45 (40.31, 60.99)$^{\dagger\pm}$	53.61 (43.84, 60.37)	52.89 (48.80, 68.0)	53.04 (48.32, 66.77)
Adiponectin	5.90 (5.96, 7.11)	5.82 (5.77, 6.95)	6.12 (5.69, 7.08)	6.06 (5.75, 6.87)	5.83 (5.62, 7.16)	6.06 (5.58, 6.93)

CRP	4.08 (4.01, 7.84)	3.73 (3.78, 6.18)	4.13 (3.67, 6.57)	3.78 (4.03, 6.77)	4.11 (4.07, 6.84)	3.68 (3.79, 6.29)
8-OHdG	1.50 ± 0.06	1.56 ± 0.05	1.50 ± 0.07	1.53 ± 0.050	1.59 ± 0.054	1.47 ± 0.05
TAS	1.40 (1.37, 1.48)	1.41 (1.36, 1.46)	1.38 (1.36, 1.43)	1.38 (1.37, 1.48)	1.42 (1.38, 1.49)	1.38 (1.38, 1.48)

Values are mean ± SEM, or median with 95% CI in parentheses (all such values). These values are presented because of nonnormally distributed model residuals; log-transformed values are analyzed in model. *Significantly ($p < 0.05$) different compared to baseline. †Tends to be significantly ($p = 0.069$) different at 12-week between groups. ‡Significantly ($p < 0.05$) different compared to Control. **Abbreviations:** HOMA-IR, Homeostatic model assessment of insulin resistance; HOMA-B%, homeostasis model assessment of beta-cell function; QUICKI, quantitative insulin-sensitivity check indexes; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol, HDL-C, high-density lipoprotein-cholesterol; Apo B, apolipoprotein B100; Apo A, apolipoprotein A; CRP, C-reactive protein; 8-OHdG, 8-hydroxy- 2'-deoxyguanosine; TAS, total antioxidant status.

Body Composition

Body composition results are presented in **Table 4**. Percent android (abdominal) fat was increased (+0.6%) at 6 and 12 weeks compared to baseline in the Control group. Android-to-gynoid ratio (abdominal fat to hip fat) was increased (0.22) in the Control group at 12 weeks. There were no significant changes noted in any time point for the Pear group.

Table 4. Body composition (DXA).

	Pear			Control		
Measures	**Baseline**	**6 weeks**	**12 weeks**	**Baseline**	**6 weeks**	**12 weeks**
Fat Mass (%)	43.5 ± 1.0	43.4 ± 1.0	reanalyzing	43.3 ± 1.0	43.7 ± 1.0	43.5 ± 1.0
Lean Mass (kg)	52.3 ± 1.5	52.4 ± 1.5	49.1 ± 2.2[b]	52.2 ± 1.5	51.3 ± 1.9	51.6 ± 1.9
Fat Mass (kg)	108.6 ± 12.8	108.2 ± 13.0	107.6 ± 13.5	107.5 ± 12.2	108.0 ± 11.3	107.4 ± 11.5
Android Fat (g)	7061.2 ± 428.0	7086.6 ± 421.3	7076.6 ± 431.6	7174.4 ± 389.6	7165.0 ± 435.0	7061.9 ± 434.5
Gynoid Fat (g)	12318.1 ± 693.6	12137.7 ± 706.9	15111.6 ± 615.1	13008.8 ± 603.2	13112.0 ± 571.3	13570.6 ± 590.8
Android Fat (%)	50.8 ± 0.9	50.7 ± 1.0	50.6 ± 1.0	50.2 ± 1.0	50.8 ± 1.0*	50.8 ± 1.0*
Gynoid Fat (%)	42.6 ± 1.3	42.5 ± 1.2	42.8 ± 1.3	42.8 ± 1.3	42.9 ± 1.3	42.8 ± 1.4
Android/Gynoid Ratio	1.22 ± 0.03	1.23 ± 0.03	1.21 ± 0.01	1.20 ± 0.03	1.89 ± 0.67	1.22 ± 0.03*

Values are mean ± SEM. *Significantly ($p < 0.05$) different compared to baseline.

Gastrointestinal Health

7-day gastrointestinal health questionnaire results are presented in **Table 5**. No improvements were noted in any of the parameters over the course of the treatment period. Pain was reported to increase at 12 weeks of treatment. There were significant differences between groups at baseline for pain and

consistency. Importantly, there was poor subject compliance with filling out and returning these questionnaires which likely the reason for these findings as there was missing data at numerous time points.

Table 5. Gastrointestinal health questionnaire.

			Pear			Control
Measures	**Baseline**	**6 weeks**	**12 weeks**	**Baseline**	**6 weeks**	**12 weeks**
Frequency, per day	1.55 ± 0.11	1.49 ± 0.12	1.39 ± 0.11	1.52 ± 0.11	1.53 ± 0.11	1.89 ± 0.45
Quantity, cups	1.54 ± 0.13	1.57 ± 0.16	1.44 ± 0.14	1.44 ± 0.12	1.50 ± 0.15	1.23 ± 0.27
Consistency	3.66 ± 0.15$^{\pm}$	3.45 ± 0.16	3.55 ± 0.18	3.29 ± 0.16^{a}	3.33 ± 0.16	3.25 ± 0.35
Straining	2.29 ± 0.18	2.53 ± 0.20	2.58 ± 0.24	2.43 ± 0.19	2.59 ± 0.17	2.47 ± 0.50
Pain	1.66 ± 0.12$^{\pm}$	1.86 ± 0.17	2.10 ± 0.21*	2.01 ± 0.17	2.09 ± 0.17	1.82 ± 0.42
Feeling of constipation	1.98 ± 0.19	2.07 ± 0.20	2.28 ± 0.24	2.10 ± 0.20	2.08 ± 0.16	2.0 ± 0.37

Values are mean ± SEM. *Significantly ($p < 0.05$) different compared to baseline. $^{\pm}$Significantly ($p < 0.05$) different compared to Control.

Discussion:

This is the first randomized controlled clinical trial conducted in the United States using fresh pears as an intervention. As such, this study is novel in that it utilized a fresh fruit rather than a dried fruit or powder, or a juice. An additional novel aspect of this study is that we utilized a crossover (within subject) design such that subjects served as their own controls.

Overall, there was excellent subject retention throughout the course of the study (7 out of 50 subjects dropped total) as well a high subject compliance (self-reported) with the treatments. Taste fatigue due to fresh pear consumption was commonly reported towards the end of the 12-week intervention. This is common in clinical studies involving daily treatment consumption (in the form of food) for an extended period of time. There were no reports of inability to tolerate the treatments. This suggests that daily fresh pear consumption if feasible for middle-age and older adults.

The major findings of this study suggest that fresh pear consumption promotes modest improvements in the cardiometabolic health of middle-aged and older adults with MetS. There were improvements in certain parameters over the course of the 12-week study period, namely systolic blood pressure, pulse pressure, triglycerides, HDL-C, leptin, waist circumference, and waist-to-hip ratio in the Pear group. However, only a between group (treatment effect) was noted for leptin. Nonetheless, improvements in these parameters were not observed in the Control group. As such, this suggests that fresh pear consumption may improve parameters of cardiometabolic health in middle-age and older adults with MetS. Additionally, leptin is a hormone produced by adipose tissue (fat) and individuals with greater levels of adipose tissue often have higher levels of leptin due to leptin resistance which leads to a reduced ability to control hunger and regulate body weight. Leptin plays an important role in satiety and hunger regulation and has pro-inflammatory effects. A reduction in leptin may be partly due to a shift in the distribution of or in the amount of adipose tissue. Additionally, this may indicate

that these individuals had improved satiety due to pear consumption as a reduction in leptin is suggestive of less leptin resistance. This cannot be confirmed at this time as satiety was not assessed in our study. However, there were no changes in self-reported energy intake throughout the course of the study.

It is important to note that MetS is a cluster of cardiometabolic risk factors. In order to be diagnosed with MetS, one needs to have 3 out of the 5 criteria for MetS (high blood pressure, high blood glucose or triglyceride levels, low HDL-C levels, or a high waist circumference. Hence, not all of our subjects had the same cardiometabolic risk factors. As such, this may be a factor contributing to the lack of a larger improvement and therefore a treatment effect. In the future, it may be of benefit to design studies using a population with more uniform metabolic syndrome or cardiometabolic risk factors (e.g. high systolic blood pressure or hyperlipidemia) to observe significant between group differences in outcome parameters.

With regard to the intervention, due to seasonal changes in pear production, we used a combination of green Anjou and green Bartlett. Because the study duration (data collection from the first subject to the last subject) occurred over the period of approximately 2 years and 4 months, there was variation in the types and quantities of each type of pear that each subject consumed. Due to the nature of the intervention (fresh pears), this variation is not something that can be controlled for a large study. It is known that the nutrient and bioactive compound composition of fresh produce can vary for multiple reasons. Also, our intervention utilized green pears rather than red pears. It is possible that red pears contain different types and quantities of bioactive compounds that may exert different or greater health effects than green pears. We are unable to determine whether the above-mentioned factors contributed to our findings; however, these factors should be considered when designing future clinical studies.

Subject compliance was reported to be good throughout the duration of the study, although there were some instances of subjects reporting issues with ripening of pears despite education about ripening throughout the course of the study. Compliance was self-reported as is done in many clinical trials, and hence there is always the possibility that subject compliance was not as good as what was reported. This a limitation of our study but is not something that can be controlled for at this time. It would be of benefit for future studies to investigate biomarkers of pear intake, e.g. a metabolite signature using metabolomics analysis that could be used to monitor intake and compliance in clinical studies. In addition, while subject compliance was very good throughout the course of the study, subjects had poor compliance with completing and returning their gastrointestinal health questionnaires. Future studies should evaluate objective measures of gastrointestinal health such as the gut microbiome.

Overall, the results of this study suggest that daily fresh pear consumption promotes cardiometabolic health in middle-aged and older men and women with MetS. Although the effects could be considered modest due to the lack of a between group (treatment effect) for the majority of the improvements observed, the findings are consistent with previous research conducted with pears and should be viewed as positive. The addition of two fresh pears into the diet was well-tolerated, promoted high compliance, and led to improvements in cardiometabolic health parameters over time that were not observed in the control group. It is likely that the addition of fresh pears in combination with other health-promoting foods to the diet or in the context of a health dietary pattern (e.g. DASH or Mediterranean diet) would contribute to significant improvements in cardiometabolic health in middle-aged and older individuals. As such, our findings can be used to promote fresh pear consumption in middle-aged and older adults with cardiometabolic risk factors. Promotion of the health benefits of fresh pear consumption on cardiometabolic health in this population could promote increased pear sales and consumption and therefore a greater demand for fresh pears.

EXECUTIVE SUMMARY

Metabolic syndrome (MetS) is a cluster of major cardiovascular risk factors including abdominal obesity, elevated blood pressure, atherogenic dyslipidemia and insulin resistance, and a pro-inflammatory and pro-thrombotic state, and is highly associated with the development of chronic diseases such as cardiovascular disease and type II diabetes. The primary treatment goals for individuals with MetS is to improve modifiable underlying risk factors such as body weight, physical activity, and diet through lifestyle changes. Pears (*Pyrus communis*) are a commonly consumed fruit and are an excellent source of soluble and insoluble dietary fiber, a good source of vitamin C and contains potassium and vitamin K, and bioactive compounds including flavonoids (e.g. anthocyanins and flavanols) and phenolic acids (e.g. gallic acid and chlorogenic acid). Although there is a paucity of clinical research that has investigated the impact of pear consumption on human health, previous research with pears supports their potential as a functional food for promoting overall health, especially with respect to the characteristics of MetS. The central hypothesis of this study was that daily consumption of 2 fresh pears for twelve weeks would improve blood pressure, lipid profiles, glycemic control and insulin resistance, inflammatory and oxidative status, body composition, and subjective measures of gastrointestinal health in middle-aged and older men and women with MetS. Fifty men and women aged 45 to 65 years with three of the five features of MetS were randomly assigned to receive either 2 medium-sized fresh pears (Pear) or 50 g pear-flavored placebo drink mix (Control) per day for 12 weeks. At the end of the 12-week period, subjects underwent a 4-week washout period and then crossed over to the other group. At baseline, 6-week, and 12-week visits, subjects underwent assessments of anthropometrics and body composition, brachial blood pressure, gastrointestinal health, food and nutrient intake, and physical activity, and blood and urine were collected. Overall, subject recruitment and overall subject retention was excellent with only 7 participants dropping from the study (14% attrition). Tolerance and compliance to treatments were reported to be very good. Laboratory and statistical analyses were performed for the 43 subjects who completed the entire study. Systolic blood pressure tended ($p < 0.1$) to be reduced and pulse pressure (difference between systolic and diastolic blood pressure) was significantly ($p < 0.05$) reduced at 12 weeks in the Pear group but not in the Control group. Triglyceride levels were significantly ($p < 0.05$) reduced and HDL-C levels tended ($p < 0.1$) to be increased in the Pear group but not in the Control group. Waist circumference was significantly ($p < 0.05$) reduced at 12 weeks and waist-to-hip ratio was reduced at 6 and 12 weeks, respectively in the Pear group while a significantly ($p < 0.05$) increase in waist circumference was noted at 6 weeks in the Control group and was sustained at 12 weeks. Percent android (abdominal) fat was significantly ($p < 0.05$) increased at 6 and 12 weeks compared to baseline and android-to-gynoid ratio (abdominal fat to hip fat) was significantly ($p < 0.05$) increased in the Control group at 12 weeks compared to baseline while no changes were noted in the Pear group. Additionally, leptin was significantly ($p < 0.05$) reduced at 12 weeks and levels were significantly ($p < 0.05$) lower than the Control group. The major findings of this study suggest that fresh pear consumption promotes modest improvements in the cardiometabolic health of middle-aged and older adults with MetS. There were improvements in certain parameters over the course of the 12-week study period, namely systolic blood pressure, pulse pressure, triglycerides, HDL-C, leptin, waist circumference, and waist-to-hip ratio in the Pear group. However, only a between group (treatment effect) was noted for leptin. Nonetheless, improvements in these parameters were not observed in the Control group. As such, this suggests that fresh pear consumption may improve parameters of cardiometabolic health in middle-age and older adults with MetS. Future studies may benefit from evaluating a population with more uniform cardiometabolic risk factors to observe significant between group differences in outcome parameters. Additionally, future studies may wish to consider the types of pears used (red vs. green) and seasonality of different pear types. Further, establishing biomarkers of pear consumption using omics methodologies (e.g. metabolomics analyses) would be of benefit in conducting and evaluating clinical and epidemiologic human studies involving fresh pear consumption. Our next step is to disseminate our findings through conference presentations and publications.

FINAL PROJECT REPORT **YEAR**: 1 of 1

Project Title: Advancing Metamitron as a chemical thinner for Bartlett pear

PI: Todd Einhorn
Organization: OSU-MCAREC
Telephone/email: (541) 386-2030 todd.einhorn@oregonstate.edu
Address: 3005 Experiment Station Drive
City: Hood River
State/Zip: OR 97031

Cooperators: Drew Hubbard

Total Project Request: Year 1: $11,779 **Year 2:** $0 **Year 3:** $0

Other funding sources: Adama- chemical product for all trials and crop destruct for additional on-farm trials.

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Russell Karow
Telephone: 541-737-3228 **Email address:** Russell.Karow@oregonstate.edu

Item	2016	2017	2018
Salaries[1]	4,357	0	0
Benefits[2]	3,006	0	0
Wages[3]	1,040	0	0
Benefits[4]	87	0	0
Equipment	0	0	0
Supplies	0	0	0
Travel	0	0	0
Miscellaneous[5]	3,289	0	0
Total	11,779	0	0

Footnotes: [1]Estimated salaries are for: 0.096 FTE for full-time technician to apply thinning compounds, conduct all measurements (fruit set, hand thinning, yield, fruit size and fruit quality attributes), hand thin and enter/collate data. [2]Actual OPE rate is 69%. [3]Wages are to cover 80 hours of part-time labor ($13/hr) to assist with harvest and data collection. [4]Benefits for part-time employees is 8.34%. [5]Miscellaneous includes per acre research plot fees: $3,104/acre and 2 months cold storage room fee (monthly fee of $0.94 per square foot for a 98.6 sq. ft. cold storage room).

Objectives

1. Evaluate the efficacy of metamitron to thin 'Bartlett' pears and inform commercialization decision.

Significant Findings, 2016

- Four trials were conducted in three different locations to evaluate the thinning efficacy of metamitron on Bartlett pear.
- Metamitron effectively thinned Bartlett pears at two locations, but not at the third where two trials were established. Incidentally, 6-BA (MaxCel) also did not thin fruits at this site. Thinning was rate-dependent. The most efficacious rates (250 to 300 ppm) reduced fruit set to 75% and 50% of untreated controls.
- Metamitron reduced photosynthesis by 70% the first four days after application. The effect diminished but was still evident 10 days after application. The response was dose dependent but saturated near 300 ppm. A strong reduction of photosynthesis was associated with fruit abscission. 6-BA did not significantly affect photosynthesis.
- Metamitron-treated trees tended to have larger fruit size compared to controls. Fruit quality at harvest and after storage/ripening was unaffected by metamitron.

Results and Discussion

2015 Results (A brief background and synopsis of significant findings): In 2015, we were funded by the pear research subcommittee to evaluate the thinning efficacy of metamitron on 'Bartlett' pear trees (please refer to the 2016 Final Report, 'Improving fruit set, production efficiency, and profitability of pears'). In that study we selected several rates of metamitron (150, 300, 600 ppm) based on a previously published trial using 'Conference' pear in The Netherlands. For each rate, we also evaluated two application timings (~6 mm and 11 mm), alone and combined. We demonstrated that metamitron reduced photosynthesis by ~50% to 90% depending on rate; an effect that lasted ~ two weeks (Fig. 1).

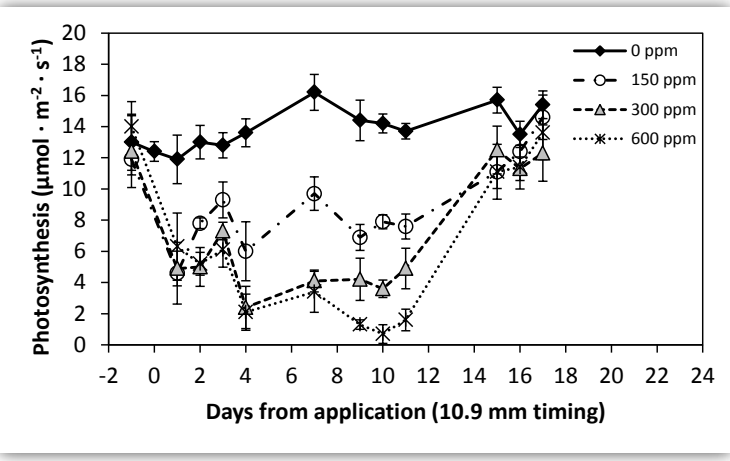


Figure 1. Effect of metamitron rates on photosynthesis of 'Bartlett' pear leaves. Applications were made at 11 mm diameter. Data points represent the mean of 4 single-tree replicates (4 leaves per replicate) and are bracketed by standard error bars.

Importantly, fruit abscission was strongly associated with the measured decrease in photosynthesis. Rates of 150 and 300 ppm reduced the crop load of untreated control trees by ~25% to 40%. The highest rate (600 ppm) did not result in significantly greater thinning than that achieved with 300 ppm. The early, 6 mm timing had relatively no thinning efficacy; therefore, the combination of early and late timings differed little from the later, 11 mm, timing. Yields reflected the relative number of fruits removed by chemical and follow-up hand thinning and fruit size improved for all 11 mm application rates as was clearly a function of crop load.

We proposed to further fine-tune metamitron rates and application timings in a new, 2016 proposal using a range of 'Bartlett' plantings (varying primarily in age and location but also in canopy architecture). Given the 2015 results, we timed our sprays for 10-12 mm fruit size, weather permitting.

2016 Results:

Trial 1. Bartlett/OH x F 97; 12-year-old commercial orchard (G. Blaine); 222 trees/acre. Application timing 11.5 mm fruit size.

Table 1.

Treatment		Fruit set	Before Hand Thinning	Hand Thinned	Yield		Avg. Fruit wt.	FF	SSC	TA
Product	ppm	(fruits/cluster)	(no. fruit/tree)	(no. fruit/tree)	(lbs/tree)	bins/acre	(g)	(lb f)	(%)	(%)
Control	0	1.06 a	732 a	227 a	202 a	41	189 c	19.8	11	0.3
Metamitron	100	0.66 b	703 a	219 a	201 a	41	196 bc	19.8	11.2	0.31
Metamitron	200	0.65 b	550 ab	162 ab	168 ab	34	206 ab	19.8	11.4	0.31
Metamitron	300	0.55 b	387 b	100 b	126 b	26	197 abc	20.4	11.2	0.34
Metamitron	400	0.59 b	424 b	101 b	146 ab	29	214 a	20.1	11.3	0.32
6-BA	100	0.44 b	415 b	115 b	136 b	27	213 ab	20.5	11.1	0.32
Pr>F		*0.0002*	*0.011*	*0.0007*	*0.05*		*0.027*	*0.3*	*0.6*	*0.5*

The above data (Table 1) show the effect of Metamitron (Adama formulation) and 6-BA (MaxCel) on fruit set, yield and fruit quality of Bartlett pear trees in Parkdale, OR. Applications were made on 7-May, 2016 to whole canopies. Data are means of 6 single-tree reps. Fruit set was based on the number of fruits per flower cluster. A minimum of 150 flower clusters were counted per rep by selecting three limbs (each containing ~50 clusters) at low, mid and high positions in the canopies. All thinning treatments significantly reduced fruit set. The number of fruits remaining after natural fruit set but prior to hand thinning, better reflected the fruit set status of entire trees compared to the flower cluster data and showed, importantly, that the thinning effect of metamitron saturated at 300 ppm. Moreover, these results agree with our 2015 findings.

Yield was projected as bins per acre based on the yield per tree and the tree density of the orchard. Yield was reduced by the higher rates of metamitron and 6-BA. While thinning reduced fruit set by roughly half, hand thinning was still required; however, hand-thinning data comprise fruits from the tops of trees which were not treated as thoroughly as the canopy below 10 feet height in an effort to mitigate potential spray drift to adjacent rows accommodating other treatments. A minimum of one guard tree and typically three, separated treatments within replications. Individual fruit weights of chemical thinning treatments were increased proportionately to whole tree crop loads. The average fruit weight for control fruit was 189 g - corresponding to a box size of 110 - compared with an average box size of 100 for thinning treatments. A shift to larger size classes was commensurate with an increase in the average fruit size for high rates of metamitron and 6-BA (Fig. 2).

Thinning agents did not affect fruit maturation (evident by similar firmness values at harvest) or fruit soluble solids concentration (SSC) and titratable acidity (TA). Further, fruit finish (russet) was

not affected by any of the treatments (data not shown). Ripening was also unaffected by thinning agents and all fruit ripened to acceptable firmness and eating quality (data not shown).

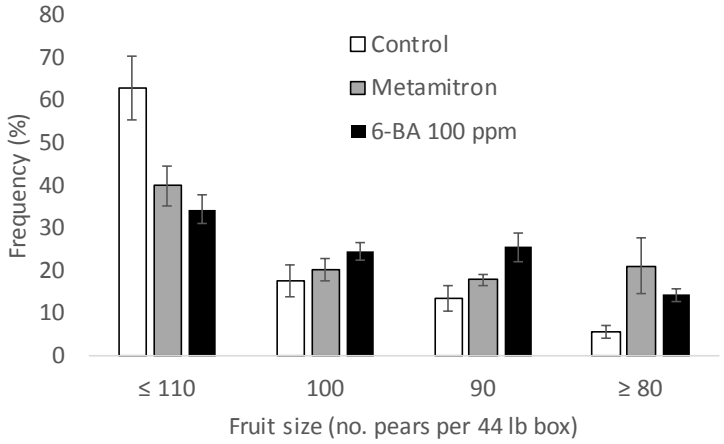


Figure 2. Distribution of fruits at harvest comprising four size classes from whole tree applications of thinning agents to Bartlett/OH x F 97, 12-year-old trees (Trial 1). Data are means of 6 reps with SE bars.

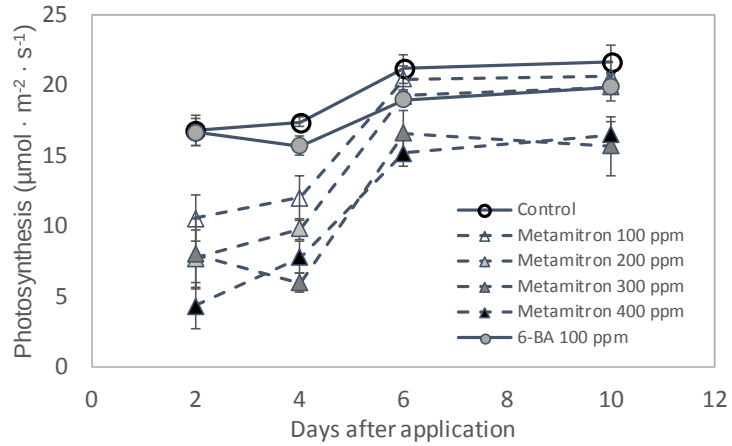


Figure 3. Photosynthesis measurements of single leaves (n=4) taken periodically after application of thinning compounds to entire Bartlett/OH x F 97, 12-year-old trees (Trial 1). Data are means of 4 replicates. Bars are ± SE.

Photosynthesis was reduced by metamitron according to rate (Fig. 3). The maximum effect was achieved at 300 ppm, as similarly observed for thinning. MaxCel had no measurable effects on photosynthesis. Metamitron had a slightly greater and longer-lasting effect in 2015; though, differences in tree age, rootstock, environmental and site factors could all have contributed to the difference. Clearly, metamitron thins by inducing carbon deficit as opposed to the hormonal regulation elicited by 6-BA.

Trial 2.

Site 1, 'Daum Block' - Bartlett/OH x F 97, 12-year-old orchard (MCAREC) 272 trees/acre. Application timing 12.3 mm fruit size.

The effect of two formulations of Metamitron (Brevis and Adama) and MaxCel (6-BA) on fruit set and production attributes of Bartlett/OH x F 97 was evaluated at OSU's MCAREC, Hood River, OR. Applications were made on April 21, 2016 to whole canopies. Table 2 data are means of

four, single-tree reps. Fruit set was not significantly reduced by any of the thinning treatments. Adama and Brevis at 500 ppm had fewer fruits per tree at harvest than other treatments, albeit nonsignifiantly (*P*, 0.088; Table 2). Trees were not hand thinned as natural fruit set was deemed appropriate to achieve commercial fruit size. Average fruit weights of 200 to 250 g equate to box sizes between 100 and 90. Numerically higher fruit weights were observed for some thinning agents but were not consistently related to formulation or rate. We observed some solubility issues with the Brevis formulation while preparing solutions, but this may not have factored into the poor thinning response since the Adama formulation and 6-BA were both ineffective at thinning.

Table 2.

Treatment		Fruit set	Yield		Avg. Fruit wt.	FF	SSC	TA
Product	ppm	(%)	(no./tree)	(lbs/tree)	(g)	(lb f)	(%)	(%)
Control	0	44.5	263.7	134.7	213.4	17.9	10.8	0.29
Brevis	125	39.6	235.5	113.4	240.3	17.5	11.3	0.29
Brevis	250	43.4	279	143.3	240	17.8	11.2	0.29
Brevis	500	33.7	150.8	82.3	238.4	18.2	11.3	0.29
Adama	125	50.7	284.3	137.6	204.5	18	11.4	0.29
Adama	250	45.7	220.5	111.1	241.4	17.8	11.4	0.3
Adama	500	36.2	205.5	114.4	253.9	18.3	11.6	0.31
Maxcel	100	53.4	231.3	107.4	244.3	17.4	11.4	0.29
Pr>F		0.748	0.088	0.061	0.055	*0.289*	*0.375*	*0.701*

Fire blight infection compromised data from trees in the fourth replicate (experimental design was a randomized complete block) following the removal of several primary scaffold limbs mid-season. Maturity and fruit quality were not affected by thinning treatments. Maximum daytime temperatures were around 60°F for a period of 10 days after applications. Nighttime low temperatures were typically between 40°F and 50°F, though on several occasions temperatures reached lows of 38°F. Collectively, fruit demand for carbon would not have been high during this period rendering thinning more difficult. On the day of application, maximum daytime temperatures were 75°F, which would have been acceptable for uptake and activity of 6-BA. Absorption of 6-BA has been shown to require temperatures >60°F and, ideally, between 65 to 75°F. The argument that temperatures for the 10-day period following applications may have increased the difficulty to thin is strengthened with photosynthesis data. Both metamitron formulations reduced photosynthesis by ~ 50% when measured one week after application (Fig. 4). These results agree with data collected from other trials and indicate that the products were in fact absorbed. 6-BA had no effect on photosynthesis. While light supplies the carbon necessary to support fruit growth, when fruit demand for carbon is low, as would be the case for the temperatures observed, thinning becomes markedly more difficult.

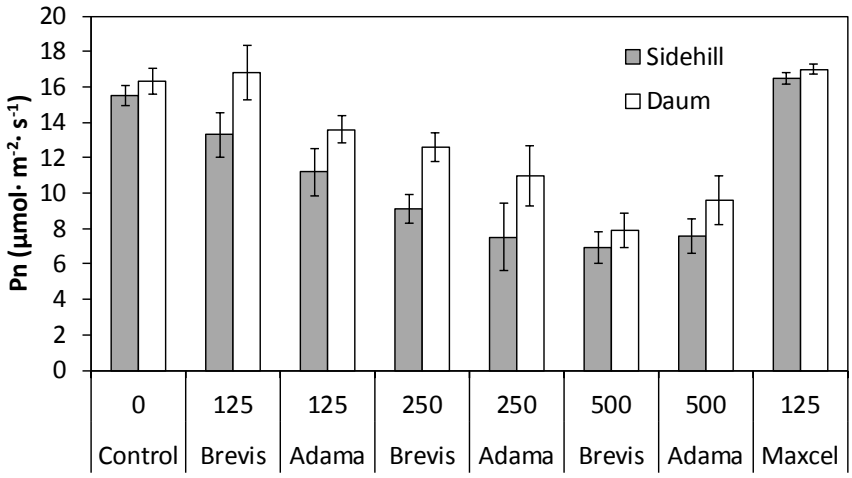


Fig 4. Photosynthesis (Pn) measurements of single leaves (n=4) on days 6 and 7 from application of thinning compounds for Sidehill and Daum trials, respectively. Both trials were performed at the OSU Mid-Columbia Agricultural Research and Extension Center in Hood River OR. Data are means of 4 replicates and indicate a persistent, rate response of Metamitron formulations on Pn. Bars are ± SE.

Site 2, 'Sidehill'- Bartlett/OH x F 87, 22-year-old orchard (MCAREC) 303 trees/acre. Application timing 12.5 mm fruit size.

Table 3.

Treatment		Fruit set	Yield		Avg. Fruit wt.	FF	SSC	TA
Product	ppm	(%)	(no./tree)	(lbs/tree)	(g)	(lb f)	(%)	(%)
Control	0	97.1	270.8	122.3	204.4	17.7	11.9	0.36
Brevis	125	68.7	267.3	122.7	207.7	18.1	11.7	0.36
Brevis	250	82.7	296.8	136.3	207.8	17.5	11.2	0.35
Brevis	500	66	234.8	116.7	224.9	18.1	11.4	0.37
Adama	125	91.2	308.8	141.9	207.9	18.1	11.8	0.35
Adama	250	98.5	254.3	120.2	213.9	17.7	11.6	0.36
Adama	500	81	271.5	122	203.4	17.9	11.5	0.36
Maxcel	100	109.4	290.3	136.1	212.2	17.7	11.5	0.35
Pr>F		*0.247*	*0.769*	*0.761*	*0.599*	*0.729*	*0.553*	*0.979*

The effect of two formulations of Metamitron (Brevis and Adama) and MaxCel (6-BA) were evaluated on fruit set and production attributes of Bartlett/OH x F 87 at OSU's MCAREC, Hood River, OR. Applications were made on April 21, 2016 to whole canopies. Table 3 data are means of four, single-tree reps. Fruit set, yield, fruit size and quality were unaffected by treatments as similarly observed at Site 1 (i.e., 'Daum'). Crop loads were nearly identical at both sites. Photosynthesis was similarly affected by high rates of metamitron at both sites (Fig. 4). Despite differences in biological and horticultural factors between sites (trees age, different rootstocks, training systems, etc.) similar responses support an overriding effect of environment on thinning as described above.

Trial 3. Bartlett/OH x F 87 4-year-old orchard (G. Blaine; 726 trees/acre). Application timing 12.7 mm fruit size.

Table 4.

Treatment		Fruit set	Yield		Avg. Fruit wt.	FF	SSC	TA	3 months PH Storage + Ripening		
									FF	SSC	TA
Product	ppm	(%)	(no./tree)	(lbs/tree)	(g)	(lb f)	(%)	(%)	(lb f)	(%)	(%)
Control	0	24.2 a	111.7 a	50.2 a	204.1 b	19.4	12.5	0.4	2.6	13.3	0.34
Metamitron	250	5.6 b	26.7 b	14.7 b	248.1 a	20.9	12.4	0.46	2.7	13.9	0.38
Metamitron	500	7.1 b	33.3 b	17.5 b	236.5 a	20.6	11.9	0.44	2.6	14	0.37
Pr>F		<0.0001	<0.0001	<0.0001	<0.0001	*0.11*	*0.18*	*0.17*	*0.63*	*0.09*	*0.29*

An additional trial was performed on a young, high-density block of 'Bartlett' trees in an expansive commercial orchard in Wamic, OR. Given that fruit were near the maximum size for thinning efficacy when we visited the site, we only applied two rates of metamitron. Treatments were selected to represent an ideal (250 ppm) and an excessive (500 ppm) rate. Applications were made to whole canopies. Table 4 data are means of 5 reps. Each replication comprised three contiguous trees. Fruit set was reduced roughly 4-fold by metamitron. The rate response was similar to our previous results (Trial 1 herein, and 2015 data). The higher fruit drop was likely associated with tree age and/or the relatively high temperatures following applications (>70°F). In fact, fruit set of control trees was relatively low when compared to Trials 1 and 2. Hand thinning was not performed since the fruits from these trees were designated as 'processing' pears. Average fruit weight was significantly increased by metamitron. Metamitron-treated fruit were slightly less mature at harvest, although not significantly. Fruit quality and ripening were not affected by treatments (Table 4).

Executive Summary:

- Five trials over two years were conducted to evaluate the thinning efficacy of metamitron on Bartlett pear. Applications coincided with 10-12 mm fruitlet diameter.
- Metamitron effectively thinned Bartlett pears in three of five trials. The two trials where thinning was not observed were established in 2016 at the same location (OSU- MCAREC). A 10-day period of low temperatures following treatments likely contributed to the poor thinning response observed at that site. Incidentally, 6-BA (MaxCel) did not thin fruits at this site either. At other sites where thinning was observed, the response was rate-dependent. The most efficacious rates (250 to 300 ppm) reduced fruit set to 75% and 50% of untreated controls.
- Depending on the year and site, metamitron significantly reduced single-leaf photosynthesis 50% to 70% within the first few days from application. The effect diminished over time but was still evident 10 to 14 days after applications. The photosynthetic response to metamitron was dose dependent but saturated near 300 ppm. The strong reduction in photosynthesis was associated with fruit abscission. 6-BA did not significantly affect single-leaf photosynthesis in trials where it was evaluated.
- Metamitron-treated trees tended to have larger fruit sizes than controls but similar to 6-BA. The effect was crop load dependent. Fruit maturity and quality at harvest was unaffected by thinning treatments and all fruit ripened to good eating quality after postharvest storage.
- Metamitron is a promising thinner for Bartlett pear. Future work could address absorption characteristics through pear leaves as influenced by temperature, humidity and surfactants.

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

PI: Stefano Musacchi
Organization: Washington State University/ TFREC
Telephone: 509-663-8181 x236
Email: stefano.musacchi@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (1): David Rudell
Organization: USDA, ARS
Telephone: 509-664-2280 x245
Email: david.rudell@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (2): Jim Mattheis
Organization: USDA, ARS
Telephone: 509-664-2280 x249
Email: james.mattheis@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Sara Serra (WSU/TFREC), Glade Brosi (Stemilt)

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

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget History 1

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 History 2

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

Overall

- Considerable variability in fruit maturity exists within the large canopy of an open vase tree.
- The use of DA meter in pre-harvest on selected trees helps to be more aware of the maturity stage and variability within the canopy to address the harvest time.
- From year to year fruit maturity distribution (accordingly to the DA meter) at 2 weeks before harvest is variable. This indicated a potential use of this tool to determine the harvest time.
- The DA meter values (I_{AD}) for internal and external canopy fruit were different at harvest. External fruit on average tend to have lower I_{AD} values compared to Internal fruit.
- At harvest, external fruit had less green background, higher red blush coverage, higher dry matter %, and higher soluble solid content than internal fruit.
- Internal fruit tend to be greener than External up to 8 months of storage.
- Crop inconsistency resulting from pear canopy position impacts most postharvest supply chain decisions.
- Fruit ripening and potentially flavor is different depending upon canopy position.
- Canopy position impacts postharvest behavior including superficial scald risk. This can affect the need to repack fruit boxes.
- Levels of natural peel chemicals we have linked with light exposure may be exploited to develop in-field or warehouse sorting tools to reduce crop variability.

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

Pre-harvest assessment and fruit maturity distribution

To assess the maturity on the 11th of August 2016 (18 days before harvest) a total of 677 fruit (included 640 good fruit and 37 of <60 mm size and/or with defects) were harvested. Total yield per tree was 121 kg and the average fruit weight was 179 g. Sunburned incidence was 1.8%, cork was 0.44% and no frost damaged fruit were observed.
By measuring I_{AD} before harvest, we determined the maturity stage of the fruit population, in fact, in 2016, more than 2 weeks before harvest, more than 95% of fruit were classified in the least mature I_{AD} classes (above 2.00 I_{AD}) and only a small percentage (0.2%) of fruit were classified in the more ripe classes (below 1.80 I_{AD}, Fig. 1).
From year to year the maturity distribution of fruit accordingly to the DA meter at 2 weeks before harvest is variable.

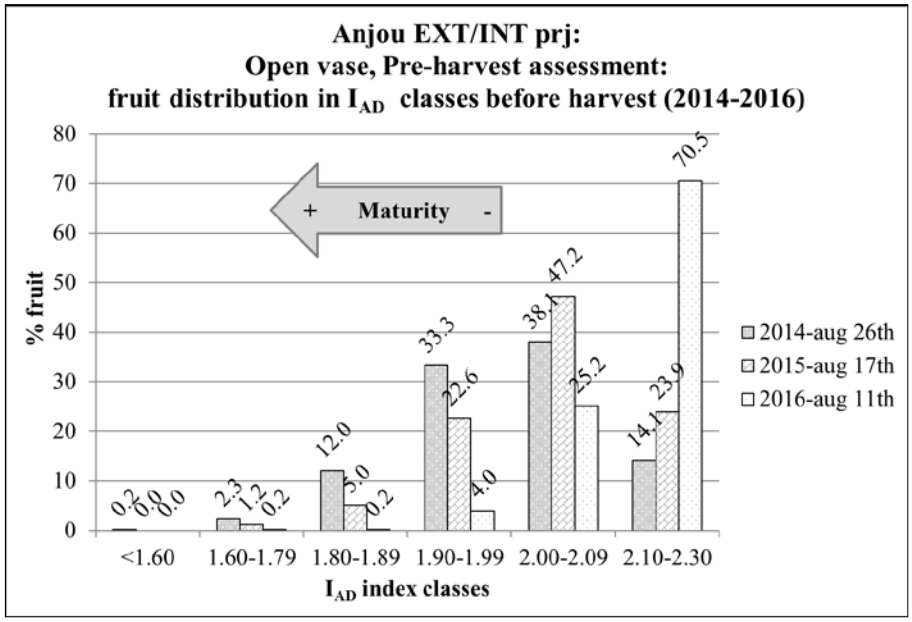


Figure 1: Pre-harvest assessment of fruit maturity distribution across the canopy of an open vase tree in 2014, 2015 and 2016 (≈2 weeks before harvest). Fruit % in each I_{AD} class of ripening is represented.

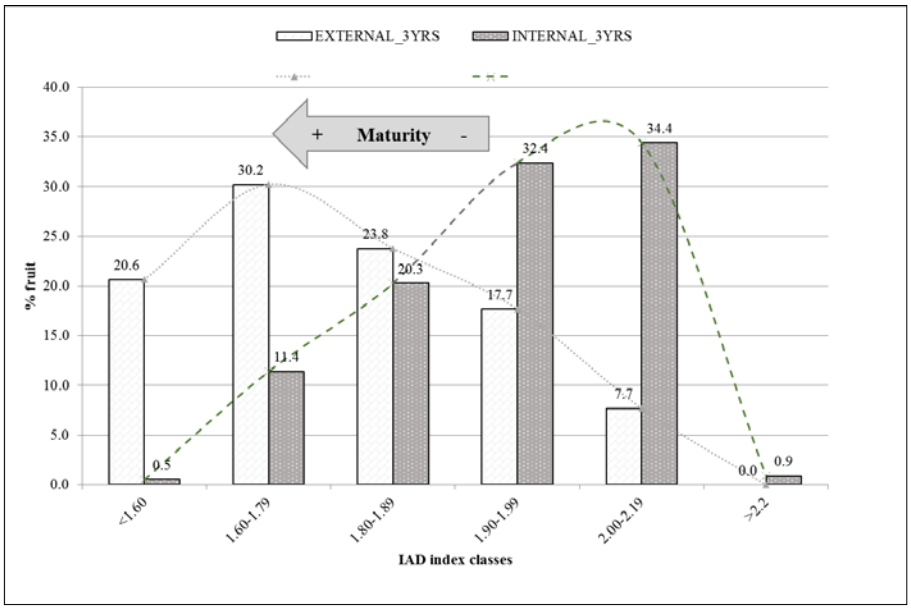


Figure 2: Distribution of fruit picked categorized by canopy position (external and internal) and I_{AD} class as well as in the 3 years, percentage are calculated on all fruit harvested in 3 yrs.

Fruit maturity distribution within I_{AD} classes at harvest divided by canopy position confirmed the observations done in the previous year where Internal fruit tend to be more unripe than the External one (Fig. 2). Looking at the distribution as all fruit harvested in 3 years, ≈34% of Internal fruit fell in the least ripe classes (I_{AD} <2.00), while only ≈8% of External fruit belonged to that class (Fig. 2). Almost 21% of the External fruit were classified in the most ripe categories (I_{AD} <1.60), while only 0.5% of the Internal ones resided in the same classes (Fig. 2).
This represents a strong example of how different are fruit belonging to those two extreme canopy positions. Harvesting as strip pick and collect all fruit in the same bin does not allow anymore to investigate canopy positions variations.

PAR measurement per single fruit and light in the canopy (2016)

PAR measurements of fruit marked for sampling allowed us to accurately choose fruit from the two canopy positions. The percentage of light intercepted by External fruit averaged 92.1% while only 1.4% by Internal fruit (Fig. 3A). Fruit belonging to light interception range from 30% to 70% were discarded. This type of precise harvest allowed us to track the behavior of the two type of pears in postharvest.
A qualitative measure of the light spectrum by a spectroradiometer (measure of photon flux in µmol s^{-1} m^{-2}) was done on 21st of July 2016 underneath one large canopy. A huge variability of light spectra hitting the trees in the four possible inner quadrants (South-West, North-West, North-East, South-East) was observed (Fig. 3B). Three quadrants on four showed lower radiation from 300 to 700 nm (PAR range) while the North-West quadrant was illuminated by direct sunlight and the trend looked similar to a full sun light spectrum (approx. External situation, Fig. 3B). Leaves in the inner part of the canopy have less energy available for photosynthesis so they may be subjected to a shortage of photo assimilates to translocate to the fruit.

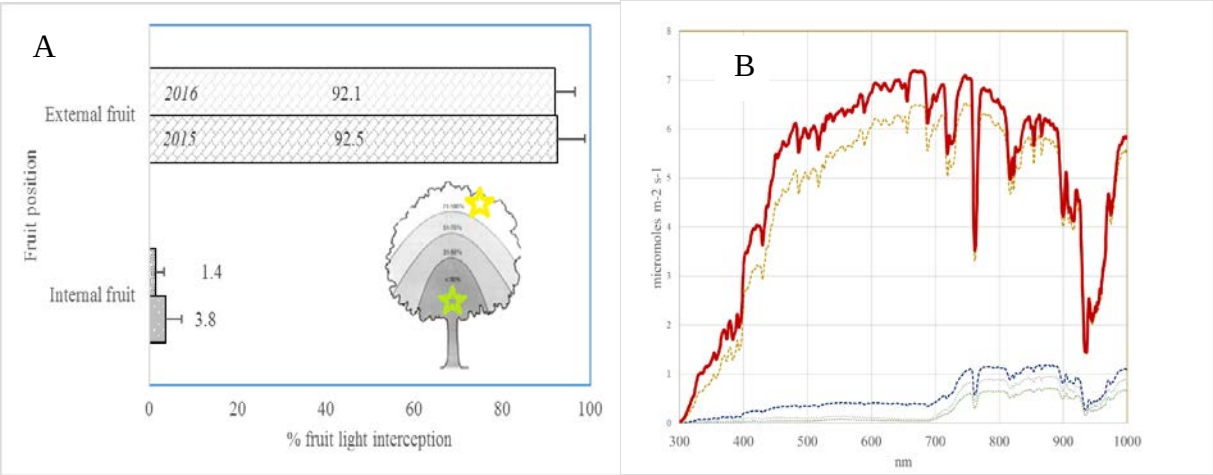


Figure 3: A) Percent of light interception of fruit harvested from the two canopy positions as determined by PAR measurement using the Q53292 quantum sensor in 2015 and 2016 (Li-Cor). Values are average ± stdDev. B) Photon flux measured in the large canopy on 21st of July 2016 between 10 am and 12 pm. Solid line is the light spectra of full sun measured above the canopy at 3.5 m from the ground, four different dashed lines are the four light spectra in the four quadrants (south-west, north-west, north-east, south-east) of a large tree at 40 cm from the trunk and 130 from the ground.

2014 fruit storage and quality assessments

Fruit quality analysis at harvest (T0) showed that External fruit were significantly heavier, larger, and had higher titratable acidity and soluble solids compared to Internal fruit at harvest. Internal were greener. No difference in chroma and firmness.

Regarding I_{AD} index decrease in storage, Internal fruit reported always higher values (less ripe fruit) than External fruit from harvest to 8 months of storage and they showed a slower I_{AD} index decrease (without any ripening post-storage) than external one where each pullout registered a significant drop in this index, suggesting a faster kinetics of ripening of those fruit. The same behavior was noticed after 7 days of ripening at room temperature, where differences between Internal and External were maintained (Fig. 4).

Regarding firmness and storage duration, we did not find differences between External and Internal fruit from harvest up to 6 months, only after 8 months Internal fruit were firmer than external immediately after removal from cold room. After 7 days ripening, Internal fruit were firmer than External except for no difference at 6 months of storage (Fig. 5).

Dry matter % was always higher in External fruit than Internal at both stages from 3 to 8 months of storage duration. In general, no big dry matter difference found among pullouts. Similar trend was reported for Soluble Solid content (SSC, Brix): External fruit showed higher SSC than Internal with or without ripening time. Correlation between dry matter % and SSC improved along storage moving from R^2=0.677 at 3 months (day 0) to R^2=0.782 at 8 months (day 0). Titratable acidity was significantly higher in the Internal fruit than External at day 0 only after 8 months, while exogenous ethylene was higher in the External than Internal at day 7 after 6 and 8 M.

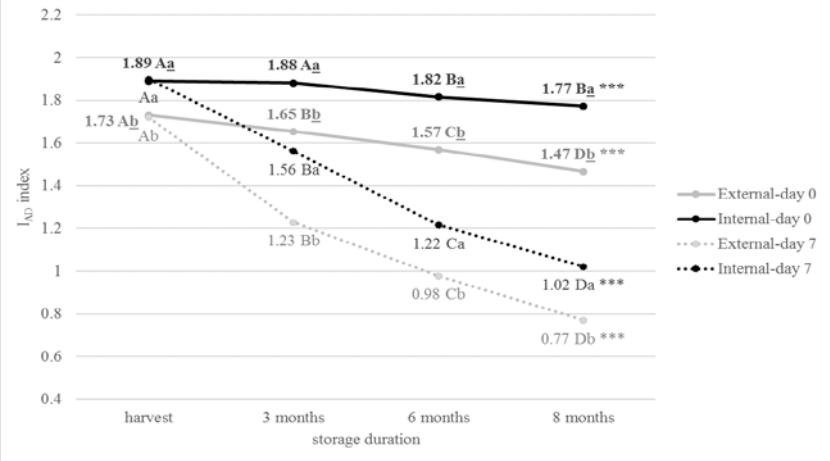


Fig. 4: I_{AD} index decrease in storage (fruit harvest 2014). Significance: p<0.05, *; p<0.01, **; p<0.001, *; ns, not significant**
Capital letters discriminate means among storage duration (horizontally), small letter between canopy position in pairs (vertically).

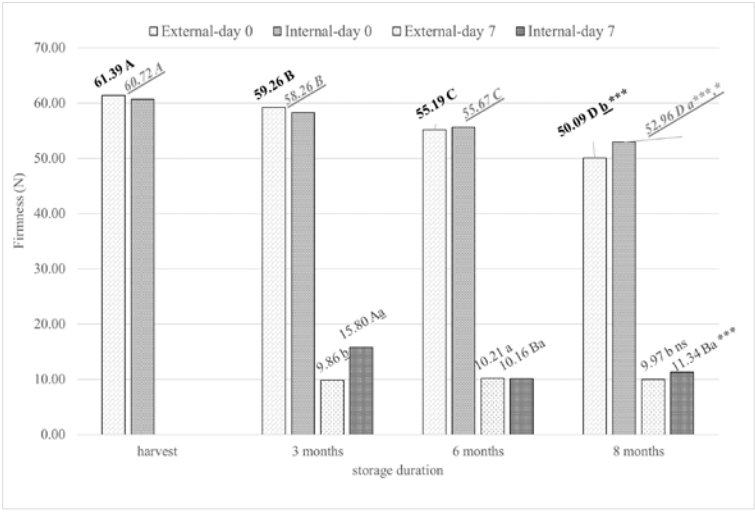


Fig. 5: Firmness decrease in storage (fruit harvest 2014). Significance: p<0.05, *; p<0.01, **; p<0.001, *; ns, not significant.**
Capital letters discriminate means among storage durations within the same canopy position (horizontally), small letter, where present, between canopy positions in pairs (vertically) within each storage time.

2015 fruit storage and quality assessments

Fruit from Internal and External canopy regions were picked separately on 31st August 2015. Fruit from each light condition were separated into two bins (containing 460 external and 486 internal pears) and immediately moved to 40°F for fruit maturity distribution analysis and sorting in DA classes.

Within each group, fruit were again classified using I_{AD} into 5 classes (I_{AD}<1.60, 1.60<I_{AD}<1.79, 1.80<I_{AD}<1.89, 1.90<I_{AD}<1.99, 2.00<I_{AD}<2.19). The first was only included in the External fruit (not present in Internal) and Internal fruit in 1.60<I_{AD}<1.79 class were not enough to cover all pullout so harvest and 8 months storage were chosen. Fruit belonging to each class were, then, equally divided into 3 groups for 0 (= harvest), 6, and 8 months CA storage. Fruit were stored in a research CA room (31°F, 2% O_2 and 0.8% CO_2). For each pullout, except for T0 at harvest, fruit were split in 2 subgroups: with or without 7 days of post-storage ripening time. Fruit quality analysis in 2015-2016 pullouts was performed in the same manner as 2014.

At harvest 2015, External fruit had less green background, higher red blush coverage (%), higher firmness, higher dry matter %, and higher soluble solid content (SSC, brix) than Internal fruit (data not shown). As reported in literature, sun-exposed 'Bartlett' pears had higher firmness than pears grown in the shade before and after ripening at room temperature probably due to the direct sun exposure (Raffo et al., 2011). This firmness difference between positions was a variation in comparison to 2014.

Within each canopy position fruit were divided accordingly to the I_{AD} index in classes and differences among them emerged. External fruit belonging to the least ripe class (2.00<I_{AD}<2.19) presented the highest background hue value (tended to more green) and the lowest SSC content (12.9 °Brix), while External fruit belonging to the most ripe class (I_{AD}<1.60) were bigger in diameter, less firm and higher SSC (14.0 °Brix). Similarly, the 2.00<I_{AD}<2.19 class for Internal fruit showed higher background hue and pH, lower SSC (11.0 °Brix), and lower acidity than the most ripe class for the same light condition (data not shown). No differences were detected in terms of dry matter %, total number of seed, viable vs dead seeds, ethylene production and weight. When all ripening classes and canopy positions were compared as combinations, significant differences of fruit weight, overcolor, dry matter %, firmness, diameter, pH and soluble solid contents, were found at harvest (Fig. 6).

After 6 months of storage in CA (T1), without any post-storage ripening time, External and Internal fruit differed for color/blush, firmness, SSC, dry matter % and pH with the most exposed fruit less green, firmer, higher in SSC and dry matter and lower pH. Same comparison done after 7 days of ripening (+6M storage + 7 days at room temperature) confirmed difference for color, SSC and dry matter. Among classes in External fruit without any post-storage ripening, 1.60<I_{AD}<1.79 class showed the highest drop in I_{AD} index, while 2.00<I_{AD}<2.19 class the lowest, confirming variation in ripening rate; similarly between 1.80<I_{AD}<1.89 class and 2.00<I_{AD}<2.19 class for Internal (1.60<I_{AD}<1.79 was absent for internal at T1). This latter class showed also the lowest SSC among Internal fruit classes (data not shown). Regarding the comparison between combinations of position and DA class after 7 days of ripening followed the 6 months of CA storage, 2.00<I_{AD}<2.19 class for Internal still showed the lowest drop in I_{AD} index in the 7 days of ripening at room temperature, the lowest SSC (13.1 °Brix) and dry matter %, the highest hue (more green), and the highest pH (Fig. 6).

After 8 months of CA storage (T2), without any post-storage ripening time, External and Internal fruit differed for weight, overcolor percentage and color, firmness, SSC, dry matter % and titratable acidity, with the most exposed fruit bigger, less green, with 15% overcolor, firmer, higher in SSC and dry matter and lower in acidity. In External fruit without any post-storage ripening, differences among classes were less than in shorter storage duration, in fact all destructive parameters like

firmness, SSC, dry matter, pH and titratable acidity did not significantly differ. Ethylene production was higher for External fruit class I_{AD}<1.60 than the other classes (less ripe fruit). Internal fruit instead after 8 months and without any post-storage ripening presented differences in the comparison between DA classes with the most ripe class showing lowest firmness and highest SSC and dry matter % (data not shown).

After 7 days of ripening (+8M storage +7 days at room temperature) the comparison between External and Internal fruit reported difference for I_{AD} index drop in the 7 days, overcolor % and color, SSC. Regarding the comparison between combinations of position and DA class after 7 days of ripening followed the 8 months of CA storage, 2.00<I_{AD}<2.19 class for Internal still showed the lowest drop in I_{AD} index in 7 days at room temperature, but the highest drop in weighs in 7 days (tendency to shriveling without proper ripening), the highest hue (still more green then the others), the lowest SSC and dry matter %, the highest hue (more green), and among the highest pH values (Fig. 6).

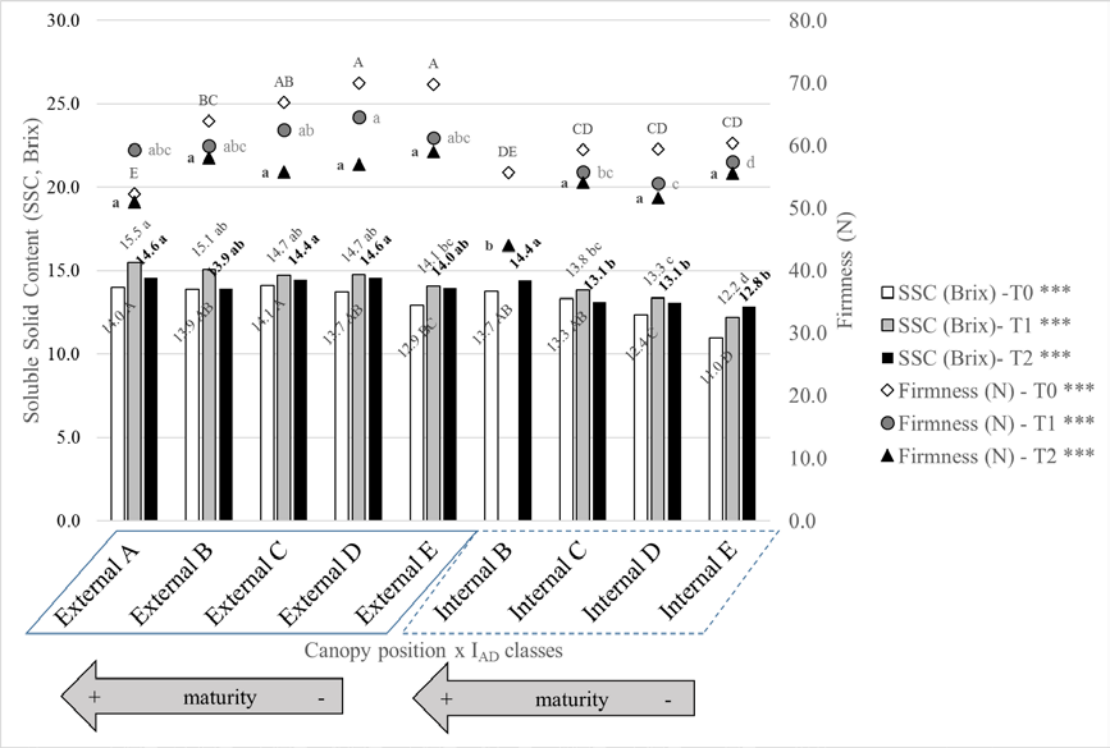


Figure 6: Comparison between combinations of DA classes and canopy position at harvest 2015 (T0), after 6 M of Ca storage (T1) and after 8 M of Ca storage (8M) for Soluble Solid Content and Firmness.

Regarding disorders observed during fruit assessment, cork incidence ranged from 10 to 14% in Internal fruit while for External fruit from 13 to 29%. Scuffing was absent at harvest (T0) in both fruit positions, while increased in the following pullouts, reaching a maximum of 96% of incidence in External fruit after 8 month of storage + 7 days of ripening (88% in the Internal fruit at the same time point). No superficial scald was noticed in the fruit from harvest up to after 8 months of CA without any post-storage ripening (day 0), while after 7 days of ripening at room temperature, superficial scald incidence was 37% in External fruit and 1.5% in Internal fruit (after 6 months) and 48% and 11% respectively (after 8 months). Superficial scald hue tended to get darker longer the storage duration but the affected area was similar approx. around 25% of fruit surface. So, in general, External fruit were more affected by superficial scald.

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

Peel chemistry changed alongside fruit appearance and other quality traits. Differences of peel chemistry were most dramatic with tree position which changed as fruit ripened during storage (Fig. 7). Results indicate the greatest impact on fruit ripening and chemistry results from tree position more than any other factor in the experiment and, accordingly, it is the greatest source of quality and ripeness variability. Differences were detected at harvest as well as throughout storage indicating the final product on the store shelf may also be different.

Differences of quality traits, including natural aroma and flavor, are clear within the chemical profile. These include sugars (sweetness), malic acid (tartness), phenolics (bitterness), and aroma volatiles. Pears may have more ripe or unripe aroma depending upon tree position, even at 8 months storage (Fig. 8). I_{AD} classification was reflected in the overall peel chemistry at harvest but this relationship declined with storage duration (data not shown). Peel chemical analysis results to date indicate that tree position will have a major impact on relative storability and eating quality.

Not only are flavor and maturity impacted by tree position but so are critical factors such as appearance. While we expect that external fruit may have more blush or, as fruit appear to ripen differentially, background color would be influenced by canopy position, there are also less obvious factors profoundly impacting finish. For instance, superficial scald incidence was higher in External fruit than Internal fruit, a factor linked with higher levels of key apple scald risk biomarkers detected in Internal peel (Fig. 9). As storage regimes and marketing strategies can be most effectively tailored to a consistent batch of fruit, it is clear that more consistent fruit at the beginning of storage would reduce losses and that these decisions are impacted by canopy position.

Shorter term strategies for reducing inconsistency of fruit going into storage may rely on the ability to "see" and sort fruit according to canopy position as that is the major contributor to inconsistency. Another outcome of our untargeted appraisal of peel chemistry are potential targets for just this task. External fruit have higher levels of compounds associated with light exposure and Internal fruit have higher levels of wax compounds involved in other pathways (Fig. 10). These metabolites associated with sun exposure are part of a fruit's natural defense to increased light exposure that are not apparent with the naked eye but can be detected using devices that focus on portions of the ultra-violet spectrum. This aspect could, potentially, be used to sort fruit in the orchard or warehouse according to tree position yielding a more consistent batch of fruit for tailored supply chain management, reducing downstream losses.

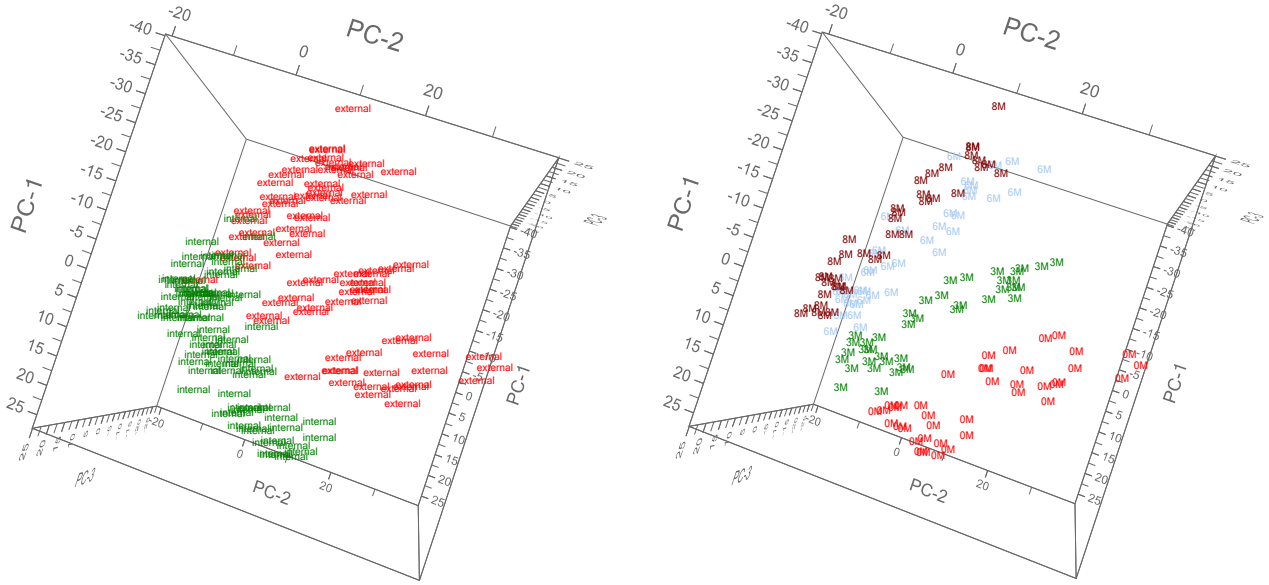


Figure 7: Principal components analysis (PCA) scores plot illustrating differences in overall natural chemical levels from Anjou pears harvested from the external or internal canopy and stored for up to 8 months in CA storage. Each point represents a summary of over 800 natural peel chemicals for a single peel sample. On the left: empty circle=internal, full square=external. On the right: full square= T0 harvest, empty circle= T1 (after 3 months CA storage), cross=T2 (after 6 months of CA storage) and full diamond=T3 (after 8 months in CA storage).

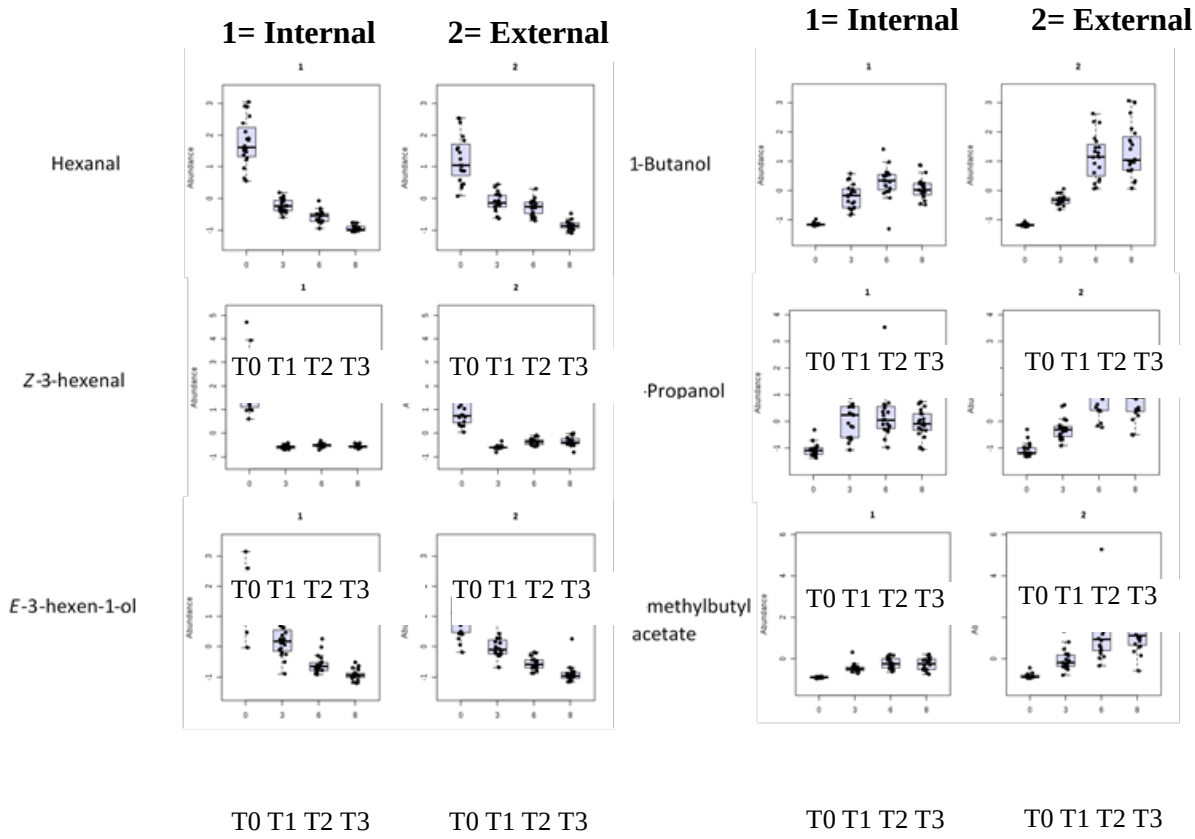


Figure 8: Changes of levels of peel chemicals different in d'Anjou pears from the Internal (1) or External (2) canopy over 8 month CA storage (from T0 to T3). Results suggest that "unripe" flavors (left) are higher in Internal fruit at harvest and are similar by 8 months while "ripe" flavors (right) are more prevalent in External fruit at the end of storage indicating fruit ripeness and quality are different depending upon tree position.

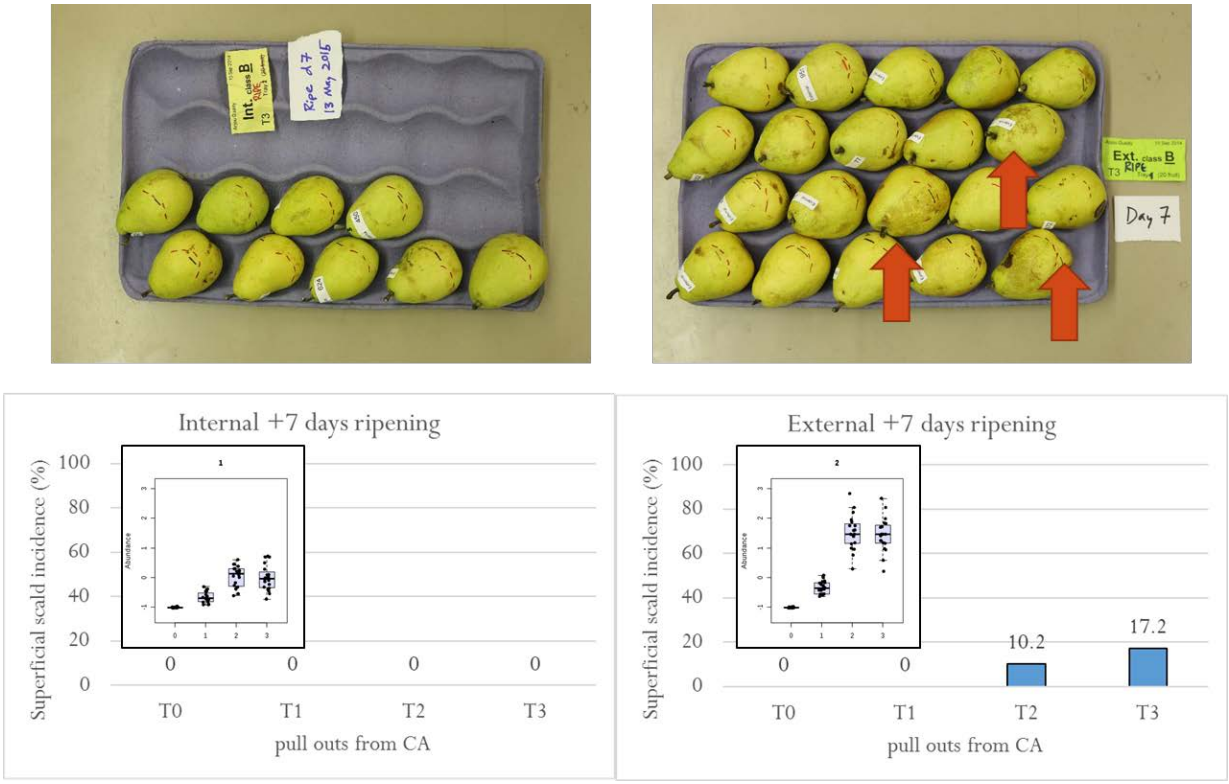


Figure 9: Superficial scald incidence (%) dependent upon canopy position for d'Anjou pears stored in CA for 8 months and left to ripen at 68 F for 1 week. In this case (orchard, year, storage conditions), External fruit (right) developed more scald than Internal fruit (left). Levels of an apple scald risk assessment biomarker (insets) were elevated in External fruit.

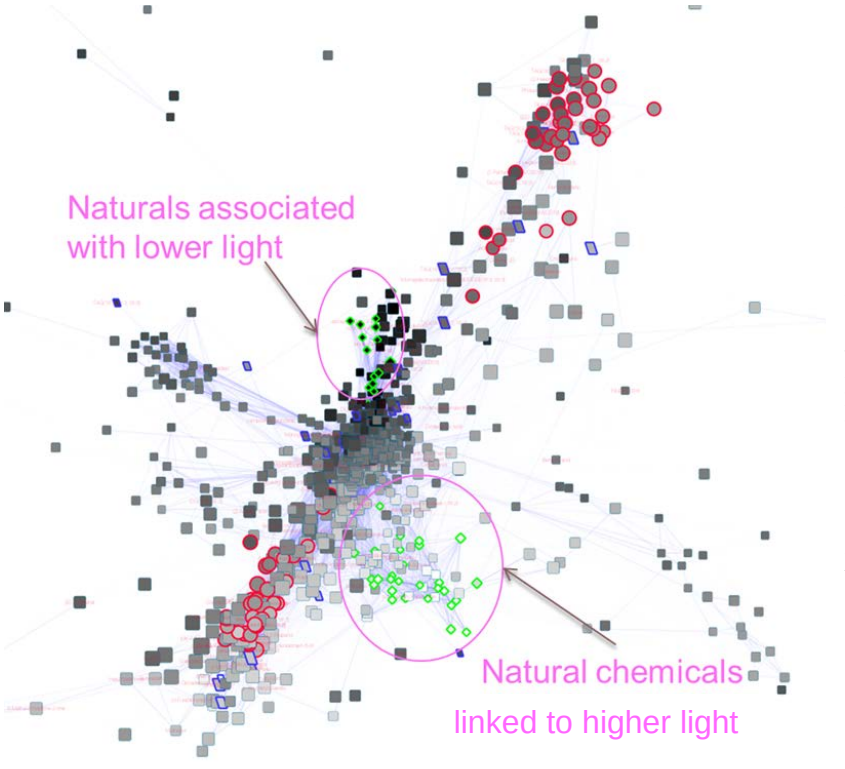


Figure 10: Associations among natural peel chemicals during 8 months CA. Chemicals (shapes) that are closer together indicate that their levels over the storage period change similarly with respect to other factors in the experiment such as tree position. Chemicals linked based on tree position are circled according to two groups: those associated with higher light environment (bottom) and lower light environment (top). Chemicals we have identified that are associated with higher light conditions include flavonol glycosides with can be detected using UV reflectance imaging and possibly exploited for in-field or warehouse pre-storage sorting.

- **Publications:**
  - Zhang J., Serra S., Leisso R.S., Musacchi S. (2016) *"Effect of light microclimate on the quality of 'd'Anjou' pears in mature open-centre tree architecture"*. Biosystems Engineering, 141:1-11.
  - Rudell D.R, Serra S., Sullivan N., Mattheis J.P., Musacchi S. (2017) *"Metabolic profiling variations within 'd'Anjou pear fruit from different canopy positions"*. In preparation to submission.

- **Presentations:**
  - Rudell D., Serra S., Sullivan N., Mattheis J., Musacchi S. *"Fruit position within pear trees impacts ripening and associated metabolism after harvest"* (oral presentation by Rudell D.). 12th Annual Conference of Metabolomics Society, Dublin, Ireland (June 2016).
  - Serra S., Rudell D., Mattheis J., Musacchi S. *"Evaluating Fruit Quality and Maturity in Large Open Vase-trained 'D'Anjou' Trees"* (Oral presentation by Serra S.) ASHS annual meeting, Atlanta, Georgia (August 2016).

Executive Summary

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

'D'Anjou has been trained for many years using an open vase. Single trees can reach 17 ft high with a very large canopy volume where fruits are distributed mostly in the upper-medium portion of the canopy. Fruit characteristics inside such a big and vigorous tree can be very different as less light can penetrate into the inside of the canopy and, consequently, light exposure can be quite different. Harvest in those orchards cannot be mechanized and is performed manually without any sorting. Consequently, many fruit quality characteristics, including maturity, can be highly variable within a single bin. This factor can dramatically impact fruit quality and storability often resulting in the need to repack to eliminate over-ripe, spoiled and scalded fruit from packed boxes.
Our preliminary work indicates a non-destructive approach using the DA-meter, which can be adopted to segregate pear fruit according to maturity by estimating associated chemical changes. We have found that fruit picked from the internal part of the canopy ripen more slowly, as estimated using the DA index, but lose weight more rapidly than fruit harvested from the outer part of the canopy. Our long-term goal is to develop tools and protocols that improve uniformity of fruit maturity and quality at harvest. Moreover, one possible long-term outcome is implementation of existing sorting technology to afford storage operators the ability to pre-sort pears by orchard or tree position/maturity. This sorting capacity would allow tailored storage regimes for improved ripening and quality consistency and reduced losses from postharvest disorders such as scald and possibly decay.

Project outcomes:

1. Method to prove that large 'D'Anjou open vase trees show inconsistency in ripening depending on light exposure.
2. Repacking problem and postharvest losses can be improved with fruit sorting at harvest and tailored storage conditions and durations.
3. New potential chemicals targets for sorting fruit accordingly to canopy position in the orchard or warehouse.

Significant Findings:

1. Crop inconsistency resulting from pear canopy position impacts most postharvest supply chain decisions.
2. Fruit ripening and potentially flavor is different depending upon canopy position.
3. Canopy position impacts postharvest behavior including superficial scald risk.

Future Directions:

1. Change 'D'Anjou trees architecture (and rootstocks) toward a narrower canopy and higher density planting and more planar canopy for more consistent crop.
2. Improve the picking process by canopy position and fruit sorting ability in the orchard.
3. Tailored storage duration depending on fruit sorted by maturity levels.

FINAL PROJECT REPORT

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

PI: W. Rodney Cooper
Organization: USDA-ARS-YARL
Telephone: 509/454-4463
Email: Rodney.Cooper@ars.usda.gov
Address: 5230 Konnowac Pass Road
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:** $5,700

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	$5,000
Benefits	$1000	$1000	$200
Wages			
Benefits			
Equipment			
Supplies	$5000	$5000	
Travel			
Plot Fees	$3000	$3000	$500
Miscellaneous			
Total	$25,000	$25,000	$5,700

Footnotes:
[1] Partial funding for a temporary employee to help with field studies

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 numbers.
4) Test the effects of defense elicitors on obligate bacterial symbionts of pear psylla.

SIGNFICANT FINDINGS

1) Both Actigard and ODC reduced pear psylla nymph populations by about 20% during peak populations.
2) Magnesium sulfate treatment reduced pear psylla numbers, but did not enhance Actigard-activated defenses against psylla beyond the effects of Actigard alone.
3) Adults collected from pear trees treated with Actigard had significantly reduced titers of the obligate symbiont, *Carsonella ruddii*, than did adults collected from untreated trees.

RESULTS AND DISCUSSION

Analysis of variance did not reveal significant week by treatment interactions for either nymphs or adults regardless of sampling year (Table 1), but counts of nymphs and adults varied by sampling week within each year (Table 1). Nymphal populations exhibited two generation peaks, which occurred in late April to early May and in June of each year (Figs. 1A-3A). The second generation of nymphs was nearly 3 to 4 times larger than the first generation in all three years (Figs. 1A-3A). Adult populations also exhibited two generation peaks, which occurred about two weeks after observed peaks in nymphal populations (Figs. 1B-3B). The relative size of the two peaks varied among years. In 2014, the second generation of adults was numerically larger than the first generation, but the second generation was small compared to the first generation in both 2015 and 2016 (Figs. 1B-3B).

Table 1. Statistical analyses examining the effects of foliar applications of defense elicitors on pear psylla populations.

Variable	2014	2015	2016
Nymphs			
Week	$F_{18, 72}$=40.6; P<0.001	$F_{16, 64}$=24.9; P<0.001	$F_{18, 72}$=32.6; P<0.001
Treatment	$F_{3, 12}$=5.7; P=0.013	$F_{3, 12}$=1.6; P=0.253	$F_{3, 12}$=4.8; P=0.020
Week × Treatment	$F_{54, 216}$=1.4; P=0.056	$F_{48, 192}$=0.8; P=0.818	$F_{54, 216}$=1.1; P=0.295
Adults			
Week	$F_{18, 72}$=58.1; P<0.001	$F_{16, 64}$=43.8; P<0.001	$F_{18, 72}$=7.3; P<0.001
Treatment	$F_{3, 12}$=6.3; P=0.008	$F_{3, 12}$=0.4; P=0.763	$F_{3, 12}$=2.6; P=0.097
Week × Treatment	$F_{54, 216}$=0.8; P=0.806	$F_{48, 192}$=0.7; P=0.900	$F_{54, 216}$=0.9; P=0.643

Analyses revealed significant differences in numbers of nymphs among foliar treatments in 2014 and 2016, but not in 2015 (Table 1). In 2014, significantly fewer nymphs were observed on trees treated with Actigard, Employ, or ODC than on untreated controls pooled over sampling dates (Fig. 1A: right panel). Although not statistically significant in 2015, nearly 20 to 30% fewer nymphs were observed on trees treated with Actigard, Employ, or ODC than on untreated controls (Fig. 2A: right panel). Paired contrasts suggested marginally significant reductions (α<0.1) of nymphs on trees treated with Actigard in 2015 compared with untreated controls (t=2.1; P=0.058; Fig. 2A: right panel). As observed in 2014, significantly fewer nymphs (Fig. 3A: right panel) were recorded from

trees treated with Actigard, Employ, or ODC than on untreated controls in 2016. Overall, results from the three sampling years were consistent and suggested that treating trees with defense elicitors leads to a modest (20-30%) reduction in populations of pear psylla nymphs. These reductions were most obvious during the second generation population peak. Results of these field trials were also consistent with our previous laboratory study, which indicated that treating pear with foliar applications of Actigard, Employ, or ODC induced systemic defenses that increased mortality of psyllid nymphs (Cooper and Horton 2015).

Significantly more adults were collected from untreated trees than from trees treated with Actigard, Employ, or ODC pooled over all sampling weeks in 2014 (Fig. 1B: right panel). As observed for nymphs, the differences in treatments were most obvious during the second generation peak, which occurred in July of 2014 (Fig. 1B). This pattern was not observed in 2015 (Fig. 2B) or 2016 (Fig. 3B), probably because the second generation both years was extremely small in all treatments. Although the overall treatment effect was not significant at the α=0.05 confidence interval in 2016 (Table 2; P=0.097), paired contrasts indicated that significantly fewer adults were collected from trees treated with Employ than from untreated trees (t=2.67; P=0.020), and marginally fewer adults were collected from trees treated with Actigard than from untreated trees (t=1.96; P=0.073) (Fig. 3B). Our previous laboratory study did not indicate that defense elicitors led to decreased adult survival, but adults did tend to settle and oviposit on untreated trees more often than on trees treated with Actigard, Employ, or ODC in choice assays (Cooper and Horton 2015). It is possible that differences among treatments observed in 2014 were due to reduced numbers of nymphs developing to adults on treated trees, and due to movement of adults to adjacent untreated trees. It is unclear whether treatment differences attributed to adult preference would be replicated if an entire orchard were treated with an elicitor product.

Results of our study demonstrate that foliar applications of Actigard, Employ, or ODC reduced densities of pear psylla nymphs under field conditions. These results are consistent with those of our previous laboratory bioassays (Cooper and Horton 2015), and with other reports that elicitors of salicylic acid-dependent defenses reduce performance of other phloem-feeding insects (Dong et al. 2004, Cooper et al. 2004, Cooper and Goggin 2005, Li et al. 2006, Boughton et al. 2006, Gao et al. 2007, Zhang et al. 2012). The modest reduction in pear psylla nymphs observed here does not warrant the use of elicitors alone for the control of pear psylla. However, elicitors are often used in pear orchards to manage fire blight, and knowledge that these products may also partially suppress pear psylla populations could be useful for system-wide integrated pest management approaches. More trials are required to evaluate the efficacy of these products applied to entire orchards and used in a spray schedule typical for fire blight management.

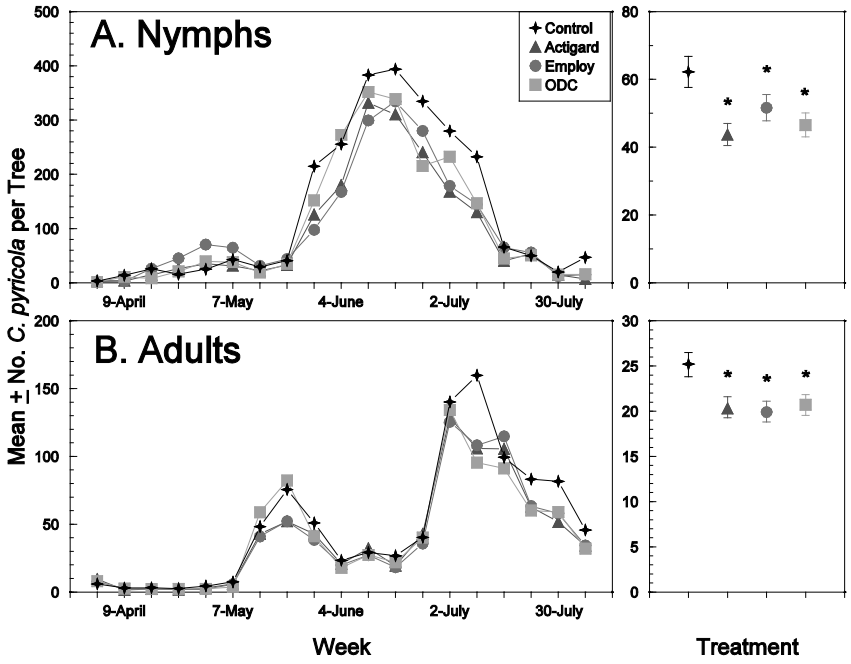


Figure 1. Mean number of pear psylla nymphs per shoot (A) and adults per beat sheet sample (B) in 2014. Dates provided on the x-axis indicate days on which foliar applications were applied. Figures on the right show the overall effects of treatment regardless of sampling week. Error bars denote standard errors and asterisks indicate that values are significantly different from the untreated control treatment.

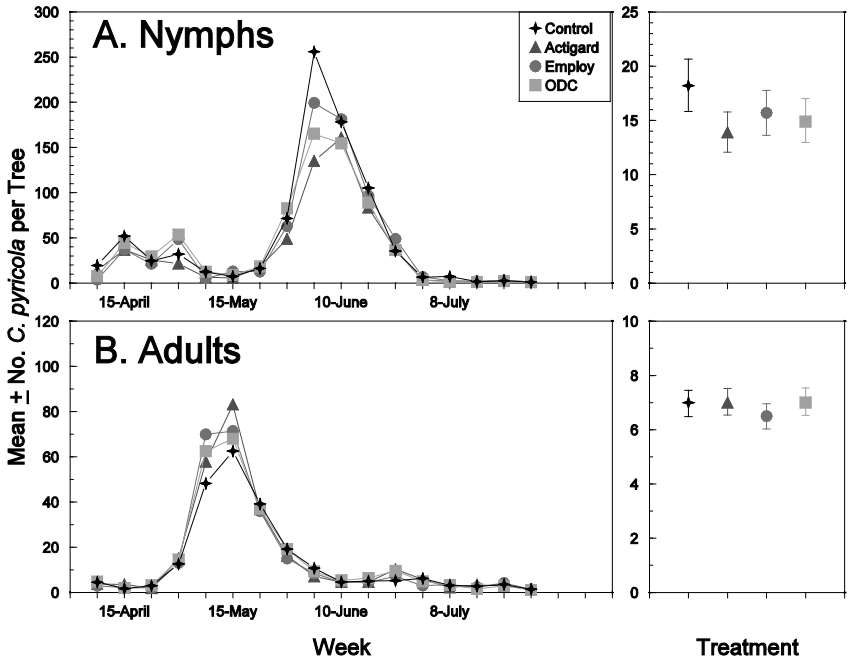


Figure 2. Mean number of pear psylla nymphs per shoot (A) and adults per beat sheet sample (B) in 2015. Dates provided on the x-axis indicate days on which foliar applications were applied. Figures on the right show the overall effects of treatment regardless of sampling week. Error bars denote standard errors.

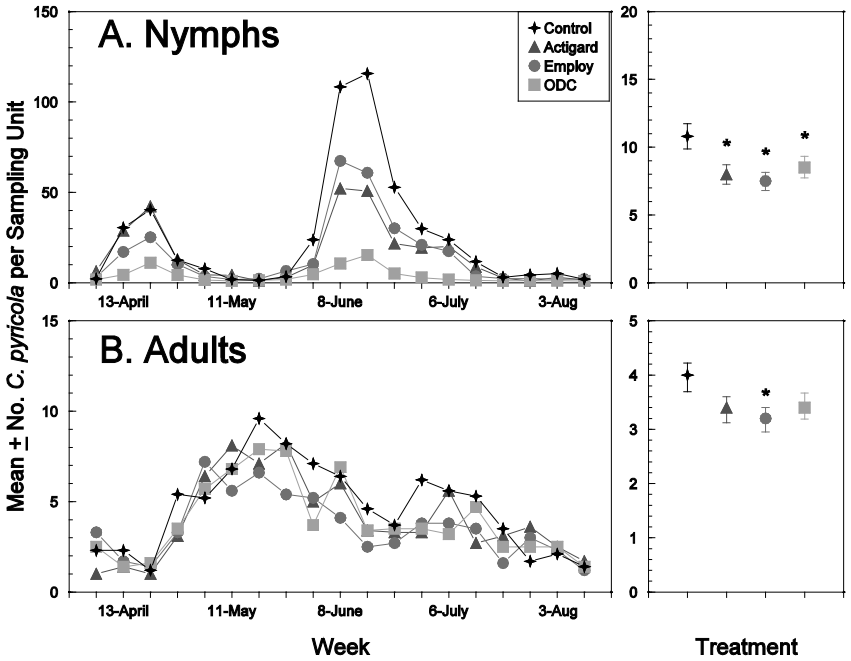


Figure 3. Mean number of pear psylla nymphs per shoot (A) and adults per beat sheet sample (B) in 2016. Dates provided on the x-axis indicate days on which foliar applications were applied. Figures on the right show the overall effects of treatment regardless of sampling week. Error bars denote standard errors and asterisks indicate that values are significantly different from the untreated control treatment.

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

We did not observe any consistent effects of defense elicitors on densities of natural enemies.

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

Greenhouse assays confirmed our previous results that Actigard treatments reduce pear psylla numbers (Figure 4). Results also revealed that foliar application of magnesium sulfate by itself also reduced pear psylla numbers (Figure 4), which is consistent with anecdotal reports on aphids. Adding magnesium sulfate to the Actigard treatment did not improve plant protection provided by Actigard alone (Figure 4). We found no evidence that potassium fertilization influences pear psylla numbers.

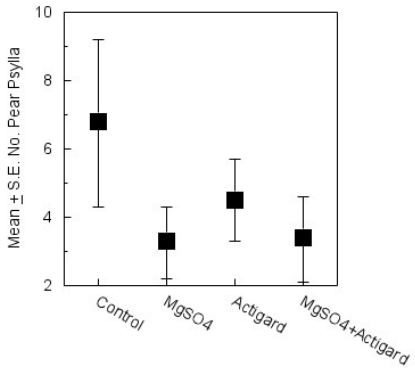


Figure 4. Effects of Magnesium sulfate and Actigard applications on pear psylla performance

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 5A inset). Our second method relies on quantitative real time PCR (qPCR) to estimate bacteria densities in whole insects. Using these methods, we showed that *Carsonella* was more abundant in females than in males (Figure 5). These results confirmed that our methods are suitable for comparing *Carsonella* among pear psylla, and showed that insect sex should be controlled in our future studies.

Because *Carsonella* varied between sexes, only females were used to examine the effects of defense elicitors on endosymbiont titers. *Carsonella* titers in whole insects were not altered by plant defenses activated by Actigard (Figure 6A). However, *Carsonella* titers in individual bacteriocytes were reduced in psylla exposed to trees treated with Actigard compared with those on control trees (Figure 6B). Results suggest that plant defenses against pathogens may reduce the obligate endosymbiont of psylla, which may explain how psylla are reduced on induced trees. However, it is not possible to discern whether *Carsonella* is directly altered by plant defense compounds, or if declining health of psylla by plant defenses leads to reductions in *Carsonella*.

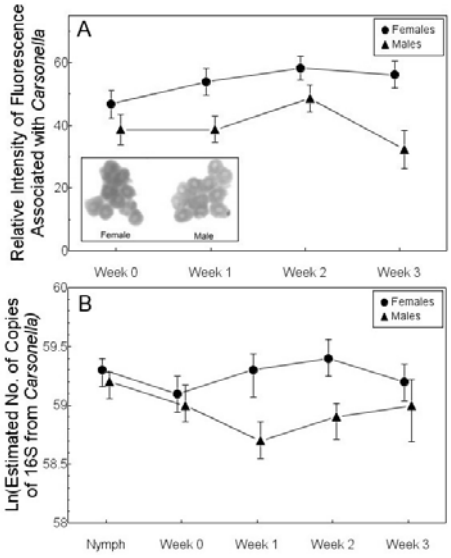


Figure 5. Comparison of *Carsonella* densities among females and males using FISH (A) and qPCR (B). Inset shows samples of bacteriocytes containing *Carsonella* labeled with a fluorescent probe; the darker cells indicate a greater density of *Carsonella*.

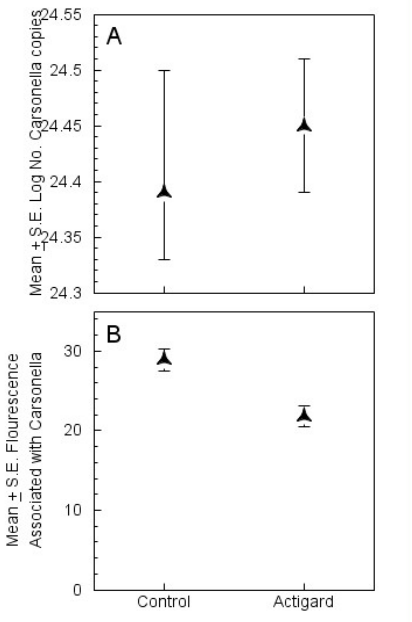


Figure 6. Effects of defense elicitors on *Carsonella* titers.

REFERENCES

Boughton, A., K. Hoover, and G. W. Felton. 2006. Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, Myzus persicae. Entomol. Exp. Appl. 120: 175-188.

Cooper, W. R., L. Jia, and G. L. Goggin. 2004. Acquired and R-gene mediated resistance against the potato aphid in tomato. J. Chem. Ecol. 30: 2527-2542.

Cooper W. R., and D. R. Horton. 2015. Effects of elicitors of host plant defenses on pear psylla, *Cacopsylla pyricola*. Entomol. Exp. Appl. 157: 300-306.

Dong, H. P., J. L. Peng, X. D. Meng, J. M. Bonasera, G. Y. Chen, S. V. Beer, and H. S. Dong. 2004. Downstream divergence of the ethylene signaling pathway for harpin-stimulated Arabidopsis growth and insect defense. Plant Physiol. 136: 3628-3638.

Gao, L., J. P. Anderson, J. P. Klingler, R. M. Nair, O. R. Edwards, and K. B. Singh. 2007. Involvement of the octadecanoid pathway in bluegreen aphid resistance in Medicago truncatula. Mol. Plant Microbe Interact. 20: 82-93.

Li, Q., Q. G. Xie, J. Smith-Becker, D. A. Navarre, and I. Kaloshian. 2006. Mi-1 mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signaling cascades.

Zhang, C. H. Shi, L. Chen, X. Wang, B. Lu, S. Zhang, Y. Liang, R. Liu, J. Qian, W. Sun, Z. You, and H. Dong. 2012. Harpin-induced expression and transgenic overexpression of the phloem protein gene AtPP2-A1 in Arabidopsis repress phloem feeding of the green peach aphid Myzus persicae. BMC Plant Biology 11:11.

EXECUTIVE SUMMARY

Defense elicitors are products that activate acquired defense responses in plants, thus making plants less susceptible to attack by a broad range of pests. We previously demonstrated under laboratory conditions that foliar applications of the defense elicitors Actigard (acibenzolar-S-methyl), Employ (harpin protein), or ODC (chitosan) to potted pear trees (*Pyrus communis* L.) each caused an increase in mortality of pear psylla nymphs, and altered the settling and oviposition behavior of the adults. The objective of the current study was to determine whether the use of defense elicitors in pear orchards reduces wild populations of pear psylla, and to determine whether these defense responses, which are primarily associated with defense against plant pathogens, may suppress psylla by reducing titers of the obligate bacterial endosymbiont, *Carsonella*.

Summary of Findings

We monitored psylla populations over a 3-year period on orchard-grown trees treated with water (untreated control), Actigard, Employ, or ODC. Fewer nymphs were observed on trees treated with elicitors compared with untreated trees in both 2014 and 2016. A similar but statistically non-significant pattern was observed in 2015 when nearly 30% fewer nymphs were observed on trees treated with elicitors versus untreated controls. Observed reductions in psyllid numbers by defense elicitors were modest, and do not warrant the use of these products alone for managing pear psylla. However, these products are often used for management of fire blight, and our observations that elicitors also reduce pear psylla populations may be useful for integrated (disease + insect) pest management approaches.

We developed two methods to estimate relative abundance of *Carsonella* in bacteriocytes and whole bodies of psyllids: fluorescence in situ hybridization and qPCR, respectively. We first compared *Carsonella* populations between female and male insects, to determine if our elicitor trials must consider sex of the psyllid specimen in the analysis. Estimations using fluorescence in situ hybridization indicated that *Carsonella* was more abundant in bacteriocytes of female psylla than in those of males. Analyses by qPCR using whole-body specimens indicated *Carsonella* was more abundant in females than in males. Thus, our study indicates that female psyllids harbor greater populations of *Carsonella* than do males, and that sex of specimens should be considered in studies which require estimations of *Carsonella* populations. Psyllid age (0 to 3 weeks after adult eclosion) had no effects on estimates of *Carsonella* numbers. *Carsonella* was observed in ovarioles of newly emerged females, and formed an aggregation in the posterior end of mature oocytes. Based on these results, we controlled for insect sex when evaluating effects of defense elicitors on *Caronella* titers. In the elicitor studies, we observed reductions in *Carsonella* numbers in psylla collected from trees treated with Actigard compared with psyllids from control trees, providing evidence that defense responses may act indirectly on psylla fitness by reducing titers of the obligate endosymbiont.

Peer-Reviewed Publications

Cooper W. R., and D. R. Horton. 2015. Effects of elicitors of host plant defenses on pear psylla, *Cacopsylla pyricola*. Entomol. Exp. Appl. 157: 300-306.

Cooper, W. R., S. F. Garczynski, and D. R. Horton. 2015. Relative abundance of *Carsonella ruddii* (Gamma Proteobacterium) in females and males of *Cacopsylla pyricola* (Hemiptera: Psyllidae) and *Bactericera cockerelli* (Hemiptera: Triozidae). J. Insect Sci. 15: 65

Cooper, W. R., and D. R. Horton. In Review. Elicitors of host plant defenses partially suppress pear psylla (*Cacopsylla pyricola*, Hemiptera: Psyllidae) populations under field conditions. Submitted to J. Insect Sci. on 5-December 2016.

FINAL PROJECT REPORT

Project Title Pesticide resistance in pear psylla

PI: Tom Unruh
Organization: USDA-ARS
Telephone: 509-454-6563
Email: thomas.unruh@ars.usda.gov
Address: USDA-ARS
Address 2: 5230 Konnowac Pass Rd
City/State/Zip: Yakima, WA 98951

Co- PI: Peter Shearer
Organization: OSU-MCAREC
Telephone: (541) 386-2030-x215
Email: Peter.Shearer@oregonstate.edu
Address: OSU-MCAREC, Horticulture
Address 2: 3005 Experiment Station Drive
City/State/Zip: Hood River, Oregon, 97031-9512

Co-PI: Richard Hilton
Organization: OSU-SOREC
Telephone: (541) 772-5165-x227
Email: Richard.Hilton@oregonstate.edu
Address: OSU-SOREC, Horticulture
Address 2: 569 Hanley Road
City/State/Zip: Central Point, OR 97502

Co-PI: Joanna Chiu
Organization: U. California, Davis
Telephone: (530) 752-1839
Email: jcchiu@ucdavis.edu
Address: U.C. Davis, Entomology
Address 2: Storer Hall 6348,
City/State/Zip: Davis, CA 95616 USA

Cooperators: Elizabeth Beers

Total Project Funding: $48,700

Budget History

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 Davis **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 a resistance survey of winterform pear psylla in WA and OR**

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

Significant Findings

- Admire, AgriMek, Delegate, Nexter, Pounce, and Warrior were screened for activity against winterform pear psylla adults from 9 sites in OR and 11 sites in WA.
- Mortality caused by Delegate and Nexter was the highest of the insecticides tested, but still averaged 56 and 49% for the populations tested.
- Mortality caused by Admire and AgriMek was 25 and 18%, respectively, indicating a high probability of resistance.
- Mortality caused by Pounce (13%) and Warrior (10%) was low overall.
- A single population with very low mortality in both Delegate and Nexter bioassays suggests the possibility of cross-resistance between the two products.
- Development of selectivity ratios (harm to natural enemies balanced against pesticidal efficacy) is important to making sustainable pest management decisions.
- Transcriptomes for 24 populations of pear psylla in Oregon or Washington were sequenced. These are the first transcriptomes produced for pear psylla and will be submitted to NCBI Genbank to facilitate psylla research.
- Mutations in genes involved in neurotransmission were identified in pear psylla populations that exhibited resistance to AgriMek and Pounce. Genetic markers can be developed to identify resistant populations and monitor the spread of resistance.

Results & Discussion

Obj. 1. Methods. We examined the relative efficacy of six insecticides commonly used for control of pear psylla. The materials included Admire Pro, AgriMek, Delegate, Nexter, Pounce, and Warrior. Active ingredients and maximum label rates are given in Table 1.

Table 1. Pesticides screened for efficacy against pear psylla

Product	AI	% AI or lb AI/gal	Maximum label rate (label units)	Maximum label rate (ppm AI)	MOA
Admire Pro	imidacloprid	4.6 lb AI/gal	7 fl oz	302	group 4A
Agri-Mek SC	abamectin	0.7 lb AI/gal	4.25 fl oz	28	group 6
Delegate 25WG	spinetoram	25%	7 oz	131	group 5
Nexter 75WP	pyridaben	75%	16 oz	899	group 21
Pounce 25WP	permethrin	25%	25.6 oz	479	group 3
Warrior II	lambda-cyhalothrin	2.08 lb AI/gal	2.56 fl oz	50	group 3

Using the insecticides listed above, we evaluated mortality of winterform pear psylla from 20 pear orchards in WA (11 orchards) and OR (9 orchards). Psylla were collected in Sept-November of 2014 and 2015. Field collections were performed with either an beating tray and aspirator or a large plastic funnel with a jar attached to the bottom (Fig. 1), speeding collection of large numbers of insects. The funnel was held beneath a branch which was struck sharply with a padded stick. This process was repeated until sufficient adults were collected. Adults were kept cool (40°F) and under short photoperiod (10L:14D) and provided with a moisture source until used in a bioassay.

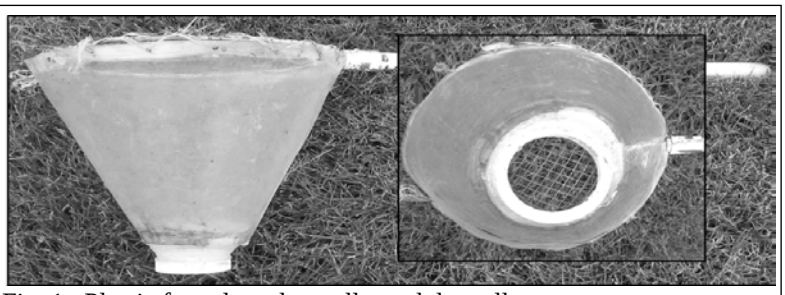


Fig. 1. Plastic funnel used to collect adult psylla

The bioassay format chosen was the slide dip so that data would be comparable to previous work. A group of 25-35 adults (unsexed) were anesthetized with CO_2 and affixed to the slide using double-sided sticky tape. After all adults for a bioassay were placed on slides, they were re-scanned and any dead adults removed. Each dosage was tested with three slides, or 50 to 150 individuals/ concentration. Depending on the numbers of adults available, 2-7 concentrations were tested. The larger number of concentrations is useful for probit analysis, while the reduced number is most appropriate for a diagnostic dose approach. All bioassays included a water check. The slide with adults was dipped in the pesticide solution (or water) for 5 seconds then held at room temperature for 48 hours. After this time, the adults were evaluated for mortality.

Fig. 2. Adult psylla on slide with double-sided sticky tape.

Obj. 1. Results & Discussion. A total of 77 bioassays were performed with psylla from various orchard and insecticide combinations. Twenty-four of those bioassays had a reduced number of doses, and are most appropriate for a diagnostic dose evaluations. The maximum label rate (MLR) was chosen as a means of comparing the various orchard populations. An additional 53 bioassays contained a wider range of concentrations, from which a probit lines were calculated (not shown). A single summary statistic was chosen that represented both bioassay types. If the MLR was included in the bioassay, the percentage mortality from this concentration was used; otherwise, the concentration *nearest* the MLR was chosen by using the minimum of the absolute value of the differences between the actual rate and 1. Two of the bioassays did not contain a concentration sufficiently close to represent the MLR, and were excluded from the summaries. Thus, the figures represent a 'best case' scenario of the mortality that would occur if the material were applied at the MLR (Figs. 3a-f). The results are arranged and color coded by the state from which the population originated.

Mortality caused by Admire was variable but generally low at the MLR (average=25% for all populations, n=12) (Fig. 3a). Average mortality for AgriMek was similarly low (12%, n=12) (Fig. 3b). Mortality caused by Delegate was considerably higher overall (45%, n=12), with only a single Washington population (OK) showing resistance to this material (Fig. 3c). Results from Nexter were

similar (50%, n=10) to those of Delegate; the same Washington (OK) populations that was highly tolerant of Delegate was also highly tolerant of Nexter (Fig. 3d). Most of the populations from Washington and Oregon were resistant to Pounce (Fig. 3e), with an overall average was 13% (n=19). Results from the Warrior bioassays were similar, with an average of 10% (n=10) mortality (Fig. 3f).

The results of these bioassays must be interpreted with a great deal of caution. Only two the materials, the pyrethroids Pounce and Warrior, are typically used against winterform adults, and thus were tested with the most appropriate target stage. Resistance to pyrethroids in pear psylla has been known from the 1970s, and was well documented for fenvalerate in the 1990s. However, neither pyrethroid in the current study was tested with piperonyl butoxide (PBO), an adjuvant commonly used to help overcome resistance mechanisms. Mortality would most likely have been higher overall for these two products with the addition of PBO.

The other four insecticides (Admire, AgriMek, Nexter, Delegate) are typically used after the dormant/delayed dormant period, when egg, nymphs, and (in later generations) summerform adults are present. Nymphs, especially the earlier instars, are likely the most vulnerable to pesticides, and therefore the primary target of these materials. Without bridging information on activity difference between winterforms and nymphs, historical levels of activity, or contemporaneous bioassays of a susceptible population, few conclusions may be drawn other than the variability among the populations tested.

Lastly, the low mortality in one population (OK) for both Delegate and Nexter suggests the possibility of cross-resistance between the two products. However, more populations would need to tested to establish this experimentally.

Selectivity. Most of the insecticides tested would be considered non-selective to natural enemies, and this presents an additional item for consideration in the choice of materials. The 'worst case scenario' is where the insecticide is no longer very effective against the target pest, but retains its toxicity to one or more important natural enemies. For instance, AgriMek is acutely toxic to a psylla parasitoid (*Trechnites* sp) and the predators *Anthocoris* and *Deraeocoris*, even at 25% of the field rate. It is also toxic to the western predatory mite *Galendromus occidentalis*, so disruption of both biological control systems can be expected. Developing a selectivity ratio, which indicates the relative harm (to natural enemies) to relative good (pesticidal efficacy) could help guide grower choices for more sustainable pest management programs.

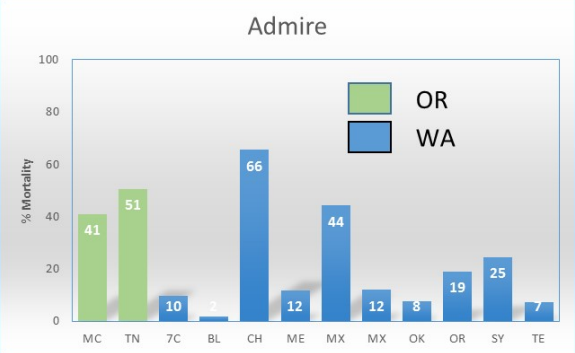


Fig. 3a. Percentage mortality of winterform psylla by Admire at or near the field rate (WA, OR).

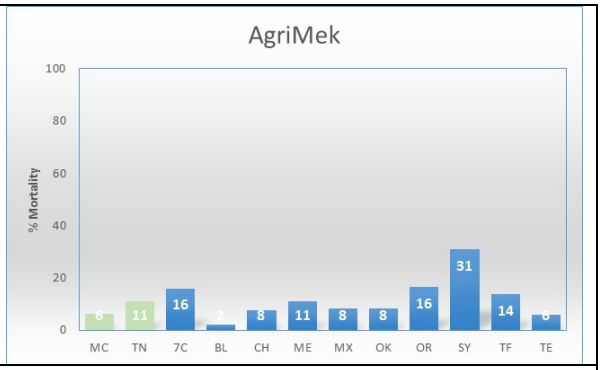


Fig. 3b. Percentage mortality of winterform psylla by AgriMek at or near the field rate (WA, OR).

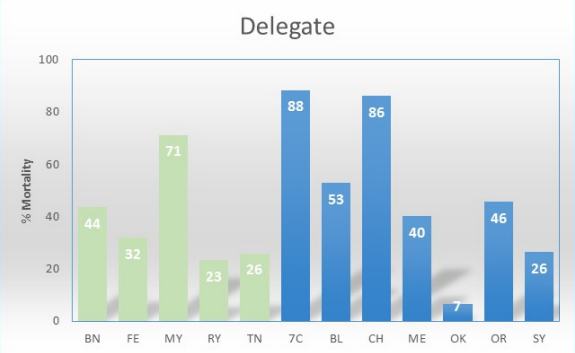


Fig. 3c. Percentage mortality of winterform psylla by Delegate at or near the field rate (WA, OR).

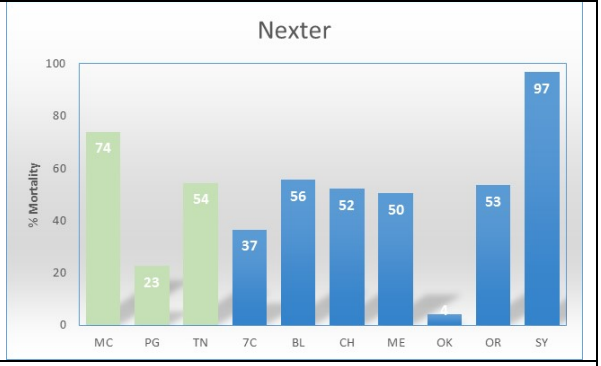


Fig. 3d. Percentage mortality of winterform psylla by Nexter at or near the field rate (WA, OR).

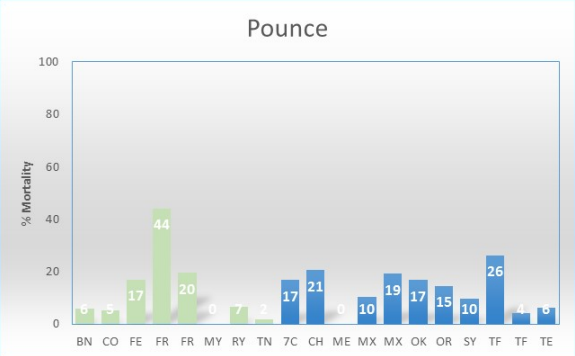


Fig. 3e. Percentage mortality of winterform psylla by Pounce at or near the field rate (WA, OR).

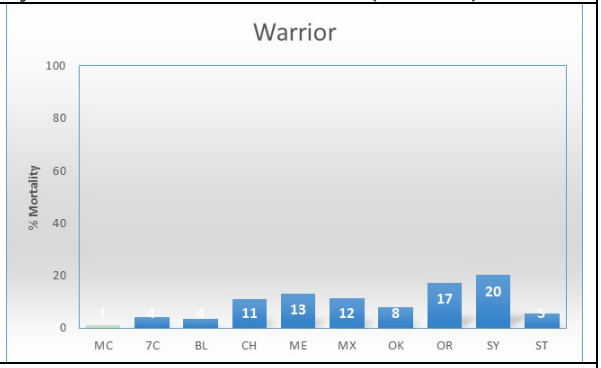


Fig. 3f. Percentage mortality of winterform psylla by Warrior at or near the field rate (WA, OR).

Obj. 2. Methods

The goal of this objective was to identify genetic mutations that could underlie resistance to specific insecticides tested in Objective 1 using RNA sequencing. Specifically, we focused our genetic analysis on AgriMek and Pounce as pear psylla populations that are either susceptible or resistant were available for RNA analysis.

RNA extraction, library preparation, and high-throughput sequencing
Total RNA was extracted from 25 individuals from each collection site using Tri-reagent (Sigma). Following polyA mRNA enrichment, which enriched for RNA from expressed genes, using the Next PolyA magnetic isolation module (New England Biolabs), paired-end sequencing libraries with an approximate average insert length of around 150bp (standard for transcriptome analysis) were created using the Next Ultra RNA library Prep Kit (New England Biolabs). Transcriptome libraries representing 24 populations (2 replicates per population) were sequenced using 100bp paired-end Illumina HiSeq at the UC Davis Genome Center Sequencing facility.

Bioinformatic analysis to identify genetic mutations underlying insecticide resistance
Since the genome sequence of pear psylla is not available, we performed *de novo* transcriptome assembly using "Trinity" (release 2013-02-25). Our experimental and bioinformatic pipeline yielded individual transcriptomes for the different psylla populations. To extract genetic information from our transcriptomes and annotate the genes, we performed comparative sequence analysis against insect genomes in the public database. Finally, we used the program "Freebayes" to identify genetic differences (single nucleotide polymorphism, SNP) between susceptible and resistant populations for (1) AgriMek and (2) Pounce. In particular, our focus was on genes that are known to be associated with insecticide target site, e.g. ion channels and neuro-receptors, or metabolic resistance, e.g. detoxification enzymes.

Obj. 2. Results and Discussion

Sequencing and annotation of pear psylla transcriptome
In addition to the value of our survey for genetic variations that may confer insecticide resistance, the psylla transcriptome resulting from this project will be submitted to NCBI Genbank and shared with other scientists to facilitate basic and applied research on pear psylla. Besides the genetic markers we can now develop to monitor insecticide resistance, especially if these mutations were confirmed in more populations, the transcriptome data can also be used to develop other molecular markers to monitor population dispersal as well as trait variations.

Identification of genetic differences that underlie the response of pear psylla to AgriMek and Pounce
Samples were available to analyze potential genetic differences between populations that were resistant and susceptible to (1) AgriMek and (2) Pounce. In the case of other insecticides, there were not enough susceptible populations to provide the statistical power necessary to identify gene mutations.

Genetic basis of Pounce resistance
Genetic differences were identified between Washington populations (ME, OK, OR, SY, TE) resistant to Pounce as compared to the Oregon TN population based on bioassays performed in Objective 1. Mutations in genes involved in neuronal function and metabolic detoxification were identified in resistant populations. Two of these mutations are non-synonymous mutations, i.e. mutations that are expected to change the sequence of the mutated proteins, and hence may either enhance or disrupt their functions. Confirmation of these mutations in causing Pounce resistance will

require biochemical analysis. We did not find KDR mutations that have been known to cause resistance to pyrethroids, indicating that the mechanisms underlying Pounce resistance in these psylla populations may be through other mechanisms.

Table 2: Select neuronal and detoxification genes that show genetic mutations in psylla populations resistant to Pounce as compared to susceptible populations

Predicted Pear Psylla Gene	E Value	Non-Synonymous?	Function
cGMP-specific 3',5'-cyclic phosphodiesterase	1.81E-86	Yes	Neuronal
cytochrome P450 4c3	7.13E-75	No	Detox
UDP-glucuronosyltransferase 2B10	4.11E-149	Yes	Detox
Kv channel-interacting protein 4	6.11E-128	No	Neuronal
sodium/hydrogen exchanger 8	0	No	Neuronal
voltage-dependent anion-selective channel	0	No	Neuronal
sodium-independent sulfate anion transporter	7.27E-152	No	Neuronal
cation-transporting ATPase 13A3	0	No	Neuronal
calcium-independent phospholipase A2-gamma	4.03E-79	No	Neuronal
piezo-type mechanosensitive ion channel component	6.37E-159	No	Neuronal

Genetic basis of AgriMek resistance

Genetic differences were identified between populations that are resistant (BL, CH, MC, ME, OK, OR, TF, TE) to AgriMek as compared to susceptible (TN) population based on bioassays performed in Objective 1. Although mutations in genes involved in neuronal function and metabolic detoxification were identified, they are synonymous mutations that are not expected to change the sequence of the mutated proteins. However, it is possible that expression level of these proteins could be influenced, even by non-synonymous mutations. This can be verified using quantitative PCR. It is expected that if more susceptible samples were available, then the identification of the causal mutations would be more likely.

Table 3: Select neuronal and detoxification genes that show genetic mutations in psylla populations resistant to AgriMek as compared to susceptible populations

Predicted Pear Psylla Gene	E Value	Non-Synonymous?	Function
cytochrome P450 4c3	4.71E-75	No	Detox
cytochrome P450 4g15	0	No	Detox
ecdysone receptor	4.16E-06	No	Neuronal
ADP/ATP translocase 2	5.42E-11	No	Neuronal
sodium/hydrogen exchanger 8	0	No	Neuronal
serine carboxypeptidase	7.48E-155	No	Detox
proton-coupled amino acid transporter 2	1.19E-44	No	Neuronal

Additional bioinformatic analysis to examine the biochemical basis of the gene mutations identified here can help to validate the causal mutations for AgriMek and Pounce resistance. Finally, more populations with varying degree of susceptibility to the other insecticides will have to be sequenced to order to identify genetic mutations underlying resistance to Admire, Delegate, Warrior, and Nexter. Our results presented here will now enable the development of genetic markers to identify and monitor the spread of pear psylla resistance populations.

Executive Summary

All of the insecticides tested produced low or moderate mortality on the average in winterform adults. Overall, the highest levels of mortality were produced with Delegate and Nexter, the two newest materials. Generally poor mortality was produced by AgriMek and Admire and, which have been used since the late 1980s and mid-1990s, respectively, in pear production. Activity of the pyrethroids Pounce and Warrior was consistently low in both Washington and Oregon populations, although they were tested without PBO.

Transcriptomes for 24 populations of pear psylla in Oregon or Washington were sequenced. These are the first transcriptomes produced for pear psylla and will be released to NCBI Genbank to facilitate psylla research. Mutations in genes involved in neurotransmission were identified in pear psylla populations that exhibited resistance to AgriMek and Pounce. Additional bioinformatic and biochemical analysis can be performed to further confirm the causal mutation that underlie resistance. Genetic markers can be developed to identify and monitor the spread of resistance populations.

The development of resistance in psylla populations despite the availability of multiple modes of action is an indication of failure of insecticide rotation as a substitute for IPM. Even with 5-6 MOAs available to pear growers, our production systems are on the brink of field failure despite the use of all possible MOAs. Without the ecosystem services of natural enemies to clean up resistant individuals, or the availability of novel MOAs, our current system is vulnerable to failure.

FINAL PROJECT REPORT

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

PI: Elizabeth H. Beers
Organization: WSU-TFREC
Telephone: 509-663-8181 ext 234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee/WA/98801

Co-PI (1): David Crowder
Organization: WSU Pullman
Telephone: (509) 335-7965
Email: dcrowder@wsu.edu
Address: PO Box 616382
City/State/Zip: Pullman/WA/99164

Cooperators: None

Other funding sources: None

WTFRC Collaborative Expenses: None

Total Project Funding: Year 1: 23,969 Year 2: 24,614

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 most populations tested, in decreasing order of strength of effect.
- Agri-Mek is expected to provide little control in the field; Acramite may still be moderately effective outside the Wenatchee River Valley.
- 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.
- There is evidence for cross-resistance between Onager (MOA 10A) and Zeal (MOA 10B). Where resistance to these materials occurred (lower Wenatchee River Valley), it was absolute.
- No evidence of resistance to Envidor was found in any population.

Results and Discussion: Obj. 1 - Survey

A total of 88 probit bioassays were performed on 9 twospotted spider mite populations, 8 collected from eastern Washington pear orchards, and 1 susceptible reference colony obtained from Cornell's Geneva Laboratory in New York. The latter has been reared in the laboratory for >15 years without exposure to pesticides. 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 considered closely related MOAs.

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. 24 °C (75 °F), and 16:8 light:dark photoperiod. Colonies were kept isolated in different rooms, and supplied with fresh bean plants every 2 weeks.

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

Each 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 dia.) 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 (Burkard Mfg, Rickmansworth, England) 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 LD_{10}, LD_{50}, LD_{90} and LD_{99} with 95% confidence intervals were retained (Table 2, Figs. 1a, b). 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 (RRs) are useful metrics in assessing the degree of resistance and likelihood for it to spread in the field. Resistance ratio 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.

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	New York TSM baseline	Calc LC_{50}	lower	upper	RR (New York)
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	C1-2014	0.004	329.82	212.821	698.98	**82,455**
Agri-Mek	C2-2014	0.004	116.05	64.10	170.53	**29,012**
Agri-Mek	D1-2014	0.004	165.67	117.072	230.602	**41,417**
Agri-Mek	O1-2014	0.004	11.31	6.235	18.371	**2,827**
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	**5**
Acramite	C1-2014	2.29	739.75	520.39	994.44	**323**
Acramite	C2-2014	2.29	2845.92	2299.65	3533.12	**1,244**
Acramite	D1-2014	2.29	125.23	86.34	188.59	**55**
Acramite	O1-2014	2.29	3.47	2.64	4.35	**2**

				95% CI		
Acaricide	TSM population	New York TSM baseline	Calc LC_{50}	lower	upper	RR (New York)
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**
FujiMite	C1-2014	1.29	8.90	6.793	11.198	**6.90**
FujiMite	C2-2014	1.29	15.19	13.09	17.43	**11.77**
FujiMite	D1-2014	1.29	4.43	3.49	5.46	**3.43**
FujiMite	O1-2014	1.29	3.84	2.57	5.24	**2.98**
Onager	C1-2013	0.014	0.51	0.37	0.74	**36**
Onager	C2-2013	0.014	0.39	0.34	0.45	**28**
Onager	C3-2013	0.014	1785.18	1573.97	1995.69	**127,513**
Onager	Y1-2013	0.014	0.42	0.29	0.51	**30**
Onager	C1-2014	0.014	2052.76	1806.379	2293.881	**146,626**
Onager	C2-2014	0.014	1182.94	1019.41	1367.30	**84,496**
Onager	D1-2014	0.014	0.15	0.11	0.18	**10**
Onager	O1-2014	0.014	0.24	0.18	0.28	**17**
Zeal	C1-2013	0.062	5.02	2.81	7.25	**81**
Zeal	C2-2013	0.062	5.77	5.00	6.47	**93**
Zeal	C3-2013	0.062	[x]			--
Zeal	Y1-2013	0.062	1.57	1.29	1.83	**25**
Zeal	C1-2014	0.062	[x]			--
Zeal	C2-2014	0.062	[x]			--
Zeal	D1-2014	0.062	0.313	0.443	0.639	**5**
Zeal	O1-2014	0.062	1.418	1.012	1.879	**23**
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	C1-2014	5.96	13.62	11.144	15.586	**2.28**
Envidor	C2-2014	5.96	6.43	5.66	7.21	**1.08**
Envidor	D1-2014	5.96	9.277	8.038	10.539	**1.56**
Envidor	O1-2014	5.96	7.559	6.311	8.809	**1.27**

[x]Unable to obtain significant mortality at 200,000 ppm AI (near limits of solubility).

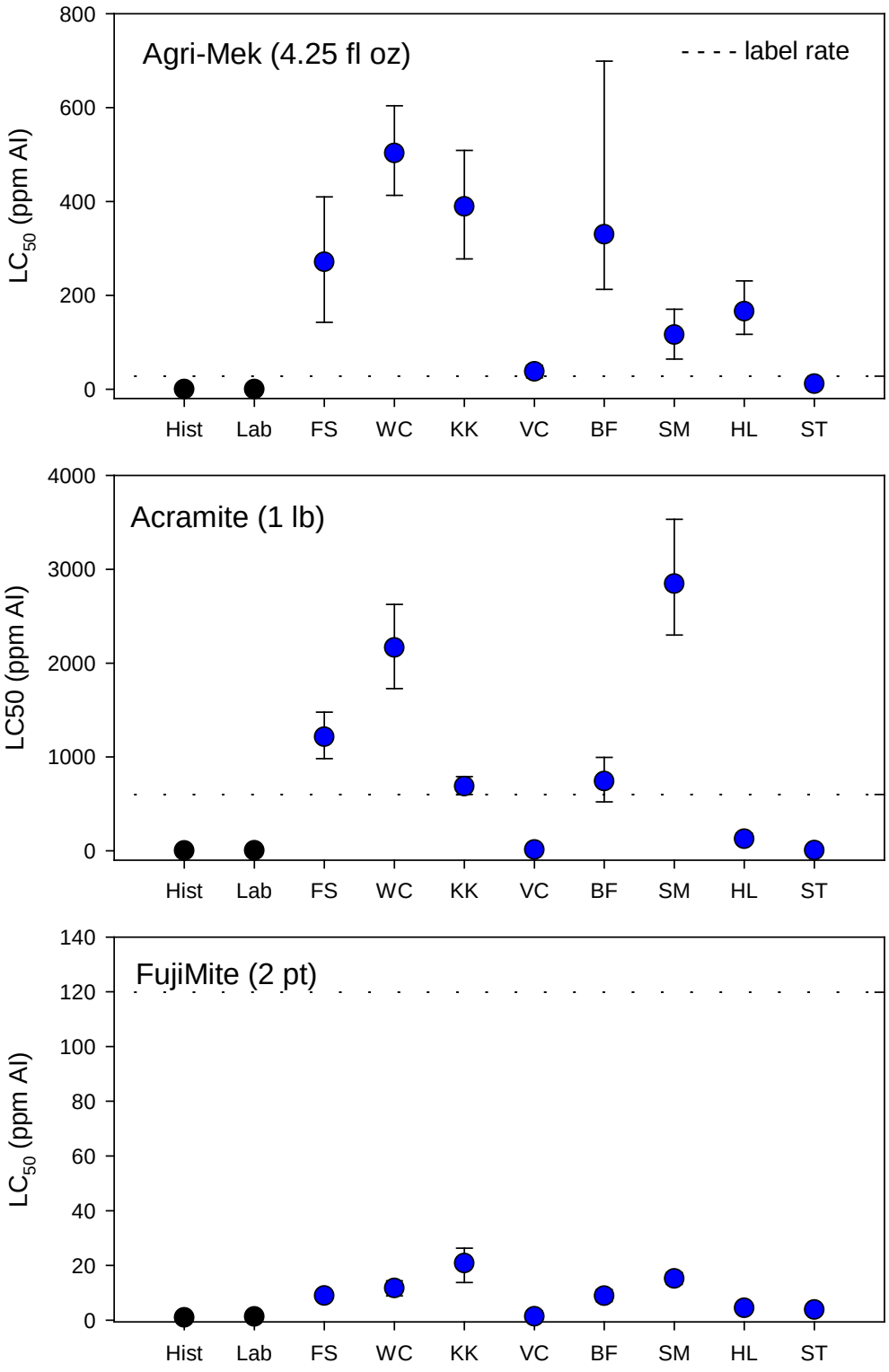


Fig. 1a. LC_{50}s of adulticidal acaricides for populations of twospotted spider mite from pear.

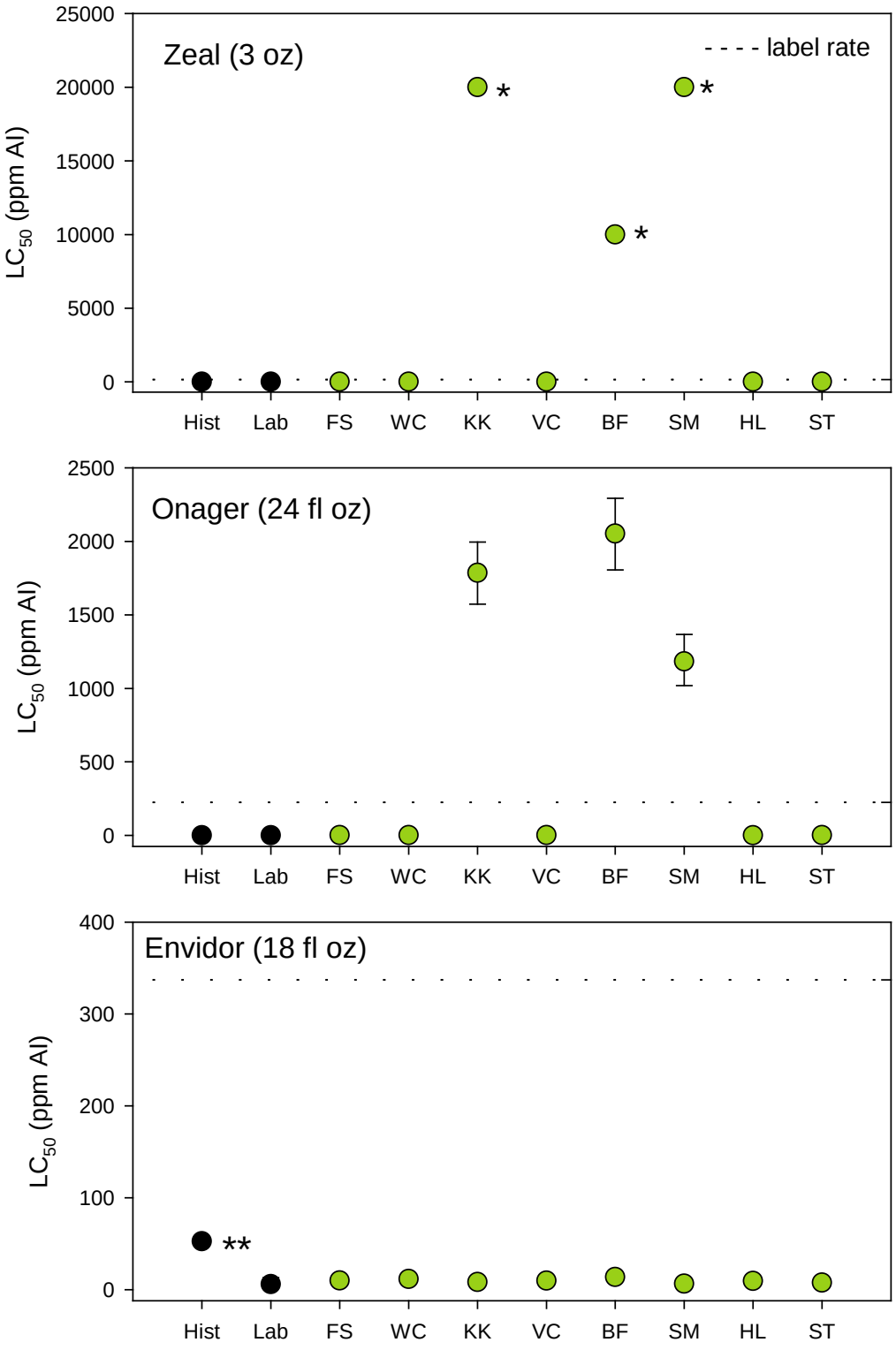


Fig. 1b. LC_{50}s of ovicidal acaricides for populations of twospotted spider mite from pear. LC_{50}s with single asterisks indicate the highest rate used when the probit bioassay failed due to resistance. The double asterisk indicates data from European red mite rather than twospotted spider mite.

Agri-Mek. The RRs for this material were extremely high for all populations tested (Table 2), ranging from ca. 2,827 to 125,760- fold increase in the LC_{50}. Of the mite populations examined, the lowest RR was from Okanogan county population; all those from Chelan County (in this case, the Wenatchee River Valley [WRV]), 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 72% (Fig. 2a).

Acramite. The RRs for Acramite were considerably lower than those for Agri-Mek (4.63-947). This material has been used for a much shorter period of time. However, with the exception of the Y1-2013 colony from Yakima and the O1-2014 colony for Okanogan county, 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 100% (Fig. 2b).

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

Onager. The RRs were quite variable for this material. Three of the populations (all from the WRV east of Dryden), were very high (84,496-146,626). Two other populations slightly to the west but still in the WRV growing region were much lower. All populations outside the WRV had low RRs (10-36), indicating some change in susceptibility. However, the predicted percentage mortality at the maximum label rate of Onager was 100% for all populations except the three resistant ones, where the predicted mortality was zero (Fig. 2d).

Zeal. The RRs for Zeal all indicated that a low to moderate level of resistance has occurred in five of the eight populations. Three of the WRV were highly resistant, such that no significant mortality was measured at 200,000 ppm AI, making the RR >3.2 million. The populations are the same ones 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 three highly resistant populations (0% predicted mortality).

Envidor. None of the populations tested showed any measureable resistance to Envidor; all RRs were <2.5. 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. All populations tested had a predicted mortality of 100% based on probit regression (Fig. 2f).

Results and Discussion: 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 moved 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.

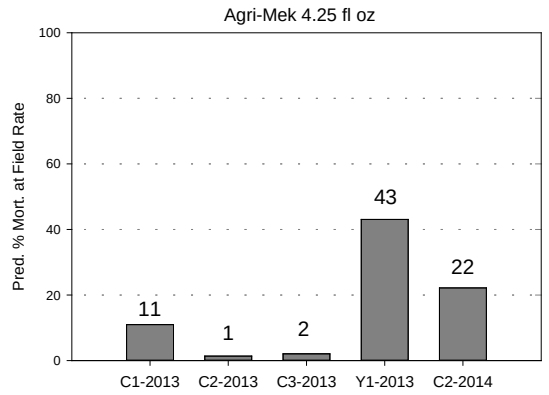


Fig. 2a. Predicted percentage mortality at the field rate of Agri-Mek (4.25 fl oz/acre).

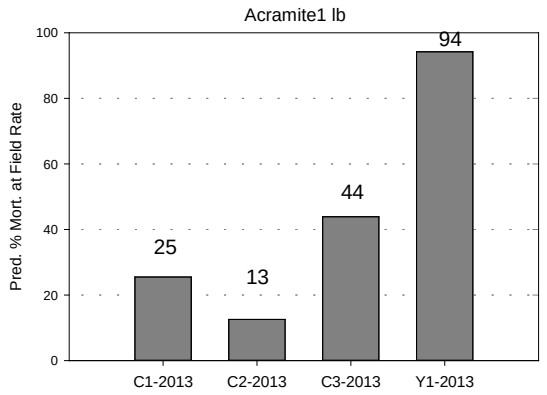


Fig. 2b. Predicted percentage mortality at the field rate of Acramite (1 lb/acre).

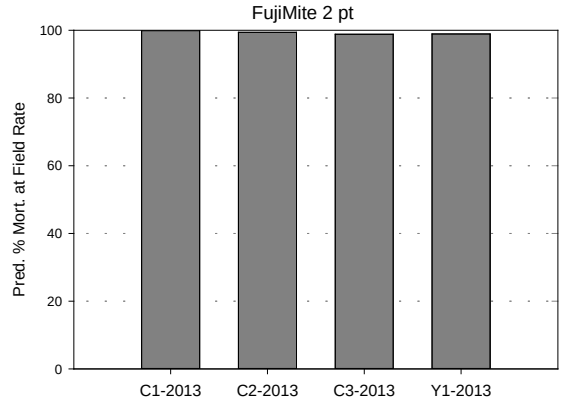


Fig. 2c. Predicted percentage mortality at the field rate of FujiMite (2 pt/acre).

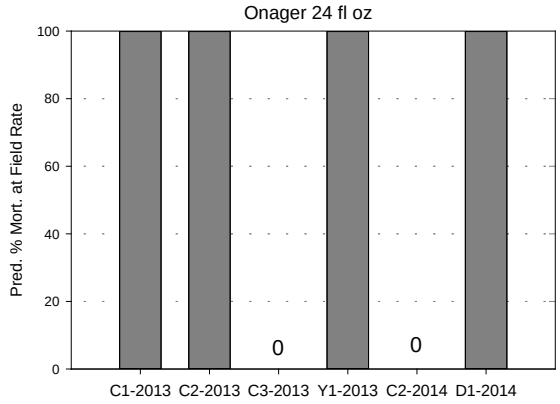


Fig. 2d. Predicted percentage mortality at the field rate of Onager (24 fl oz/acre).

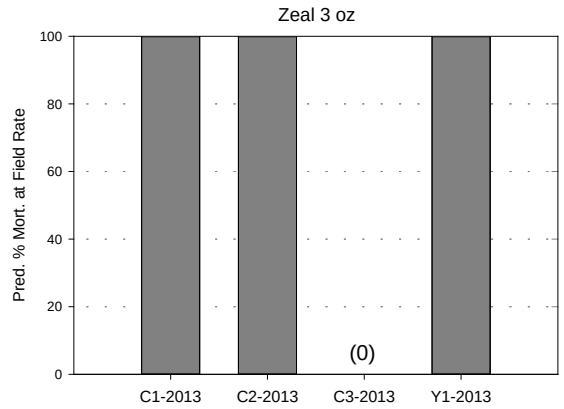


Fig. 2e. Predicted percentage mortality at the field rate of Zeal (3 oz/acre).

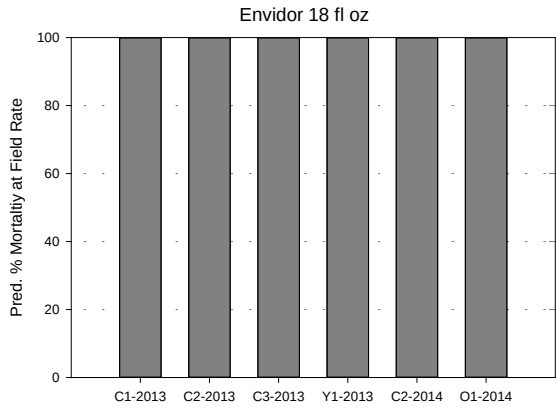


Fig. 2f. Predicted percentage mortality at the field rate of Envidor (18 fl oz/acre).

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 F_1 *T. urticae* females were placed on each disk. 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 F_1 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 set at 44.8 kPa using the intermediate nozzle.

Adulticide bioassays were held in a growth room at 22 °C (72 °F) and evaluated every 24 h for 3 days after treatment (DAT). Mites were counted as live, dead, runoff, or moribund. All juveniles from hatched eggs were 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. 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.

Crosses were performed with two of the resistant pear populations (FS and KK) with the lab (susceptible) colony, and the progeny were assayed with FujiMite. 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.

Twospotted spider mites are a haplodiploid species, which means males are produced from unfertilized (i.e., haploid) eggs and females are produced from fertilized (i.e., diploid) eggs. This differs from most generalist predators that feed on twospotted spider mites and are diploid, where both males and females are produced from fertilized eggs. The implications of haplodiploidy for resistance evolution are well known. If you assume resistance is controlled by two alleles, where R is recessive and S is susceptible, then diploid species have three genotypes: RR, RS, and SS. This is important for resistance evolution because most of the resistance alleles in diploids are carried by heterozygotes (i.e., RS). If these individuals are killed by the pesticide because dominance is recessive then resistance will evolve slowly. However, in haplodiploid species more resistance alleles are carried by homozygotes (i.e., male R or female RR), and this speeds up resistance evolution.

As an example of the impacts of haplodiploidy, assume that a pesticide kills >90% of susceptible individuals and 0% of resistant individuals (such as Agri-Mek), and the initial frequency of resistance is low (1 allele out of 1,000). If the pesticide is sprayed on 90% of orchards then a diploid species will take more than 100 generations to evolve resistance under these conditions, while a haplodiploid species will evolve resistance in less than 10 generations. Moreover, if our hypothetical pesticide is used on only 50% of pear acreage then the diploid species would not be expected to evolve resistance in over 1,000 generations while the haplodiploid species would be expected to evolve resistance in

20-30 generations. These differences are staggering, and indicate that the genetic pre-disposition of haplodiploid species to evolve resistance to pesticides is one reason species such as mites and whiteflies are such major crop pests.

Table 3. Percentage mortality ± SE for the offspring of KK ♀ crossed with NY♂ treated with FujiMite.

Conc (mg AI/liter)	% Mortality 1 DAT	2 DAT	3 DAT
75	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
12	81.89 ± 6.60	83.00 ± 5.15	88.89 ± 5.09
9	74.00 ± 7.97	73.24 ± 6.07	85.74 ± 6.24
7	65.00 ± 2.24	67.68 ± 6.43	74.89 ± 7.84
4.55	52.00 ± 7.00	42.42 ± 6.80	45.00 ± 6.89
0.06	5.00 ± 1.58	9.16 ± 3.31	17.00 ± 4.06
0	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 2.22

Table 4. Percentage mortality ± SE for FS ♀ crossed with NY♂treated with FujiMite.

Conc (mg AI/liter)	% Mortality 1 DAT	2 DAT	3 DAT
23	98.82 ± 1.18	100.00 ± 0.00	100.00 ± 0.00
6	96.95 ± 2.01	95.89 ± 1.96	98 ± 1.22
0.6	38.97 ± 3.87	37.04 ± 4.10	41.53 ± 3.69
0.06	8.33 ± 3.79	5.23 ± 2.74	11.56 ± 4.38
0	2.23 ± 1.37	4.11 ± 1.89	5.22 ± 2.30

Table 5. Survival (*W*) and dominance of inheritance (*h*) calculations for doses of FujiMite tested against the KK cross.

Conc (mg AI/liter)	W_r	W_s	W_h	*h*
75	0.05	0.01	0.00	0.00*
12	0.75	0.09	0.11	0.03
9	0.85	0.12	0.14	0.03
7	0.92	0.15	0.25	0.13
4.55	0.97	0.21	0.55	0.44

Table 6. Survival (*W*) and dominance of inheritance (*h*) calculations for doses of FujiMite tested against the FS cross.

Conc. (mg AI/liter)	W_r	W_s	W_h	*h*
7	0.76	0.17	0.02	0.00*
4.55	1.00	0.61	0.58	0.00*
0.06	1.00	0.94	0.88	0.00*

* Values rounded up to 0.0

Executive Summary

A survey of twospotted spider mite from pear orchards indicated that resistance to several acaricides is present in moderate to high levels. As a group the adulticides were more affected by resistance than the ovicides. Of the adulticides, Agri-Mek (a material used for both mites and pear psylla since the late 1980s) had the highest resistance ratios (RRs), and mite control at the field rate is predicted to be poor in most orchards. Acramite also had had high levels of resistance in all populations in Chelan County, while those from Yakima and Okanogan counties showed only a minor increase in resistance. FujiMite overall had the lowest RRs, and is predicted to give good control at the field rate.

Of the ovicides, only three populations from the Wenatchee River Valley showed a significant level of resistance to Onager and Zeal; all other populations appeared to be susceptible. However, the populations that were resistant to Zeal and Ongager were virtually immune to this product. The resistance to Zeal and Onager appear to be related, which is not surprisingly given that they are have closely related MOAs. All populations tested were susceptible to Envidor, the most recently introduced miticide. However, it is in the same MOA group as Ultor, which has also been widely adopted in pear production, and caution is advised in its use.

With the exception of Agri-Mek, all of the acaricides tested are limited by their labels to a single application per year, presumably for the purposes of resistance management. Despite this, the development of resistance in spider mite populations appears to be progressing rapidly in pear orchards.

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

Project Title: Delivering quality pear fruit to consumers

PI: Yan Wang
Organization: MCAREC
Telephone: 541-386-2030 x38214
Email: yan.wang@oregonstate.edu
Address: 3005 Experiment Station Dr
City/State/Zip: Hood River, OR97031

Cooperators: Steve Castagnoli, Todd Einhorn, David Sugar, Paul Chen
Drs. Yu Dong, Xingbin Xie, Shunchang Cheng, Yingli Li, Shaoying Zhang

Total Project Budget: Year 1: 25,725 **Year 2**: 26,390 **Year 3**: **27,073**

Other funding sources: None

Budget 1
Organization Name: Agricultural Research Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2015	2016	2017
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	672[4]	692	712
Equipment			
Supplies	3,500[5]	3,500	3,500
Travel	500[6]	500	500
Miscellaneous			
Total	25,725	26,390	**27,073**

Footnotes:
[1]Postdoctoral Research Associate: 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: 10% of the wage, with a 3% annual increase.
[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. Elucidate the cell metabolic mechanisms and pre/postharvest factors affecting the development of buttery-juicy melting texture (BJMT) during ripening of pears.
2. Study pre/postharvest factors influencing the chilling requirement for ripening capacity (CRRC) of pears.
3. Develop conditioning protocols for 1-MCP treated 'Anjou' pear.

SIGNIFICANT FINDINGS

1. Mechanisms of developing buttery-juicy melting texture (BJMT)
    a. Water soluble polyuronides (WSP), the metabolic product of pectin, is positively correlated with BJMT. WSP are hygroscopic and give consumer the BJMT feeling.
    b. Ethylene synthesis is the trigger of pectin metabolism.
    c. A BJMT index is developed based on extractable juice (EJ, mL/100g). BJMT index = (100-EJ)/10.
2. Factors affecting the development of BJMT
    a. Accumulated cold unit (ACU = hours < 50°F during 42d prior to harvest). The higher ACU (0-300), the greater BJMT.
    b. Fruit tissue Ca content. The higher Ca content (500-850ppm), the greater BJMT.
    c. Harvest maturity. FF=15-16 lb: good BJMT but less flavor; FF=14-15 lb: excellent BJMT and flavor; FF=12-13 lb: inferior BJMT and flavor.
    d. CA storage. Anjou pear fruit developed excellent BJMT following 3-5 months in RA; 3-8 months in regular CA (1.5% O_2 + <0.05% CO_2); 3-10 months in Low-O_2 CA (0.8-1% O_2 + <0.05% CO_2).
3. Factors affecting chilling requirement for ripening capacity (CRRC)
    a. ACU (0-300) affects CRRC of 15-30d at the optimum harvest maturity. The higher ACU, the less CRRC.
    b. Ca content (500-850ppm) affects CRRC of 15-20d. The higher Ca content, the longer CRRC.
    c. Harvest maturity (FF=15-11 lb) affects CRRC of 20-90d. More mature, less CRRC.
    d. Ethylene conditioning. Ethylene at 100ppm for 72h at 68°F can eliminate CRRC. However, the BJMT is inferior in the ethylene conditioned than chilled fruit.
    e. However, the BJMT of the fruit using late-harvest, intermediate storage temperature, ethylene conditioning, or their combinations for reducing or eliminating CRRC is inferior to the fruit that are harvested at 15-14 lb and stored at 30°F after 60-90d.
4. Conditioning protocols for 1-MCP treated 'Anjou' pear
    a. Late-harvest at FF=12-13 lb helps ripening of the 1-MCP treated Anjou pear while controlling scald.
    b. A post-storage ethylene conditioning (PSEC) (100ppm for 72h at 68°F) improved ripening capacity of the 1-MCP treated Anjou pear after a long-term RA or CA storage (i.e. > 7-8 months).

METHODS

See protocol

RESULTS

1. Mechanisms of ripening capacity and BJMT

a. Cell-wall pectin metabolism. Harvested at 14-15lb, Anjou pear had no ripening capacity in 7d at 68°F following 1-2 months, developed excellent BJMT following 3-5 months, but had a dry-coarse texture following 6-8 months in RA storage at 30°F. We found that water soluble polyuronides (WSP) content was positively correlated with the development of BJMT (Fig. 1). Total pectin, EDTA soluble pectin, and alkali soluble pectin were not found to be correlated with BJMT (data not shown).

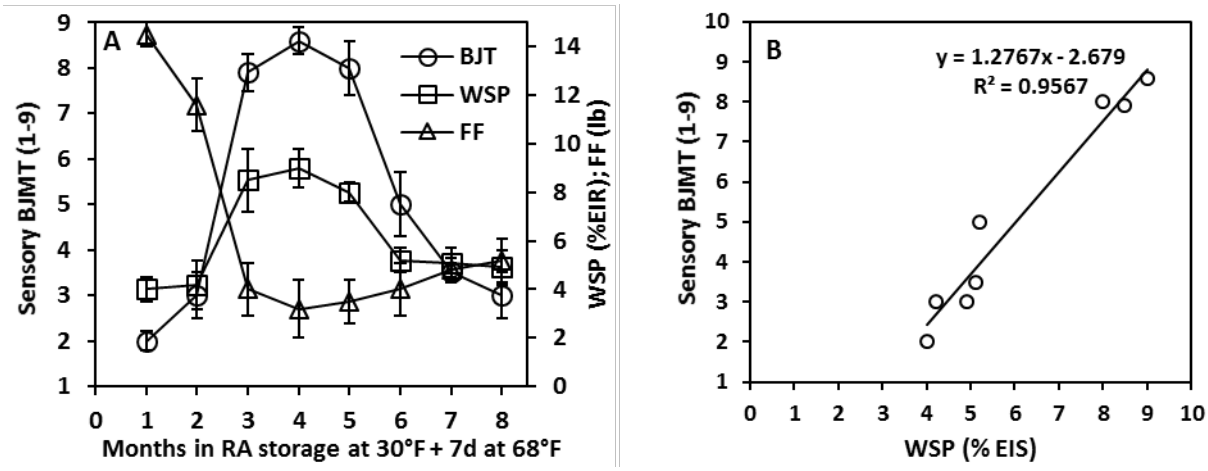


Fig. 1. Sensory buttery-juicy melting texture (BJMT) score, water soluble polyuronides (WSP), and fruit firmness (FF) (A) and the correlation of BJMT with WSP (B) of Anjou pear in 7d at 68°F following 8 months storage in regular air (RA) at 30°F.

b. Ethylene. Ethylene synthesis is the trigger for developing ripening capacity and pectin metabolism. *PcACO1* is the critical gene for developing ripening capacity and a good indicator for WSP production and BJMT of Anjou pear (Fig. 2).

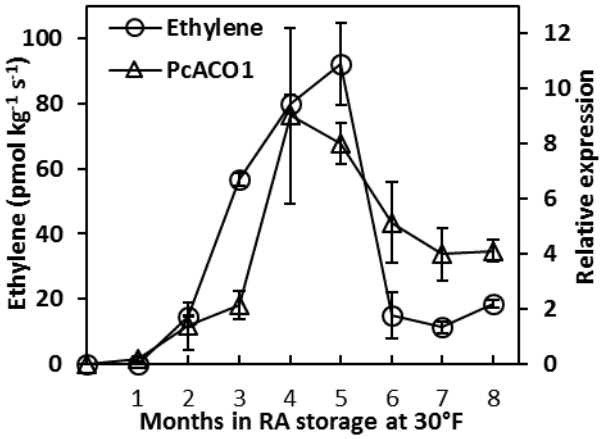


Fig. 2. Ethylene synthesis and expression of the gene PcACO1in Anjou pear following 8 months storage in regular air (RA) at 30°F.

c. BJMT index. Softening in Anjou pear may occur without development of optimum dessert quality (Fig. 1A). Measuring WSP is tedious. Measuring extractable juice (EJ) is relatively simple

and EJ is negatively correlated with WSP and BJMT of Anjou pear (Fig. 3A). A BJMT index is developed based on EJ (ml/100g) and is an objective measurement for BJMT: BJMT index = (100-EJ)/10. The BJMT index is positively correlated with sensory BJMT score (Fig. 3B).

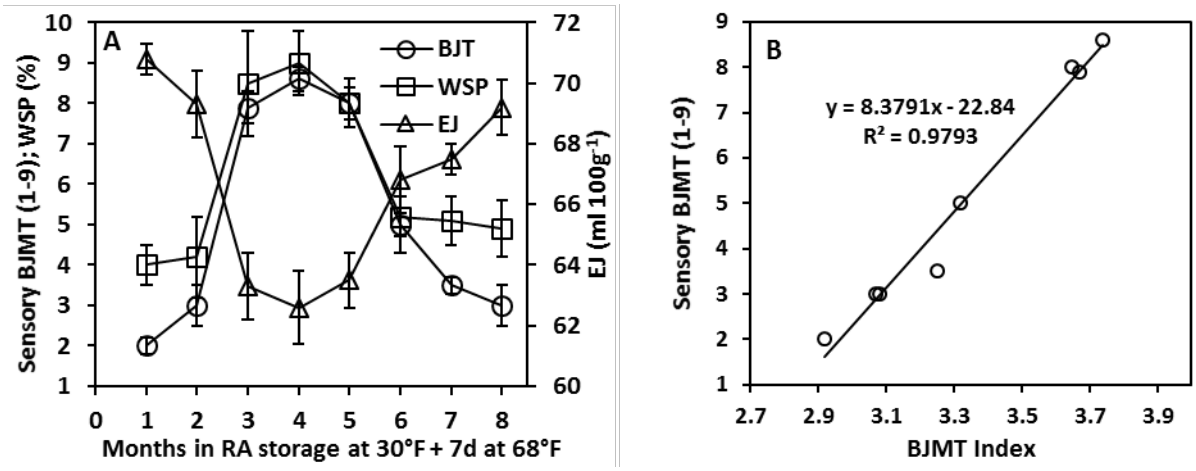


Fig. 3. Sensory buttery-juicy melting texture (BJMT) score, water soluble polyuronides (WSP), and extractable juice (EJ) (A) and the correlation of sensory BJMT score with BJMT index (B) of Anjou pear in 7d at 68°F following 8 months storage in regular air (RA) at 30°F.

3. Factors affecting BJMT

a. ACU. Fruit were harvested at ~15lb from orchards of different elevations from ~500 to ~2,000ft with received ACU from 0 to 269. In general, the higher ACU, the greater WSP content and BJMT index (Fig. 4). The fruit received ACU > ~200 developed significant higher WSP and BJMT index than fruit with ACU < ~200.

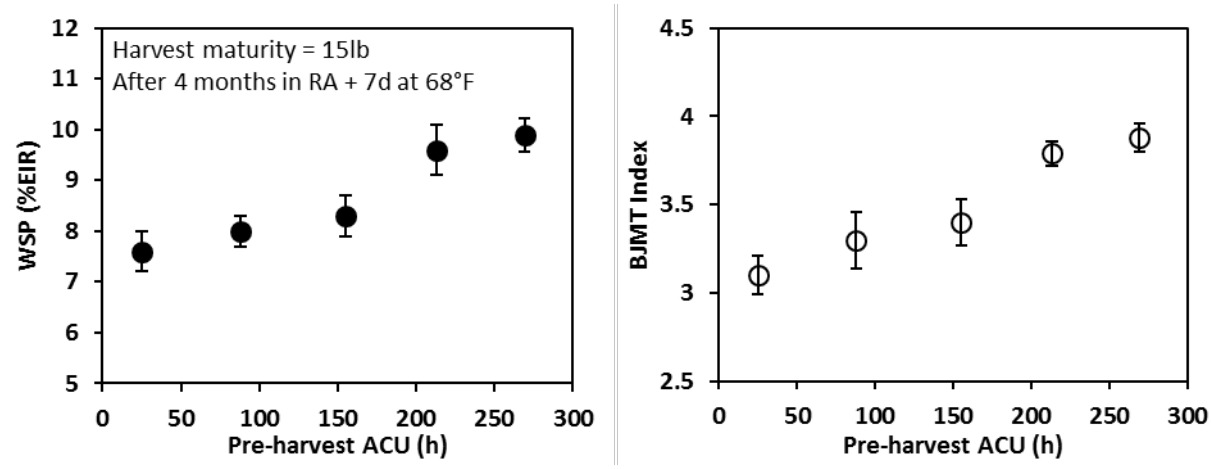


Fig. 4. Water soluble polyuronides (WSP) and buttery-juicy melting texture (BJMT) index affected by pre-harvest accumulated cold unit (ACU) of Anjou pear after 4 months storage in regular air (RA) at 30°F plus 7d at 68°F.

b. Ca content. (data will be reported in the final report)

c. Harvest maturity. Anjou pear harvested at 13-12lb had a shorter buttery-juicy texture storage life, such as 3-4 months in RA or 5-6 months in CA. Anjou pear harvested at 15-14lb developed better buttery-juicy texture than that harvested at 13-12lb in 7d at room temperature

following 3-5 months in RA and 3-8 months in regular CA at 30°F. Fruit harvested at 17-16lb developed good buttery-juicy texture, but inferior flavor (taste and aroma) and unacceptable shriveling during storage.

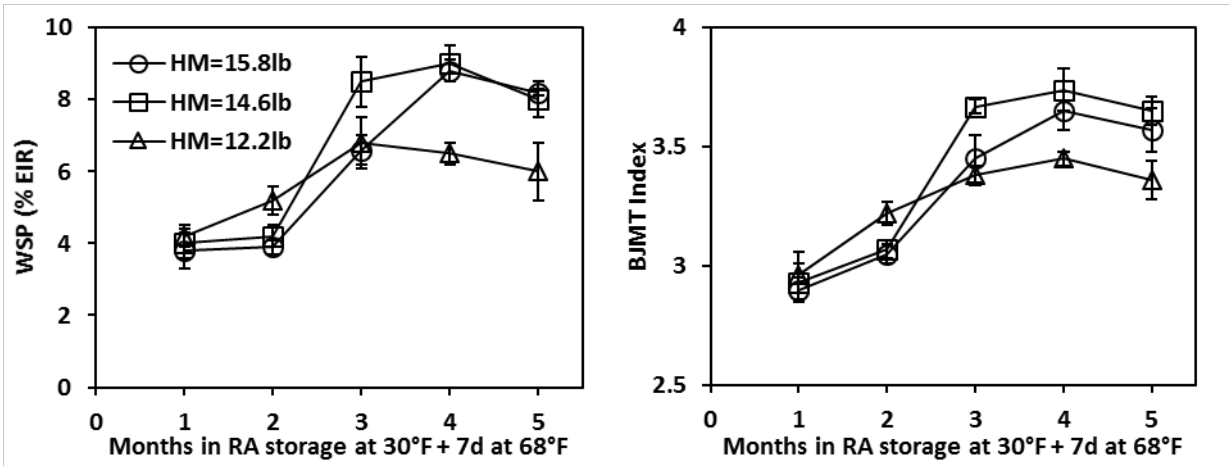


Fig. 5. *Water soluble polyuronides (WSP) and buttery-juicy melting texture (BJMT) index affected by harvest maturity of Anjou pear in 7d at 68°F following 5 months storage in regular air (RA) at 30°F.*

b. CA storage. Sensory and objective evaluations indicated that Anjou pear harvested at 15-14lb from MCAREC (500ft, Mid-Columbia area) developed BJMT in 7d at 68°F following 3-5 months in RA; 3-8 months in regular CA (1.5% O_2 + < 0.05% CO_2); and 3-10 months in Low O_2 CA (0.8-1.0% O_2 + < 0.05% CO_2) at 30°F (Fig. 6). They developed a dry-coarse mealy texture after 6 months in RA, 9 months in regular CA, and 11 months in Low-O_2 CA.

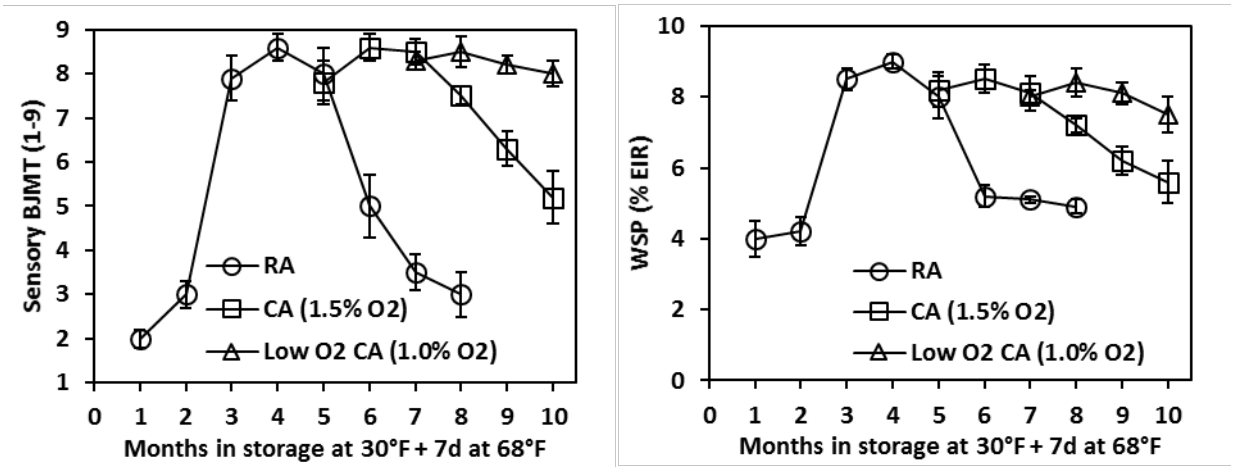


Fig. 6. Sensory buttery-juicy melting texture (BJMT) and water soluble polyuronides (WSP) content of Anjou pear in 7d at 68°F following 1-10 months storage in regular air (RA), regular CA (1.5% O_2 + < 0.05% CO_2), and Low O_2 CA (0.8-1.0% O_2 + < 0.05% CO_2) at 30°F.

3. Factors influencing chilling requirement for ripening capacity (CRRC)

a. ACU and harvest maturity. Production elevation and harvest maturity influenced CRRC significantly (Fig. 7A). Fruit from low elevation (i.e., 500ft) required longer CRRC than that from higher elevation (i.e., 2,000ft) at the same harvest maturity. For examples at harvest maturity of 15lb, it needs 85, 60, and 55 days at 30°F to induce ripening capacity for fruit produced at 500, 1,000, and

2,000 ft, respectively. When harvest at 12lb, it needs 45, 35, and 30 days at 30°F to induce ripening capacity for fruit produced at 500, 1,000, and 2,000 ft, respectively.

A preliminary analysis of the first year data indicated that the ACU affects CRRC in Anjou pear (Fig. 7B). ACU and CRRC will be collected from different orchards at varied elevations (from ~500 to ~2,000ft) in multiple years (2015, 2016, and 2017). A model may be developed to predict 'Anjou' pear CRRC at the time of harvest based on ACU.

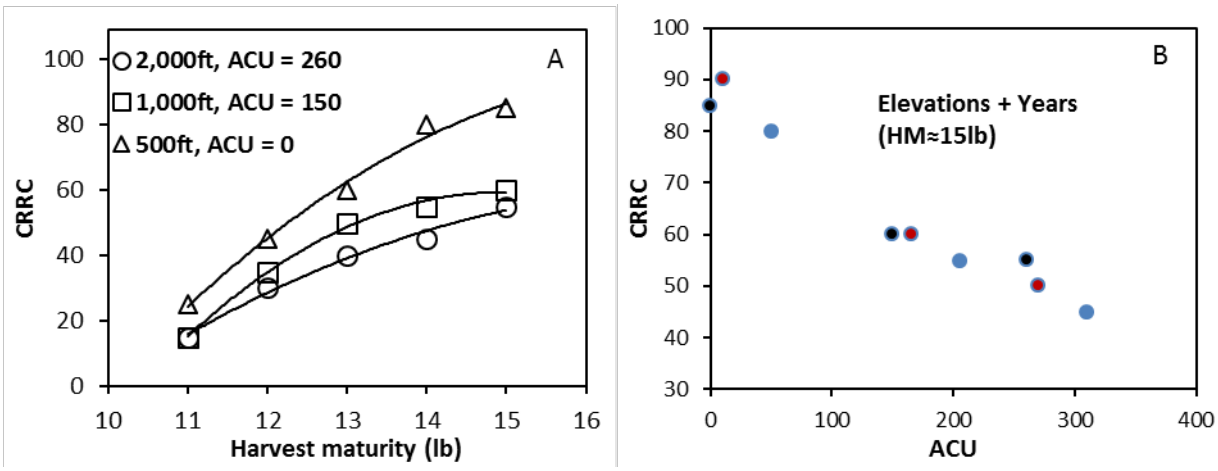


Fig. 7. Relationship of the chilling requirement for ripening capacity (CRRC) with harvest maturity, production elevation, production year, and accumulated cold unit (ACU) in Anjou pear.

b. Ca content. (data under analysis)

Preliminary analysis indicated that Anjou pear with low Ca content (i.e. < 500-600ppm dw) requires shorter CRR, but reduces storability significantly. Anjou pear with high Ca content (i.e., ≥ 900ppm) requires ≥ 90d CRR.

c. Temperature and ethylene conditioning. (data under analysis)

3. Conditioning protocols for 1-MCP treated Anjou pear (data under analysis)

a. Late-harvest at FF=12-13lb helps ripening of the 1-MCP treated Anjou pear while controlling scald.

b. The combo treatment of 1-MCP (300ppb) + ethylene (300-600ppb) improves ripening capacity of the 1-MCP treated Anjou pear while controlling scald.

c. A post-storage ethylene conditioning (PSEC) (100ppm for 72h at 68°F) improved ripening capacity of the 1-MCP treated Anjou pear after a long-term storage (i.e. > 7-8 months).

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

Project Title: Evaluation of potential, new pear cultivars for the PNW

PI: Todd Einhorn
Organization: Michigan State University
Telephone: 517 353 0430
Email: EinhornT@MSU.edu
Address: 1066 Bogue St
City: East Lansing
State/Zip: MI 48824

Co-PI (1): Tom Auvil
Organization: WTFRC
Telephone: (509) 665-8271
Email: Auvil@treefruitresearch.com
Address: 1719 Springwater Ave
City: Wenatchee
State/Zip: WA 98801

Co-PI (2): Richard Bell
Organization: USDA-ARS
Telephone: 304 725 3451 x 353
Email: Richard.Bell@ars.usda.gov
Address: 2217 Wiltshire Road
City: Kearneysville
State/Zip: WV 25430

Budget: **Year 1:** $12,578 **Year 2:** $17,334 **Year 3: $19,415**

Cooperators: Kate Evans

Other funding sources: None

Budget 1: Todd Einhorn
Organization Name: MSU **Contract Administrator:** Greta McKinney
Telephone: **Email address:** mckin134@anr.msu.edu

Item	2015	2016	2017
Salaries[1]	2,291	4,720	4,862
Benefits	1,535	3,162	3,257
Wages[2]	0	0	1,040
Benefits	0	0	104
Equipment	0	0	0
Supplies[3]	500	500	500
Travel	0	0	0
Miscellaneous[4]	1,552	1,552	1,552
Total	5,878	9,934	**11,315**

Footnotes: **[1]Salaries are calculated as 5% of technician time (2.5 weeks) in year 1 and 10% of technician time in years 2 and 3 (5 weeks). The increase in salary in year 2 reflects a 3% rate increase. Benefits are calculated using OPE rate of 66%. [2]Wages are for part-time employee help harvesting fruit and general maintenance during the season; 80 hours at $13/hr. Part-time employee benefits are calculated at 10%. [3]Supplies are for tree training. [4]Miscellaneous costs account for MCAREC plot fees at a rate of $3,103/acre, prorated to 1/2 acre for field on-site field trials.**

Budget 2: Tom Auvil
Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
Telephone: 509-665-8271 **Email address:** Kathy@treefruitresearch.com

Item	2015	2016	2017
Salaries	3,000	3,500	4,000
Benefits	1,200	1,400	1,600
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	1,000	1,000	1,000
Travel[1]	500	500	500
Miscellaneous[2]	1,000	1,000	1,000
Total	6,700	7,400	**8,100**

Footnotes: **[1]Ten trips to Wapato/Dryden from mid-August through mid-Oct. [2]RCA cold storage room charges.**

Objectives:

1. To test five new scion selections from the USDA-ARS pear breeding program in small-scale plantings in WA and OR.

2. To test two new pear cultivars from Prevar, Australia, in medium-scale plantings in WA and OR.

Significant Findings:

Objective 1

- 2016 was the second cropping year for fourth-leaf USDA-ARS scion selections. All scions fruited at Hood River. Only one of the two WA locations had fruit, but not all scions had a sufficient number of fruit to evaluate. Trees of 84907-078 had high mortality at the WA sites.
- 84907-166, which flowered profusely and produced attractive, blushed fruit in 2015, continued to produce similar yields and fruit size as Bartlett.
- Fruit size differed between locations despite being harvested at similar maturity (based on flesh pressure). In all cases, fruit size was markedly larger at Hood River. The exception was scion 84907-078, which was small at both sites (~140 g).
- We were informed by Dr. Richard Bell (USDA-ARS) that all selections tested positive for viruses. Consequently, information gleaned from these trial evaluations may not adequately represent the selections attributes in a 'virus-free' condition.
- With the exception of 014 (Gem) and 84907-166, these selections appeared to lack promising attributes (yield, fruit quality, harvest timing, etc.) that distinguish them from the current suite of commercially produced cultivars. The exception being fire blight resistance.
- Based on the two preceding points, we propose to terminate Objective 1. Potentially, 84907-166 could be heat-treated to produce virus-free material for future evaluation.

Objective 2

- Tree growth in Hood River continued to be strong in 2016 (3rd leaf) despite small tree sizes at planting and poor growth in the establishment year. Tree growth at both WA sites was recovering.
- Fruiting did not occur at the WA sites. Minimal fruiting (~6 fruits/tree) occurred in 2016 at Hood River. Given the large, multi-tree replicates, enough fruits were produced to evaluate on two separate pick dates.
- Fruit size of 0118 which harvested ~2 weeks before 'Bartlett', was small (~135 g) and did not improve between the first and second pick (~1 week apart).
- 0131 harvested ~3 weeks after 'Bartlett'. Fruit size was relatively larger than 0118, but on the small end of commercial range (180 g). After 2 months of cold storage and 7 days of ripening conditions, fruit did not ripen to dessert quality (~6 lbs), indicating that this cultivar may require additional chilling to attain ripening competency. Alternatively, 0131 could potentially be consumed crisp, as a ready-to-eat pear but its quality in this condition was not evaluated.

Results:

1. USDA-ARS cultivars. For most selections, tree size was about 2/3rds the size of Anjou trees and similar or slightly smaller than 'Bartlett'. Selection 069, however, appears to be a weak tree (~50% of 'Bartlett'); this condition is likely a result of virus infection. In Hood River, we observed a wide range

of precocity among the four scions evaluated in 2015 (3rd leaf); 166 >> 038 = 069 > 078. In 2016, all scions produced a fair amount of flowers; the exception being 078. However, 078 still produced double the flowers as Anjou. All scions bloomed with Anjou, except 166, which bloomed with Bartlett. Fruit set was highest for 078, followed by 069 and 166. Fruits were not hand thinned as was performed in 2015 since the crop loads were deemed adequate for tree sizes. Fruit maturity was monitored weekly via firmness measurements beginning mid-July based on preliminary data from 2015 and information from Dr. Richard Bell. Anjou did not have sufficient fruit to evaluate; all other selections produced enough fruit and, in OR, fruit of 038 and 069 were divided over two pick dates. WA picked all fruit when Bartlett reached commercial harvest maturity. Fruit size was variable between sites and genotypes. 038 is a small-fruited genotype- too small for commercialization. 078 trees performed poorly in WA and fruits were unattractive at harvest in Hood River. Additionally, 078 was not precocious in 2015 compared to other selections. 166 had large fruit and produced yields similar to 'Bartlett' in OR. In WA, fruit size of 166 was small.

2016, 4th leaf production for 4 USDA-ARS pear selections compared to standard cultivars at OSU- MCAREC, Hood River, OR.

Genotype	Tunk size (cm^2)	Flower clusters (no./tree)	Fruits/cluster (%)	Harvest (date)	Yield/tree (no. fruit)	Fruit wt. (g)	SSC (%)	TA (%)	FF (lbs)
69426-038	27.9	149	19.82	21-Jul	27.4	131.36	12.6	0.3326	13.49
69426-038				28-Jul		156.54	12.3	0.3039	12.18
84907-069	16.3	124.2	43.58	28-Jul	37.2	215.82	11.7	0.3166	13.36
84907-069				4-Aug		241.6	11.3	0.3125	12.94
84907-078	29.6	82.8	112.24	3-Aug	59.6	193.92	12.3	0.3489	11.81
84907-166	25.6	115	37.86	3-Aug	37.6	269.18	11	0.388	14.49
Anjou	40.8	34	10.95	n.a.	3.5				
Bartlett	31.2	180	29.12	3-Aug	44.8	275.78	12.2	0.3751	18.42
Bosc	6.0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

2016, 4th leaf production for 4 USDA-ARS pear selections compared to standard cultivars at Blewitt Pass, WA.

Genotype	Yield/tree		Fruit wt.	Trunk size	SSC	TA	FF	Yield Effic.
	(no. fruit)	(lbs)	(g)	(cm^2)	(%)	(%)	(lbs)	(kg/cm^2 tca)
84907-166	40.8	14.1	160.5	12.9	8.8	0.3	14.6	0.46
69426-038	20.0	6.2	148.6	18.3	10.5	0.21	12.4	0.17
84907-078	4.6	1.4	111.2	18.4	9.4	0.2	20.1	0.03
84907-069	17.5	5.9	173.3	11.0	8.7	0.2	13.8	0.21
71655-014	56.4	21.6	185.5	21.3	11.2	0.35	11.5	0.46
Bartlett	32.3	16.9	203.7	19.5	10.0	0.32	19.6	0.37

In Hood River, all selections attained moderate SSC (~ 12%); however, in WA SSC was extraordinarily low. Titratable acidity was fairly low for all scions, but higher in OR. Although crop loads were not high in WA, clearly issues related to tree health/photosynthesis limited carbohydrate partitioning to fruit as evident by the small fruit sizes and low SSC and TA. Given that maturity was at the correct range (with the exception of 078), we attribute these issues to virus infection.

Given that all scions are considered summer pears (R. Bell, personal communication), we expected that they would ripen to a soft juicy texture after a few months of cold storage (i.e., below 4 lbs flesh pressure). Despite 2 months of postharvest cold storage in OR, most selections barely reached an acceptable firmness level (see table on next page). In WA, only 2 weeks of cold storage was provided prior to ripening. While these selections are all considered 'summer' pears, they would benefit from longer cold conditioning. In WA, 014 and 166 did not soften to acceptable dessert quality. 014 (aka, Gem) requires 30 days of chill to soften (Einhorn and Wang, *Journal of the*

American Pomological Society, 2016), so ripening would not have been expected after 2 weeks. An informal evaluation of flavor was performed after ripening. In Hood River, fruits of all scions were generally considered acceptable and possessed a relatively similar flavor profile as Bartlett. WA evaluated fruit using a 3-point scale where a value of 1 represented good flavor, a 2 represented no flavor, and a 3 represented off-flavor. Generally, the selections ranked around 1.5 and were similar to Bartlett.

	2 months RA cold storage			+ 7 days at room temp.		
Genotype	SSC (%)	TA (%)	FF (lbs)	SS (%)	TA (%)	FF (lbs)
69426-038	13	0.24	12.5	12.7	0.17	4.3
84907-069	12.8	0.27	12.9	13	0.26	3.5
84907-078	13.3	0.19	10.4	13.7	0.18	3.8
84907-166	12.3	0.61	14.1	12.7	0.47	6.0
Anjou	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bartlett	12.6	0.35	18.6	13.1	0.38	2.5
Bosc	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

In light of the above and in an effort to save valuable time and resources, we propose to discontinue this objective. 166 is a selection with promising attributes: precocity, consistent productivity, attractive finish, and fire-blight resistance. Hence, 166 should be submitted to Clean Plant Network to undergo heat therapy so virus free material can be developed for future evaluation.

2. Australian (Prevar) cultivars.

We propose to continue with training and development of the Prevar cultivars. WA did not receive the 0131 trees expected this spring from the nursery. We will meet with a Prevar representative to determine the timeline to build these trees. All trees were exceptionally small when planted in 2014 and despite limited growth during the establishment year (at all sites), good growth was observed in 2016 in Hood River, OR. In WA, no mortality was reported but continued poor growth in 2015 limited production in 2016. In OR, trees of both selections yielded roughly 6 fruits per tree. Given the multi-tree reps, this enabled multiple picks in an effort to identify optimal harvest timings under PNW conditions. The first harvest was based on flesh pressures identified in Australia to represent maturity.

0118 is an early-maturing cultivar, harvesting ~ 2 weeks prior to Bartlett. Fruit size, however, was quite small, in the range of 'Seckel' or 'Forelle'. The parentage of both selections is 'Corella', which resembles and is closely related to 'Forelle'. Providing an additional week on the tree did not improve fruit size of 0118. 0131 is a later-maturing cultivar, which harvested ~ 3 weeks after 'Bartlett (i.e., Anjou timing). Fruit size was equivalent to a 110 box size. SSC and TA levels at harvest were moderate.

2016, 3rd leaf production for two Prevar, Australian pear selections at OSU- MCAREC, Hood River, OR.

Genotype	Tunk size (cm^2)	Flower clusters (no./tree)	Fruits/cluster (%)	Harvest (date)	Yield/tree (no. fruit)	Fruit wt. (g)	SSC (%)	TA (%)	FF (lbs)
118	16.3	4.9	154.9	21-Jul	6.3	132.0	12.4	0.31	12.9
118				28-Jul		134.3	12.4	0.31	10.4
131	14.3	7.6	98.5	18-Aug	7.4	175.1	12.8	0.46	14.9
131				24-Aug		180.6	12.0	0.40	13.4

Following 2 months of RA cold storage, fruit were assessed for quality and then exposed to a 7-day ripening treatment and evaluated for their ripened quality. 0118 fruits softened to acceptable dessert texture. 0131 fruits did not soften to a soft-buttery texture. 0131 has been characterized as a 'ready-to-eat' European pear. We did not sample pears directly at harvest or prior to ripening given the relatively low fruit volume this year. We will, however, assess this cultivar's attributes in both the fresh and ripened condition in 2017.

2016, 3rd leaf PH quality of Prevar, Australian pear selections at MCAREC, OR.

	2 months RA cold storage			+ 7 days at room temp.		
Genotype	SSC (%)	TA (%)	FF (lbs)	SS (%)	TA (%)	FF (lbs)
118 Harvest 1	13.2	0.34	11.0	13.6	0.29	3.3
118 Harvest 2	13.3	0.28	9.6	13	0.25	3.1
131 Harvest 1	13.5	0.50	15.1	14	0.50	6.1
131 Harvest 2	14	0.34	14.1	13.8	0.44	8.8

Given that 'Corella' is in the parentage of both cultivars, we have concern regarding its susceptibility to fire blight. We are monitoring blight incidence and to date we have not observed natural shoot strikes. Depending on the availability of resources and time, controlled *Erwinia* inoculations will be performed.

Plant material, Sites and Planting Designs:

1. USDA-ARS cultivars. Five European pear scion selections from USDA-ARS were established in 2013 at two sites in Washington (Wapato, Chuck Peters; and, Wenatchee, Josh Koempel) and one site in Oregon (Hood River, MCAREC) via a 3-year project entitled, 'Pear scion trials in the Pacific Northwest' (see Evans et al. 2015 Final Report). At all sites, 5 single-tree replicates were randomized in high-density, modern training systems with 'd'Anjou', 'Bartlett', and 'Bosc' trees as controls. At Wenatchee, trees were planted 3 ft. in-row x 12 ft. between rows (1,210 trees per acre) without a trellis. Trees will be positioned ~70° from the vertical in year 4. At Wapato, trees were spaced 4 ft. in-row x 12 ft. between rows (908 trees per acre); each tree was tipped opposite its neighbor in a narrow V trellis. At MCAREC, spacing is 5 ft. in-row x 12 ft. between rows (726 trees per acre) and trained to a V, similar to Wapato.

2. Australian (Prevar) cultivars. Two bi-colored, Australian cultivars were to be established in medium-scale plantings in WA and OR in 2014. 'Lanya' (ANP-0118) was planted at two Washington sites (Dryden, Josh Koempel; and, Wapato, Chuck Peters) and at one site in Oregon (Hood River, MCAREC). Each site had a minimum of ~80 trees. At Dryden, trees were planted in a double-row design spaced 3 ft. x 12 ft. (1,210 trees per acre). At Wapato, trees are trained to a tall spindle and spaced 4 ft. x 12 ft. (908 trees per acre). In Hood River, trees were planted and trained identical to the USDA-ARS selections described above. The second cultivar, 'Deliza' (ANP-0131), however, was only established at MCAREC (40 trees) due to a shortage of nursery material. Additional trees were budded and cultured by a nursery collaborator for 2016 delivery (funding provided from the previous grant).

ONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-15-105

Project Title: Pear rootstock breeding

PI: Kate Evans
Organization: Washington State University
Telephone: 509 663 8181 x245
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee 98801

Co-PI (2): Amit Dhingra
Organization: Washington State University
Telephone: 509 335 3625
Email: adhingra@wsu.edu
Address: P O Box 616414
City/State/Zip: Pullman WA 99164

Cooperators: David Neale (UC-Davis); Stefano Musacchi (WSU-TFREC); Richard Bell (USDA-ARS WV); Joseph Postman (USDA-ARS Corvallis).

Total Project Request: **Year 1:** $63,499 **Year 2:** $112,138 **Year 3: $97,616**

Other funding sources

Agency Name: PNW Pear Bureau
Amt. awarded: $66,586 (2014-2017)
Notes: "Establishing NW-acclimated *Pyrus* rootstock breeding material" PI Dhingra, Co-PI Evans. Synergistic project to develop and establish pear rootstock seedlings.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

WTFRC Collaborative Expenses: None

Budget
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509 335 2885/509 663 8181 **Email address:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2015	2016	2017
Salaries[1]	29,064	67,666	58,406
Benefits[1]	10,501	22,116	17,463
Wages[2]	5,760	5,990	6,230
Benefits[2]	1,094	3,786	3,937
Equipment & Supplies Pullman	6,500	6,500	6,500
Equipment & Supplies TFREC	6,000	2,500	1,500
Travel[3]	4,580	3,080	3,080
Plot Fees	0	500	500
Total	63,499	112,138	**97,616**

Footnotes:
[1]Salaries for Nathan Tarlyn (Research intern, Dhingra lab) and researcher to be appointed (Evans lab);
[2]Wages for time-slip labor for orchard management and trait phenotyping;
[3]In-state travel between collaborators and year 1 trip to Corvallis, OR for collection of propagating wood.

OBJECTIVES

1. Phenotyping USDA-ARS Corvallis accessions for dwarfing and rooting.
2. Phenotyping established seedling populations for dwarfing.
3. Establish the Pear Rootstock Breeding Program.

This project aims to build on recent (and concurrent) research to develop a long-term, dedicated pear rootstock breeding program at the Tree Fruit Research and Extension Center, Wenatchee. Diverse germplasm collected from USDA-ARS Corvallis and seedlings derived from previously performed crosses, currently growing in Pullman, will be transferred to Wenatchee for establishment in the orchard and development of high quality phenotypic data essential to exploit the genomic data being generated in the Neale project (*PR-14-111*) and others. New germplasm will be produced using the traditional breeding method of crossing and selection. Parents for crossing within this 3-year proposal will focus on *Pyrus;* however, it is expected that should the breeding program continue, parents will also be sourced from other species, for example *Amelanchier* and Quince (*Cydonia oblongata*).

SIGNIFICANT FINDINGS

- 25 of the 64 accessions from the USDA-ARS Corvallis collection were successfully established in tissue culture to enable propagation. Remaining accessions will be sampled and re-established in spring 2017.
- A selected subset of the seedling populations have been propagated for small scale replicated trials and will be planted in Wenatchee spring 2017.
- First crosses made specifically for establishing pear rootstocks in 2016.

METHODS

Objective 1: Phenotyping USDA-ARS Corvallis accessions for dwarfing and rooting.

a. Greenhouse phenotyping of rooting potential.

A diverse subset of accessions from the US pear germplasm repository (Corvallis, OR) has already been selected for genotypic analysis in the Neale project (*PR-14-111*). Hardwood cuttings of this set (plus commercial controls and as many other accessions as possible) will be collected straight after leaf fall of the germplasm to be tested. The absolute number of accessions tested will depend on the availability of sufficient propagating wood and on the size and number of wooden bins that we are able to obtain. Following removal of spines, the cuttings will be bundled into 50's and the ends cut flat and dipped into rooting hormone. Tops of the cuttings will also be sealed to stop dehydration. The bundles will be placed upside down in wooden bins lined with black plastic liners and filled with peat moss and maintained at temperatures around 15ºC (59F) until root callus starts to form (usually by the following January). Appearance of callus will be scored as an indication of rooting potential. Callused cuttings can be potted into soil-less media or stored at 4ºC (39F) until ready to plant. After 3 months of growth, plants will be uprooted, medium removed and extent of rooting and architecture documented.

Accessions that fail to produce roots as hardwood cuttings will be micropropagated to provide rooted shoots for (Objective 1b, below). Although typically in the breeding program these would be selected against, this germplasm may provide valuable parental alleles for size control of the scion. Although new micropropagation facilities are available at the TFREC (Musacchi lab), making use of the considerable expertise and resources available in the Dhingra lab for micropropagation of *Pyrus* should expedite this process.

b. Phenotyping of dwarfing potential.
Ten rooted cuttings from each of the accessions rooted in Objective 1a (above) will be budded with a standard scion variety (to be determined, but most likely d'Anjou) and grown in pots in the greenhouse prior to planting in the field in a randomized block design. It is expected that this will be in two waves of planting, the accessions that root from hardwood cuttings would be the first wave followed by those that require micropropagation.

Trees will be grown in the field for the remainder of the project and shoot length and trunk diameter (and precocity if relevant) will be assessed as a measure of vigor. One problem that may be encountered is incompatibility of the scion to the rootstock. If this is the case, an alternative scion variety will be considered.

Depending on how fast we can determine a good dwarfing phenotype (which may be beyond the time frame of this project), we will also test the genomic loci previously reported to be involved in dwarfing (pear - *PcDw* locus [Wang et al., 2011]; apple - *Dw1* and *Dw2* loci [Celton et al., 2009, Rusholme Pilcher et al., 2008, Fazio et al., 2014]) to determine whether or not there is a good correlation in this germplasm. If well-correlated, these DNA-based tools will be a useful indication of dwarfing in new populations of seedlings. Should new DNA-based tools be developed from other projects within the timeframe of this project, we will also attempt to incorporate them where relevant.

Objective 2: Phenotyping established seedling populations for dwarfing.
Seedlings will be selected using the growth habit, precocity and floriferousness data generated in the Dhingra/Evans project and will be propagated *in vitro* and budded with a standard scion cultivar (most likely 'd'Anjou'). These seedlings are predominantly derived from the crosses 'Barlett' × 'd'Anjou' and 'Bartlett' × 'Comice' (reminder: the true parentage of OH×F 87 was recently identified as 'Old Home' × 'Bartlett'). The most dwarf individuals (short inter-noded) will form the bulk of those selected but some individuals from medium and high vigor groups will also be selected (up to a maximum of 50 individuals). Budded trees will be planted in the field; shoot length and trunk diameter (and precocity if relevant) will be assessed as a measure of vigor. Seedlings derived from the irradiated pollen that can be rescued in the Dhingra/Evans project will also feed into this phenotyping when available.

Objective 3: Establish the Rootstock Breeding Program.
A crossing program will be initiated to generate seedlings focused on the principal targets determined in the earlier PNW-funded project of size-controlling, precocity, good fruit size and finish, resistance to fire blight and pear decline, ease of propagation and winter hardiness.

Crosses will be made in year 1, fruit harvested and seeds collected in the fall. Those seeds will be vernalized and then germinated in the TFREC greenhouse in spring of year 2. Seedlings will be planted at close spacing in the orchard in Wenatchee (year 2) and budded with a popular scion cultivar (most likely 'd'Anjou') in year 3. Crosses will also be made in year 2 and year 3.

These seedlings would form the basis for an on-going, long-term breeding program. They will be grown using standard orchard practices and assessed annually (beyond the scope of this project) for vigor by measuring shoot length and trunk diameter. Bloom date and amount will be recorded annually to determine the precocity of the seedling rootstock. Fruit data recorded will include harvest date, yield, size, skin finish, firmness, titratable acidity and ^{o}Brix. Seedlings that are selected as dwarfing and precocious will be cut back to remove the scion and earthed up to promote the

production of rooted suckers. This method has been successfully used by PI Evans in her previous rootstock breeding program at East Malling Research, UK.

RESULTS AND DISCUSSION

Objective 1a: Greenhouse phenotyping of rooting potential.

Hardwood cuttings of 78 accessions from the Corvallis collection were collected for rooting potential tests failed to root. This experiment was repeated and was still unsuccessful. The decision was taken to initiate the establishment of some of this germplasm into tissue culture. The resulting additional labor requirement in the Dhingra lab was funded with the salary funds initially allocated for the Evans lab. Two collecting trips were taken to Corvallis and of the 64 accessions originally targeted, 25 are now in tissue culture and available for propagation as required. Another collecting trip is planned in spring 2017.

The explant material, representing 64 accessions, collected from Corvallis was divided into three groups. The oldest, most dormant, material was sterilized and placed in "maintenance tissue culture medium" at 40^{o} F. This material was monitored for a few months however, given the time and stage of collection, none of this material survived. The second set of explant material was intermediate in its developmental stage and an attempt was made to directly induce rooting, a method that we call bench cloning. With over 160 cuttings made through this process, there was very limited success and only about 1% of the sticks rooted (Figure 1).

Figure 1: A representative set of explant material collected from USDA Corvallis that rooted directly in the greenhouse.

The third group of explants was the one considered most amenable for introduction in tissue culture. The bud sticks were sterilized and divided into nodes and cultured aseptically in tissue culture media. Despite the fact that several lines did not respond, we were able to successfully establish 25 accessions in tissue culture (Figure 2).

Table 1 summarizes the number of plants at various stages of the cloning process:

	Accession number	Rooted dormant in cold room	Non-rooted dormant in cold room	Rooted from TC in greenhouse (pre-dormant)	Direct rooted, post-dormant growing in Greenhouse	T.C. chamber, number of plantlets (not rooted)	T.C. refrigerator, dormant number of plantlets (not rooted)
1.	2-23			2	1		10
2.	3-15				1		
3.	4-19						5
4.	10-13					8 (1 rooted)	7
5.	15-19			2			11
6.	19-11		4	2	1	13	6
7.	19-17						3
8.	22-7			2		4	7
9.	21-43			8	3	5	26
10.	NF23-15	2		6		14	2
11.	23-31				3	4	
12.	25-29	1				3	5
13.	26-25			2		14	3
14.	27-1						5
15.	NF28-9			6		17	25
16.	29-53	2			1		
17.	31-19				1	8	7
18.	NF34-2	2			1		
19.	NF34-7	1					
20.	47-5				1		
21.	NF52-1					25	
22.	65-17					4	
23.	67-7						14
24.	67-9	4				3(dormant)	12
25.	67-17				1	4	

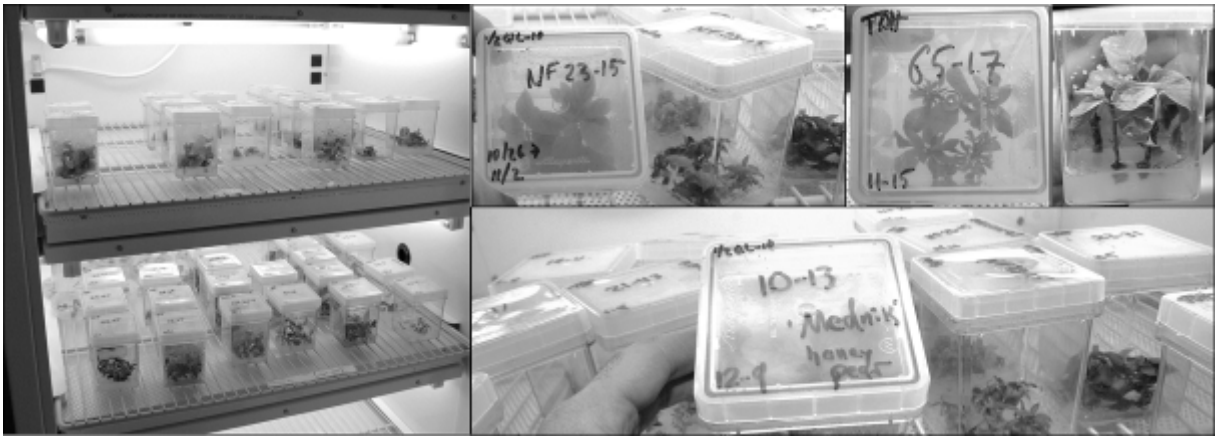


Figure 2: A representative set of explant material collected from USDA Corvallis successfully established in tissue culture. Some of the material is currently being cloned for producing material to be used in subsequent experiments.

Objective 2: Phenotyping established seedling populations for dwarfing

A subset of 13 individuals was selected for propagation from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' and three trees of each will be planted in a randomized complete block design at the Columbia View orchard, Wenatchee, in spring 2017. The trees will be budded with a standard scion in August 2017. Vigor data will be taken in 2018 and 2019.

Currently these plants are in a dormant state (Figure 3) and will be moved to Wenatchee in early spring. A summary of the selected individuals is presented in Table 2.

Table 2 Propagated seedlings for planting in Wenatchee

Cross	Number of plants
B × A 12-13	4
B × A 12-26	4
B × A 12-6	9
B × A 12-21	6
B × A 12-32	3
B × A 12-60	6
B × A 12-9	3
B × C 12-10	4
B × C 12-79	5
B × C 12-69	4
B × C 12-71	2
B × C 12-42	2
B × C 12-37	2

Figure 3: Selected F1 seedlings maintained in a dormant state in the cold room at 40^{o} F.

Objective 3: Establish the Rootstock Breeding Program.
Five crosses were made in spring 2016 using parents such as 'Bartlett', OHF333, 'Old Home', two dwarf *P. communis* varieties and three interspecific hybrids. Just over 3000 seeds were extracted of which approximately 1000 are currently receiving cold treatment prior to germination.

Outreach

Amit Dhingra's article 'The pear industry has unlimited potential and is ripe for a revolution' was published in the Good Fruit Grower, September 2016 (http://www.goodfruit.com/the-age-of-the-pear/ September 14, 2016) and his research on pears was featured in an article in The Atlantic, June 2016 (The push to make pears the new apples). The Atlantic. http://www.theatlantic.com/science/archive/2016/06/battle-of-the-pomes/488687/ June 27, 2016.

Kate Evans presented the outline of the breeding program at the Washington State Horticultural Association Show, Wenatchee in a talk entitled 'Update on pear rootstock breeding'.

CONTINUING PROJECT REPORT **YEAR: 3 of 3 (no cost extension)**
Project Number: PR-14-104

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

PI: Stefano Musacchi
Organization: WSU/ TFREC
Telephone: 509-663-8181 x236
Email: stefano.musacchi@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (1): Elizabeth H. Beers
Organization: WSU/ TFREC
Telephone: 509-663-8181 x234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (2): Jim Mattheis
Organization: USDA, ARS
Telephone: 509-664-2280 x249
Email: james.mattheis@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

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.

WTFRC Collaborative Expenses: None

Budget
Organization Name: WSU **Contract Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 **Email:** arcgrants@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 and physiological measurements

- Regardless of rootstock, more material was removed in winter pruning than fall, while regardless of the pruning treatment, OHF97, OHF69, and OHF87 did not differ in weight pruned.
- Trunks of winter pruned trees were significantly larger than fall pruned trees for all rootstocks and, OHF97 trunks were the largest and OHF87 were the smallest ($p<0.001$) regardless of pruning time. There was no significant difference between annual trunk growth of trees pruned in different seasons. However, OHF97 trunks grew the most and OHF87 trunks the least.
- OHF87 had the most fruit set per branch and OHF69 had the least when considering both pruning treatments together ($p<0.05$).

Yield (2016) and quality (2015)

- In the 2016 harvest, winter pruned trees had significantly more and heavier fruit, higher yield efficiencies and crop loads, but more fruit with sunburn and cork than trees pruned in the fall.
- There was no significant difference between the three rootstocks for productivity, average fruit weight, and incidence of sunburn and cork; however, OHF97 had significantly lower yield efficiencies and crop loads than the semi-vigorous rootstocks.
- After 7 months, fruits from the winter pruning treatment were riper (by I_{AD} index) than fall+summer fruit: they lost significantly more weight in storage, ripened significantly faster and were less firm (only significant at 5 months) than fall+summer fruits.
- Winter pruned fruit from 2015 had more cork than fall+summer fruit after 5 and 7 months of storage. However, there were no differences in calcium content for pear tissue after 5 or 7 months of storage.

Psylla and Mite Densities

- Adult psylla densities were high (up to 30/tap) before the delayed dormant spray, but were less than 4/tap throughout the rest of season. Nymph densities were also low (<0.05/leaf) throughout the post-bloom period. Mites were almost non-existent in this plot.
- No differences in seasonal densities for mites or psylla were found among pruning treatments or rootstocks.
- Fruit damage from insects (psylla, mealybugs, rust mite) was very low, although significantly higher pear rust mite russetting occurred in the winter-pruned treatment.

METHODS

The trial was carried out in an Anjou orchard trained at central leader and 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 and physiological measurements
Half of the experimental rows were winter pruned (2 Mar 2016) following traditional farm pruning practices, removing big branches and trying to promote renewal for the following year. The other half of the rows were fall pruned (no summer pruning in 2016) and trimmed only after harvest (Oct. 2015) and not in summer with the aim to remove big and vertical branches, competing limbs and to promote flower buds for the following years' production. These pruning treatments were repeated exactly as done the previous years. The decision to not apply summer pruning was taken with the idea to completely avoid the fruit removal and observe the natural fruit development and crop up to harvest. For each pruning time, cut wood (and leaves for fall pruning) from each tree was collected and weighed. Trunk circumference at 20 cm above ground was measured per single tree to calculate TCSA (trunk cross sectional area) in March and in November 2016. In March 2016, counting of flower buds per m^3 on both sides of the trees was performed on 10 trees per rootstock and per pruning technique (total 60 trees) to assess if the fall pruning technique had an effect on the flower bud formation. A 1 m^3 PVC structure was hung on the tree at the same height from the ground to assess the buds counting. A branch about 5ft from the ground was chosen on each of the flower bud trees to follow fruit set and buds. Healthy fruit were counted from the base of the branch to the tips.

Yield 2016
Pre-harvest assessment of 2016 fruit maturity was carried out one week before harvest on one tree per each pruning treatment (OHF87 as reference) to observe ripening levels for the coming harvest. Fruit from the pre-harvest were not assessed for quality for this report. Harvest 2016 was done (on Aug 18^{th} -19^{th}) by tree with 10 trees per each rootstock, for a total of 60 trees. Fruit disorders were assessed at harvest as % of sunburned fruit, frost and cork.

Fruit quality (harvest 2015)
Fruit belonging to 2015 harvest were pulled out after five (T1) and seven months (T2) of air storage at -1°C, fruit quality and maturity were assessed keeping fruit divided accordingly to I_{AD} classes (Z, A, B, C, and D: <1.60,$1.60< I_{AD}<1.79$, $1.80<I_{AD}<1.89$, $1.9<I_{AD}<1.99$, and $2.00<I_{AD}<2.09$ respectively, class Z and D were absent in T2). Skin color parameters (L, a, b), red blush, overcolor percentage, weight, firmness, soluble solids content (SSC), exogenous ethylene concentration, cork incidence, % dry matter, acidity, and pH were assessed at each pull out after 7 days of ripening at room temperature.

Calcium analyses
Samples of pear flesh tissue (3 reps x 3 I_{AD} classes x 2 pruning treatments =18) were collected, frozen and ground for calcium, nitrogen and other nutrients content analysis by enzymatic digestion (Best Test Analytics, Moses Lake, WA).

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 late-April through the late August. After leaves had fully expanded, leaf samples were used to assess psylla and mite 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 28 April. 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).

Fruit damage. Fruit damage was assessed on 24 August on 46 fruit per subplot. 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 and physiological measurements

Regardless of rootstock, significantly (2.4 times) more material was removed in winter pruning than in fall (Fig. 1), and regardless of the pruning treatment, OHF97, OHF69, and OHF87 did not differ in weight pruned (approx. 9.3 kg/tree, data not shown). For winter pruned trees, there was no difference in the amount of material removed per tree among rootstocks (Fig. 2). In fall pruned trees, significantly more pruned material was removed in OHF69 and 97 rootstocks than OHF87 (Fig. 2). Trunks of winter pruned trees were significantly larger than fall pruned trees for all rootstocks and, regardless of pruning time, OHF97 trunks were the largest and OHF87 were the smallest (Fall pruning-OHF87 was significantly lower than all of the other combinations, Fig. 2). There was no significant difference between trunk growth of trees pruned at different times (Fig. 1), however OHF97 trunks grew the most and OHF87 trunks the least. After considering both pruning treatments, this difference is due to the behavior of the rootstocks in the fall treatment because there was no significant difference for the winter pruned trunks (data not shown).

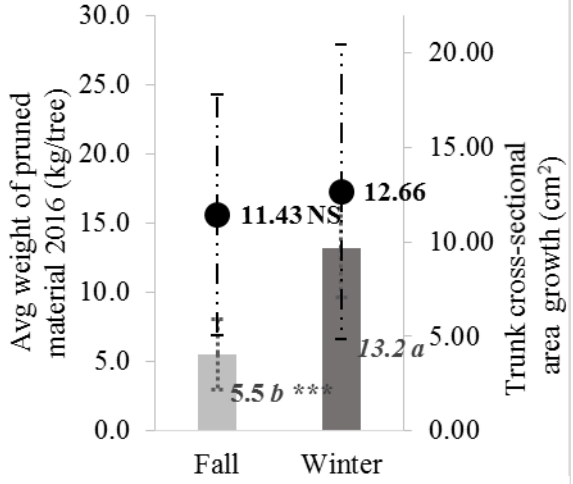


Figure 1: Comparison between weight of wood (and leaves only in fall) removed per tree (kg) in 2016 and trunk growth (2015- 2016) for each pruning treatment. *Significance: * p<0.05, ** p<0.01, ***p<0.001, ns= not significant for Type III sums of squares model significance; Student-Newman-Keuls post-hoc test to assign letter groups to arithmetic means where model was significant. Error bars are ±SD*

Pruning treatment and rootstock did not have a significant impact on average flower bud counts per m^3. Fall pruned trees reported 25 flower buds/m^3 while winter pruned had 21 flower buds/m^3, the resulting difference was not statistically significant. Also in the comparison by combinations (pruning time x rootstock), there was no significant difference but OHF87 fall pruned showed 31 flower buds/m^3 versus 20 flower buds/ m^3 in

OHF87 winter pruned. In 2016, we noticed a general reduction in flower buds/m^3 in comparison to 2015, when they were 32 and 25 buds/m^3 for Fall+summer and winter pruned trees, respectively (difference not significant in 2015 as well). The fruit set (percentage of total flowers that set to fruit) per branch count showed no differences between pruning time, while significant differences were found between rootstocks. OHF87 had the highest percentage of fruit set per branch and OHF69 had the lowest when considering both pruning treatments ($p<0.05$). This difference is due to the behavior of the rootstocks in the winter treatment because there was no significant difference in fall (Fig. 3). OHF87 winter pruned trees had 1.8 times higher percentage of fruit set than OHF69 (Fig. 3).

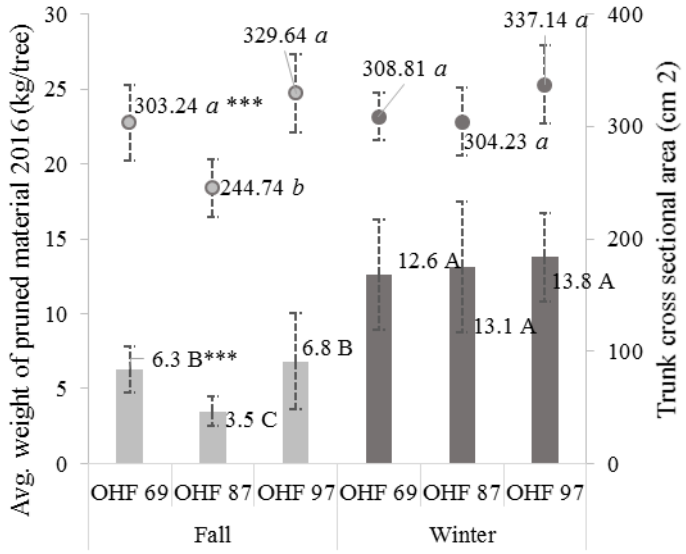


Figure 2: Comparison between weight of wood (and leaves fall only) removed per tree (kg) and trunk area (cm^2) in 2016 for rootstock NS pruning treatment. *Significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns= not significant for Type III sums of squares model significance; Student-Newman-Keuls post-hoc test to assign letter groups to arithmetic means where model was significant. Small letters indicate differences in the TCSA, capital letters for avr. weight of prunet material. Error bars are ±SD.*

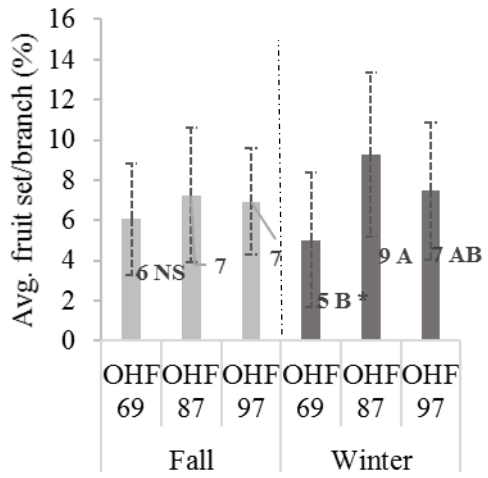


Figure 3: Comparison between fruit set (%) on a branch for each rootstock by pruning treatment. *Significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns= not significant for Type III sums of squares model significance; Student-Newman-Keuls post-hoc test to assign letter groups to arithmetic means where model was significant. Error bars are ±SD.*

Yield 2016

The pre-harvest fruit ripening assessment on OHF87 rootstock and both pruning treatments one week before harvest revealed that the majority of fruit (approx. 39%) were classified as $2.00<I_{AD}<2.09$ (class D) for both treatments, while fall pruned trees seemed to have riper fruit in $1.90< I_{AD}<1.99$ (class C) than fruit on winter pruned trees. This behavior is opposite than that observed in the previous two years (Fig. 4).

Yield in 2016 had significantly more and heavier fruit from trees pruned in the winter than those in the fall (Table 1). The difference between treatments was around 35 lb/tree or 71 fruit/tree (Table 1). The average fruit weight for winter pruned trees was 7g higher than fall pruned trees and they were commercially sized between 90-100 fruit/box and 100-110 fruit/box, respectively (Fig. 5). Winter pruned trees had significantly higher yield efficiencies, crop loads, but more fruit with sunburn and cork than trees pruned in the fall, like in 2015. No frost damage was detected in 2016. There was no significant difference between the three rootstocks for productivity, average fruit weight, and incidence of sunburn and cork. However, OHF97 had significantly lower yield efficiencies and crop loads than the less vigorous rootstocks (Table 1).

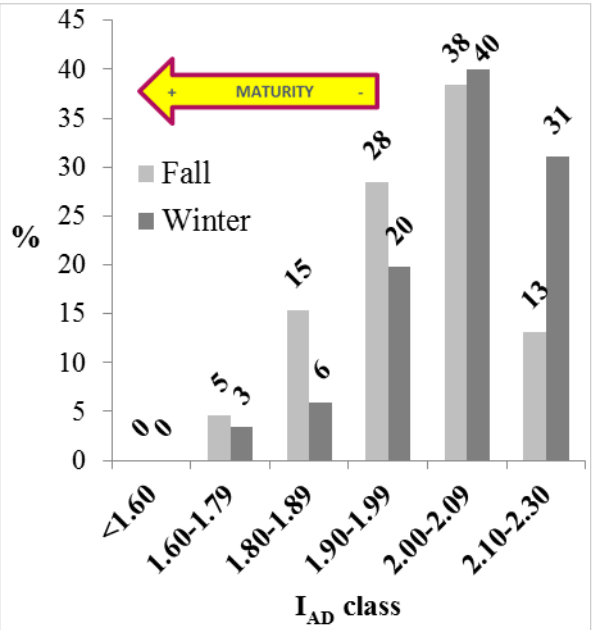


Figure 4: Fruit distribution in I_{AD} classes one week before harvest in fall and winter pruned trees in 2016.

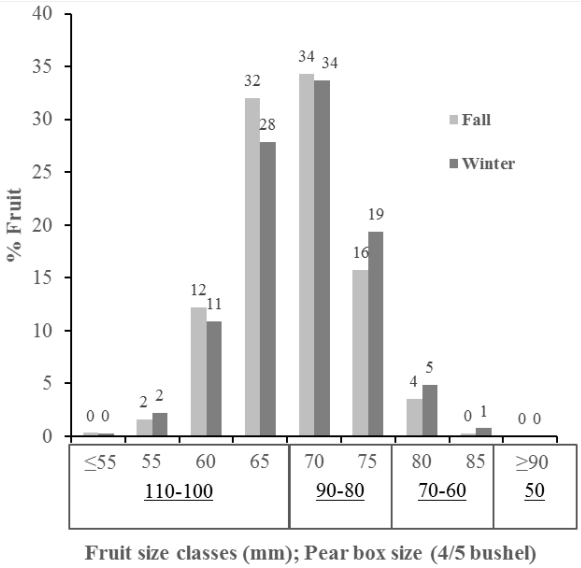


Figure 5: Fruit size distribution (in mm diameter) for fall and winter pruning at harvest 2016. Correspondence in 4/5 bushel pear box underlined below.

Table 1: D'Anjou yield and disorders in Cashmere, WA in August 2016.

Treatment	Count fruit /tree		Net yield (lb /tree)		Fruit weight (g)		Yield efficiency (lb /TCSA)		Crop load (num. fruit /TCSA)		Sunburned fruit (%)		Fruit with cork (%)	
Pruning season														
Fall	251	**B**	108.8	**B**	198	**B**	0.38	**B**	0.88	**B**	0.74	**B**	0.08	**B**
Winter	322	**A**	143.6	**A**	205	**A**	0.46	**A**	1.04	**A**	1.77	**A**	0.20	**A**
Significance	***		***		*		**		*		***		*	
Rootstock														
OHF69	295		131.1		205		0.43	**A**	0.98	**AB**	0.87		0.16	
OHF87	294		129.5		201		0.47	**A**	1.08	**A**	1.62		0.03	
OHF97	269		118.1		199		0.36	**B**	0.82	**B**	1.22		0.24	
Significance	NS		**NS**		**NS**		**		*		NS		NS	
Signif. Prun.XRoot.	**NS**		**NS**		**NS**		**NS**		**NS**		**NS**		**NS**	

*p<0.05, *; p<0.01, **; p<0.001, ***; NS, not significant for Type III sums of squares model significance.*
Student-Newman-Keuls post-hoc test to assign letter groups to arithmetic means where model was significant.

Fruit quality (harvest 2015)

Fruit from 2015 harvest on OHF87 rootstock had differences in post-storage quality between pruning treatments. After 5 months, fruits from the winter pruning treatment ripened significantly faster (according to the I_{AD} drop) and had a lower firmness than fall+summer pruned trees (Table 2). Winter fruits also lost significantly more weight and ripened faster after 7 days of ripening than fall+summer fruits after 7 months of storage. At harvest, fruits from both treatments were similar in hue (color) and chroma (shade), but fall+summer pruned fruit were significantly greener color after 7 months of storage than winter fruit (Table 2). At harvest fruits from both treatments were similar in firmness,

but fall+summer fruits were significantly firmer after 5 months of storage than the winter fruit and the trend continued (although not significant) in the 7th month pullout. At harvest, fall+summer pruned trees had significantly more soluble solid content (SSC) than winter, but after storage there was no significant difference among the treatments (data not shown). At harvest and after 5 months, fall+summer fruits showed lower titratable acid (TA, $p<0.05$) than winter fruits and after 5 months higher pH than winter fruit. Incidence of cork was similar at harvest among the pruning treatments, but winter fruit had more cork after 5 and 7 months of storage than fall+summer fruit. The I_{AD} ripening classes were distinguished at harvest and the ripest class in both treatments ripened the most and was the most yellow after 5 and 7 months in storage. The opposite was observed for the most unripe class. At 5 months for both treatments, the ripest class (Z) was the least firm, had the highest SSC, and winter only had the highest percentage of dry matter. At 7 months considering both treatments, the second and third ripest classes (B, C) was least firm and classes A and B had the highest dry matter %.

Samples of pear flesh tissue from T1 and T2 (harvest 2015) were analyzed for calcium, nitrogen, and other macro and micronutrients and there were no significant differences between winter and fall+summer pruned fruit except for a higher percentage of potassium (K%) in winter fruit than fall (data not shown).

Table 2: Fruit quality parameters (Anjou/OHF87 fruit harvested in 2015 and stored up to 7 months) T1 =5 months of storage, and T2= 7 months of storage on quality.

Storage 2015	Treatment	Weight drop (g) after storage	Weight drop (g) after 7 days of ripening + storage	IAD index drop after storage	IAD index drop after 7 days of ripening + storage	Color parameter: hue	Color parameter: chroma	Firmness (lb) avr of 2 faces	SSC (Brix)	pH	Titr. Acidity (% malic ac.)
5 months (T1)	Fall +sum pr.	5.7	7.2	0.28 **B**	0.19	108.5	41.9 **B**	7.82 **A**	14.2	3.89 **A**	0.26
	Winter pr.	5.9	7.5	0.32 **A**	0.21	107.6	42.8 **A**	6.49 **B**	14.3	3.73 **B**	0.27
	Significance	NS	NS (5.3)	**	NS	NS	***	***	NS	***	NS
7 month (T2)	Fall +sum pr.	7.0 **B**	8.4 **B**	0.47 **B**	0.41 **B**	105.8 **A**	42.6	4.27	14.4	3.66	0.20
	Winter pr.	8.0 **A**	9.0 **A**	0.52 **A**	0.46 **A**	104.2 **B**	42.2	3.79	14.1	3.68	0.22
	Significance	***	**	*	**	***	NS (5.2)	NS (5.2)	NS	NS	NS

*Pr = pruning p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant for Type III sums of squares model significance*
Student-Newman-Keuls post hoc test to assign letter groups to arithmetic means where model was significant.

Psylla and Mite Densities

Overwintering psylla adult densities were high (15-30/tap) on the first three sampling dates (29 February and 17 March) before the first insecticide applications were made. They remained low (<4/tap) throughout the season. Neither the main effects (treatment and rootstock) or the treatment x rootstock interactions were significant for an index of the seasonal densities, cumulative insect-days (CID). Spider mites and rust mites were close to zero throughout the season, with no significant treatment/rootstock differences.

Fruit damage (russeting) from psylla was moderate (ca. 15% overall), with slightly higher percentage damage in the OHF87 rootstock (no differences among pruning treatments). Mealybug and codling moth damage were both near zero, but leafroller damage was significantly higher in the winter-pruned treatment. Despite the low number of pear rust mite on the leaves, fruit russeting (presumably by pear rust mite) affected a high percentage (89% overall) of the fruit, although most received the lowest rating of 1 (1-10% of the fruit surface russeted). The winter-pruned/OFH97 trees had a significantly higher percentage of fruit with rust mite damage than the other treatments.

CONTINUING PROJECT REPORT **YEAR:** 1 of 2
WTFRC PROJECT NUMBER: PR-16-105

Project title: Dry matter assessment in pear and consumer perception

PI: Sara Serra
Organization: WSU -TFREC
Telephone: 509 663 8181 (251)
Email: sara.serra@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Co-PI: Stefano Musacchi
Organization: WSU -TFREC
Telephone: 509 663 8181 (236)
Email: stefano.musacchi@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Co-PI: Carolyn Ross
Organization: WSU (Pullman)
Telephone: 509 335 2438
Email: cfross@wsu.edu
Address: Food/Nutrition 122
City/State/Zip: Pullman, WA 99164

Cooperators: Alex Goke (WSU –TFREC)

Total Project Request: Year 1: $ 51,655 **Year 2**: **$ 56,172**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 **Email:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	2017
Salaries[1]	24,000	24,960
Benefits[2]	8,414	8,750
Wages[3]	2,880	2,995
Benefits[4]	289	300
Equipment	0	0
Goods/Services[5]	14,572	17,667
Travel[6]	1,500	1,500
Plot Fees	0	0
Miscellaneous	0	0
Total	51,655	**56,172**

Footnotes:
[1] Salary for a new hire 50% Research Intern (Serra-Musacchi) paid the other 50% on other grant.
[2] Benefit on salary at 31.5%
[3] One non-Student temporary for 12 wks: 20hrs/wk at $12/hr (Serra-Musacchi).
[4] Benefits on temporary at 10% (Serra-Musacchi).
[5] Labware/consumable, fruit sample reimbursement (Serra-Musacchi), sensory panel costs (consumable and incentive advertising), electronic tongue: sensors, chemicals and glassware (Ross), publication (all).
[6] 2778 miles/year for domestic travel ($0.54/mile) to go to the orchard and to Pullman to meet co-pi and deliver fruit.

OBJECTIVES

1) Determine the reliability of the Felix F-750 Produce Quality Meter and therefore if this non-destructive dry matter assessment tool can be used as at harvest sorting step for more consistent fruit quality categories.

2) Assess if higher dry matter in pear translates into greater consumer liking and acceptability through consumer preference and sensory analysis studies.

SIGNIFICANT FINDINGS

- Accumulation of fruit dry matter % up to harvest can be predicted using the Felix F-750 Produce Quality Meter directly on the tree starting several weeks before harvest.

- The Felix F-750 can be used to non-destructively predict dry matter % in fruit postharvest, saving a lot of time in comparison to the traditional and destructive method and allowing the evaluation of a larger number of fruit.

- Lower dry matter % classes tended to have fruits that were more firm, had a lower SSC (Brix), and higher I_{AD} index. In general, this is related to different fruit exposure to the light and, therefore, to a ripening variability in the canopy.

- An Anjou dry matter model was created and it performs reliably with an R^2 (goodness-of-fit) of 0.947 for the calibration dataset.

- With the new Anjou model, the Felix F-750 was able to predict whole-fruit dry matter with an R^2 of 0.909.

- In Bartlett, an older Anjou model predicted dry matter with an R^2 of 0.79 between predicted and destructive dry matter.

- A preliminary consumer panel showed that high dry matter fruits were rated higher overall than medium and low dry matter fruits. They were significantly higher in perceived sweetness and juiciness than medium or low dry matter fruits.

METHODS

1)Determine the reliability of the Felix F-750 Produce Quality Meter and, therefore, if this non-destructive dry matter assessment tool can be used as at harvest sorting step for more consistent fruit quality categories.

Step 1 - Orchard 1

In a block of Anjou/OHF87 trained to central leader (planted in 1998 at 4.30 m x 2.45 m) we chose four trees (where possible) for each of the four treatments under evaluation for dry matter (DM) accumulation. Different timing to practice pruning in the orchard were compared:

- *Winter pruning 2016 (2 March 2016) + NO summer pruning 2016* =*WP*
- *Winter pruning 2016 (2 March 2016) + Summer pruning 2016 (6 June 2016)* =*W+SP*
- *Fall pruning 2015 (20 Oct 2015) + NO summer pruning 2016* =*FP*
- *Fall pruning 2015 (20 Oct 2015) + Summer pruning 2016 (6 June 2016)* =*F+SP*

For 10 weeks (June 13th to August 15th), fruit diameter was measured weekly (mm) on labeled fruit on the trees. For six weeks, starting from July 15th, the same selected fruit were measured by the Felix F-750 with two readings/fruit (blush and shade cheeks) on the tree using a preliminary model built initially with only two temperatures (April 2015). Harvest 2016 was done by tree on Aug 18th -19th (15 total) and number of fruit/tree counted and weighted. All fruit were immediately stored in the cold room (1°C=33.8°F) for sorting purposes. All fruit were sized for fruit size distribution and approx. 500 fruit per treatments (total≈2000 pears) were measured (two readings per fruit) by Felix F-750 for a non-destructive dry matter % (DM) and SSC (Brix) prediction. All fruit were sorted by DM from the lowest to highest % in six classes (from 11 to 13% where possible). According to availability, fruit were divided in three groups: T0q (fruit quality analysis at T0, done 15-16th September 2016), T1q (fruit quality analysis at T1= after five months of storage at 0.5°C, beginning of February 2017) and T1CT (fruit for Consumer Test purpose, they will be delivered to Pullman in February). Fruit quality analysis at T0 assessed skin color parameters (L, a, b), red blush, over-color percentage, weight, I_{AD} index, firmness, soluble solids content (SSC), exogenous ethylene concentration, cork incidence, % dry matter, titratable acidity, and pH after seven days of ripening at room temperature.

Step 1 - Orchard 2

In a block of Anjou on seedling trained to open vase (planted in 1970's at 6 m x 6 m) we chose fruit belonging to two extreme light interception positions within the large canopy. 200 External exposed fruit and 200 Internal shaded fruit were chosen within 19 homogeneous trees and tagged properly to locate them at harvest. On the 15th of August light intercepted by each fruit was measured using the PAR quantum Q53292 sensor (LI-COR) by placing the sensor perpendicular to the ground on the south face of each pear. Measurements (expressed in µmol s^{-1} m^{-2}) were carried out on sunny days at solar noon ± 1.5 h. All fruit were classified in two canopy positions by percentage of actual light intercepted (Internal: <30% light and External: 70-100% light). We picked the labeled fruit by position on August 29th, kept them separately and stored in the cold room (1°C=33.8°F). Each position type of fruit was measured (2 readings per fruit, blush and shade) by Felix F-750 for a non-destructive dry matter % (DM) and SSC (Brix) prediction for a total of 400 fruit. According to fruit DM % distributions by canopy position, it was not possible to have the same high and low classes for Internal as well as External, so a larger-range dry matter class from 14.00 to 15.99% to be in common between External and Internal was built up in order to compare the fruit position within the same dry matter class. Additionally, we will be able to investigate lower DM classes in the Internal fruit and higher DM classes for the External fruit and try to correlate quality traits with consumer acceptability and liking. Each DM class was divided in three groups: T0q (fruit quality analysis at T0, done 22th September 2016), T1q (fruit quality analysis at T1= after five months of storage at 0.5°C, beginning of February 2017) and T1CT (fruit for Consumer Test purpose planned for February 2017).

Step 1 - Orchard 3

Bartlett pears were harvested on August 5th, 2016 from a block in Monitor, WA. The orchard was planted on OHF87 in 2012 at 3.5 m x 1.5 m and trained to spindle. 65-70mm size pears were selected for fruit quality analysis and storage. After one month of normal air (33ºF) storage non-destructive (at day 0 and day 7 after 7 days of ripening) and destructive parameters (at day 7) were

assessed. Among all the quality parameters investigated, here attention is focused on dry matter prediction by the Felix F-750 using the first Anjou model with three temperatures (July 2016).

Step 2 (Creating a pear Dry Matter Model for the Felix F-750)

Anjou pears harvested from two blocks (different age, rootstocks, and training systems) in Cashmere in 2016 exhibiting a wide range of morphologies and maturity were selected for use in improving the Felix F-750's dry matter predictive power beyond its manufacturer-equipped capabilities. To build the model, fruits were scanned with the F-750 instrument across three internal fruit temperatures (41, 68, and 88°F) to collect their emission spectra and create an instrument calibration set. After collection of these spectra, the two cheeks on each fruit corresponding to their F-750 scan were traditionally destroyed and assessed for dry matter % (DM) and SSC (Brix).

A new Anjou dry matter and Brix prediction model was created by regressing the collected emission spectra across the three fruit temperatures against the known DM and SSC values for each cheek of each fruit obtained destructively. The range of spectral wavelengths and the number of principle components used in the regression was selected to achieve the best performing model based on its linear goodness-of-fit (R^2) value provided by the Felix F-750 Model Builder software.

2)Assess if higher dry matter in pear translates into greater consumer liking and acceptability through consumer preference and sensory analysis studies (reporting activity by Serra S. and Musacchi S.).

We conducted a preliminary consumer panel study to test our new Anjou model to sort fruit and evaluate consumers' opinions. Fruits set aside from the Felix F-750 model improvement effort (described above) were measured on opposing cheeks with the Felix "new Anjou 3 temp. model" to predict their average dry matter % (DM). Four predicted dry matter classes were developed for the panel study; 13.00-13.99% (low DM), 14.00-14.99 and 15.00-15.99% (medium DM), and 16.00-16.99% (high DM). Pears from each predicted dry matter class at both three and eight days ripened were vertically sliced and served to panel participants. Panelists were asked to judge each slice using a 1-9 Likert-style scale anchored by "low" and "high intensity" on the basis of firmness, crispiness, juiciness, sweetness, bitterness, aroma, appearance, and overall rating. Additionally, panelists were asked how much they would be willing to pay for a pear such as the one that produced the tasted sliced based on USDA average retail prices for organic and conventionally-grown Anjou, either $0.97, $1.72, or $2.47/lb. A total of 29 individuals acted as panel participants.

A large-scale consumer panel will be conducted in February of 2017 by Ross C.'s group. Demographic data will also be collected to describe the composition of each panel. Consumers will evaluate the DM sorted samples from Orchard 1 and 2, using a 9-point hedonic scale, for acceptance of appearance, aroma, taste/flavor, texture and overall. For evaluation, a pear wedge will be presented to each. On each panel day, a control pear will also be evaluated to allow for comparisons among the different treatments. Differences among treatments will be visualized using Principal Components Analysis (PCA). Data will also be analyzed using cluster analysis to determine if any groups of consumers can be identified.

RESULTS AND DISCUSSION

Objective 1
Step 1 - Orchard 1

Dry matter accumulation of Anjou pears from July showed a higher DM in fruit from the "no summer pruned" trees than the "summer pruned" and a higher net DM accumulation was calculated for the "no summer pruned" (data not shown). DM difference was significant since the beginning of the measurement period up to August 16th (Fig.1). An increase in DM in the fruit was non-destructively measured by Felix F750 after the beginning of August and up to three days before

harvest. DM accumulation did not stop before harvest (Fig. 1). Difference in fruit DM distribution between the four treatments was confirmed at harvest when all trees were strip-picked and 500 fruit for each treatment were measured for DM with the Felix F-750. Fall pruning (FP) showed a highest % of fruit in the higher DM classes (from 15-16% and above), while Winter pruning (WP) reported a higher presence of fruit in the lowest DM classes (13-14 % and below, Fig. 2). This trend is linked to the typical vigor reduction of the fall pruning. "Summer pruned" pears (F+SP and W+SP) have a lower dry matter than the "no summer pruned" (FP and WP), particularly clear between Fall pruning vs Fall+Summer pruning (>10% more fruit in 16-17% DM class in FP respect to F+SP).

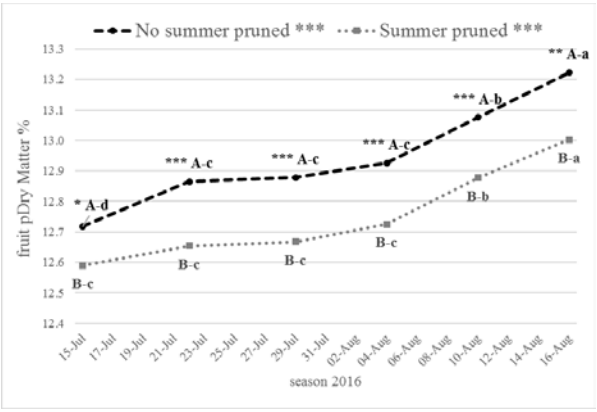


Figure 1: Seasonal Dry Matter % accumulation in Anjou pears in 6 weeks from July 15th to August 15th 2016 (Orchard 1): comparison between summer pruned trees versus no summer pruned trees.

Fruit quality data analyzed by DM classes, regardless of pruning treatment, revealed significant differences among them. Pears in the lowest class (11.00-12.99%) were found to be smaller by size (data not shown) and weight than all other dry matter classes (Table 1). Fruits in the lower two classes (11.00-12.99 and 13.00-13.99%) had significantly higher I_{AD} index immediately after storage and the smallest decrease in I_{AD} index in seven days of ripening at room temperature than all other classes, suggesting lower maturity. Lower DM% classes tended to have fruits that were more firm and had lower SSC (Brix).

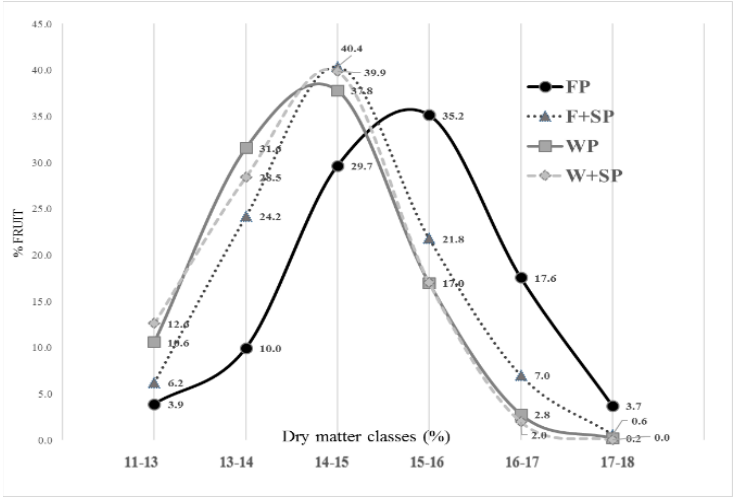


Figure 2: Predicted Dry Matter % distribution of Anjou pears at harvest 2016 on > 2000 pears from Orchard 1: comparison between 4 pruning treatments.

Table 1: Anjou pears quality 1 month after harvest 2016 from Orchard 1: comparison between 6 Dry Matter classes regardless any pruning treatments for the main quality parameters assessed.

DM% Class	Weight (g) day 0		I_{AD} index day 0		Firmness (kg) day 7		SSC (Brix) day 7		pDM % by FelixF-750 day 7		trad.-destr. DM% day 7		Titrat. acidity (% malic ac.) day 7	
11.00-12.99	167	D	2.06	A	7.49	AB	11.61	B	13.04	F	13.90	F	0.33	BC
13.00-13.99	205	C	2.02	A	7.64	A	12.67	AB	14.02	E	15.18	E	0.35	ABC
14.00-14.99	217	ABC	1.95	B	7.42	AB	13.56	AB	14.93	D	15.93	D	0.36	A
15.00-15.99	230	A	1.87	C	7.31	AB	15.35	A	15.77	C	16.67	C	0.35	AB
16.00-16.99	226	AB	1.75	D	7.28	AB	14.92	A	16.65	B	17.52	B	0.32	C
17.00-18+	209	BC	1.60	E	7.08	B	15.92	A	17.58	A	18.58	A	0.29	D
Significance	***		***		*		***		***		***		***	

*Significance: p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant for Type III sums of squares model significance. Student-Newman-Keuls post hoc test to assign letter groups to arithmetic means where model was significant.*

Step 1 - Orchard 2

Fruit distribution in DM classes by canopy positions (Figure 3) showed that only few classes were in common between the two types of fruit. The most representative classes in common for both External and Internal fruit in 2016 were DM 14.00-14.99% and 15.00-15.99%, while the lowest and highest DM classes were exclusive to internal (<13%) and external fruit (>15%), respectively. This confirmed the strong difference existing between those extreme types of fruit within a same large canopy, already observed in our previous studies.

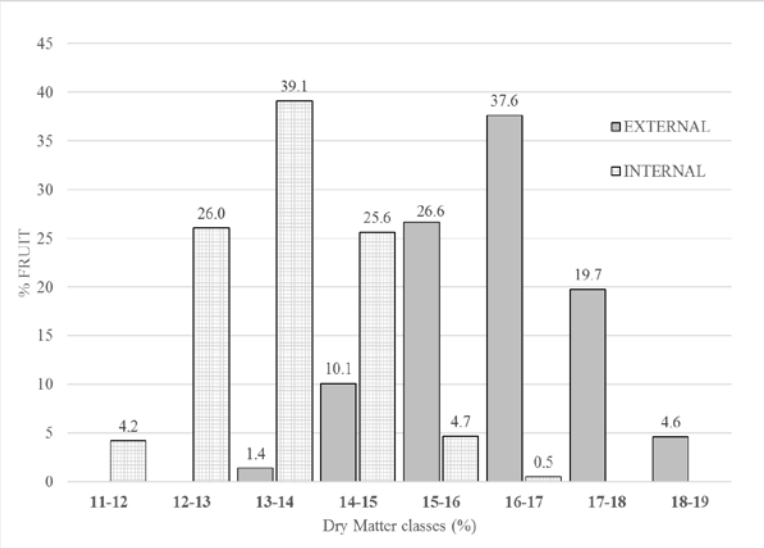


Figure 3: Predicted Dry Matter % distribution of Anjou pears at harvest 2016 (Orchard 2): comparison between 2 canopy positions (External – Internal), 2015 fruit/position.

At T0, External fruits were significantly larger by weight, more ripe (having lower I_{AD} index), had higher percentages of overcolor and higher (destructive) dry matter % and SSC (Brix) than internal fruits (data not shown).

Between predicted dry matter classes, both external and internally-positioned fruits were significantly different in "traditional/destructive" dry matter % and SSC (Brix) at T0. Fruit from higher predicted dry matter classes had higher "traditional/destructive" dry matter % and SSC values. Comparing fruit by position within the same "common" DM% class (14.00-15.99%, see Fig. 3) revealed significant differences among them: External fruit showed higher SSC, lower ripening I_{AD} index, lower titratable acidity than Internal, suggesting diversity in maturity (data not shown).

Step 1 - Orchard 3

A DM% prediction on Bartlett pears was obtained by Felix F-750 using the first Anjou model with three temperatures (July 2016). A linear regression between predicted and real ("traditional/destructive") Dry Matter in Bartlett pears was found with a R^2 value of 0.792 (Fig. 4). While not the best coefficient, it is acceptable considering the model was developed on a different variety. The next step will be developing a Bartlett-specific model for the Felix F-750 with three temperatures to adopt in 2017.

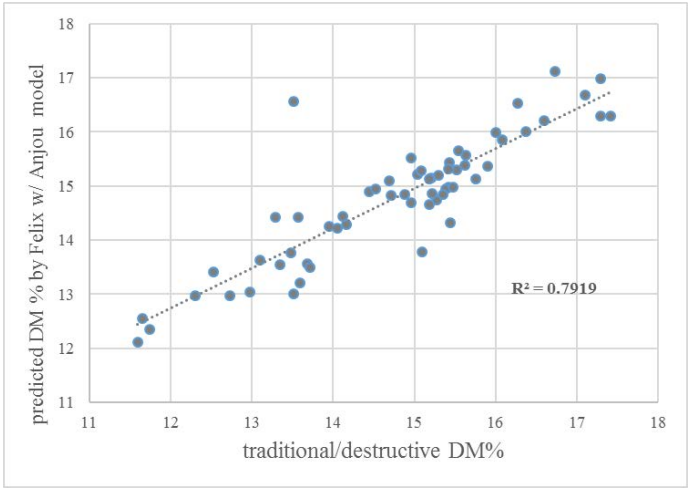


Figure 4: Correlation between predicted Dry Matter % in Bartlett fruit by Felix F-750 with an Anjou model and actual Dry Matter % values destructively assessed.

Step 2 (Improvement of the Felix F-750 pear model)

We were able to train the Felix F-750 produce quality meter to reliably and non-destructively predict dry matter in the cheeks of Anjou fruits using a new prediction model with a 0.947 coefficient of determination (R^2) and a root mean square error (RMSE) of 0.34 for dry matter, and 0.907 coefficient of determination (R^2) and a root mean square error (RMSE) of 0.42 for brix. This was an improvement of approximately 0.03 and 0.01 in coefficient of determination (R^2) for dry matter and SSC (Brix), respectively, from an earlier iteration of the Anjou model (July 2016), and a vast improvement from the preliminary dry-matter only Anjou model created in April 2015 on two temperatures only.

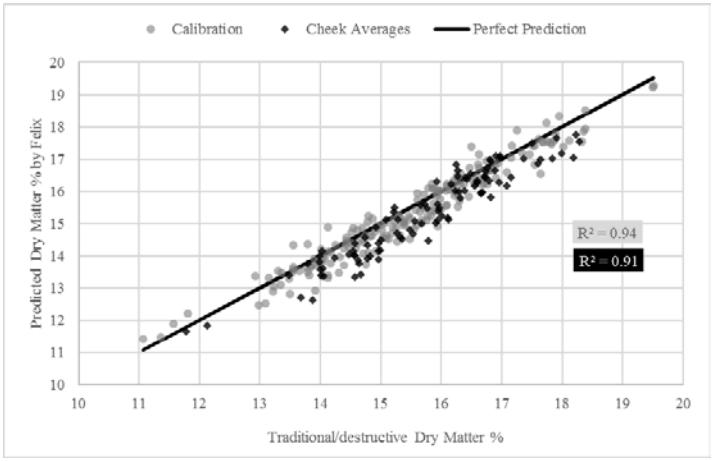


Figure 5: Predicted dry matter vs. actual Dry Matter values of fruit cheeks (cheeks of fruits used in model creation, n=200), and predicted whole-fruit dry matter % (average of two cheeks) vs. actual whole-fruit Dry Matter values of fruit cheeks (fruits used in model creation, n=100).

Averaging two opposing cheek dry matter predictions (blush and shade side), "whole-fruit" dry matter accounting for DM gradient within the fruit (vertical slice across the core) was well-predicted with a coefficient of determination of 0.909 (R^2) and a root mean square error (RMSE) of 0.60. This method led to a 0.43% under-prediction of whole-fruit dry matter on average.

Objective 2 - Preliminary consumer panel study (reporting activity by Serra S. and Musacchi S.).

Consumers overwhelmingly perceived higher dry matter fruits to be sweeter and juicier, regardless of ripening stage of the fruit (Fig. 6). Overall fruit rating was best associated with perceived fruit slice sweetness and juiciness, but not firmness or crispiness. The fruit price that consumers were willing to pay was best correlated with perceived juiciness and sweetness ratings, and thus consumers were willing to pay premium price for a high dry matter fruit due to its high perceived juiciness and sweetness. Finally, high dry matter fruits were rated significantly higher overall than lower dry matter fruits. These results suggest that dry matter may be an important parameter in predicting consumer acceptance of pears- perhaps more so than fruit firmness.

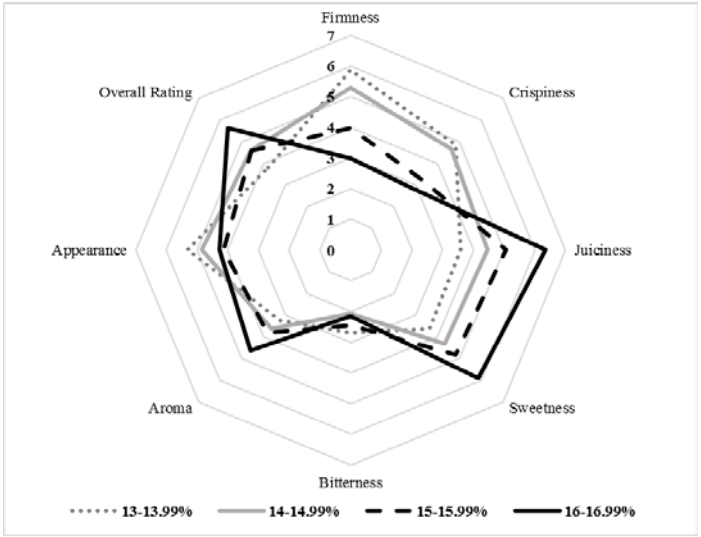


Figure 6: Average consumer rating of perceived fruit quality parameters and overall fruit rating for four predicted dry matter classes.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2 (No-cost extension)

Project Title: Molecular gut content analysis to pinpoint where psylla overwinter

PI:	W. Rodney Cooper	**Co-PI**:	David Horton
Organization:	USDA-ARS, Wapato	**Organization**:	USDA-ARS, Wapato
Telephone:	509/454-4463	**Telephone**:	509/454-5639
Email:	Rodney.Cooper@ars.usda.gov	**Email**:	David.Horton@ars.usda.gov
Address:	5230 Konnowac Pass Road	**Address**:	5230 Konnowac Pass Road
City/State/Zip:	Wapato, WA 98951	**City/State/Zip**:	Wapato, WA 98951

Total Project Request: **Year 1:** $29,000 **Year 2: $0**

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	2016	2017	
Salaries	$7500		
Benefits	$2500		
Wages			
Benefits			
Equipment			
Supplies	$17,500		
Travel			
Miscellaneous			
Plot Fees	$1500		
Total	$29,000	0	

Footnotes: Supplies include PCR reagents, TA cloning reagents, vector growth media, gene sequencing costs, and shipping costs.

OBJECTIVES

1. Design PCR primers to detect shelter plant DNA.
2. Determine the number of sequences required to identify previous shelter hosts.
3. Determine how long the plant DNA signal persists in winterform psylla.
4. Develop and test flight interception traps that (a) effectively trap returning psylla before they have entered the orchard and fed extensively on pear, but (b) do not compromise the plant (DNA) signal within the guts of those returning insects.

SIGNFICANT FINDINGS

1) PCR primers for the P6 loop of the chloroplast gene, *trnL*, amplify a short variable region of plant DNA suitable for identification of shelter plants used by overwintering psylla.
2) Preliminary results suggest that a large number of sequences will be required to identify previous shelter hosts.
3) Brown or olive green 3D printed traps successfully capture winterform psylla directly into preservative.

METHODS

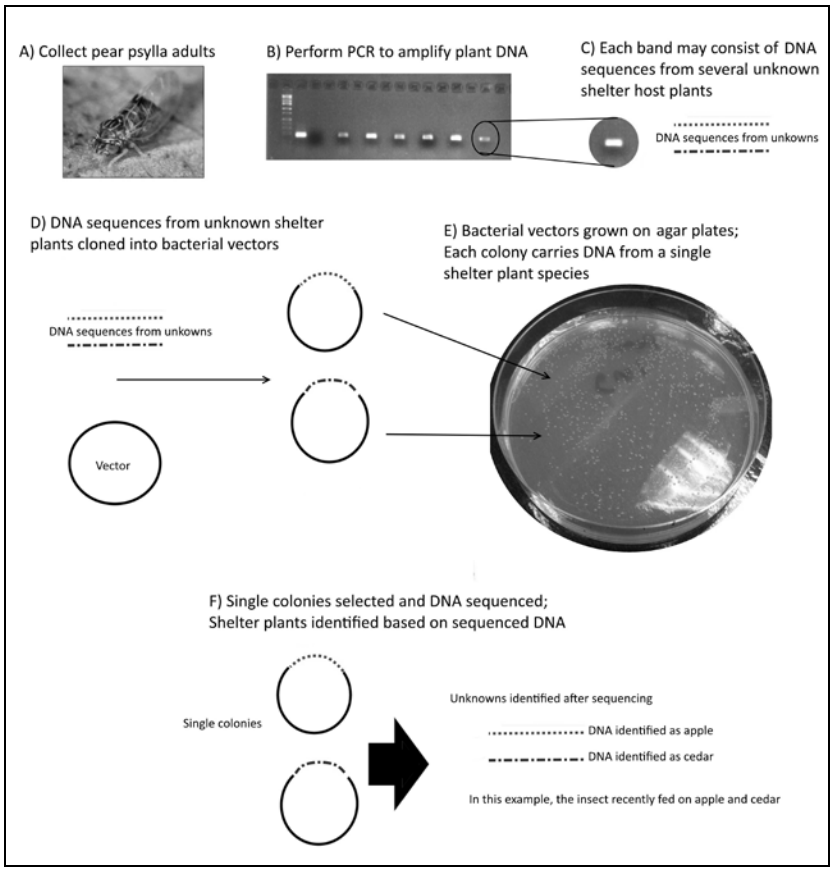


Figure 1. Basic process for identifying previous shelter plants fed upon by winterform pear psylla

The basic process for identifying previous host plant use by winterform pear psylla is summarized in Figure 1. Universal PCR primers will be used to amplify short regions of chloroplast DNA from the guts of winterform pear psylla (Figure 1A). Specimens will include psylla having a partially known dietary history (i.e., psyllids collected directly from shelter plants) and specimens

having an unknown dietary history (i.e., psyllids collected in traps or from non-plant sources; see Results). The resulting PCR bands will be excised from the agarose gels and purified (Figure 1B). Since each psylla may feed from multiple shelter plants, each PCR band may be comprised of DNA from several shelter plant species (Figure 1C, unknowns). PCR products from unknown shelter plants will be cloned into *E. coli* vectors (Figure 1D) and grown on an agar growth medium (Figure 1E). Each resulting bacterial colony will harbor DNA from a single shelter plant. Multiple bacterial colonies will be selected from each plate and grown in liquid media. Plasmid DNA will be harvested from bacterial cells, and restriction enzyme digestion will be used to confirm the presence of shelter plant DNA in each subsample. The DNA clones will be sequenced by a commercial sequencing service (MC Laboratories, San Francisco, CA) (Figure 1F). The sequences will be identified using the BLASTn analysis available from the NCBI website and by comparing sequences with those obtained from potential shelter plants. Although the basic process for molecular gut content analysis has been established, techniques specific to pear psylla and their shelter plants need to be developed.

Objective 1: Design PCR primers to detect shelter plant DNA

This objective is finished. We are now examining utility of these primers by assaying field-collected winterforms having a partially known dietary history and winterforms having an unknown dietary history (see Methods and Results for Objective 2).

Objective 2: Determine the number of sequences required to identify previous shelter plants

Because winterform pear psylla are highly mobile and thus may visit multiple shelter plants, sequences from multiple gene-clones will be required to identify recently visited plants (Figure 1C-D). The number of gene-clones required depends largely on how mobile winterform psylla are and the relative amount of feeding that occurs on each shelter plant. Pear psylla adults will be collected in winter using beat trays from pear and apple orchards, from coniferous windbreaks, from other shelter plants, and from traps and other non-plant sources (see Table 1 summary in the Results). Shelter plant DNA will be amplified using primers developed in Objective 1, and amplicons will be cloned into bacterial vectors (Figure 1). Sequences from 15 clones (bacterial colonies) from each insect will be sequenced to estimate the diversity of shelter plants detected from winterform psylla. The number of sequences required to identify recently visited shelter plants will be determined based on diversity of sequences. For example, if all 15 clones from each individual psylla are largely from a single shelter plant, then very few clones will be required to determine the feeding history of wild psylla. On the other hand, if a diversity of shelter plants are identified, more sequenced clones may be required from each psylla to better represent the diversity of shelter plants visited by winterform psylla.

After the submission of this proposal in 2016, the ARS location in Wapato purchased a Denaturing Gradient Gel Electrophoresis machine (DGGE). The DGGE apparatus separates DNA fragments not only by size, but also by charge. Thus, sequences of identical size can be separated if they have minor variations in base pair sequences, which would drastically cut costs of cloning and sequencings because the number of bands observed will equal the number of plants fed upon by a psyllid. DGGE will be assessed to determine if plant sequences amplified from psyllids can be separated on DGGE, and whether direct sequencing (without cloning) can identify the amplified products.

Objective 3: Determine how long the plant DNA signal persists in winterform psylla

The purpose of this objective is to determine whether shelter plants visited by psylla in November or December can readily be detected in adults returning to pear orchards in February and March. This experiment will be conducted during the winter/spring of 2017, and will employ molecular techniques developed in Objectives 1 and 2. In early November, December, and January, pear psylla adults collected from a pear orchard will be confined to shoots of shelter plants for 2 weeks. Shelter plants will be selected based on preliminary results from experiments conducted

during the winter of 2016 (Objective 2). Following the 2-week exposure to shelter plants, the psylla will be transferred to shoots of pear located at the USDA experimental orchard in Moxee. Psylla will be collected in late February and March to determine whether the shelter plant DNA (DNA other than pear) is still detectable.

Objective 4: Develop and test flight-interception traps that (a) effectively trap returning psylla before they have entered the orchard and fed extensively on pear, but (b) do not compromise the plant (DNA) signal within the guts of those returning insects.

We will compare several flight-interception traps to develop a trap that is effective at capturing psylla, but that also allows psyllids to be removed from the trapping medium fairly easily and with no substantial loss of the plant DNA signal. Our major issue with currently used interception traps is that these traps include heavy layers of tangle-trap. The tangle-trap tends to fully coat trapped insects, and this leads to substantial difficulties in salvaging psylla for later molecular work. We will examine four types of traps that we hope will avoid the messiness of currently-used traps: mesh traps (our standard trap used in our field-testing of the psylla pheromone), but coated only very lightly with (1) a sprayable form of tangletrap or (2) with a thin layer of horticultural oil; (3) interception traps composed of clear, low-tack tape attached to wooden frames; and (4) a prototype psyllid trap manufactured with 3D printer technology, designed to collect psyllids directly into preservative. Traps will be placed on the perimeter of pear orchards. We will select areas of pear orchards adjacent to the following non-pear habitats, to allow testing for several types of target plant DNA: rangeland (targeting evergreen shrubs such as sagebrush and rabbitbrush); apple orchard; cherry orchard; poplar windbreak; coniferous windbreak. Traps will be set-out at the beginning of the re-entry period (February) and examined daily for psylla. Psylla will be removed from traps, moved immediately into alcohol, and examined later for plant DNA.

RESULTS AND DISCUSSION

Objective 1: Design PCR primers to detect shelter plant DNA

Our previously published primers for chloroplast DNA (Cooper et al. 2016. Environ. Entomol. 45: 938-944) amplify sequences from plants within the Solanaceae with high efficiency, but do not adequately amplify sequences from other plant Families. Several other universal primer sets were tested, but most did not consistently amplify plant DNA from psylla. A primer set that efficiently amplifies the P6-loop of *trnL* (chloroplast) from a wide variety of plants species will be used for gut content analysis of pear psylla. Although this region of DNA is highly variable among plant species, we have observed similar sequences among unrelated plants which could complicate identification of sequences to species. Regardless, this primer set consistently amplifies plant DNA from psylla, and produces product suitable for TA cloning.

Objective 2: Determine the number of sequences required to identify previous shelter plants

Diapausing/dispersing winterform psylla were collected in November-December 2015 and 2016 from a number of shelter plant species (Table 1: (1)-(4)). Because specimens were collected directly from shelter plants, they have a partially known dietary history and are being used extensively in our methods development and examination of the *trnL* (chloroplast) primer set. A second set of diapausing/dispersing psylla having an unknown dietary history were collected to eventually test our methods using specimens for which we have no previous idea of feeding history (Table 1: (5)). Our initial collections of winterform psylla from the Moxee farm (Table 1: (1)) and assays of those specimens showed that psylla had sampled a large number of highly diverse plant species, including annual weeds. Assays are continuing with other specimens having partially known dietary histories, collected from a diverse array of shelter plant hosts (Table 1: (2) – (4)).

Because of the apparently large number of shelter hosts or feeding hosts visited by psylla, a large number of sequences will be required to fully assess the dietary history of dispersing psylla if these sequences are still present when psylla return to orchards in late winter/early spring. The use of

DGGE could drastically reduce the number of products requiring cloning and sequencing, and is the purpose of our non-funded extension of this project. We now have a large number of winterform psylla in storage having an unknown dietary history (Table 1: (5)), with which to examine using DGGE as necessary. Additional specimens having an unknown dietary history are to be collected in February-March 2017 using traps placed near a pear orchard (Objective 4).

Objective 3: Determine how long the plant DNA signal persists in winterform psylla

This objective will be performed during the winter and spring of 2017.

Objective 4: Develop and test flight interception traps that (a) effectively trap returning psylla before they have entered the orchard and fed extensively on pear, but (b) do not compromise the plant (DNA) signal within the guts of those returning insects.

Efficiency of several interception traps for capture of winterform adults were compared in spring of 2016. Interception traps with low-tack tape were not effective at capturing psylla, and will not be suitable for capturing psylla for gut content analysis. Mesh traps treated with horticultural oil were very effective at capturing psylla, but were messy to work with. Brown and olive green 3D psyllid traps successfully captured winterform psylla. Since these traps capture psylla directly into preservative, there is no need to remove horticultural oil or sticky trap residue from psylla before DNA extraction. We will continue work this winter with mesh traps and 3D traps, and determine whether trapping methods compromise the plant DNA signal.

Table 1. Winterform psylla were collected mid-November to early-December 2015 and 2016 from miscellaneous orchard and shelter plants at four locations. Collections (1)-(4): specimens were collected directly from shelter plants and are being used to confirm the utility of our molecular methods for psyllids having a partially known dietary history. Collection (5): dispersing winterforms were collected in mid-November from the side of a house in West Yakima, located some 2 miles from the nearest pear orchard; these specimens will allow us to examine our methods for psylla having an unknown dietary history.

	Numbers of winterforms collected and now in storage (-80 ^{o}C)
(1) Known plant sources (Moxee farm; winter 2015-2016)* Pear orchard, apple orchard, coniferous windbreak	(specimens already assayed)
(2) Known plant sources (West Yakima; Nov-Dec 2016)**	
Juniperus windbreak	22
Mixed creekside vegetation (*Rosa, Populus, Salix, Cornus*)	6
Ponderosa pine (*Pinus ponderosa*)	5
Weeping Nootka false cypress (*Chamaecyparis nootkatensis*)	7
Unidentified coniferous	3
Golden currant (*Ribes* sp.)	2
Unknown ornamental fir (*Abies* sp.)	9
Lilac bush (*Syringa vulgaris*)	3
Gold Cone Cedar (*Cedrus deodara*)	28

(3) Known plant sources (YARL-Wapato; Nov-Dec 2016)**	
Butterfly bush (*Buddleja* sp.)	41
Unknown ornamental fir (*Abies* sp.)	35
Western Cedar (*Thuja plicata*)	42
Weeping Nootka false cypress (*Chamaecyparis nootkatensis*)	64
Ponderosa pine (*Pinus ponderosa*)	30
Oregon grape (*Mahonia aquifolium*)	30
(4) Known plant sources (Naches region; Nov-Dec 2016)**	
Ponderosa pine (*Pinus ponderosa*)	1
Douglas fir (*Pseudotsuga menziesii*)	4
Western cedar (*Thuja plicata*)	14
Mugo pine (*Pinus mugo*)	9
(5) Unknown dietary history (West Yakima)***	258
Planned: Unknown dietary history (traps placed on perimeter of orchard)	(February 2017)

*Specimens were collected in 2015 and used in initial development of methods; completed.
** To be used in further testing of trnL primer set as well as proof-of-concept confirmation of winter feeding; specimens were collected Nov-Dec 2016.
*** Specimens were collected from the four sides of a house located in West Yakima. The specimens are to be used in examining our methods for psylla that have an unknown dietary history; specimens were collected Nov-Dec 2016. Additional specimens will be collected during the 2017 re-entry period using interception traps that have been placed on the perimeter of a pear orchard.

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

Project Title: Improved late- and post-bloom sanitation of fire blight pathogen

PI: Ken Johnson
Organization: Oregon State University

Telephone: 541-737-5249
Email: johnsonk@science.oregonstate.edu
Address: Dept. Botany & Plant Pathology
2082 Cordley Hall
Corvallis, 97331-2902

Other funding sources

Agency Name: USDA NIFA ORG
Amt. awarded: $495K to Johnson, Elkins, Granatstein and Smith 10/14 - 9/17
Notes: Objectives of this proposal are supplemental to objectives for the above project.

Budget
Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Russ Karow
Telephone: (541) 737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	**2016-17**	**2017-18**	
Salaries Faculty Res. Assist. 2 mo.	9,200	9,384	
Benefits OPE 58%	5,336	5,443	
Undergraduate labor (&OPE 12%)	1064	1,085	
Equipment			
Supplies	1,250	1,275	
Local Travel	250	255	
Miscellaneous			
Plot Fees	1,000	1,020	
Total	$18,100	**$18,462**	

***Footnotes: Total Budget** **Year 1**: $36,200 **Year 2**: $36,924 (2% inflation)
50% by WTFRC Apple Crop Protection, 50% by FPC/WTFRC Pear.

OBJECTIVES

1) Evaluate EPA-registered materials for their ability to reduce epiphytic populations of the fire blight pathogen, *Erwinia amylovora*, on pear and apple trees, and to kill *E. amylovora* in laboratory-based dose-response experiments.

2) Evaluate the mineral material, alum ($KAl(SO_4)_2$), for fire blight control, to reduce epiphytic populations of *E. amylovora* on pear and apple trees, and to kill *E. amylovora* in laboratory-based dose-response experiments.

3) Evaluate and characterize the abilities of near-commercial preparations of *E. amylovora*-specific phage and protective amendments (sunscreens and carrier strains) for fire blight control, to reduce epiphytic populations of *E. amylovora* on pear and apple trees, and to kill *E. amylovora* in laboratory-based experiments dose-response experiments.

SIGNIFICANT FINDINGS

- In both pear and apple, epiphytic populations of the fire blight pathogen on flowers were still increasing at one week after petal fall.
- Acidifying oxytetracycline with half rate of buffer protect (pH 4) appeared to improve the level of inoculum sanitation and fire blight control from this antibiotic.
- In general, materials that suppress infection also reduce pathogen inoculum.
- Among EPA-registered materials for non-antibiotic fire blight control, a three-quart rate of Previsto soluble copper and the antibiotic Kasumin stood out as an effective materials for both inoculum sanitation and infection suppression.
- Under weather conditions highly conducive for fire blight, numerous materials were only poor to fair at inoculum sanitation including Serenade Opti, a three-quart rate of Cueva soluble copper, and experimental phage-based materials.
- Among various (mostly disappointing) phage-based materials, a preparation of phage pre-infected into *Pantoea agglomerans* show significant inoculum and infection suppression.
- Alum at 1% (8 lbs./100 gal) provided very good inoculum sanitation and excellent fire blight control.
- Late bloom treatments of lime sulfur (2%) provided good inoculum sanitation and fire blight control and improved fruit finish of pear.

METHODS

Rationale. In recent years, there has been a rapid increase in the number of biopesticide materials available to control fire blight. Many have become EPA-registered with only a limited number of field trials that demonstrate efficacy. Consequently, we are making a comparative investigation of the various materials registered for fire blight control in conventional and organic systems. In addition, we are investigating several experimental materials near commercialization. We seek to better understand on a comparative scale: the effects of a material on epiphytic pathogen populations on inoculated trees, their ability to suppress infection, and the material dose/pathogen killing relationship.

Experimental design. Objectives were addressed in experimental orchards located at Oregon State University's Botany & Plant Pathology Field Laboratory in Corvallis. Experiments were arranged in a randomized complete block design with 4 replications. During early morning, treatment suspensions and pathogen inoculum were sprayed to near runoff with backpack sprayers.

Measurement of pathogen populations: Eight flower clusters were sampled from each replicate tree on each of three dates: full bloom, petal fall, and one-week post petal fall. Each flower cluster sample was washed in sterile phosphate buffer. After washing, dilutions of wash were spread on CCT medium to selectively enumerate *Erwinia amylovora*. We also spread the washes on Pseudomonas

agar F amended to enumerate total cultural bacteria populations and on potato dextrose agar to enumerate yeast (*A. pullulans*) populations.

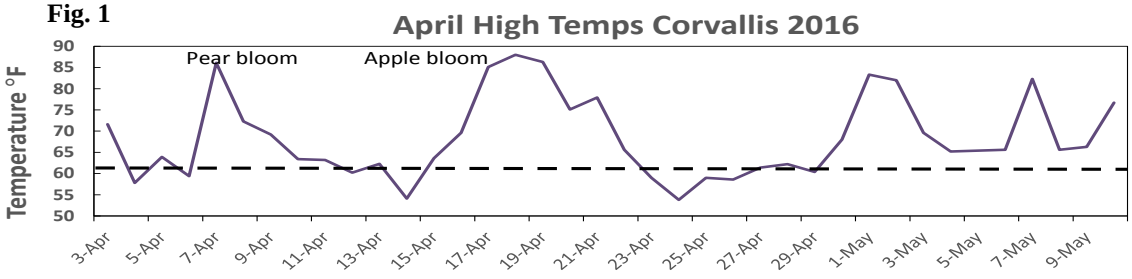
Disease and fruit assessment. Incidence of fire blight was determined by counting blighted flower clusters from each tree 2- to 4-weeks after bloom. Number of blighted flower clusters were divided by total clusters per tree, which was determined pre-bloom. In August, percent fruit russet was scored with a modified Horsfall-Barratt rating scale.

Lab-based dosed response experiments. Laboratory-based assays were designed to develop logistic-decline dose-response curves for effect of biopesticides on *E. amylovora* survival. The assay exposed pathogen cells to a dose of biopesticide for a period of time (60 min). Pathogen cells were recovered by filtration, rinsed in buffer, then dilution plated on nutrient agar to determine survivorship.

RESULTS & DISCUSSION

Weather in spring 2016. Temperatures were exceptionally favorable for epiphytic growth of *E. amylovora* on both pear and apple flowers. Fire blight risk as determined by the heat unit model, COUGARBLIGHT, was high to exceptional during bloom of both tree species. Average maximum daily high between 7 and 22 April was 72ºF with high temperatures of 86 and 87ºF on 7 and 19 April, respectively (Fig. 1). Consequently, epiphytic populations of the fire blight pathogen and incidence of fire blight were very high in all four orchard trials. For orchards used in objective 1, the number of strikes per tree on the water treated control averaged 673 and 315 in Bartlett pear and Golden Delicious apple, respectively; for orchards used for objective 2 and 3, strikes per tree on the water treated control averaged 319 and 197 in Bartlett pear and Gala apple, respectively.

Fig. 1



Obj. 1. Evaluate EPA-registered materials for their ability to reduce epiphytic populations of the fire blight pathogen, *Erwinia amylovora*, on pear and apple trees, and to kill *E. amylovora* in laboratory-based dose-response experiments.

Fire blight control. For pathogen-inoculated trials, disease intensity was high with fire blight infections on water-treated trees averaging 76% and 55% of total clusters in Bartlett pear and Golden Delicious apple, respectively (**Table 1**). Antibiotic standards (streptomycin (FireWall) once, and oxytetracycline (FireLine) twice) were among the best performing materials in both trials (72 to 88% control). In apple, compared to the water-treated control, percent control from two applications of Blossom Protect plus Buffer Protect (88%), one application Blossom Protect plus Buffer Protect then OxiDate twice (70%), Previsto twice (78%) were statistically similar to the antibiotic standards. In pear, these materials showed an intermediate performance along with the soluble copper material, Cueva. In both trials, low to intermediate levels of control (<50%) were observed with Serenade Opti applied three times or Serenade Opti (once) in a program with Cueva (twice). In apple, disease control obtained with Previsto was statistically superior to control obtained with Cueva, which we speculate is attributable to the amount of metallic copper in each material (2.9 and 1.8%, respectively). In lab-based bioassays, copper-based materials, OxiDate (hydrogen dioxide, peroxyacetic acid), acidified solutions, lime sulfur, streptomycin and kasugamycin were effective materials for killing *E. amylovora* (data not shown).

Table 1. Evaluluation of EPA-register non-antibiotic materials for fire blight control in Bartlett pear and Golden Delicious apple, Corvallis, 2016.

Treatment	Rate per 100 gallons water	Bloom stage of treatment* 70%	Full	Petal Fall	BARTLETT PEAR Percent blighted floral clusters**		GOLDEN D. APPLE Percent blighted floral clusters**	
Water		---§	**X**	**X**	**76.3**	**a**	**55.0**	**a**
FireWall	8 oz.	---	X	---	10.4	f	7.3	ef
FireLine	16 oz.	---	X	X	20.9	ef	6.9	f
Serenade Opti (plus BioLink)	20 oz.	X	X	X	60.0	abc	30.5	cd
Serenade Opti (plus BioLink)	20 oz.	X	---	---	72.4	abc	41.6	bc
then Cueva (2 quarts)	64 fl. oz.	---	X	X				
Buffer Protect	150 oz.	X	---	---	65.2	abc	44.5	bc
Blossom Protect	21.4 oz.	X	---	---	69.7	abc	31.5	cd
Blossom Protect	21.4 oz.	X	---	---	54.3	bcde	23.6	de
Buffer Protect	150 oz.	X	---	---				
Blossom Protect	21.4 oz.	X	X	---	40.8	de	6.7	f
Buffer Protect (twice)	150 oz.	X	X	---				
Blossom Protect	21.4 oz.	X	---	---	42.4	cde	16.0	ef
Buffer Protect	150 oz.	X	---	---				
then OxiDate	128 fl. oz.	---	X	X				
Cueva (3 quarts)	96 fl. oz.	X	X	---	45.9	bcde	38.7	bcd
Previsto (3 quarts)	96 fl. oz.	X	X	---	35.6	ef	12.0	ef

*** Trees inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at 5 x 10^5 CFU/ml on 30 March (pear) and 6 April (apple). ** Trees used in the experiments averaged 871 and 507 flower clusters per tree for pear and apple, respectively. For each treatment, percent blighted flower clusters was transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. § X indicates material was sprayed on that specific date; --- indicates material was not applied on that specific date. # Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$.**

Epiphytic pathogen populations. Measured epiphytic populations of pear and apple flowers were generally correlated with incidence of disease. The highest epiphytic population size was measured on the water-treated control and the lowest was measured on flowers that received streptomycin. The soluble copper material, Previsto, applied at 70% and full bloom effectively suppressed *E. amylovora* populations through petal fall (**Fig. 2A&D**). In contrast, FireLine (oxytetracycline) and Cueva (soluble copper) provided intermediate levels of suppression of the fire blight pathogen. Serenade Opti showed only slight suppression of epiphytic pathogen populations (**Fig. 2B&E**), which also correlated with the low level of disease control obtained with this material. The exception to the correlation of pathogen population size and disease incidence occurred with Blossom Protect plus Buffer Protect treatments, either applied twice or applied once and followed in a program with OxiDate (applied twice). Populations of the pathogen were not markedly suppressed by these treatments (**Fig. 2C&F**), but the corresponding levels of disease control were intermediate (pear) to outstanding (apple) (**Table 1**).

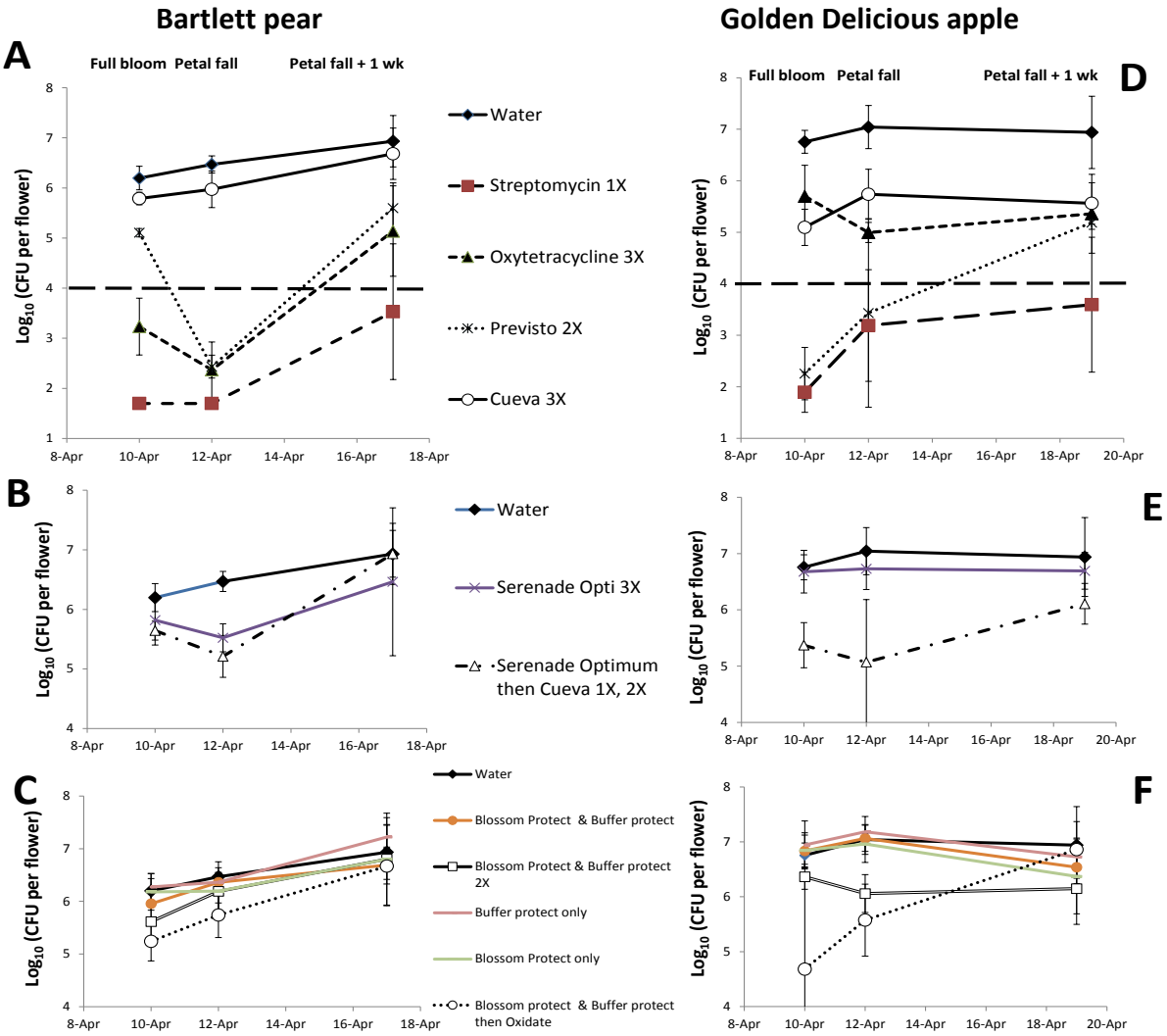


Fig. 2. Effect of treatments for fire blight suppression on *E. amylovora* populations on flowers during pear and apple bloom, April 2016. Orchards were located near Corvallis, OR with each treatment applied to four replicate trees. Pathogen populations were determined by bulk sampling five flower clusters (~25 flowers) from each tree; each sample was washed in 25-ml of sterile phosphate buffer followed by dilution plating onto tryptic soy agar. Panels A (pear) and D (apple): antibiotics and soluble copper materials; Panels B (pear) and E (apple): Serenade Opti with and without Cueva soluble copper; and Panels C (pear) and F (apple): Blossom Protect and Buffer Protect with and without OxiDate. Horizontal dashed line in Panels A and D indicate the bottom of y-axis scale in Panels B, C, E and F.

Obj. 2. Evaluate alum ($KAl(SO_4)_2$) *E. amylovora*-specific phage for fire blight control and ability to reduce epiphytic populations of *E. amylovora* on pear and apple trees, and to kill *E. amylovora* in laboratory-based dose-response experiments.

Obj. 3. Evaluate and characterize the abilities of near-commercial preparations of *E. amylovora*-specific phage and protective amendments (sunscreens and carrier strains) for fire blight control, to reduce epiphytic populations of *E. amylovora* on pear and apple trees, and to kill *E. amylovora* in laboratory-based experiments dose-response experiments.

Fire blight control. In previous trials, alum ($KAl(SO_4)_2$), a low pH salt used at the rate of 2% (w:w), has effectively suppressed fire blight infection and has not contributed to fruit marking in several russet evaluations. In 2016, 2% alum (16 lbs. per 100) again effectively suppressed fire blight in both pear and apple. Moreover, 1% alum was nearly as effective as the higher concentration. In contrast, with 0.5% alum, fire blight suppression fell off compared to higher rates of the material.

Materials containing phage (bacterial viruses) to specifically attack *E. amylovora* generally provided poor fire blight suppression. Poor performance is possibly attributable to the need for phage to efficiently infect their prey, which is a difficult task when weather conditions allow the pathogen's epiphytic population size to expand rapidly. An exception was Ag Canada's #2 phage prep that was pre-infected into *Pantoea agglomerans* strain E325 (the EPA-registered strain in Bloomtime Biological first selected by Dr. Larry Pusey, ARS, Wenatchee). We term this strategy of phage deployment a 'Trojan horse' because the viruses that attack *E. amylovora* can increase inside a closely related, beneficial bacterium (with suppressive properties of its own) when populations of the fire blight pathogen are small. Research with Ag Canada's strategy will continue in 2017.

Table 2. Evaluluation of EPA-register altenative materials for fire blight control in Bartlett pear and Gala apple, Corvallis, 2016.

Treatment	Rate per 100 gallons water	Bloom stage of treatment*			BARTLETT PEAR Percent blighted floral clusters**		GALA APPLE Percent blighted floral clusters**	
		60%	Full	Petal Fall				
Water		---§	X	X	**57.3**	**a#**	**33.1**	**a#**
Streptomycin	8 oz.	---	X	---	1.0	h	6.4	ghi
Kasumin 2L	64 fl. oz.	---	X	X	2.4	h	6.4	hi
FireLine plus	**16 oz.**	---	**X**	**X**	-not tested-		**2.9**	**i**
Buffer Protect (half)	**75 oz.**	---	**X**	**X**				
Alum 2%	**267 oz.**	---	**X**	**X**	**4.6**	**gh**	**7.9**	**efghi**
Alum 1%	**134 oz.**	---	**X**	**X**	**13.1**	**fgh**	**6.8**	**fghi**
Alum 0.5%	67 oz.	---	X	X	37.1	bcde	19.9	abcd
Buffer Protect (full)	150 oz.	---	X	X	56.2	ab	18.0	bcde
Alum 0.5% plus	67 oz.	---	X	X	34.7	cde	17.9	cde
Buffer Protect (half)	75 oz.	---	X	X				
Serenade Opti (BioLink)	20 oz.	X	X	X	39.7	abcd	24.2	abcd
Pantoea agglomerans C9-1	10^8 cfu/ml	X	X	X	-not tested-		17.2	cde
Ag Canada *Pantoea* spp. #1	10^8 cfu/ml	X	X	X	-not tested-		23.1	abcd
Ag Canada *Pantoea* spp.	10^8 cfu/ml	X	X	X	32.7	cde	20.8	abcd
plus selected phage #1	~	X	X	X				
Ag Canada *Pantoea* spp.	**10^8 cfu/ml**	**X**	**X**	**X**	**19.3**	**ef**	**16.5**	**cdef**
plus selected phage #2	**~**	**X**	**X**	**X**				
Fire Quencher A	~	X	X	X	51.1	abc	29.4	abc
Fire Quencher A	~	X	X	X	35.5	cde	22.2	abcd
plus Serenade Opti (BioLink)	20 oz.	X	X	X				
Fire Quencher B (tryptophan)	~	X	X	X	48.7	abc	32.5	ab
Fire Quencher C	~	X	X	X	48.0	abc	23.5	abcd
plus Serenade Opti (BioLink)	20 oz.	X	X	X				
Lime sulfur 2%	**256 fl. oz.**	**---**	**X**	**X**	**21.9**	**def**	**12.2**	**defgh**
OxiDate 1%	128 fl. oz.	---	X	X	46.6	abc	12.7	defgh
Oxycom CA	64 oz.	---	X	X	35.7	cde	16.7	cdefg

*** Trees inoculated with *E. amylovora* strain Ea153N (streptomycin-sensitive) at 5 x 10^5 CFU/ml on 1 April (pear) and 6 April (apple). ** Trees used in the experiments averaged 584 and 574 flower clusters per tree for pear and apple, respectively. See footnote of Table 1 for other callouts and description of statistical analysis.**

Epiphytic pathogen populations. Again, measured epiphytic populations on pear and apple flowers generally correlated with incidence of disease. Alum showed a strong dose response relationship on pear but less so on apple (**Fig. 3**), and on both pear and apple, Ag Canada phage prep #2 stood out from other phage treatments and the water-treated control. Interestingly, on apple, suppression of the pathogen's population size with phage prep #2 was similar to observed with 1% and 2% alum, but alum had a larger effect on suppression of infection. We speculate that this is related to the lower pH of alum; corresponding levels of disease control with this alum were intermediate (pear) to outstanding (apple) (**Table 1**).

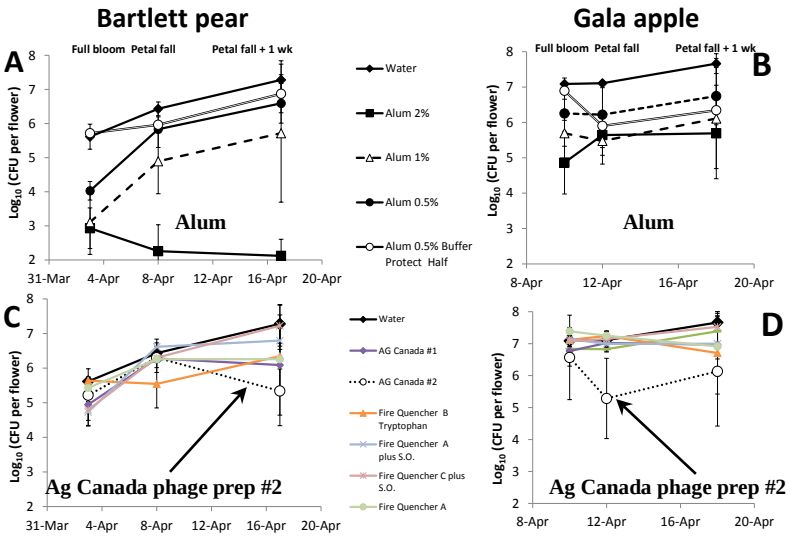


Fig. 3. Effect of alum and phage treatments for fire blight suppression on *E. amylovora* populations on flowers during pear and apple bloom, April 2016. Sampling protocol described under Fig. 2. Panels A (pear) and B (apple): various rates of alum ($KAl(SO_4)_2$) compared to water –treated control; panels C (pear) and D (apple): various formulations of phage materials including 'naked' phage prep with and without sunscreens (FireQuencher, BYU University) and 'trojan horse' prepa

Other notable observations. FireLine (oxytet) amended with a half label rate of Buffer Protect was the best treatment in the Gala apple trial (**Table 2**). This treatment is notable because the pathogen's population size at one week post-petal fall was lower than we have observed previously (compare oxytet result in **Fig. 4** to oxytet results in **in Fig. 2**). Acidification of select treatments with Buffer Protect (e.g., oxytet, Serenade Opti) will be a focus of 2017 experiments.

Lime sulfur (2%) was another notable observation as it significantly suppressed fire blight (**Table 2**), suppressed pathogen populations size (**Fig. 4**), and resulted in the least russeted pear fruit in the alternative materials trial (**Fig. 5**). In the other pear trial (**Table 1**), Blossom Protect treatments <u>increased</u> fruit russet (data not shown), thus we believe the effect of lime sulfur shown in **Fig. 5** is the result of suppressed yeast populations. In apple, some central WA advisors are now using lime sulfur (up to 4%) for fire blight control in late bloom. Higher rates of lime sulfur will be a research focus in 2017.

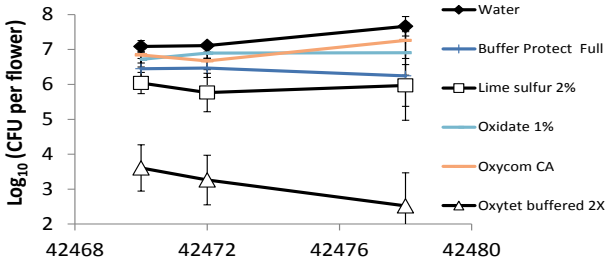


Fig. 4. Effect of alternative treatments for fire blight suppression on *E. amylovora* populations on flowers during pear and apple bloom, April 2016. Sampling protocol described under Fig. 2.

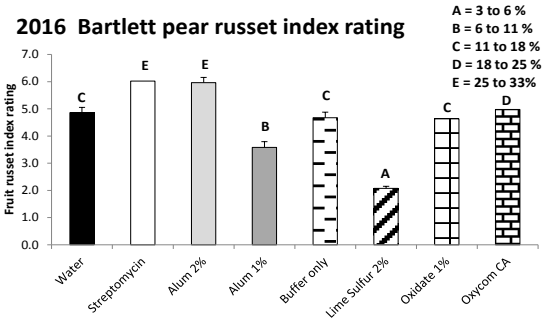


Fig. 5. Effect of alternative treatments for fire blight suppression on fruit russet of Bartlett pear, Aug 2016.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-16-103

Project Title: Enhancement of postharvest decay management in pear

PI: Achour Amiri
Organization: WSU-TEFREC
Telephone: 509-663-8181 ex 268
Email: a.amiri@wsu.edu
Address: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

Co-PI (2): Richard Kim
Organization: Pace Int. LLC
Telephone: 925-357-6708
Email: Richard.kim@paceint.com
Address: 5661 Branch Road
City/State/Zip: Wapato, WA 98951

Cooperators: Yan Wang, OSU-MCAREC, Kelly Wallis (Oregon), multiple packers in WA and OR, Craig Christensen (Cashmere, WA).

Total Project Request: **Year 1:** $32,284 **Year 2: $33,284** **Year 3:** $34,323

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization name: WSU-TFREC **Contact Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 x221 **Email:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	2017	2018
Salaries[1]	17,550	18,252	18,982
Benefits[1]	7,434	7,732	8,041
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[2]	4,100	4,100	4,100
Travel[3]	2,000	2,000	2,000
Miscellaneous	0	0	0
Plot Fees[4]	1,200	1,200	1,200
Total	32,284	**33,284**	34,323

Footnotes:
[1] Salaries for a research intern (Laxmi Pandit, 0.65 FTE) at 42.4% benefit rate.
[2] Supplies include Petri dishes, multi-well plates, microbiological media for fungi growth and fungicide sensitivity tests.
[3] Travel to multiple packinghouses in WA and OR for fruit collection.
[4] Plot fees for an experimental orchard to be used for field studies.

OBJECTIVES

1- Conduct a general disease survey to identify and quantify major postharvest rots.

2- Conduct a general resistance monitoring program across multiple pear orchards and packinghouses in WA and OR to TBZ, pyraclostrobin, boscalid, fludioxonil and pyrimethanil.

3- Evaluate the efficacy of fungicides applied by thermofogging and investigate the possibility of reducing fungicide input.

4- Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.

SIGNIFICANT FINDINGS

Objective 1: *Conduct a general disease survey to identify and quantify major postharvest rots*

- 23 grower lots from 5 packinghouses in WA and 2 packinghouses in OR were surveyed in May of 2016. Blue mold, mucor rot and gray mold were predominant and accounted for 34, 17 and 9% of total decay, respectively.
- The "export" quarantine pathogens Bull's eye rot and *Phacidiopycnis piri* were found at about 6 and 4% of total decay, respectively.

Objective 2: *Conduct a general resistance monitoring program across multiple pear orchards and packinghouses in WA and OR*

- A total of 160 isolates of *Penicillium expansum* (blue mold) and 45 isolates of *Botrytis cinerea* (gray mold) were collected from the different packinghouses surveyed in objective 1. These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), pyrimethanil (Penbotec) and fludioxonil (Scholar) for both *P. expansum* and *B. cinerea* and to pyraclostrobin + boscalid (Pristine) and fluxapyroxad (Merivon) for *B. cinerea* only.
- Resistance of *P. expansum* to thiabendazole (Mertect) and pyrimethanil (Penbotec) was found in about 39% and 24% of the total isolates tested, respectively.
- Resistance of *B. cinerea* to pyrimethanil was about 9% and interestingly, resistance to TBZ was not found. About 4% of *Botrytis* isolates were resistant to Pristine but not to fluxapyroxad (Merivon).
- Populations of *B. cinerea* and *P. expansum* with reduced sensitivity to fludioxonil were found at 11 and 3%, respectively. These populations are controlled by the label rate of the fungicide. However, continuous use of Scholar and related products can cause these populations to become actually resistant.
- A decay and resistance profile was created for each grower lot surveyed and results were sent to the participating packers and growers before the beginning of the new season to allow them change strategies and spray regimes based on decays and resistance found at their locations.

Objective 3: This trial will be conducted in 2017 and results will be shared accordingly.

Objective 4: *Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.*

- A field trial was conducted in the summer of 2017 in a commercial D'Anjou orchard in Dryden (Craig Christensen) including 6 different pre-harvest treatments. Fruit are stored at 33°F at room atmosphere at TFREC and will be evaluated for decay after 5 to 6 months of storage.

METHODS

Objective 1. *Conduct a regional decay survey program.*

The survey started in 2016 was done on a limited number of packinghouses late in the season. In 2017, we plan to start in February and include a larger number of grower lots from Washington and Oregon. For this, 50 decayed fruit will be sampled on the packing line. Ten grower lots (orchards) will be surveyed from each single packinghouse. Fruit will be sampled between February and May and will be placed in clamshells to avoid crashing and cross contamination and transported to the Pathology lab at WSU-TFREC for decay identification and culturing on agar media. Decay identification will be done based on symptoms, spore shape and colony morphology on agar plates. If needed, some pathogens will be identified molecularly.

Objective 2. *Conduct a multiyear regional resistance monitoring program.*

Fruit collected for decay survey (objective 1) will be used to conduct a fungicide resistance monitoring. We will test *Penicillium*, *Botrytis*, and *Neofabraea* (Bull's) isolates from each orchard lot. All *Botrytis* and *Neofabraea* isolates will be tested for sensitivity to boscalid, and fluxapyroxad (Merivon), from the same chemical group (FRAC7), and to difenoconazole, TBZ, pyrimethanil, and fludioxonil whereas *Penicillium* will be tested for the last four fungicides only. Results from the second year will be compared to those from 2017 to produce a map with location-specific resistance profiles to help understanding resistance development and spread. Because storage room can harbor tremendous amount of airborne fungal population, we will survey resistant population of *Penicillium* in storage room atmospheres using an Air-Test sampler. This will help in understanding the buildup and spread of resistance inside storage rooms.

Objective 3. *Evaluate the efficacy of fungicides applied by thermofogging and investigate the possibility of reducing fungicide input*

In recent years, the pear industry has adopted fogging as a new method to apply fungicides in postharvest. Currently, 5 formulations, i.e. Shield-Brite TBZ 99WP or Deccozole A for TBZ, ecoFOG-160 for pyrimethanil, and eFOG-80 or Scholar EZ for fludioxonil, are available for postharvest applications. We will evaluate the efficacy of the pyrimethanil and fludioxonil based formulations in select commercial packinghouses in the Cashmere area, WA. Fifty bins of fruit stored in rooms fogged with the aforementioned fungicides will be evaluated at the end of cold storage. Bins will be run through packing lines to determine decay incidence on multiple grower lots. Because of potential logistical difficulties, if a commercial packinghouse is not identified, smaller-size trials will be conducted at Pace International facilities in Wapato.

To determine potential impact of the different treatments on fungicide resistance development, symptomatic fruit from each treatment/rep will be used to collect fungal isolates that will be evaluated for fungicide sensitivity as described in objective 2.

Objective 4. *Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.*

A field trial was initiated in the summer of 2016 at a commercial D'Anjou pear orchard in Cashmere, WA. The objective was to evaluate six different fungicide rotation programs on disease development in postharvest and potential for resistance development. Fruit were harvested in September and will be evaluated after 6 months of storage at 33°F. A second-year trial will be conducted in 2017 to start earlier in the season and include additional treatments based on results from 2016.

RESULTS AND DISCUSSION

Objective 1. *Postharvest diseases prevalence*

Blue mold with almost 35% of total decay was predominant (Figure 1A) and was found in all lots surveyed with frequencies ranging from 17% to 68% (Figure 1B). Mucor rot represented 16% of total decay and was found in 73% of lots surveyed at frequencies ranging from 4% to 60%. The third major decay was gray mold found in 45% of lots surveyed with an overall frequency of 9% ranging from 4% to 34%.

Besides these three major decays, the "export" quarantine pathogens Bull's eye rot and *Phacidiopycnis piri* were found in 64% of lots surveyed at frequencies of 6 and 4% of total decay, respectively. *Neonectria* is a pathogen known to cause cankers on trees and to cause minor decay on fruit represented 3% of total decay (Figure 1A).

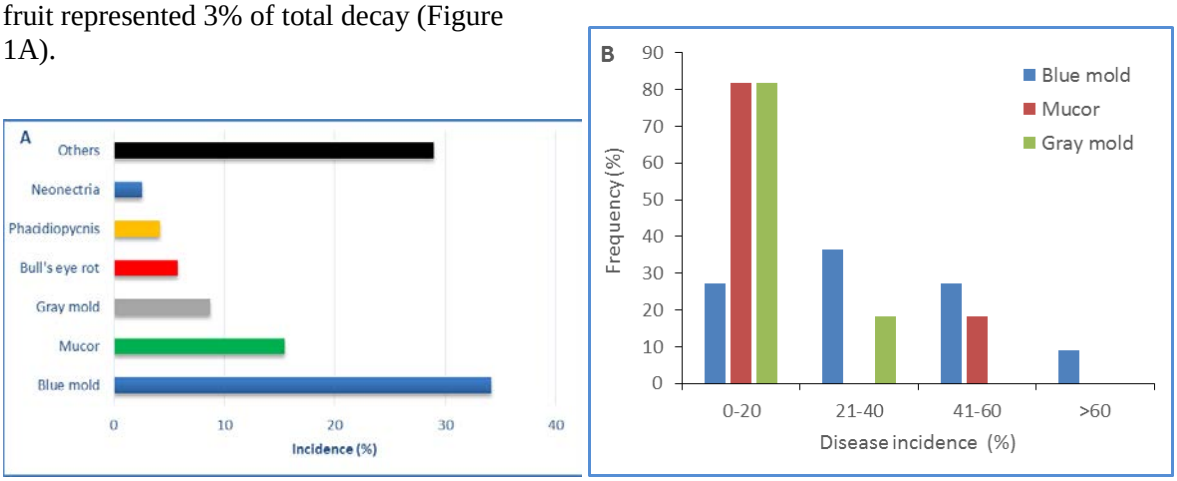


Figure 1. Overall incidence of major postharvest diseases found in in 2016 (A) and incidence distribution of blue mold, gray mold and mucor rot among grower lots (B).

The low number of lots surveyed in 2016 does not allow us to make general conclusions with regard to the diversity of pathogens and their frequency. However, the major decays, such as blue and gray molds and mucor rot, usually encountered on pear seem to be persistent. The fact that the survey was done late in the season may explain why mucor rot was more frequent than gray mold, believed to be more problematic on pear.

The frequency of Phacidiopycnis rot is significantly lower in 2016 compared to when it was first reported in 2005 (30%). The smaller sample size and better management strategies may explain the low presence of this quarantine pathogen.

Objective 2. *Fungicide resistance occurrence and frequencies*

Resistance of *P. expansum* to thiabendazole (Mertect) and pyrimethanil (Penbotec) was found in about 39% and 24% of the total isolates tested, respectively (Figure 2A). Resistance of *B. cinerea* to pyrimethanil was about 9% of total isolates. Interestingly, resistance to TBZ was not found in the gray mold fungus from pear (Figure 2B). About 4% of *Botrytis* isolates were resistant to Pristine, but resistance to fluxapyroxad (Merivon) from the same chemical group (7) was not detected.

Populations of *B. cinerea* and *P. expansum* with reduced sensitivity (tolerance) to fludioxonil were found at 11 and 3%, respectively (Figure 2A&B). These populations are controlled by the label rate of the fungicide. However, continuous use of Scholar and related products can cause these populations to become actually resistant.

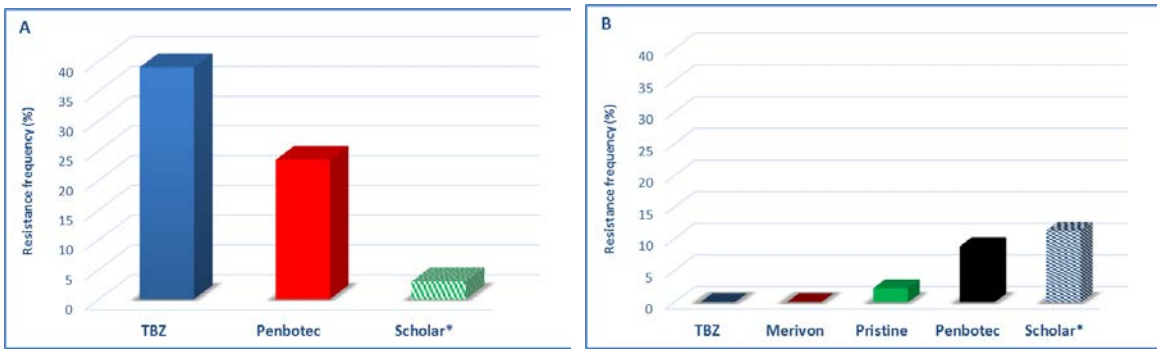


Figure 2. Overall resistance frequencies to major pre- and postharvest fungicides in blue mold (A) and gray mold (B) observed statewide in 2016. * indicate tolerance or reduced sensitivity to Scholar (fludioxonil) bur not actual resistance.

While resistance to TBZ was expected, it seems to be higher than what was seen on apples in 2016 in the blue mold fungus. Resistance to Penbotec (pyrimethanil) is surging (>20%), especially in blue mold. This is certainly because the later has been used more frequently in recent years compared to the two other fungicides. Interestingly, resistance to TBZ was absent in the gray mold fungus (*B. cinerea*). The low number of isolates tested (45) is not enough to get a clear view of the real resistance distribution which we hope to evaluate more accurately in 2017 with a larger population size of the pathogens.

Another element that requires continuous monitoring is the emergence of populations of *P. expansum* and *B. cinerea* tolerant to Scholar (fludioxonil). This fungicide is known to have a lower risk for resistance development compared to TBZ and Penbotec. However, the surge of such populations warrant careful use and rotations of existing fungicides. Indeed, continuous use of Scholar for 2 or more continuous seasons can make these tolerant populations actually resistant and uncontrollable by the fungicide. Therefore, we recommend a one year rotation between scholar and Penbotec. Topsin-M may be used preharvest to control gray mold as the resistance frequency seem to be low. However, TBZ should be avoided postharvest unless mixed with Scholar when risks for bull's eye rot are expected in certain grower lots and susceptible cultivars.

Objectives 3 and 4. Ongoing and results will be shared upon evaluation.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR16-104

Project Title: Integrated fruit production for pears

PI: Elizabeth H. Beers
Organization: WSU-TFREC
Telephone: 509-663-8181 x 234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: None

Total Project Request: **Year 1:** $105,424 **Year 2: $121,474** **Year 3**: $125,811

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/J. Cartwright
Telephone: 509-335-2885/509-663-8181 **Email:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	**2017**	2018
Salaries[1]	63,597	75,054	78,056
Benefits[2]	21,932	26,250	27,300
Wages[3]	6,240	6,490	6,749
Benefits[4]	626	651	677
Equipment	0	0	0
Supplies[5]	4,000	4,000	4,000
Travel[6]	3,529	3,529	3,529
Miscellaneous	0	0	0
Plot Fees[7]	5,500	5,500	5,500
Total	105,424	**121,474**	125,811

Footnotes:
[1]Research Intern, 7 months (year 1), 12 months (years 2 and 3) 0.40 FTE. Post-Doc, 3 years
[2]Benefits for Research Intern 38.6%, Post-Doc 33.5%.
[3]Wages for time-slip help, 1.0 FTE, summer.
[4]Benefits for time-slip 10%.
[5]Supplies – office and lab supplies, electronics, statistical consulting.
[6]Travel to plots – motor pool rental.
[7]5.5 acres total: 2.7 acres (TF8,9), 2.8 acres (WSU Sunrise)/yr x $1,000/acre, 3 years.

Objectives:

1. Evaluate selective pesticides and non-insecticidal tactics for supplementing broad-spectrum insecticides for pear pests
2. Determine the potential for the use of insect growth regulators (IGRs) as pre-bloom and post-harvest sprays for reducing overwintering psylla populations
3. Evaluate tree washing techniques for control of pear psylla and mites
4. Evaluate non-target effects on the predatory mite *Galendromus occidentalis* for commonly used pear miticides
5. Evaluate pesticide efficacy for specific pesticide and pest issues
6. Communicate project results as they become available using electronic outlets (websites, email lists)

Significant Findings

- Psylla adults were higher in soft vs conventional plots from May through August
- Psylla nymphs were higher in June in the soft plot, but similar to conventional during other parts of the growing season
- Psylla nymph numbers were higher in Bartlett than in Anjou in both soft and conventional plots
- There were no consistent differences in soft vs. conventional adult lacewing and syrphids caught in plant volatile sticky traps; earwigs and spiders in cardboard trunk traps were much higher in the soft plot
- FujiMite was acutely toxic to female Typhs, with no survival at the field rate. Agri-Mek was also acutely toxic, and few live larvae were produced. Acramite was not toxic, and caused only a slight reduction in live larvae

Obj. 1. Evaluate selective pesticides and non-insecticidal tactics for supplementing broad-spectrum insecticides for pear pests.

Methods: Two research blocks at WSU's Sunrise orchard were used to test a soft vs a conventional program. The conventional program was applied in SRO8, and the soft program in the neighboring block, SRO7. Both blocks consisted of a mixed planting of 'Anjou' and 'Bartlett' pears planted in 2007 (9 years old). The two cultivars were planted in alternating groups of two rows. Both plots had mating disruption dispensers (Isomate C+, 400 ties/acre).

The soft program used insect growth regulators (IGRs) and materials with a physical mode of action (oil, kaolin) for psylla to the extent possible. The prebloom program consisted of a delayed dormant spray of Surround, Esteem, Microthiol and oil, followed by a popcorn spray of Centaur, Esteem and Vendex (for rust mites). Blossom Protect and Agri-Mycin were applied during bloom during a fire blight infection period. The petal fall spray contained Centaur, Vendex and Intrepid (codling moth ovicide); no materials for psylla were used after petal fall with the exception of 1% oil in the codling moth cover sprays. A fairly complete codling moth program was applied because of the high pressure on the farm. The Intrepid was followed by two Altacor+oil cover sprays for the 1st generation, and the same sequence was used for the 2nd generation. Two applications of CM-GV (Cyd-X) were applied in August against the 3rd generation. Fungicides (Fontelis and Flint) were applied in May, and a routine program of nutrients (zinc, boron) and herbicides (Goal, Alion, Matrix, Venue, Gly-Star) was followed.

The conventional block received a delayed dormant spray of Cobalt Advanced (chlorpyrifos + lambda-cyhalothrin), a PBO (Exponent), Microthiol, and oil. The popcorn spray consisted of Centaur, Assail, Rimon and Agri-Mek, and the petal-fall spray of Ultor, Agri-Flex (abamectin + thiamethoxam), Rimon and oil. The codling moth program consisted of two cover sprays of Altacor for the first generation (plus the Rimon in the petal fall spray); two cover sprays of Delegate against

the 2nd generation, and an application of Imidan against the 3rd generation. The fungicide, bactericide, nutrient and herbicide program was the same as in the soft block.

The soft and conventional blocks were sampled for pests and natural enemies throughout the growing season. The plots were divided into 10 sampling areas per plot, with a sampling area consisting of two rows of the same cultivar (5 reps of Anjou, 5 reps of Bartlett). All samples were taken from these replicates. Beating tray samples were done ca. weekly to evaluate pear psylla adults and natural enemies. Spur samples (8-10/rep) were taken prebloom (March and April) to evaluate pear psylla eggs and nymphs. After bloom when the leaves had expanded, a composite sample of 25 leaves per replicate was brushed with a leaf brushing machine, and all stages of psylla and mites were counted. The adults of syrphids and lacewings were monitored with plant volatile traps (GMP lure; geraniol, methyl salicylate, and 2-phenylethanol clipped to a 5 x 9 inch white sticky panel). One trap per replicate was counted and replaced every 1-2 weeks from mid-May through mid-October; lures were changed every 4 weeks. Earwig and spider densities were monitored using 4 x 10 inch rolls of cardboard tied to the trunk with flagging tape (one/replicate). The traps were placed in a self-sealing plastic bag, frozen, and the earwigs and spiders counted.

Results and Discussion. Overwintering adult psylla populations were low to moderate in March prior to the delayed dormant (DD) treatment, and were very low throughout April after the DD and popcorn treatments. Populations increased throughout the growing season, with peak periods of activity in late May, mid-June, mid-July, and early August, with the highest peak (max. ca. 15 adults/tap) in mid-July (Fig. 1). Adult densities were consistently higher in the soft plot from early May through late August, and were similar in the two plots through the rest of the fall (<1/tap). Surprisingly, nymph densities were poorly correlated with adult densities for much of the season, with a single peak population in early June (Fig. 2). This occurred in both plots, but to a far greater extent in Bartlett/soft; densities in the Anjou/soft were similar to the conventional plot, and only slightly higher than the threshold of 0.3 nymphs/leaf. Interestingly, the cumulative number of nymphs was higher in the Bartletts in both soft and conventional plots. The high seasonal levels in the Bartlett/soft densities is based on three count dates in early June; counts were more similar to the other plots during the rest of the season.

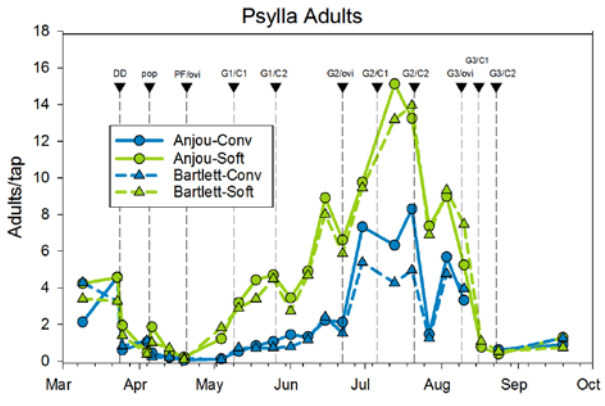


Fig. 1. Psylla adult counts, soft and conventional programs, Anjou and Bartlett (SRO 7 and 8), 2016.

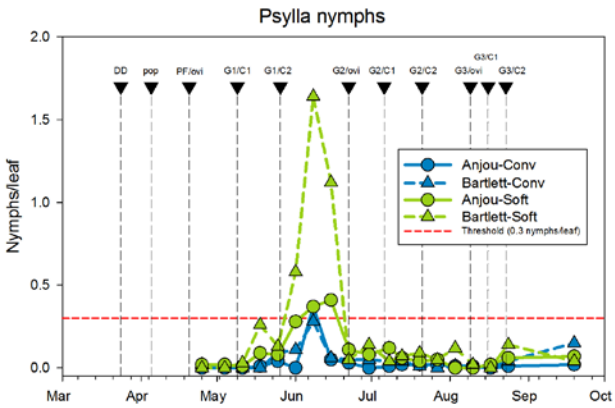


Fig. 2. Psylla nymph counts, soft and conventional programs, Anjou and Bartlett (SRO 7 and 8), 2016.

Mites. Spider mites and rust mites were near zero in both plots throughout the growing season. The soft plot had two applications (popcorn, petal fall) for rust mite (Vendex), and the conventional plot had two applications of Agri-Mek at the same timings. Given the high levels of resistance to Agri-Mek and Vendex in spider mites, rust mites were likely the only species controlled by these sprays.

The reason for the low spider mite levels throughout the season is unknown; predatory mites were also zero during the same period.

Plant volatile traps. There were no consistent differences in lacewings and syrphids between the soft and conventional plot throughout the season. Syrphid densities were low throughout the season, with the highest levels on a single date in late October, most likely a reflection of the 49-day deployment period (as opposed to 7-14 days during the rest of the season). The adults of these two predators are highly mobile, and lack of differences may be due to the relatively small plot size.

Earwig traps. There was a striking difference between the soft and conventional plots in seasonal earwig densities. Earwigs were virtually absent in the conventional plot, and maintained moderate levels in the soft plot (Fig. 3). Earwigs are known to be effective psylla predators, and this may have helped keep nymph populations low in the soft plot. The use of Delegate for codling moth in the conventional plots may have been responsible for the observed low levels of earwigs. Spiders were also reduced in conventional plots in comparison to soft plots, but not to the same degree as earwigs. Although the seasonal cumulative index as only about half in the conventional plots, spiders were able to survive the conventional program to some extent.

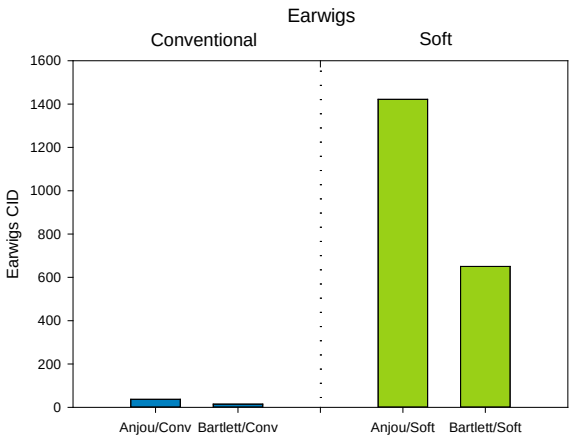


Fig. 3. Earwig population (cumulative insect days), soft and conventional programs, Anjou and Bartlett (SRO 7 and 8), 2016.

Beating tray. *Deraeocoris* and spiders tended to be lower in the conventional plots compared to the soft plots, although not dramatically so. This difference is consistent with the information on natural enemies in the earwig traps.

Fruit damage. Fruit from the soft plot had considerably fewer fruit free from pear psylla damage, about 27% averaged across the two cultivars (Fig. 4). This is in contrast to the conventional plot, which had 67% of the fruit free from damage. However, the difference is less if 0 and 1-10% categories are added, with 94 and 87% for the conventional vs soft. The Anjou in the soft program had 21% of the fruit that was placed in categories 2-4, and likely subject to downgrading. This is the reverse of what might be expected given the higher level of nymphs in the Bartletts. The majority of the fruit was rated as 0 (no damage) or 1 (1-10% damage) for pear rust mite, with no differences between soft and conventional programs. Most of the fruit exhibited a slight amount of russeting. About 96% of the fruit was free from codling moth damage, again with no differences

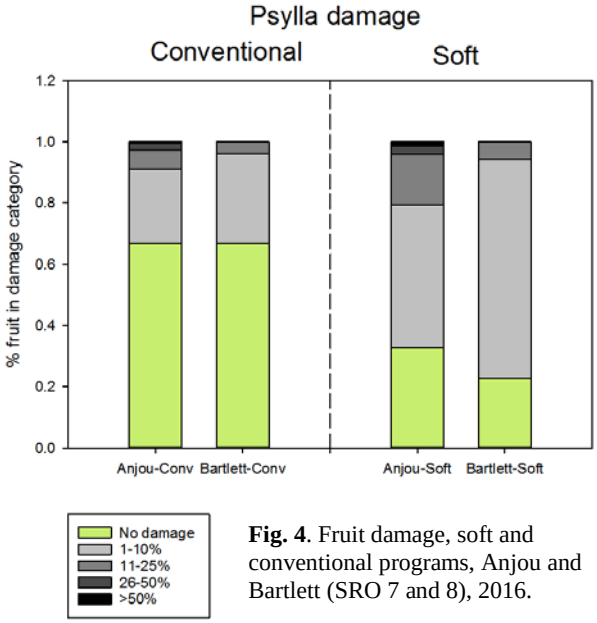


Fig. 4. Fruit damage, soft and conventional programs, Anjou and Bartlett (SRO 7 and 8), 2016.

between the soft and conventional programs. Given the high level of pressure in this research orchard, this is a good level of control. Mealybug and leafroller damage in these orchards was negligible.

Conclusions: nymph populations and fruit damage was lower than might be expected given the absence of dedicated control of the 1st summer generation, which was the only one that exceeded the economic injury level of 0.3 nymphs/leaf. This generation usually occurs in early July, but due to the precocity of the 2016 season, optimal timing was 3-4 week earlier than typical. Targeting this generation with selective controls may help reduce damage at harvest

Obj. 2. Determine the potential for the use of insect growth regulators (IGRs) as pre-bloom and post-harvest sprays for reducing overwintering psylla populations.

Methods: A post-harvest test was deployed in September to determine the effect of Esteem, Dimilin, and Rimon (three IGRs) on overwintering survival and reproductive success of pear psylla. Applications were made using an airblast sprayer to single tree plots in SRO 7 with 4 replicates per treatment. After sprays had dried, the trees were caged and 100 winterform psylla (50 males, 50 females) were released into the cages. Trees untreated during the post-harvest season served as a check.

A windstorm in mid-October fractured about half of the PVC frames of the cages; in one case, the tree was also broken off (Fig. 5). An attempt was made to salvage the rest of the experiment by removing the upper half of the rigid frame, thus reducing the wind shear caused by the fine net of the covering. However, subsequent period of high winds also caused damage to the cages, and the experiment was abandoned.

Tree cages

Cage after windstorm

Broken tree

Fig. 5. Damage to tree cages and broken tree in SRO 7, 2016.

A bioassay was conducted to determine the minimum effective rate of lime-sulfur used for post-harvest sprays. This test used winterform female psylla collected from a commercial orchard. The treatments were 4 rates of Rex Lime sulfur plus an untreated check. Each treatment was replicated 5

times. The adult psylla were anesthetized and sprayed with the appropriate solution of lime sulfur in a laboratory sprayer, and evaluated for mortality after 2 days.

The 10%, 7.5% and 5% concentrations all produced high and not significantly different levels of mortality (92-98%). Mortality was significantly lower in the 2.5% rate (71%) (Fig. 6).

Obj. 3. Evaluate tree washing techniques for control of pear psylla and mites. This objective was deferred until 2017.

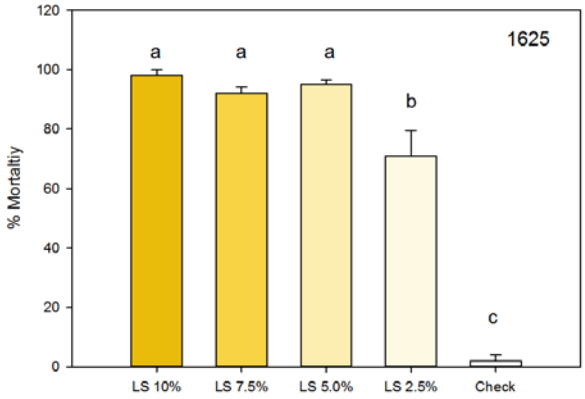


Fig. 6. Bioassay results, minimum effective rate of lime-sulfur used for post-harvest sprays, 2016.

Obj. 4. Evaluate non-target effects on the predatory mite Galendromus occidentalis for commonly used pear miticides.

Methods: A laboratory bioassay was conducted on adult female *G. occidentalis* from a colony collected from a pear orchard in the spring of 2016. We tested three adulticidal acaricides and compared them to an untreated check. In the first part of the bioassay, we measured mortality and fecundity, and in the second part, egg viability and short-term larval survival. The production of live larvae from the treated females is regarded as good summary measure of both lethal and sublethal effects.

A single adult female was transferred from the colony to a bean leaf disk 3.5 cm diam. with ample prey in the form of twospotted spider mite eggs and larvae. Fifty arenas per acaricides treatment were tested. The arenas with *G. occidentalis* and prey were sprayed with the field rate of three acaricides (FujiMite, Agri-Mek, and Acramite), plus a check sprayed with distilled water. Mortality and the number of eggs laid were evaluated after three days, at which time the females were removed from the disk, retaining prey. The *G. occidentalis* eggs were allowed to hatch, at which time the viability (% hatch) of the eggs and the number of live larvae were counted.

Results and Discussion. There were no surviving females in the FujiMite treatment after 3 days, and poor survival (15%) in the Agri-Mek treatment (Fig. 7). Net fecundity was greatly suppressed by these two treatments, along with the production of live larvae. Survival was only slightly impacted in the Acramite treatment (88.6%), with corresponding reductions in fecundity and live larvae. Overall, Acramite is the most selective of the miticides tested to date. The second group to be tested is the ovicidal miticides (Zeal, Envidor, Onager).

Obj. 5. Evaluate pesticide efficacy for specific pesticide and pest issues.

Methods: A series of bioassays was done with Nealta, a newly registered miticide, to determine it relative efficacy and baseline sensitivity. The twospotted spider mites tested were from a colony collected from pear in 2015. The treatments consisted of the field rates of four miticides (Nealta, Vendex, Acramite, FujiMite) plus an untreated check. This colony has been screened and exhibited resistance to multiple miticides, and would be considered typical of mite population in the Wenatchee River Valley. Twenty adult female mites were transferred to a bean leaf disk, with five replicate arenas per treatment. The arenas and mites were sprayed with a laboratory sprayer and evaluated for mortality after 2 days.

Nealta, FujiMite and Acramite all caused high levels of mortality (96-100%) after two days, indicating this new material is similar in efficacy to existing ones. Vendex killed only 42% of the adult mites, significantly less than the other materials. Resistance to organotins such as Vendex has

been demonstrated since the 1990s, and it is likely that this resistance persists in many mite populations.

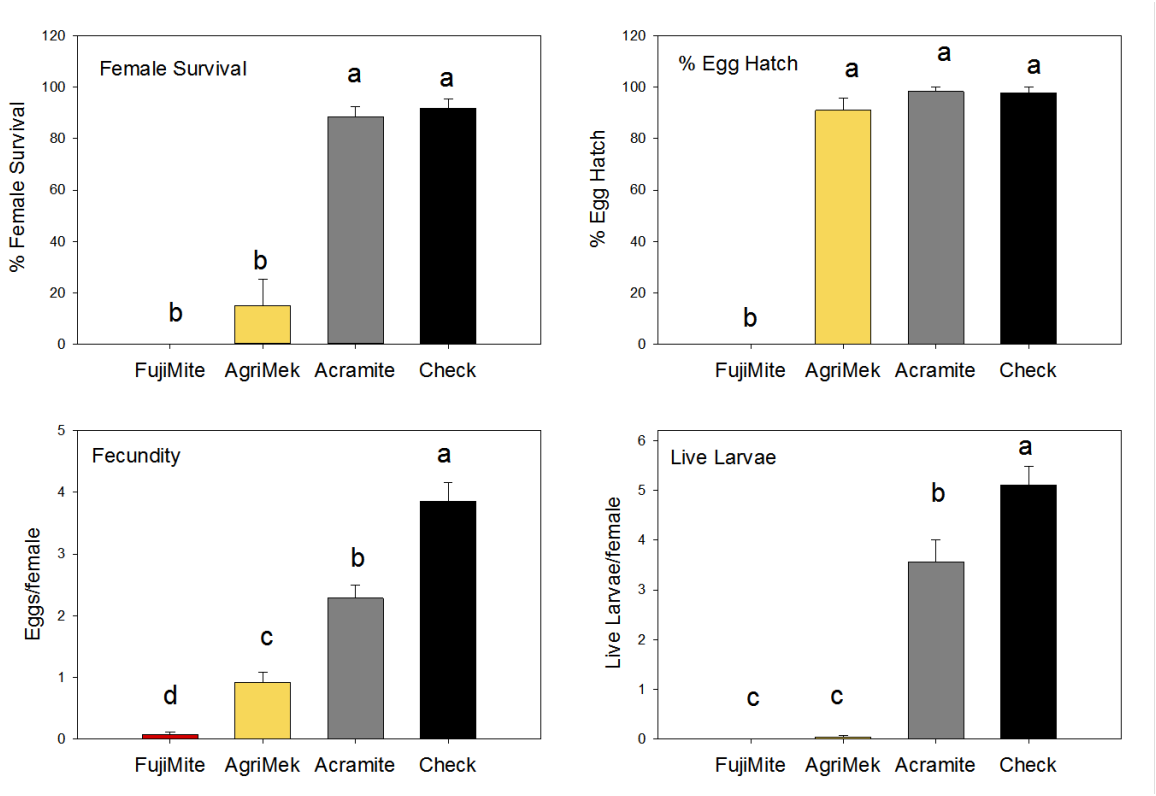


Fig. 7. Non-target effects on the predatory mite Galendromus occidentalis, 2016.

A second bioassay was performed with the same miticides on diapausing females collected directly from a pear orchard in the upper Wenatchee River Valley in April of 2016. Theoretically, diapausing females are more difficult to kill than those that are actively feeding and laying eggs. The results of this bioassay were almost identical to those with non-diapausing females, giving little support to the notion that they are more difficult to kill. It should be noted, however, that although they still retained their orange coloration indicative of diapause, oviposition was evident in the field, thus diapause was in the process of terminating when the tests were done.

Lastly, a baseline bioassay was performed with Nealta using a susceptible laboratory colony. This baseline will serve as a reference for detection of resistance to this material in the future. The rates tested were too high for this very susceptible population, and will have to be reduced and re-tested.

Obj. 6. Communicate project results as they become available using electronic outlets (websites, email lists).

Methods: This objective was deferred until a post-doc could be hired to manage the project, and the beginning of the companion project (2017 field season) funded by the WSDA-SCBG, which emphasizes outreach and implementation.