Northwest Pear Research Review

Yakima Convention Center, Yakima

Wednesday, February 19: Yakima Pom Club meeting, 5:30pm at Zesta Cucina, West Yakima.

			Thursday, February 20, 2020	
Time	Page	Presenter	Title	Yrs
8:00		Schmidt	Welcome/Housekeeping	
	1	McClain	California Pear update (written report only)	
			Continuing Projects	
8:45		Mendoza	WTFRC technology projects (See Appendix)*	20
8:55	3	Crowder	Acoustically based mating disruption of winterform psylla: ***NCE***	18-20
9:05	11	Cooper	Using transcriptomics to target key behaviors of pear psylla	19-21
9:15	17	Northfield	Enhancing pear psylla biological control through predator recruitment: ***NCE***	20
9:25	20	Schmidt-Jeffris	Incorporating trechnites into a psylla biocontrol program	20-22
9:35	28	Nottingham	Improving pear pest management with integrated approaches	20-22
9:45	35	KC	Epidemiology and management of pear gray mold in the PNW	20-22
9:55	43	Johnson	Refinement of fire blight control strategies: buffered oxytetracycline : **NCE**	19-20
10:05			***Break***	
10:20	50	Einhorn	Field evaluation of pear cultivars on cold hardy quince rootstocks**: *Videoconference***	19-21
10:30	58	Dhingra	Evaluating the dwarfing capacity of 65 diverse pear accessions	20-22
10:40	65	Kalcsits	Optimizing irrigation frequency and timing to improve fruit quality	19-21
10:50	73	Thompson	Fine tuning calcium application rates for cork spot	20-22
11:00	80	Rudell	New active ingredients for pear superficial scald control	20-22
11:10	87	Evans	Pear rootstock breeding	20-22
		Murray	IPM strategic planning for pears in WA and OR (no report): ***NCE***	20
11:30 - 1:00			**Working lunch - Industry discussion of proposed pear scion breeding program**	
			Final reports	
1:00	94	Nottingham	Integrated fruit production for pears	17-19
1:10	106	Honaas	Functional genomics of ‘D’Anjou’ pear fruit quality and maturity	18-19
1:20	118	Postman	Interstem grafts to evaluate pear germplasm for dwarfing potential/status of replacement	18-19
1:35	128	DuPont	Fire blight management: new products and effective rates	18-20
1:50	138	Dhingra	Greenhouse screening of 49 dwarf rootstock candidates	18-20
2:05	144	Dong	Mechanisms and practical solutions to control scald of pears	18-20
2:20	155	Cooper	Erythritol: an artificial sweetener with insecticidal properties	20
2:35	163	Hanrahan	Food safety update	
2:45	167	KC	Epidemiology & management of postharvest decay on pears	17-19
2:55 - 3:30		**Amiri/KC**	**Research Showcase: Postharvest Pathology - Current efforts & future directions**	

2019 California Pear Harvest – Industry Outlook

Given the oversupply of Bartlett's created by Seneca Foods leaving the California processing business in early 2018, fresh shippers were encouraging their growers to chemically thin because the packing houses were maxed out in packing capacity and the remaining processors were advising they were buying about the same tonnage as 2018. Nearly every Bartlett grower in the State applied MaxCel to some of their acreage. The results of thinning applications were mixed at best with some growers realizing very little thinning, others observed over thinning resulting in short crops and a very few who felt they reduced the numbers and obtained good size as a result. Weather conditions at the time of application was a factor in the mixed results along with the availability of MaxCel at the critical timing for optimal thinning.

At the estimating meeting on June 20th, most growers were resigned to leaving some percentage of their crop on the trees. Bartlett harvest started in the Sacramento River district on July 18 with all River growers in full harvest swing by July 22. This start date is a week to ten days later than normal, leaving less of a sales window for the normally earlier California fresh crop.

It became apparent about a week into harvest that the Sacramento River district was not going to pick out to anywhere near originally estimated. Size was the issue. Processing pears in California are inspected and paid for by size, 2 ¾ inches and larger being paid the higher processing price. After the first ten days of harvest on the River grower lots was running at 28 to 34% 2 ¾"+. On a normal year percentage of 2 ¾ inch Bartlett's run about 58 to 64% in a grower truck load lot.

Demand for processing Bartlett's greatly increased in all districts after the short fall was recognized in the River district. Very few acres went unharvested - mostly due to maturity issues.

The fresh Bartlett pack-out fell short of the estimate by about 260,000 36# tite-fill equivalents due to processor demand and very poor returns on sales.

There are about 300 acres of highly productive Bartlett's that were pushed out last fall. Judging from the number orchards already fully and/or partially pruned, we don't expect to see any Bartlett's pushed before the 2020 harvest.

Best regards,

Bob McClain

2020 CA Pear Research Proposals

		Budget Request	CPAB Amount	PPMRF Amount
	Entomology			
	Female Codling Moth Lure to Improve Monitoring and Management - Alan Knight, InStar Biologicals; Broe Zoller, Pear Doctor - Lake County; Rachel Elkins, UCCE Lake County	15,276		
2	Monitoring Brown Marmorated Stink Bug in the Sacramento River Delta Pear Orchards and North Coast Counties - Jhalendra Rijal, UCIPM Advisor Stanislas County; Cindy Korn, UCIPM North Coast; KareyWindbiel-Rojas, UCIPM Capital Corridor; Rachel Elkins, UCCE Lake County	22,176		
	Subtotal:	**37,452**		
	Plant Pathology			
3	Evaluation of New Bactericides for Control of Fireblight of Pear Caused by *Erwinia amylovora* - Jim Adaskaveg, UC Riverside	21,500		
4	Detection of Fungicide Resistance in Populations of Pear Scab *(Venteria pirina)* in California Pear Orchards - Akif Eskalen, UC Davis	16,600		
5	Screening Potential Antagonists for Fire Blight Control - Rachel Vannette, UC Davis No-cost Extension			
	Subtotal:	**38,100**		
	Orchard Systems/ Pear Germplasm			
6	Rootstocks and Orchard Systems for European Pears - Rachel Elkins, UCCE Lake County	30,622		
7	Website in Support of the Pear Genomics Research Group - Carlos Crisosto, UC Fruit and Nut Research and Information Center: http://ucanr.edu/sites/peargenomics/	3,000		
8	Maintenance and Conservation of Pear *(Pyrus)* Germplasm - Sugae Wada, OSU	19,760		
9	Planting at UC Davis of Tissue Cultured **ARS** Core Collection Propagated and Grown by Wada and Montanari - No-cost Extension			
10	The Effect of Cover Crop Mixtures on Iron Deficiency in Pears -Astrid Voider, UC Davis	5,545		
	Subtotal:	**58,927**		
	Farm Advisor Research Travel	3,000		
	Printing and Web Page Costs Associated with Research	2,500		
	Total:	**139,979**		

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Acoustically based mating disruption of winterform psylla

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Co-PI (3): David Crowder
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City/State/Zip: Pullman, WA 99164

Total Project Request: **Year 1:** $52,761 **Year 2:** $49,733 **Year 3:** $53,166 **Year 4:** $0

Other funding sources: Awarded (Jan. 1, 2020-Dec. 31, 2021)
Amount: $21,000
Agency Name: USDA-ARS, Innovation Fund

Notes: To purchase equipment (speakers, minishakers, laptop computers) for field tests of disruption

Budget 1

Organization Name: WSU Pullman **Contract Administrator:** Ben Weller
Telephone: 509-335-0052 **Email address:** grants.fsclark@wsu.edu

Item	6/1/2017 to 5/31/2018	6/1/2018 to 5/31/2019	6/1/2019 to 5/31/2020	6/1/2020 to 5/31/2021
Salaries[1]	$28,417	$29,554	$30,736	
Benefits[2]	$2,580	$2,683	$2,791	
Wages[3]	$11,040	$11,251	$11,471	
Benefits[4]	$1,124	$1,145	$1,168	
Supplies[5]	$6,000	$3,000	$3,000	
Travel[6]	$3,600	$2,100	$4,000	
Total	$52,761	$49,733	$53,166	$0

Footnotes:

[1] Salary for the PhD student for the academic year
[2] Benefits for the PhD student for the academic year include health insurance and fringe
[3] Wages for the PhD student for the summer; also includes a time-slip employee who will work 40 hours a week for 12 weeks each summer during the project
[4] Fringe benefits for the PhD student and time-slip employee during the non-academic year
[5] Yr 1 – acoustics equipment for conducting the vibrational studies (Objective 2). Yrs 2 and 3 - Experimental supplies for Objectives 3 and 4
[6] Yr 1 – Funds will support travel to the USDA-ARS facility in Gainesville, FL. Yrs 2/3 - Vehicle lease through the state motor pool; this vehicle will be used to complete field research objectives

OBJECTIVES

1. Recruit Ph.D. student (co-supervisors D. Crowder, E. Beers, and D. Horton).
2. Describe vibrational signals used by psylla in mate location activities.
3. Show (in large cage studies with potted trees) that it is possible to slow or disrupt mating by mechanically transmitting these signals to the tree substrate.
4. Show that it is possible to slow or disrupt mating in a field setting by mechanically transmitting signals through the support wires of a trellised pear orchard.

SIGNIFICANT FINDINGS

Years 1-2

- Ph.D. student (Ms. Dowen Jocson) arrived in summer 2018 (Pullman campus).
- Pear psylla colonies were established at the Pullman location.
- Acoustics equipment was purchased and set-up at the Pullman location. Assays with summerforms and winterforms began in December 2018.
- Acoustic signals from male summerform psylla detected, quantified, and described. This is the first evidence that this species communicates acoustically. The signal is superficially similar to that in a closely related pear psyllid (the European pear psylla; *Cacopsylla pyri*).

Year 3 (current year)

- Playback tests confirmed that the male signal induces female acoustic response (duetting).
- Females respond with pulses only after waiting for males to finish a complete signal (a set of pulses and a syllable).
- Males are more likely to sing when they have recently encountered or sensed female presence. Possibly a chemical cue produced by females triggers male acoustic activity. Psylla pheromone was obtained from Dr. Jocelyn Millar for tests in 2020 to determine if the compound triggers male singing.
- Male song syllables increase in pitch (frequency in Hz) as temperatures get warmer.
- Applied for Innovation Fund grant (USDA-ARS); the proposal was funded for $21,000. Funds are to be used in purchasing equipment (speakers, minishakers, laptop computer) for testing field-disruption.

METHODS

Source of insects and plants. We are using field-collected and lab-reared winterforms and summerforms in recording acoustic cues and for eventual testing of synthesized mimics of those cues. Insects in culture are reared on pear whips purchased from a nursery. Pear whips as well as field-cuttings of shoots are being used in assays.

Objective 1. Recruitment of Ph.D. student. Completed (see Results and Discussion).

Objective 2. Describe vibrational signals.

Detecting and recording vibrational signals. Assays are being done in a semi-soundproof room at the Pullman campus (Fig. 1A). We are recording vibrational signals of pear psylla using an accelerometer (Fig. 1B-C). The accelerometer detects vibrations in the plant surface produced by signaling insects much as a seismograph is used to detect earth tremors. The insect-signal is detected by the accelerometer and sent to a computer, where the signal is then translated into a readable form. Analysis of signals is then done using freely available software (Raven, Audacity). *Previous exposure of males to females*. Irregular calling by males prompted us to examine whether exposure to females led to improved calling rates. We examined whether males removed from mixed-sex cultures were more likely to call than males from single-sex cultures. *Temperature effects*. Temperature is likely to affect how rapidly the stridulatory structures of singing psylla vibrate and therefore affect properties of the vibratory signal. Male assays were conducted across a range of temperatures (74 to 90 ^{o}F) to test whether this environmental factor affected signal characteristics.

Playback tests of signal. Playback trials began in summer 2019. Recordings from live summerform males were sent through pear stems using a Linear Resonance Actuator (the same technology that is used to make your cellphone vibrate) connected to a computer, with the other end attached to the stem of a plant hosting one or more female psylla. Our objective was to induce females to respond acoustically to the male signal. Synthetic mimics of male-produced vibrational signals will also be assayed in playback tests (beginning spring 2020) to confirm that we can re-create the male signal sufficiently well to prompt vibrational response by female psyllids (duetting).

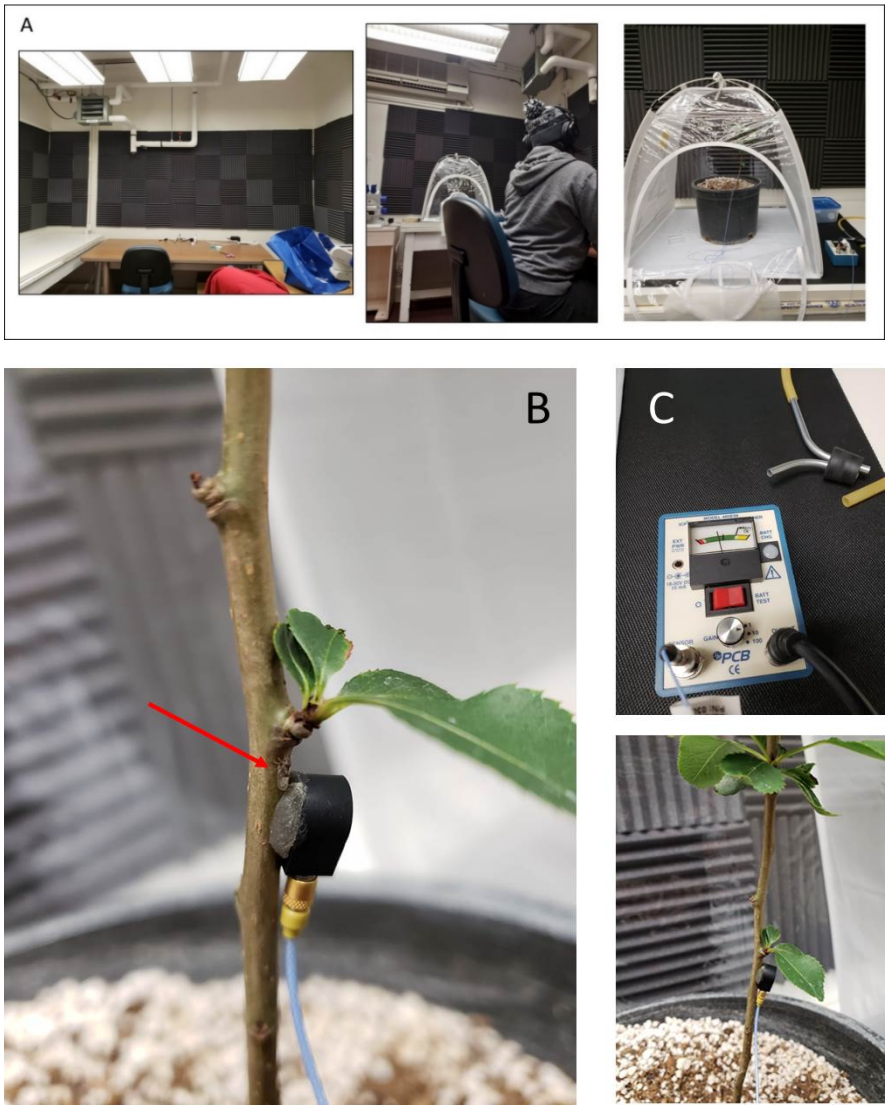


Figure 1. (A) Semi-soundproof room at Pullman location being used in our acoustics assays. (B) Head of accelerometer attached to pear whip (red arrow shows location of a psyllid). (C) Signal conditioner used to power accelerometer head and translate the vibrations.

Objective 3. Large cage studies to prove disruption. We will use a cage study to examine the effects of synthetic mimics of vibrational signals on mating success of psylla. The tests will be done out-of-doors in large "Bugdorm" cages (6 x 4 x 4 foot) each containing a potted pear tree 4-5 foot in height. A minishaker will be used to transmit the female-signal to trees. Fifty virgin female winterforms will be introduced into each cage and allowed to settle on trees. After 48 hrs, 50 male winterforms will be added to each cage, and the buzzer apparatus activated. Females will be collected from each cage after 2 days and dissected to determine mating status. Control cages will be treated identically to treatment cages, with the exception that no buzzer system will be present. These trials are tentatively planned to begin in spring 2020.

Objective 4. Field tests in trellised pear orchard. We will conduct a field test of the disruption concept under an orchard situation. Tests will be done in late winter/early spring at a high density pear orchard under a wire

trellis system. Electromagnetic minishakers attached to trellis wires will be used to disseminate the acoustic signals to trees. A laptop computer will control the minishakers and signal production. We will collect winterforms from target trees (those receiving the signal mimics) and control trees located a few rows away. Females will be dissected to determine mating status.

RESULTS AND DISCUSSION (YEARS 1-3)

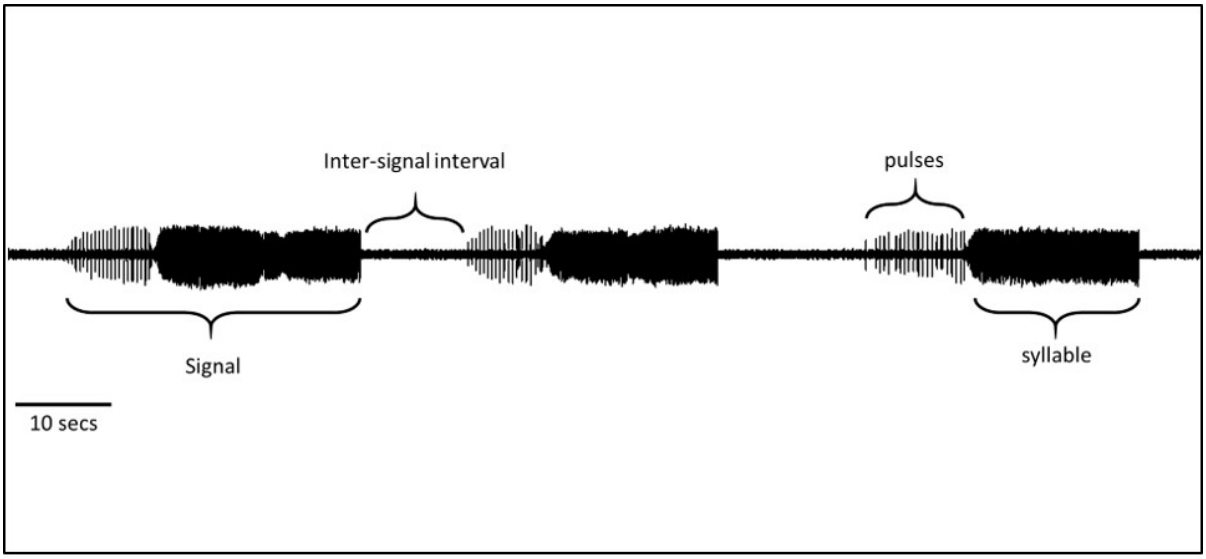
Objective 1: Recruitment of Ph.D. student (Year 1). Ms. Dowen Jocson arrived at the Pullman campus in summer 2018 to begin a Ph.D. program in Entomology.

Objective 2: Describe vibrational signals (Years 2-3). ***Male acoustic signal (Year 2).*** We have successfully described the summerform male signal (Fig. 2: upper panel), as shown in last year's Report. The male signal consists of a series of 15-25 "pulses" lasting about 10 seconds, followed by a longer phrase of more tightly packed syllables (Fig. 2: upper panel). The female signal occurs only in response to the male signal, and is described below (see ***Playback tests***). We are now assaying winterforms following the same protocols as developed for the summerform. *Previous exposure of males to females (Years 2-3)*. Inconsistent calling by males prompted us to see if exposure of males to females preceding the assay improved calling probabilities. We saw a 4-fold increase in calling probabilities for males previously exposed to females compared to calling by isolated males (Fig. 3). Female synthetic pheromone was sent to Dowen by Dr. Jocelyn Millar (UCR) for tests of whether the compound triggers male singing. *Temperature (Years 2-3)*. Pitch of the male signal increased with increasing temperature between 74 and 90 ^{o}F (Fig. 4). This result is likely caused by an increase in how rapidly the psyllid stridulatory apparatus vibrates at the higher temperatures.

Playback tests of male signal and description of the female response (Year 3). Our practical aim for this project is to show that a mimic of the female signal, transmitted through pear trees under field conditions, disrupts the mate-seeking behavior of males. For that purpose, we need a description of the female signal. Eben et al. (2014) showed that females of the European pear psyllid rarely signaled spontaneously but required the male signal to induce her acoustic reply. Our assays showed that female summerforms responded to male recordings by sending out vibrational pulses similar to the male song pulses (Fig. 5). Females waited for males to signal and then responded with their own song. The female-song is less complex than the male song, and consists only of a series of pulses (highlighted in Figure 5). Females did not always respond to the male playback. Variable female response may indicate: (a) females were choosy about the quality of the male signal due either to traits of the male signaler or because some unknown host or environmental conditions affected signal quality; or, (b) some females were just not in a receptive condition (for unknown reasons).

Reference

Eben, A. et al. 2014. First evidence of acoustic communication in the pear psyllid *Cacopsylla pyri* L. (Hemiptera: Psyllidae). J. Pest Sci. DOI 10.1007/s10340-014-0588-0.



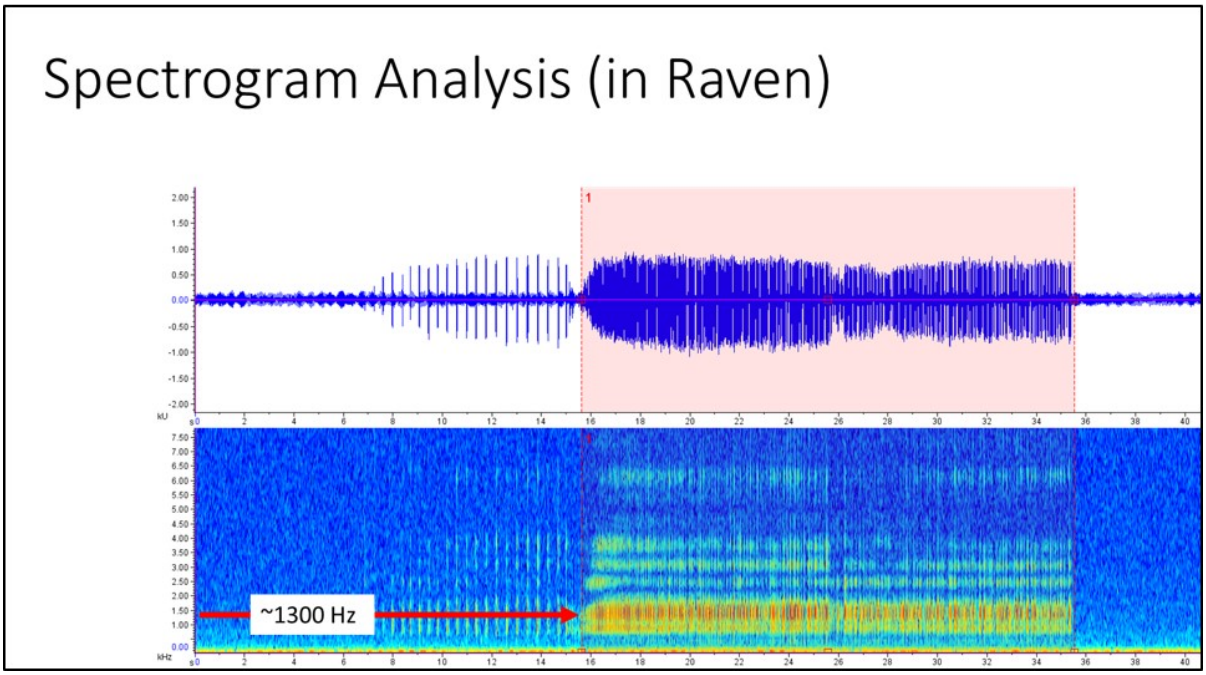


Figure 2. Upper panel shows oscillogram for signaling male summerform pear psylla; lower figure shov ignal frequency concentrated at 1300 Hz.

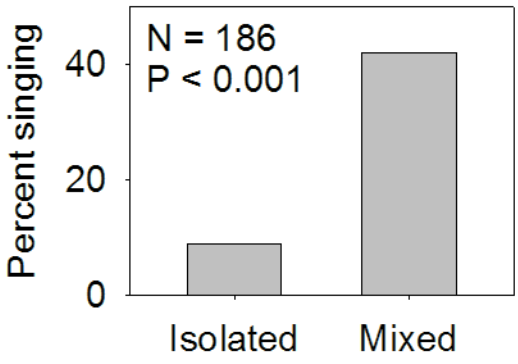


Figure 3. Probability a male summerform will sing as a function of pre-exposure to females. "Isolated": males from single-sex culture; "Mixed": males from mixed-sex culture.

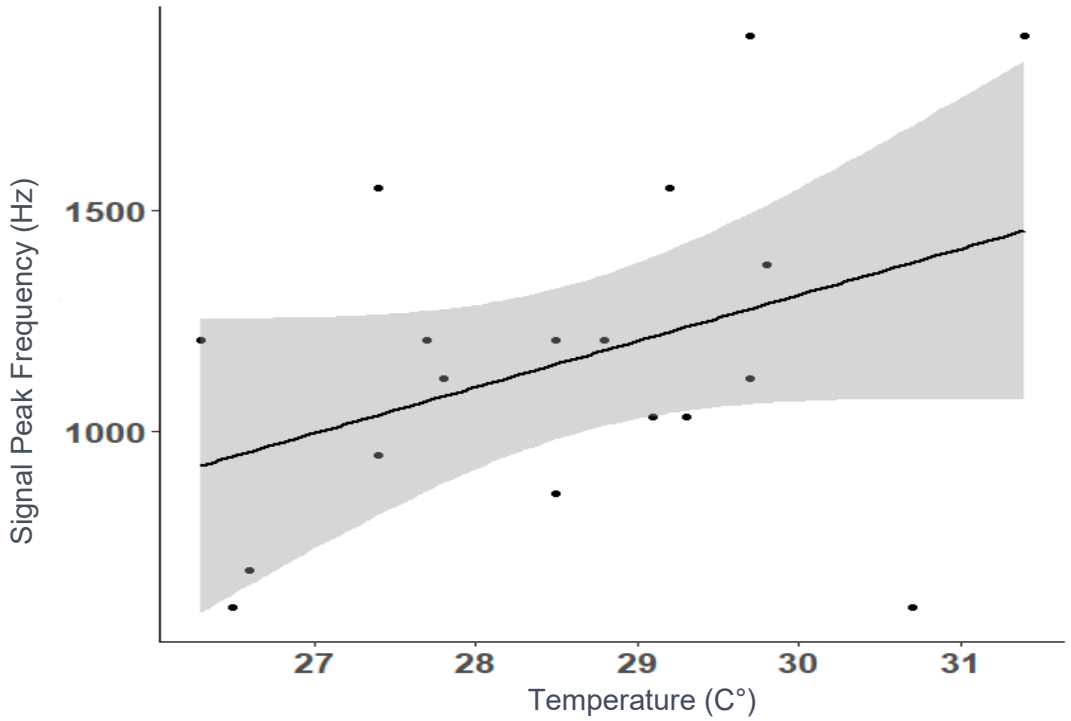


Figure 4: Linear regression that shows that there is a trend of increasing pitch (frequency in Hz) as temperature increases (from 74 to 90 °F). The gray bar shows the variability around the predicted line. Summerform males.

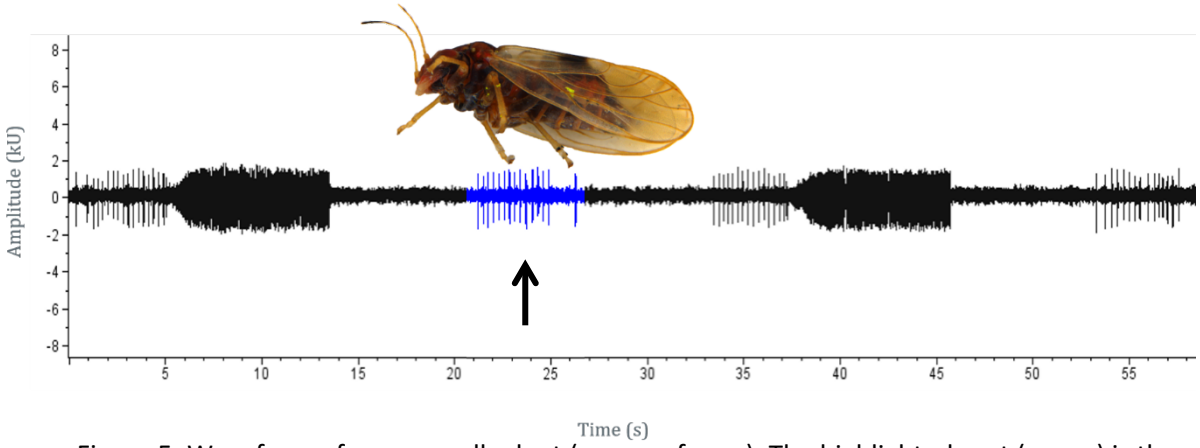


Figure 5: Waveform of a pear psylla duet (summerforms). The highlighted part (arrow) is the female response to the male signal that preceded it.

CONTINUING PROJECT REPORT **YEAR**: 3

Project Title: Using transcriptomics to target key behaviors of pear psylla
PI: W. Rodney Cooper
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CO-PI: Surya Saha
Organization: Boyce Thompson Institute, 533 Tower Road, Ithaca, NY 14853
Telephone: 662 312 3227
Email: ss2489@cornell.edu

Cooperators: David Horton, USDA-ARS in Wapato, WA; William Walker, Swedish University of Agricultural Sciences

Budget: **Year 1**: $12,000 **Year 2**: $10,000 **Year 3**: **$10,750**

Other funding sources

Agency Name: USDA-ARS Research Associate Program
Amt. awarded: $163,635
Notes: Funding for a USDA-ARS Research Associate

Agency Name: Northwest Potato Research Consortium
Amt. requested: $36,000
Notes: Study on potato psyllid saliva

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

Item	2018	2019	2020
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$11,000	$9,000	$750
Travel			
Miscellaneous			
Plot Fees	$1000	$1000	
Total	$12,000	$10,000	**$750**

Footnotes: Funds requested for the purchase of qPCR primers and reagents

Budget 2
Organization Name: Boyce Thompson Institute **Contract Administrator:** Regina Holl

Telephone: 607 254 1249 **Email address:** rch275@cornell.edu

Item	2018	2019	2020
Salaries			$8,045
Benefits			$2,955
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total			**$10,000**

Footnotes: Salary funds are requested to support continued collaboration between USDA and Dr. Surya Saha of the Boyce Thompson Institute. Dr. Saha is Senior Bioinformatics Analyst at Boyce Thompson Institute for Plant Health with powerful bioinformatics computers that can extract far better information, both quantitative and qualitative, from the pear psylla transcriptomes, and can compare transcriptomes with those of other psyllid pests including citrus psyllid and potato psyllid.

OBJECTIVES

1. Compare gene expression among summerform, diapausing winterform, and post-diapause winterform pear psylla.
2. Compare gene expression profiles between winterform that emigrate from pear versus those that remain in pear.

SIGNIFICANT FINDINGS

- Genes involved in reproduction, photoreception, muscle, and immunity were more highly expressed in summerform psylla than in winterform psylla, consistent with previously documented biological differences between these populations.
- Genes associated with bacterial endosymbionts were more highly expressed in winterform psylla than in summerform psylla, which is consistent with the previous report that endosymbiont infection is higher in winterforms.
- Genes that are homologous to odorant-binding protein chordotonal receptor genes were identified from the pear psylla transcriptome. The putative identification of these genes will enable further study on how psylla locate host plants and mates.

METHODS

Collection of summerform, diapausing winterform, and post-diapause winterform pear psylla. We have collected adult pear psylla monthly from a pear orchard located at the USDA experimental farm near Moxee, WA (Figure 1) since August of 2017, and will continue to collect psylla until at least July of 2018. Summerform and winterform psylla will be separated during autumn when populations of the different morphotypes overlap. The specimens will be stored in -80°C in RNAlater to preserve the RNA. These collections will provide us with about 4-5 months of summerform collections, 3-4 months of diapausing winterform psylla, and 4-5 months of post-diapause winterform psylla (Table 1).

Collection of winterform psylla from overwintering shelter hosts. Post-diapause winterform psylla will be collected from pear trees (non-dispersing) and from various shelter hosts including Juniper, Pine, Spruce, *Salix*, and apple in early-February. Collections will be made from plants located at the USDA experimental farm near Moxee, WA (Figure 1). Winterform psylla have been collected from these shelter hosts in previous years, and results of gut content analysis indicate that psylla visit and feed upon these trees at this location (see 2018 Final Report by Cooper and Horton). Specimens will be stored in -80°C in RNAlater until they are processed for analyses.

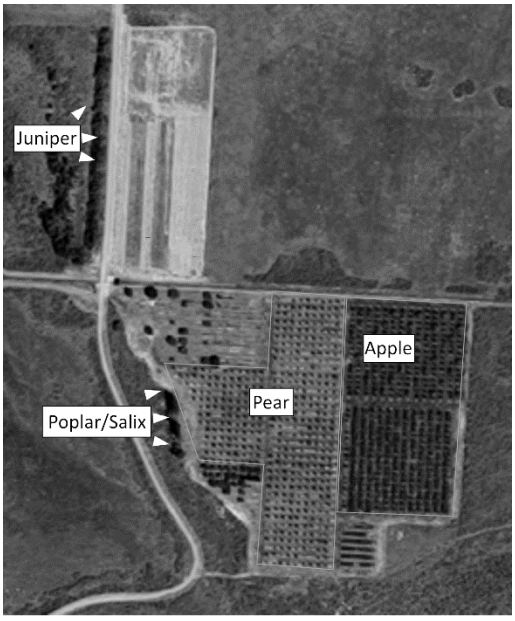

Figure 1. Winterform pear psylla will be collected from a pear orchard at the USDA experimental farm, and from surrounding shelter hosts including apple, Juniper, Poplar, and Salix.

Transcriptomics. RNA from whole bodies of at least 10 pear psylla in RNAlater will be extracted using a commercial kit. Two replications will be included for each treatment (12 months for Objective 1, at least five overwinter hosts for Objective 2). Samples will be shipped to Novogene for RNA sequencing (www.novogene.com; cost of $225 per transcriptome). Whole transcriptomes will be assembled using the online bioinformatics software, EGassembler. Annotation of all transcripts will be performed using Blast2GO software (Conesa et al. 2005) that categorizes putative biological functions to genes by identifying similar sequences of known function within publicly available databases. Quantitative analyses of transcript expression levels will be determined with standard abundance expression software, such as RSEM, and differential expression analysis will be conducted with DESeq to assess expression levels across samples.

Differential expression of at least 10 genes will be confirmed using quantitative real time PCR (qPCR). Based on gene annotations and homologies to other insects, qPCR analysis will be performed on genes that are predicted to be involved with diapause, sensing (visual or olfactory), or basal immunity. Primers and probes specific for each target gene will be designed from sequences obtained from the transcriptome. cDNA libraries will be constructed from RNA from each sample. qPCR will be performed on cDNA using a Roche Lightcycler real-time PCR machine located at the ARS laboratory in Wapato. Ribosomal protein 3 and Actin gene will be used as control genes to standardize gene expression among samples.

Table 1. Summary of psylla collections and transcriptome comparisons.

Collection	Description	Phenological traits
Objective 1		
Sept. - Nov.	Diapausing winterform -Morphotypes overlap in September	-Reproductive diapause; lack of mating and ovarian development -Attracted to the color of foliage -More susceptible to insecticides
Dec - Feb.	Post-diapause winterform -January collection may include a mixture of diapausing and post-diapausing adults	-Reproductive development is slow due to cold temperatures -Not attracted to the color of foliage
March		-Mating and egglaying activities -Not attracted to the color of foliage
April		-Mating and egglaying continue -Attracted to the color of foliage
May - Aug.	Summerform	Reproductive, attracted to pear and the color of foliage
Objective 2		
Pear	Non-dispersing winterform	Overwinters on developmental host
Apple	Dispersing winterform	Overwinters on deciduous fruit tree
Juniper Pine		Overwinters on conifer
Salix		Overwinters on deciduous wind break

RESULTS & DISCUSSION

We previously reported variable gene expression among summerform, diapausing winterform, and post-diapause winterform pear psylla based upon single replications. Those results were consistent with observed differences among the populations in behavior and biology (Table 1; Ullman and McLean 1988, Krysan and Higbee 1990, Horton et al. 1998, Horton et al. 2007, Civolani et al. 2011, Cooper et al. 2017). In collaboration with Dr. Surya Saha with the Boyce Thompson Institute, we increased our replication size to three replications per population with 10 insects within each replication. Results confirmed that there are indeed large differences in gene expression among the psylla populations (Fig. 2). As expected, the largest variance in differentially expressed genes was observed between the summerform (reproductive) and diapausing winterform (non-reproductive) populations, with 9,290 differentially expressed genes (Fig. 2A). The summerform and post-diapausing winterform populations exhibited similar gene expression profiles with only 1,505 differentially expressed genes (Fig. 2B), which might be expected since both populations are reproductive. The post-diapausing winterforms and diapausing winterforms exhibited intermediate gene expression profiles with 3,195 differentially expressed genes (Fig. 2C). Initial analyses of these differentially expressed genes thus far are consistent with our initial study that was summarized in the 2019 continuing report; many identified genes have likely roles that explain the biological and behavioral differences among these populations.

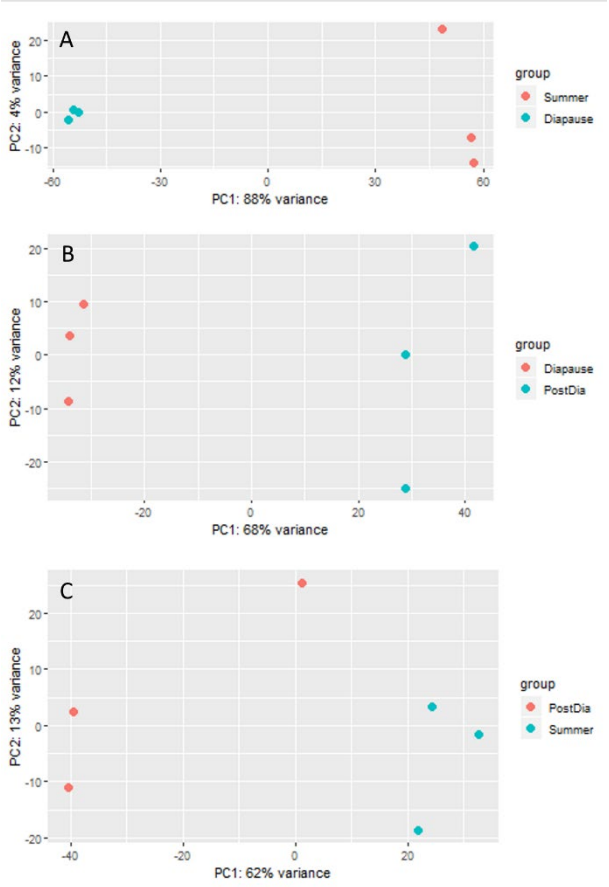

Figure 2. Plots demonstrating the variance in gene expression profiles between the summerform and diapausing winterform populations (A), diapausing and postdiapausing winterforms (B), and postdiapause winterform and summerform populations (C). Each point represents a single replication. Greater variance on the x-axis denotes greater differences in overall gene expression profiles. Gene expression profiles were similar among replications within populations, yet highly different between populations.

Ongoing progress. Analyses of differentially expressed genes will continue in 2020, in collaboration with Dr. Surya Saha. Surya Saha is the Senior Bioinformatics Analyst at Boyce Thompson Institute for Plant Research in Ithaca, NY. He operates powerful computer pipelines that will allow us to extract far more detailed information from the transcriptomes than we can currently process with the resources available to us at the USDA laboratory in Wapato. He has worked extensively on genomics and transcriptomes of citrus psyllid and potato psyllid, has resources including the molecular libraries and computing power and expertise, to compare pear psylla transcriptomes with those of other Hemipteran pests. Dr. Saha has provided us services in 2019 in an informal capacity. We request additional funds of $10,000, all salary, in year 3 to support continued collaboration between USDA and Boyce Thompson Institute.

In addition, we will design qPCR primers to specifically amplify biologically important differentially expressed genes, including genes involved in reproduction, immunity, olfaction, and sight (Table 1). The primers will be used to confirm differences in gene expression, to examine for differences in gene expression between winterform psylla that stay on pear versus those that disperse from pear, and to investigate the mechanisms and timing of behavioral changes between summerform and winterform pear psylla.

Anticipated benefit to the industry. Although changes in behaviors and phenotypes associated with summerform, diapausing winterform, and post-diapause winterform psylla are well-documented, the timing for these behavioral changes and mechanisms controlling behaviors are not currently understood. The results of our studies will allow us to pinpoint the exact timing for these changes and will contribute to our long-term goal of providing a better understanding of winterform biology and improve management of this bottlenecked population.

Results have potential to lead to practical tools for the pear industry. Researchers are on the cusp of developing novel gene-based tools to manage agricultural pests including citrus psyllid. The genomic resources produced by our study will allow us to adapt these developing technologies for use against pear psylla.

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CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Enhancing pear psylla biological control through predator recruitment

PI: Tobin Northfield
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Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

Cooperators: Louie Nottingham (WSU), Vince Jones (WSU)

Total Project Request: Year 1: $51,325 **Year 2: $0**

Other funding sources: None

Budget 1
Organization Name: WSU TFREC **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803
Email address: arcgrants@wsu.edu/shelli.tompkins@wsu.edu

Item	2019	**2020**
Salaries[1]	23,750	0
Benefits[2]	8,723	0
Wages	5,760	0
Benefits[3]	92	0
Equipment	0	0
Supplies[4]	8,000	0
Travel[5]	5,000	0
Miscellaneous	0	0
Plot Fees	0	0
Total	51,325	**0**

Footnotes:
[1] Postdoctoral associate 50% FTE (Y1 -12 months, Y2 – 12 months)
[2] Postdoctoral associate (36.73%)
[3] 1.6%
[4] Includes lab and field supplies.
[5] In state travel.

OBJECTIVES

1. Evaluate the indirect effects of thrips on psylla abundance in the presence and absence of anthocorid predators

SIGNIFICANT FINDINGS

This project is focused on spring pear psylla control, particularly the effects of thrips combined with predators to reduce psylla abundance early in the season. Because the funding arrived mid-summer 2019, the project's first spring will occur in 2020 when we will conduct the proposed experiments.

To get a jump start on the project and simulate spring conditions during late summer months, we did attempt an experiment with potted pear trees and colony-raised thrips in a growth room. However, the growth room had not been used in several years and was not functioning properly, killing all of the plants prior to experiment initiation. We also conducted a small field experiment focused on psylla predation late in the summer as a pilot experiment to develop methods.

METHODS

We set out to conduct an inexpensive pilot study in July 2019 to develop methods for the following spring. We conducted the experiment in the pear orchard at the WSU TFREC in Wenatchee, WA. First, we conducted a survey of the plot to identify the most abundant predators, and we designed an experiment focused on these predators to evaluate which combination of predators were most impactful on pear psylla abundance. At this time thrips were not as abundant in the orchard as they were earlier in the season. Therefore, we did not include thrips in the experiment. We appeared to observe overlapping psylla generations, such that there was high variation in psylla reproduction that overwhelmed experimental manipulation. Nonetheless, we describe this experiment below.

We set up a sleeve-cage experiment where sleeves made of fine mesh approximate 2 feet long were placed over the tips of branches including 20 adult psylla and a predator treatment or no-predator control. To set up the cages, on July 24th 2019 we first removed all insects on the branches and added the sleeve. Next (on 7/24/2019), we used beat sheets to collect adult psylla and added 20 adult psylla to each branch. We allowed the psylla 48 hours to establish, after which we counted the psylla by looking through the closed sleeve cages and added predators. We sampled every tree in 2 middle rows of trees for predators, and focused treatments on these predators.

The most common predator species were *Dereocoris* sp. bugs (D), *Harmonia axyridis* ladybeetles (H), and *Adalia bincutata* lady beetles. Spiders were present too, but there were not enough of the same species to include in an experiment. Thrips were not abundant at this time. We next designed an experiment to determine which combination of these predators provided the best control of psylla. Each cage included two individuals of either a single predator species, or a pairing of one individual from each of the three species listed above. We also included no-predator controls, and each treatment was replicated 4 times. Predators were introduced on July 26th 2019, and psylla abundances were estimated by peering through mesh sleeve cages, to avoid disruption of psylla treatments by opening cages. We introduced predators immediately after time zero psylla counts. Then, we broke down the experiment on August 12th and counted all psylla and predators.

RESULTS & DISCUSSION

We found that in July the most abundant predators were *Dereocoris* sp. bugs and two species of lady beetles. While adult psyllas were abundant, we observed very few thrips. The experimental approach worked well, except we found very little reproduction. The four no-predator controls had very few psylla in cages, suggesting that reproduction was very low (mean of 3.5 psylla/cage). Numbers of psylla in other cages were highly variable, ranging from 0 to 18 psylla in the predator treatments. Discussion with Louie Nottingham suggested that this was due to a combination of aging adults from the previous generation that were not

reproducing, and newly emerged adults from the next generation. This solidified the benefit of studies early in the season when there is a single generation of psylla, such that psylla reproduction is similar across treatments.

This spring we plan to conduct the objective 1 experiment evaluating potential interaction between thrips and predators on psylla abundance, as described in the proposal.

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

Project Title: Incorporating *Trechnites* into a psylla biocontrol program

PI: Rebecca Schmidt-Jeffris
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Co-PI (4): Richard Hilton
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Co-PI (5): Louis Nottingham
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Cooperators: Steve Castagnoli/Christopher Adams (OSU-MCAREC)

Total Project Request: **Year 1:** $39,839 **Year 2: $39,542** **Year 3:** $39,769

Other funding sources

Agency Name: WSDA SCBC
Amt. requested: $245,974
Notes: This grant was submitted using Year 1 data from this project as preliminary data.

Budget 1

Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers

Telephone: 510-559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2019	2020	2021
Salaries[1]	$17,404[2,3,4]	$17,839[2,3,4]	$18,286[2,3,4]
Benefits	$4,529[2,3,4]	$4,642[2,3,4]	$4,759[2,3,4]
Supplies[5]	$8,500	$7,500	$7,000
Travel[6]	$500	$500	$500
Total	$30,933	**$30,481**	$30,545

Footnotes:

[1]All salaries include 2.5% COLA increase per year

[2]8 weeks ($23.56/hr) for PCR technician at 32% benefits (Cooper)

[3]~6 weeks for trap collection/psylla dissection technician at 32% benefits (Horton)

[4]Summer technician (GS-3) to work 40 h/wk×12 wk×$12.74/hr assisting all other technicians with the project at 15% benefits rate (Schmidt-Jeffris)

[5]Funds to purchase PCR reagents and other PCR supplies, trapping supplies, pesticide non-target effects bioassay supplies

[6] Travel to commute to orchards and scout for native psyllid host plants

Budget 2

Organization Name: OSU-ARF **Contract Administrator:** Russ Karow

Telephone: (541) 737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2019	2020	2021
Salaries[1]	$2,510[2,3]	$2,572[2,3]	$2,638[2,3]
Benefits	$2,046[2,3]	2,096[2,3]	$2,150[2,3]
Travel[4]	$200	$200	$200
Total	$4,756	**$4,868**	$4,988

Footnotes:

[1]All salaries include 2.5% COLA increase per yea

[2]Technician at OSU-SOREC ($15.68/hr*80hr) at 81.5% benefits

[3]Technician at OSU-MCAREC ($15.68/hr*80hr) at 81.5% benefits

[4]Travel to commute to orchards and scout for native psyllid host plants

Budget 3

Organization Name: WSU **Contract Administrator:** Katy Roberts/Kim Rains

Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrants@wsu.edu/kim.rains@wsu.edu

Item	2019	2020	2021
Salaries[1]	$1,560[2]	$1,599[2]	$1,639[2]
Benefits[3]	$145	$149	$152
Travel[4]	$2,445	$2,445	$2,445
Total	$4,150	**$4,193**	$4,236

Footnotes:

[1]Salary includes 2.5% COLA increase per year

[2]Summer technician at $15/hr×8 hr/wk ×13 wks

[3]Benefits: 9.3%

[4]Travel: 50% use of motor pool vehicle for 26 wks ($1,057) and 50 mi/wk with pro-rated total fuel cost=$1,388

OBJECTIVES: Goals, Year 2 activities, and expected results

1. Improve methods for monitoring adult *Trechnites* and for estimating percent parasitism.

We will continue testing monitoring methods and collecting percent parasitism data in Year 2. In order to improve modelling, we will also collect data on abundance of pear psylla nymphs (by instar) on random leaf counts within each plot. Our preliminary exploration of the data indicates we need to account for this information to understand percent parasitism. Percent parasitism will now be estimated using a combination of parasitoid emergence and PCR of pear psylla nymphs that do not mature to adults. This method is more time/cost efficient and will allow us to identify non-*Trechnites* parasitoids to species more easily, as sequences for most of these species are not available.

Expected results. Preliminary results from trap catch, dissections/emergence, and PCR will be summarized in the winter following each year of catch. Determination of the most efficient method for trapping *Trechnites* and which trap best reflects percent parasitism at conclusion of Year 3.

2. Define the relationship between counts of adult *Trechnites* and parasitism of psylla nymphs

We will continue collecting data in order to refine our model building process.

Expected results. Development of a model describing the relationship between adult trap catch and percent parasitism at conclusion of Year 3. Results from objectives 1-2 will be combined for both a peer-reviewed publication, an extension publication, and an update of the *Trechnites* section in Orchard Pest Management (http://treefruit.wsu.edu/crop-protection/opm/, OPM).

3. Screen additional IPM and organic chemicals for effects on parasite survival and life history.

We will begin conducting these assays in Year 2. Attempts to rear *Trechnites* in abundance in Year 1 were unsuccessful, likely due to the difficulty of keeping an adequate number of the correct instars of pear psylla available at all times. Instead, we will use freshly collected adult *Trechnites* from orchards where we have identified abundant populations (Wenatchee area). This winter, we tested a method for collecting overwintering mummies. We will use this method next winter to obtain adequate numbers of mummies to conduct non-target effects testing.

Expected results. Summary of pesticide non-target effects will be updated annually, with differences in adult mortality, percent emergence from mummies, percent parasitism, and movement pattern differences between a pesticide and water check as the main results.

4. Examine native psyllids from multiple locations for *Trechnites*.

We will continue examining native psyllids for *Trechnites* parasitism. Results from Year 1 were promising (see significant findings/results below). Additionally, we are seeking funding from the WSDA to expand this work so that we can survey a larger area of Washington for native psyllids and parasitoids.

Expected results. Year 1 results indicate that *Trechnites* does parasitize native psyllids. We will better determine the extent of this in Years 2-3, especially if awarded funding through the WSDA. If *Trechnites* regularly parasitizes native psyllids, planting native plants that host these psyllids near pears may improve biological control of pear psylla.

SIGNIFICANT FINDINGS

Large *Trechnites* larva observed via dissection of pear psylla

- 3D-printed tube traps and screened sticky cards were successful at capturing *Trechnites* and monitoring pear psylla. Beat trays were a poor measure of *Trechnites* abundance.
- Parasitism levels tend to peak ~2 weeks after the adult *Trechnites* population peaks in each generation.
- PCR detected *Trechnites* parasitism at a higher rate than dissection. Preliminary work indicates that this is likely because PCR can detect young (small) stages of *Trechnites* more accurately than dissection.
- *Trechnites* mummies can be easily captured using overwintering bands. This will allow us to collect larger numbers of individuals for bioassay work.
- *Trechnites* (6 individuals) were reared from *Cacopsylla alba* nymphs collected from *Salix exigua* (willow). Many individuals of *Prionomitus mitratus* and *Prionomitus tiliaris* were reared from willow and current *Cacopsylla* spp. Both parasites are important natural enemies of pear psyllids in Europe, but of unknown importance here in North America

METHODS (updates included)

1. **Improve methods for monitoring adult *Trechnites* and for estimating percent parasitism.** (Participating organizations: USDA-ARS Wapato, OSU-MCAREC, OSU-SOREC, WSU-TFREC)

Adult Trechnites. At each of the four locations, five plots will be laid out in an orchard. Collection of all data will occur from April-late September at all locations. In Year 1, we monitored adult *Trechnites* in our two Washington orchards (where *Trechnites* was more abundant) throughout the entire season, as opposed to just peak populations. We will change orchards monitored in Medford and Hood River, as these orchards had very low *Trechnites* populations in Year 1.

Within each plot, there will be one screened sticky card, changed/removed after one week. Work in Year 1 indicated that screened sticky cards were an effective method for monitoring *Trechnites*; these will replace the unscreened sticky cards at all locations. Beat tray samples, which were conducted in Year 1, will be discontinued, as they did not adequately reflect *Trechnites* abundance. Leaf samples will consist of up to 20 leaves that are found to contain psylla nymphs, when sufficient quantities are present. This is increased from 10 in Year 1, as there were often dead (potentially predator consumed) psylla in Year 1 samples, which could not be dissected. An additional sample of 25 leaves will be randomly collected from each plot to determine the age distribution of psylla nymphs (new in Year 2 for all locations except Moxee). We obtained enough 3D-printed tube traps in Year 1 to include one per plot. We will continue to use these traps to sample for *Trechnites*.

Percent parasitism. In Year 2, we will use emergence cages to monitor percent parasitism instead of dissection. Ten psylla from each plot at a location will be placed inside a cage on a detached pear leaf. Emergence of parasitoids will be monitored. Parasitoids will be identified to species. Any psylla that die prior to reaching adulthood will be examined for the presence of *Trechnites* DNA using PCR to

allow us to determine if *Trechnites* causes "hidden mortality". This will allow us to have adult specimens of any parasitoids that emerge, which will facilitate species-level identification. Our previous methods did not allow us to identify dissected parasitoid larvae to species (except for *Trechnites*). If we obtain WSDA funding, this project will be expanded to include additional monitoring sites, which will improve the robustness of the model we develop in Obj. 2.

2. **Define the relationship between counts of adult Trechnites and parasitism of psylla nymphs.** (Participating organizations: USDA-ARS Wapato

The percent parasitism data will allow us to model how counts of the adult parasitoid in orchards via the three different methods (sticky cards, tray counts, traps) relate to actual percent parasitism in the field, improving grower understanding of what level of control to expect when they are scouting for adult *Trechnites*. Counts from each method will be compared to percent parasitism to determine if the relationship is consistent between locations and which trap type most closely predicts parasitism levels.

3. **Screen additional IPM and organic chemicals for effects on parasite survival and life history.** (Participating organizations: USDA-ARS Wapato)

A total of at least ten products (Bexar, Centaur, Malathion, lime sulfur, Delegate, Envidor, Altacor, Actara, Tritek, and Neemix) will be tested over the three years of the project. For each pesticide tested, we will examine effects on sprayed adults (% mortality) and mummies (% emergence) compared to a water sprayed control. A minimum of 20 replicates will be tested. For materials which have adult survival, a subsample of sprayed adults that survive will also be tested for sublethal effects, including ability to parasitize psylla and changes in searching behavior, which will be monitored using a computer-based motion tracking system (Ethovision). Here, a minimum of 10 replicates will be tested.

4. **Examine native psyllids from multiple locations for *Trechnites*** (Participating organizations: USDA-ARS Wapato, OSU-MCAREC, OSU-SOREC, WSU-TFREC)

Each year, we will locate *Salix scouleriana*, *Salix prolixa*, and *Ribes* patches in early spring and *Salix exigua*, *Purshia tridentata,* and *Cercocarpus ledifolius* (Medford only) in spring and summer. This work focused on the Yakima area in Year 1, but will be expanded in Year 2. These plant taxa host native psyllids that are related to pear psylla, and thus could be sources of parasites (including *Trechnites*) that attack pear psylla. Beat tray samples will be used to determine if adult psyllids are present. When adults are found, shoots infested with immature psyllids will be collected and shipped to USDA-ARS. From these samples, psyllid mummies will be isolated and the emerging parasites and psyllid host will be identified. Collection will occur 2-3 times per season, with the timing focused on life cycles of known psyllid species that feed on these plants. We will also record any hyperparasites of *Trechnites* that are found in collected psyllids.

RESULTS AND DISCUSSION

Obj. 1. Beat tray sampling proved to be very poor for estimating *Trechnites* abundance; *Trechnites* were rarely captured on beat trays, even when our other traps were catching large numbers of them (Fig. 1-2). Other "look-alike" encyrtids were frequently captured on beat trays and could not be distinguished from *Trechnites* with the naked eye. Growers should not use beat trays as their sole measure of *Trechnites* abundance, as there is strong potential that they will severely underestimate *Trechnites* abundance. For instance, based on beat tray samples from the Moxee and Wenatchee orchards, growers would conclude that they had virtually no *Trechnites* in their orchards, despite high

abundance of adults and high parasitism levels.

Use of tube traps and screened sticky cards reduced trap bycatch and increased the ease of counting samples, without significantly decreasing trap catch (Fig. 1-3). Screening consisted of glittery gold tulle (Setamou et al. 2019); screened traps were not included in the original sampling protocol, but were tested at the Moxee and Hood River sites. The tube trap was by far the most promising monitoring method. Trechnites catch in these traps was equivalent to that obtained on small sticky cards (Wenatchee location) and only slightly reduced from that obtained by large sticky cards. The small openings leading into traps greatly reduced catch of larger non-target insects, increasing processing efficiency. Moreover, because insects caught by the tube trap fall into a vial containing preservative, which can be emptied and examined under a microscope, specimens are easier to separate and are in better condition than specimens from sticky cards. Finally, use of preservative allows these samples to be easily processed for DNA-based research, including species identification. Both screened sticky cards and tube traps are very promising for growers and researchers to monitor *Trechnites* and other small parasitoids in their orchards.

We were able to detect all stages of *Trechnites* inside pear psylla via dissection. We believe that we are the first to photograph immature stages of *Trechnites*. There was a trend for the "peak" count of adults in each generation to be followed two weeks later by the highest levels of psylla parasitism. PCR is being conducted for each dissected pear psylla nymph to directly compare detection rates between methods. PCR detected *Trechnites* more frequently than dissection on most sampling dates (Fig. 1). It is likely that PCR is better at detecting earlier (smaller) stages of *Trechnites*. This was indicated by our results, which showed that psylla found positive for *Trechnites* via PCR, but not by dissection, were younger than psylla where PCR and dissection results agreed

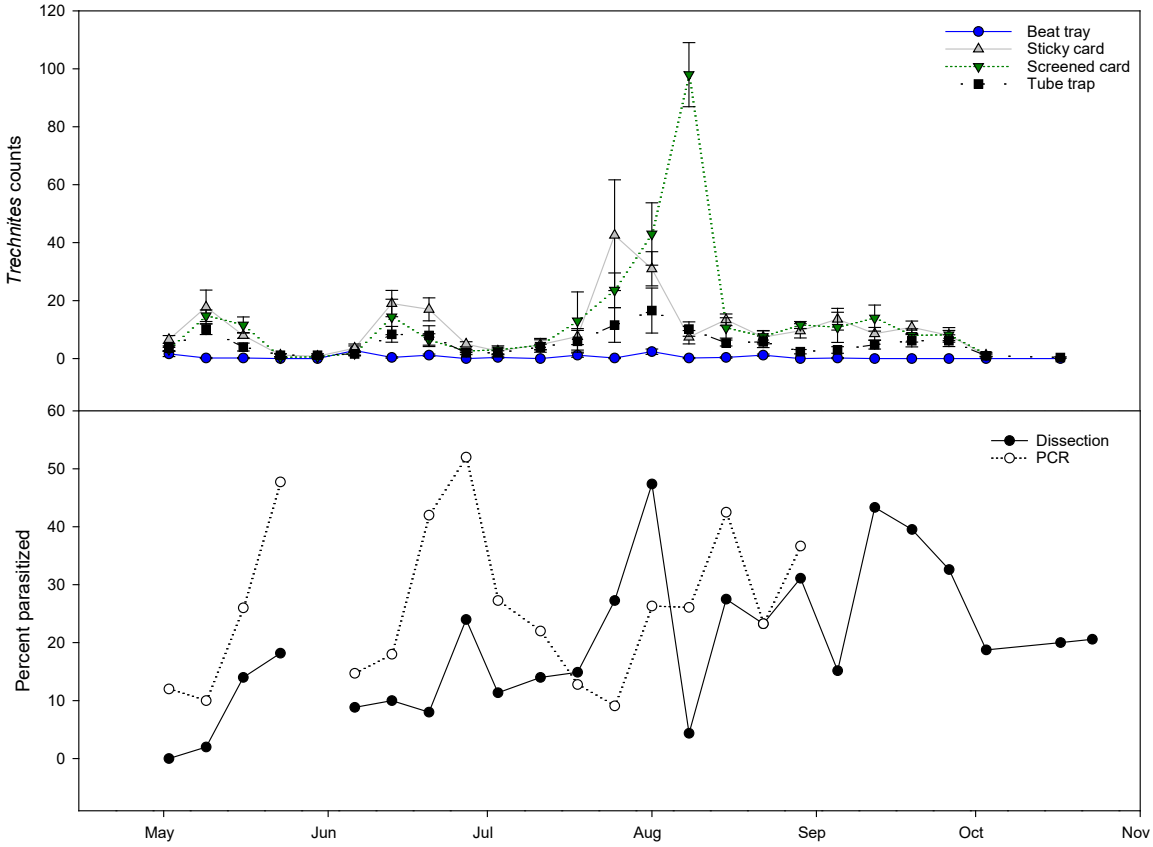

Fig. 1. Trechnites abundance measured by four methods and percent parasitism of pear psylla measured by two methods in Moxee,

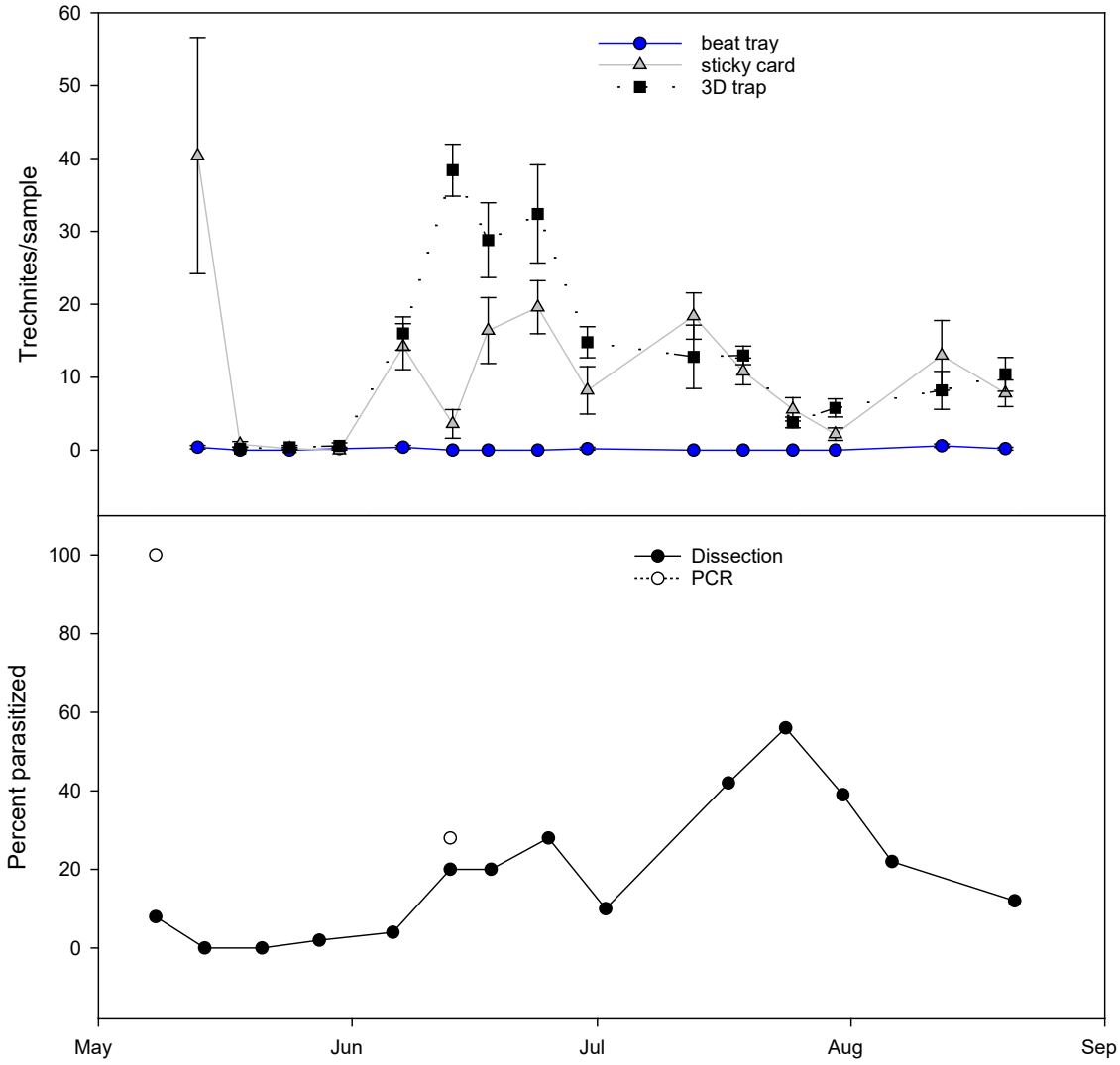

Fig. 2. Trechnites abundance measured by three methods and percent parasitism of pear psylla measured by two methods in Wenatchee, WA in 2019. [Note: PCR samples still being processed. First four dissection samples were from frozen material, likely

(Table 1). The younger psylla likely hosted younger and smaller stages of *Trechnites*. Additionally, a much higher percentage of PCR+/Dissection- individuals had faint bands in PCR gels compared to +/+ individuals (Table 1). The faint bands could indicate less DNA was extracted, potentially due to the smaller size of the *Trechnites* individuals.

Trechnites were nearly absent at the Hood River orchard; only one individual was captured by any of the trapping measures for all eight dates sampled. Very few *Trechnites* were

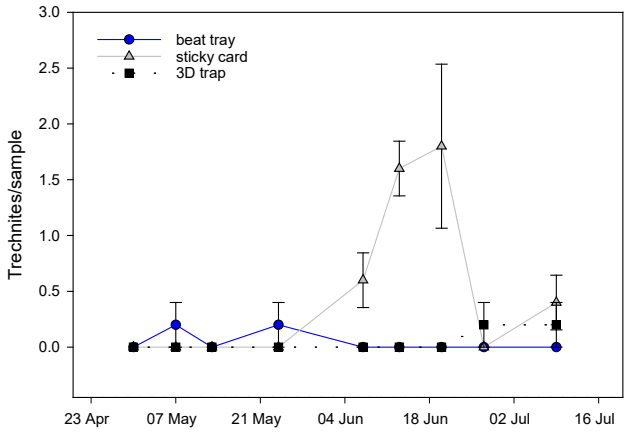

Fig. 3. Trechnites abundance measured by three methods in Medford, OR

captured at the Medford orchard for the first 4-week sampling period and populations were low during the second sampling period (Fig. 3). We will change orchards for both areas in Year 2.

Table 1. Comparison of individual pear psylla found to be parasitized by *Trechnites* via both dissection and PCR. Mean instar of psylla in each category. Comparison of percent faint bands in PCR gels when PCR was positive when PCR results either disagreed or agreed with dissection results.

Diss/PCR	#	%	instar	# faint bands	% faint bands
-/+	120	16	3.0	62	52
+/-	45	6	3.3	-	-

Obj. 2. We determined that additional information is needed to adequately model the relationship between *Trechnites* adult capture and levels of psylla parasitism. In Year 2, we will collect random leaf samples to determine pear psylla age distribution. We are also applying for funding through the WSDA to increase the number of sites included in the sampling work to improve our models.

Obj. 3. We have not yet conducted pesticide non-target effects bioassays. We were unable to rear *Trechnites* in sufficient numbers to begin this objective. However, we have identified orchards to collect adults from to perform this objective in Year 2. Additionally, we placed cardboard bands in the research orchards in Moxee and Wenatchee in Fall 2019. We determined that parasitized psylla nymphs used these bands as overwintering sites and form mummies within the bands. We are in the process of collecting data on the emergence of parasitoids from the mummies in these bands. Pending the results from this study, in Year 2, we will use mummies collected from overwintering bands for the "mummy testing" portion of this objective.

Table 2. Parasitoid wasps emerging from *Cacopsylla* spp. mummies collected from native plants.

Location	Date	Host Plant	Psyllid species	Parasitoids
Fulbright Park, Union Gap	6-May	*S. exigua*	*C. alba*	3 *Prionomitus* spp.
	12-Jun		*C. alba*	1M *T. insidiosus*
Yakima Canyon	30-May	*S. amygdaloides*	*Cacopsylla* spp.	1F *Prionomitus mitratus*
				2M *Prionomitus tiliaris*
	18-Jun	*R. aureum*	*C. ribesiae*	1 *P. mitratus*
	9-Jul	*S. exigua*	*C. alba*	12F, 12M *P. mitratus*
				1F, 4M *T. insidiosus*
				1 *Pachyneuron* spp.
				2 figitids
				1 *Syrphophagus* spp.
West Yakima	21-May	*S. prolixa*	*Cacopsylla* spp.	1 *P. tiliaris*
	27-May			5 *P. tiliaris*
	2-Jun		*Cacopsylla* spp.	1 *P. tiliaris*
	24-May	*S. amygdaloides*		7 *P. tiliaris*
	2-Jun		*Cacopsylla* spp.	1M, 1F *P. tiliaris*
	9-Jun			1M *P. tiliaris*
			Cacopsylla spp.	2 *Pachyneuron* spp.
				1 figitid

Obj. 4. We found *Trechnites* emergence from willow psyllids (*Cacopsylla americana* and *C. alba*) collected from *Salix rigida/prolixa* and *S. exigua*, the first records world-wide that this parasitoid attacks willow-associated psyllids (Table 2). *Trechnites* were also collected by tube traps placed near native willows. While we did see some slight morphological differences between "willow *Trechnites*" and "pear *Trechnites*", initial limited analysis of DNA sequences suggests that they are the same species. This work is the first to demonstrate that native, non-pest psyllids in North America might be reservoirs of *Trechnites*, and this opens a new avenue for implementing *Trechnites*-based biological control of pear psylla. Planting or managing willow populations near pear orchards may be a very effective strategy for maintaining *Trechnites* in or near orchards even when pear psylla populations are low. To maximize success of this approach, research must confirm regular presence of *Trechnites* in native non-pest psyllids and must also determine taxonomic or biotypic composition of *Trechnites* populations in non-orchard and orchard habitats**.**

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

Project Title: Improving pear pest management with integrated approaches

PI: Louis Nottingham
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Cooperators: Tianna Dupont, Rebecca Schmidt-Jeffris, W. Rodney Cooper, David Horton, Richard Hilton, Tobin Northfield, Vince Jones, Nathan Moses-Gonzales, Robert Orpet

Total Project Request: $344,327
Year 1 (2019): $110,363 **Year 2 (2020): $114,718** Year 3 (2021): $119,247

Other funding sources: Industry gift grants

WTFRC Budget: None

Budget 1

Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Shelli Tomkins
Telephone: (509) 335-2885/293-8803
Email address: arcgrants@wsu.edu/shelli.tompkins@wsu.edu

Item	2019	**2020**	2021
Salaries[1]	70,200	73,008	75,928
Benefits[2]	20,498	21,318	22,171
Wages[3]	7,800	8,112	8,436
Benefits[4]	725	754	785
RCA Room Rental			
Shipping			
Supplies[5]	1,500	1,500	1,500
Travel			
Plot Fees[6]	9,640	10,026	10,427
Miscellaneous			
Total	110,363	**114,718**	119,247

Footnotes: [1]Research Assistant Professor, 12 months (year 1,2,3), [2]Benefits for Research Ass. Prof. 29.2%. [3]Wages for time-slip help, 1.0 FTE, summer. [4]Benefits for time-slip 9.3%. [5]Supplies – office and lab supplies, electronics, statistical consulting. [6] 3 years x $2,500/year (total acreage maintenance) + $2,100/acre (fees) on 3.4 acres

Objectives:

1. Determine lethal and sublethal effects of common insecticides and particle films to psylla natural enemies.
2. Compare particle films effects on pear psylla and natural enemies.
3. Evaluate potential for augmentative releases of earwigs for psylla control.
4. Examine novel strategies for psylla control including soil/root systemic insecticide applications, insecticide-infused netting, and reflective ground covers.
5. Determine baseline toxicities for new insecticides on two stages of pear psylla. Evaluate efficacy of other materials against pear pests *ad hoc*.

Significant Findings, Methods and Accomplishments:

- EthoVision camera and software package have been established in lab and trialed with particle films on earwigs. Initial experiments suggest that kaolin clay (Surround) has minimal impact on mobility and foraging behavior or earwigs.
- Residual contact bioassays on earwigs revealed that Tolfenpyrad (Bexar) was toxic to earwigs (30% mortality, 50% moribund), but less-so than lambda-cyhalothrin (Warrior II) (60% mortality, 30% moribund) .
- Lab and field studies suggest that Surround provides the best overall control of psylla in terms of repellency and juvenile developmental suppression, while Celite 610 (diatomaceous earth) is a close second. Microna (calcium carbonate) resulted in marginal-at-best repellency of psylla adults in the lab and field; however, it did suppress juvenile psylla development in the lab and field.
- Microna field plots exhibited significant increase in pear rust mite densities compared with Celite and Surround plots.
- No particle film products left detectable residues on harvested pear fruit from plots sprayed three times at 50 lb/acre from late May through early June.
- Corrugated cardboard bands wrapped around the circumference of pear trees caught significantly more earwigs than rolled corrugated cardboard traps in the field, suggesting that bands will be better for collecting earwigs for releases.
- Earwigs densities increased in most commercial orchard release sites 2 weeks after releases. In control sites in the same orchards, earwig densities decreased.
- One soil drench of thiamethoxam (Platinum) on potted pear trees resulted in 90% reduction in psylla surviving from egg to nymphs. Soil applied spirotetramat (Movento) did not affect psylla survival.
- Deltamethrin infused netting did not result in a significant mortality of psylla adults.
- Extenday placed in orchard drive rows from 3 April to 13 May reduced psylla eggs densities by 41% compared with control plots. Adults and nymphs were also reduced significantly.
- We successfully acquired matching grant funding from two other agencies: 1. USDA NIFA Crop Protection and Pest Management Grants ($323,622) and 2. Washington State Commission on Pesticide Registration ($19,437). These projects will be used to provide supplemental funding for objectives 1, 2, and 5. Another proposal has passed the first stage of review and is now in final review with Western SARE, which will provide supplemental funding for objective 3.

1. Determine lethal and sublethal effects of common insecticides and particle films to psylla natural enemies.

Methods: An EthoVision camera and software package was purchased from Noldus in May 2019. Some time was taken to build an infrared lightbox frame and camera stand to provide reliable, high quality tracking of insects in areas. By mid-August the system was built (Fig. 1), and we were running various trials to troubleshoot methods. By the end of September, we developed multiple a bioassays with earwigs (which should work for other predators such as *Deraeocoris*) to assess the effects of pesticides and particle films on mobility, motor coordination and predatory feeding behavior. Sixteen insects can be tracked per session. Insects can be exposed to materials prior to experiments or during experiments. For example, insects may be first exposed to insecticide residues on leaves, then added to Petri dishes for tracking. EthoVision is programed to track each insect's movement over 1 hour and analyze distance traveled, acceleration, velocity, vertical and horizontal distance among treatments. Another assay incorporates repellency, making it especially useful for particle film non-target effects trials. It involves treating half of the circular arena with a particle film or water for control, then putting a food source (*Ephestia* eggs) on top of the treated area. In EthoVision, the treated area with food is labeled as a 'zone', and the amount of time the insect spends in the zone is analyzed among treatments.

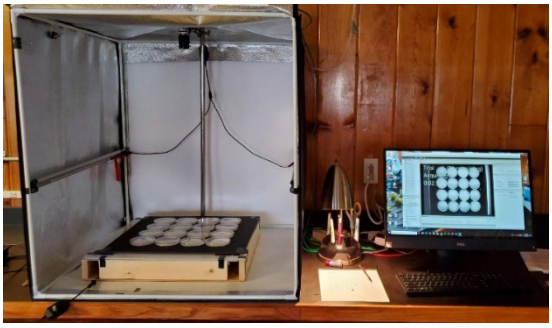

Fig. 1. EthoVision system used for non-target effects testing: above-mounted camera, infrared light box, 16 Petri dish insect arenas, and video tracking software.

Results: In preliminary trials, earwig feeding activity was not inhibited by kaolin covered surfaces and walking on kaolin residues did not affect their general mobility or coordination.

2. Compare effects of particle films on pear psylla and natural enemies.

Surround (kaolin clay), Celite 610 (diatomaceous earth), and Microna AG (calcium carbonate) were tested in multiple experiments in the field, lab and greenhouse. Lab and greenhouse tests also included additional comparisons to Cocoon (kaolin clay, generic), IAP 440 horticultural oil, and Bexar (positive control).

Field trials. Methods: Exp. 1. Surround CF, Celite 610, Microna AG and an untreated control were compared on single-tree field plots. For each treatment, there were six replicate plots, three Anjou and three Bartlett. Each particle film treatment was applied to trees using a pressurized handgun sprayer at 50 lb/acre. Treatments were applied on 15 May, 21 May, and 5 June, targeting the second generation of juvenile psylla and 3rd generation of adults. Sampling for psylla, mites and natural enemies was conducted weekly, beginning immediately prior to the first application on 15 May through 3 September, via beat trays, leaf collections and earwig traps. At Bartlett and Anjou harvest, four pears per plot (96 total) were collected and taken to the Blue Star packing facility in Cashmere, WA. Pears were inspected, washed, and respected to determine if particle film residues would be result in rejection.

Exp. 2. Early season residue decline on branches was examined for Surround CF, Celite 610, Microna and Cocoon. In March, before green tissue was present on trees, each particle film (surround, Celite,

Microna, Cocoon), 4% IAP 440 oil and a water (check) were applied to field ground Anjou trees. Six branches per treatment were excised 24 hours after application, photographed individually (Fig. 3), then analyzed for whiteness using ImageJ greyscale tool. Seven days after application, during which one heavy rain event occurred, another six branches were excised per treatment and processed using the same methods.

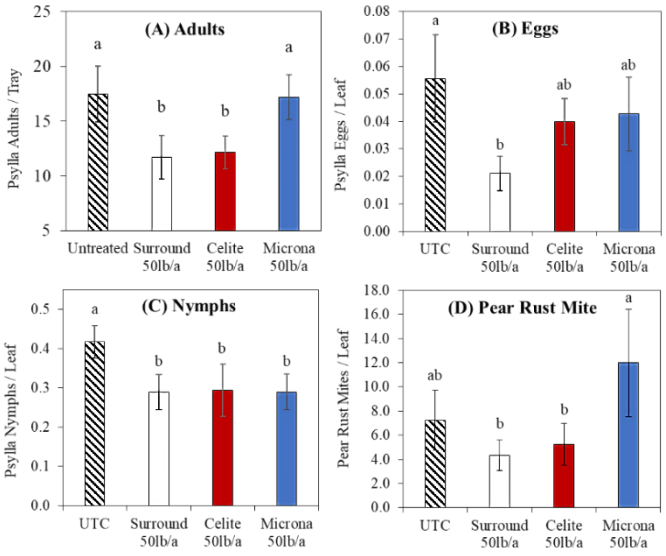

Fig. 2. Pear pest densities occurring in field plots treated with 50 lb/acre of either Surround CF, Celite 610, or Microna or untreated.

Results: Exp. 1. Surround and Celite both significantly reduced psylla adult densities similarly, by ca. 40%; Microna did not affect adult densities (Fig. 2A). Only Surround resulted in significant egg reduction, ca. 65%, while Celite and Microna had similar numerical but non-significant reductions (Fig. 2B). Combined early and late nymphs densities were significantly reduced in all three particle film treatments; however, all treatment reductions were only around 30% (Fig. 2C). Interestingly, pear rust mite densities were significantly greater in Microna plots than Surround and Celite, but the check had intermediate rust mite densities to all treatments (Fig. 2D). Effects of particle on natural enemies were minimal. Minor reductions occurred for certain species and treatments, but they appeared commensurate with reductions in psylla. As for residues on pear fruit, some fruit had very faint particle film residues in the calyx or stem bowls prior to washing, however, none were considered culls by packing house inspectors. The commercial washing line removed and lingering residues, and were all pears were considered acceptable for packing.

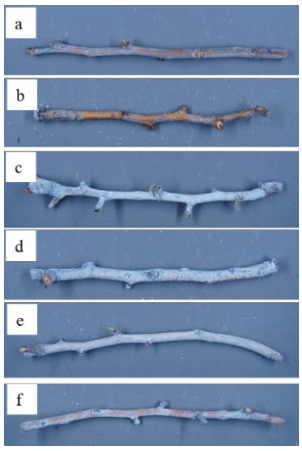

Fig. 3. Branches one day after treatment with particle films: a. check, b. 4% oil; c. Surround CF, d. Celite 610; e. Cocoon; f. Microna. Photographs are analyzed with ImageJ

Exp. 2. Twenty-four hours after application, all branches sprayed with particle films were significantly whiter than the check, while oil at 4% made branches significantly darker than the check. Surround CF resulted in the whitest branches overall: significantly whiter than all other particle films except Celite 610. Although Surround was not significantly white than Celite, Celite was intermediate between Surround, Microna and Cocoon. Microna and Cocoon were significantly whiter than the check. Seven days and one rain even after application, Surround CF, Celite and Cocoon were statistically the same, and significantly whiter than the check and Microna. Microna was whiter than the check. Oil at 4% remained darker than all treatments and the check.

Lab experiments. Methods: Potted Bartlett seedlings were used to examine suppression of pear psylla by particle films via two mechanisms: repellency and juvenile mortality. For repellency evaluations, five seedlings for each treatment were sprayed with a particle film at 50 lb/acre (30g/500ml), 0.5% oil, or water (check) using hand-pump spray bottles, then allowed to dry for 24 hours. Five psylla adults, three females and two males, were caged to each seedling in a mesh drawstring bag and left on seedings for seven days. After seven days, bags and adults were removed, and eggs were counted. For juvenile mortality, the same methods were used except adults were caged over seedlings before treatments were applied. Eggs were counted on seedling, then treatments were allocated so that all treatments began with similar numbers of eggs. Treatments were applied via hand-pump spray bottles and

seedlings were left in the greenhouse until counts occurred. Nymphs were counted 13 and 22 days after treatment applications.

Results: For adult repellency, Surround and Celite resulted in the greatest repellency of psylla, having significantly fewer eggs than the check. Microna, Cocoon, and 0.5% oil were intermediate to the check, Surround and Celite. For juvenile mortality, all four particle films and 0.5% oil resulted in significantly fewer surviving nymphs than the check. Mortality was similar among all particle films and oil, averaging between 75-85%.

3. Evaluate potential for augmentative releases of earwigs for psylla control.

Methods: Exp. 1. An experiment was conducted to help determine the optimal trap type and trap size for earwig collecting. We tested four traps: 1. Small corrugated carboard rolls (6x12"); 2. Large rolls (6x18"); 3. Small corrugated cardboard bands (single trunk wrap); and 4. Large corrugated cardboard bands (double trunk wrap). Treatments were placed in pear, apple and stone fruit orchards throughout the summer, and collected 7-14 days after deployment.

Exp. 2. We performed no-choice bioassays with earwigs to determine which fruit they would injure. Fruit tested were pears, cherries, peaches, and nectarines. Fruit were tested at two developmental stages: developing (about 2" in diameter) and harvest (except cherries, which were only tested at harvest). Five earwigs were placed into plastic cup containers with a single fruit; for a check, fruit were placed into containers without earwigs. Earwigs were left in containers for 7 days, then fruit were rated for injury.

Exp. 3. Earwigs adults were released in four pear orchards in late August to test the potential to inoculate orchards with low initial population densities. Sites were chosen if they had fewer than 1 earwig per trap on average, determined from scouting data (DuPont and Strohm, 2017-2019) or past experiments (L. Nottingham). Earwigs were collected throughout the summer as either nymphs or adults from the TFREC pear and apple orchards and a stone fruit orchard near Monitor, WA. Earwigs were stored in 5 gallon buckets with corrugated cardboard and fed a diet of dry dogfood and occasional greens (collards and kale). Releases occurred on 22 and 23 August. Each site utilized four orchard acres divided into two blocks, one for earwig releases and one for a check (Fig. 4). Prior to releases, the baseline earwig population for the orchard was determined by deploying rolled corrugated cardboard traps throughout the orchard, which were sampled two weeks later. Baseline psylla populations were determined via beat trays and leaf sampling. Earwigs were releases at six locations (individual trees) on the release side of each orchard site. At each release tree, 200 earwigs were releases (1200 per orchard). We returned to each site to sample earwigs and psylla two weeks following releases. In 2020, we will sample the same sites for earwigs and psylla to compare establishment, dispersal and control of psylla. In 2020, half of the release and control site trees will be provisioned with earwig shelters (pink circles, Fig. 4), in the form of rolled newspapers or carboard bands (TBD), to determine if shelters can increase earwig densities in trees. The provisioned shelters will be removed during weeks when sampling is being conducted to prevent the shelters from competing with traps.

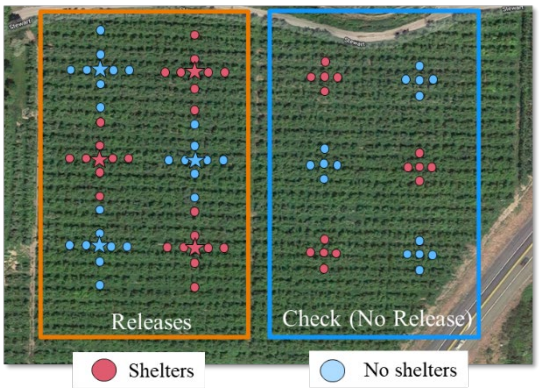

Fig. 4. Earwig release map for 1 of 4 orchards. Releases occurred in late August 2019.

Results: Exp. 1. Bands caught approximately 40% more earwigs than rolls. The size of bands or rolls did not have a significant effect.

Exp. 2. Earwigs did not injure either young or harvestable pears and peaches. Earwigs injured both young and harvestable nectarines. Earwigs injured harvestable cherries (young cherries were not tested).

Exp. 3. Earwig populations were significantly greater in release locations than non-release locations two weeks after releases. Average numbers of earwigs per trap in focal release trees increased by 55% from the pre-count, while trees adjacent to release sites had the same number of earwigs as at the pre-count. Interestingly, earwigs in non-release sample sites (both focal and adjacent trees) decreased by 70%, suggesting that wild populations were declining at this point in the season. Therefor, the effects of releases may have be even greater than the counts suggest.

4. Examine novel strategies for psylla control including soil/root systemic insecticide applications, insecticide-infused netting, and reflective ground covers.

Soil Applications. Methods: Platinum (thiamethoxam), Movento (spirotetramat) and an untreated check (water) were examined for control of psylla via soil applications. All treatments were mixed with Regulaid at 1 pint/acre. Potted Anjou scions on O.H. x F. rootstocks with no psylla were used. Prior to soil drenches, the trees were not watered for 48 hours to ensure that they would uptake the chemical. One liter of insecticide solution was applied to the soil at high label rates: 0.35g/liter for Platinum and 0.89ml/liter for Movento. Three days following soil drenches, ten psylla adults (five males and females) were bagged onto shoots. Bags with adults were left on shoots for 18 days, then living and dead adults, eggs and nymphs were counted.

Results: Significant egg lay occurred on all treatment and check trees, with high variability from 20 to 240 eggs per tree. Treatment and check trees exhibited variable egg lay, suggesting that insecticides did not affect adult oviposition. Therefore, treatment effects were measured as survival from egg to nymphs (living nymphs/initial eggs). One soil drench of Platinum (thiamethoxam) resulted in 90% reduction in survival of psylla from egg to nymphs. Soil applied Movento (spirotetramat) did not affect psylla survival.

Insecticide infused netting. Methods: Psylla adults were collected in April 2019 from an untreated orchard by aspirating adults into plastic fly tubes. Each tube received 5 males and five females (judged by size in the field), and 10 tubes were used (100 adults total). Pieces of deltamethrin infused mesh netting (Permanet 2.0, Vestergaard, Inc.) was cut into 4 x 4" squares and placed into five fly tubes with psylla adults, and untreated netting was placed into five other tubes for a check. Adults were left in tubes for 72 hours, then rated for mortality.

Results: Insecticide screen did not cause significant mortality. Both controls and treated tubes exhibited between 15-30% mortality. Psylla in the Wenatchee Valley are known to be tolerant to pyrethroids, which is likely why no affect occurred. However, the company now has a new product, Permanet 3.0, that has the addition of PBO, which may yield better mortality.

Reflective Ground Covers (Extenday). Methods: Our previous experiments demonstrated that reflective plastic mulch can suppress colonization and oviposition by winterform psylla by ca. 85%. In 2019, we tested if Extenday, a reflective textile which can be reused for multiple seasons, may offer similar repellency. We tested this is at the TFREC research pear orchard, which has old large trees and high psylla pressure. There were three replicate plots of Extenday and three untreated check plots. Each plot was 16 trees total, in 4 rows (4 x 4 trees). Only the center 4 trees where used for sampling. Each Extenday plot used 3 sheets of Extenday cut to 50 ft each (150 ft of Extenday per plot). Extenday was installed on 3 April and removed on 13 May 2019. Sampling for psylla adults, natural enemies, eggs and nymphs was conducted weekly throughout this timeframe using beat trays, fruiting bud inspections and leaf collections.

Results: Prior to installation of Extenday, there were no differences in psylla adult densities. One week after Extenday was installed, psylla adult densities in Extenday plots were 65% lower than the

check, and two weeks after they were 75% lower than the check. Adults densities evened by the third sample on 22 April. Eggs densities on fruiting buds remained ca. 30-55% lower in Extenday plots than check for the four samples between 9 April and 29 April (Fig. 5). Young nymph counts on leaves were reduced ca. 23% in Extenday plots on 6 May and ca. 28% on 13 May. Due to these positive results in small plots, in 2020 we will compare Extenday and reflective mulch on larger commercial plots.

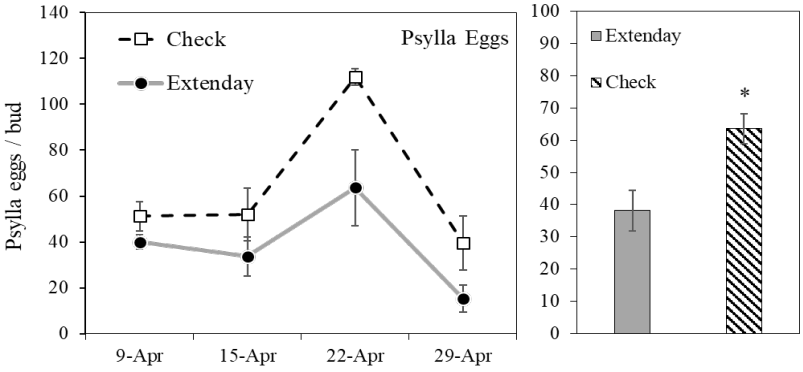

Fig. 5. Pear psylla eggs densities as daily averages (left) and cumulative season averages (right) in pear orchard field plots with Extenday placed in drive rows or untreated controls.

5. Determine baseline toxicities for new insecticides on two stages of pear psylla. Evaluate efficacy of other materials against pear pests ad hoc.

Baseline Toxicity Testing. Baseline toxicity assays for Bexar on psylla adults and nymphs will be conducted in the spring of 2020 using leveraged funding from Washington State Commission on Pesticide Registration. Five populations will be tested: Wenatchee, Yakima, Okanogan, WA, Hood River and Medford, OR. For each population we will conduct diagnostic dose probit bioassays to determine LC10, 50 and 90 values for winterform adults and first generation nymphs.

Pesticide efficacy testing. Methods: Multiple insecticide efficacy experiments were conducted, therefor, the methods will be kept general and we will only report notable findings herein.

Pesticide efficacy was tested for multiple products, both conventional and organic in both field and laboratory bioassays. Field experiments involved 4 tree plots with at least four replicates per treatment. Lab bioassays involved treating psylla infested pear seedlings, then counting survivors at various life stages.

Results: **Thyme Guard**: Thyme Guard (thyme oil) at 0.5% resulted in significant but not impressive psylla adult mortality, at ca. 44% after 72 hours. Thyme Guard at 0.25% and the check were not different, both resulting in 4% mortality of psylla adults. IAP oil at 0.25% resulted in 100% mortality of psylla adults. Thyme Guard at 0.5% resulted in significant but marginal mortality of twospotted spider mite (TSM) after 72 hours at 44% mortality compared with the lower rate, the check, and 0.25% IAP oil, all resulting in 0% mortality of TSM. The 0.5% Thyme Guard rate also reduced TSM ovipostion compared to all other treatments by about 65%.

Cinnerate: Lab bioassays suggested that Cinnerate is most effective at killing psylla as hatching eggs or adults, and efficacy is dose-dependent. Cinnerate at 32 fl oz/100 gal did not provide control of eggs, while 64 fl oz/100 gal caused ca. 70% mortality. Cinnerate at 40 and 60 fl oz/100 gal resulted in nearly 100% mortality of adults; however, mortality occurred much faster at the higher rate.

Bexar and Ultor : Lab bioassays examining Bexar (tolfenpyrad) at 27 fl oz/acre applied to psylla eggs resulted in ca. 90% reduction in development to young nymphs, and 95% reduction in development to old nymphs (i.e. mortality occurred rapidly). When treatments were applied to young nymphs in a second bioassay, Bexar resulted in 100% mortality. Ultor (spirotetramat) at 14 fl oz/acre applied to psylla eggs resulted in 60% reduction in development to young nymphs, and 94% reduction in development to old nymphs (i.e. mortality occurred slower than Bexar, but with similar efficacy). Ultor applied to young nymphs resulted in 55% mortality.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: 9-20-p

Project Title: Epidemiology and management of pear gray mold in the PNW

PI:	Achala KC	**Co-PI:**	Achour Amiri
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Address:	569 Hanley Rd.	**Address**:	1100 N. Western Ave.
City/State/Zip:	Central Point/OR/97502	**City/State/Zip**:	Wenatchee, WA, 98801

Cooperators: Dr. Peever, WSU-WA; Dr. Ashley Thompson, OSU-OR; Dr. Richards Kim -Pace International; Christensen and Spanjer Orchards in Cashmere, WA, Duckwall and Stewart Orchards in Hood River, OR, Naumes and Bear Creek Orchards in Medford, OR.

Total Project Request: **Year 1:** $99,768 **Year 2:** $108,781 **Year 3:** $111,248

Other funding sources
KC lab:
Agency Name: Chemical company contracts. **Amt. awarded:** $34,000
Amiri lab:
Agency Name: Specialty Crop Block Grant program-USDA-WSDA. **Amt. awarded:** $170,195.
Notes: "Strategies to enhance pre- and postharvest management of gray mold in pome fruit" PI: Amiri, co-PI: Tobin Peever. This grant is split 70% and 30% for apple and pear, respectively.

WTFRC Collaborative expenses: None

Budget 1: Achala KC

Organization Name: OSU Ag. Res. Foundation **Contract Administrator: Russ Karow**

Telephone: 541-737-4066 **Email address: russell.karow@oregonstate.edu**

Item	(2019-20)	(2020-21)	(2021-22)
Salaries[1]			
Post-Doctoral research associate 6 mo	25,000	25,750	26,523
Undergraduate labor (800 hrs @ $13.00)	6,240	10,400	10,712
Benefits[1]			
Post-Doctoral research associate	15,775	16,248	16,735
Undergraduate labor	749	1,248	1,285
Equipment	0	0	
Supplies[2]	1,500	1,545	1,591
Travel[3]	500	1,000	1,060
Hood River Plot Fees[4]		3,000	3,000
Total	**49,764**	**59,191**	**60,906**

Footnotes:

[1] Salaries for a Post-Doctoral research associate @ $50,000/month for 6 months, and 63.1% benefit rate. Salaries for an undergraduate research assistant at $13.00/hr for 800 hrs and 12% benefit rate. The hours request for undergraduate labor is increased for year 2 based on the requirement from 2019 samples collection and processing time. We anticipate the same amount of sample collection and processing for year 2 of this study.

[2] Materials to collect and process samples, plates and media to isolate pathogens, reagents for DNA extraction and qPCR analysis, chemicals and reagents for in vitro analysis for year 1 and 2; labels and field supplies for year 3.

[3] Travel to experimental and commercial orchards for year 1; Travel to Hood River for PI and FRA during trial set up and spray application for year 2 and 3.

[4] Plot fees for trials in Hood River @ $3,000 per acre.

Budget 2: Amiri

Organization Name: WSU **Contract Administrator:** Katy Roberts/Kim Rains

Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu / kim.rains@wsu.edu

Item	2019-20	2020-21	2021-22
Salaries[1]	30,240	31,450	32,708
Benefits[1]	11,884	12,360	12,854
Supplies[2]	6,700	4,600	3,200
Travel[3]	1,180	1,180	1,580
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	**50,004**	**49,590**	**50,342**

Footnotes:

[1] Salaries for a Research Associate at $3,600/ month for 12 months, 0.7 FTE and 39.3% benefit rate.

[2] Supplies include chemical and reagents needed to culture fungi and material for pathogenicity tests and Molecular detection and sequencing of Botrytis from pear samples.

3 To travel to experimental and commercial orchards and to packinghouses in WA and Hood River, OR to conduct trials and collect data at about 1,200 miles/season @$0.58/mile. At the end of Year 2, travel is budgeted for the PI to travel to Medford to meet with co-PI for Extension and result discussion

OBJECTIVES

1. Understand the epidemiology of *Botrytis* infections and *Botrytis* causal species in orchards and their impact on gay mold development in storage

2. Identify new approaches to manage gray mold in pear

2.1. Continued testing of registered and new fungicides for the control of gray mold disease
2.2. Evaluate epidemiology-based spray programs for gray mold management

3. Conduct an outreach program to update pear growers/packers in the PNW

SIGNIFICANT FINDINGS:

- In trials conducted in WA and Hood River, *Botrytis* was detected on flowers and fruits collected throughout the season from bloom to harvest.
- In trials conducted in Southern Oregon, *Botrtyis* was detected in all stages and largely present in samples at petal fall.
- *Botrytis* was detected in orchard atmospheres throughout the season from bloom to harvest at low and variable frequencies between locations in WA and Hood River.
- In all locations, the size of *Botrytis* inoculum was greater in organic orchards compared to conventional orchards.
- In WA and Hood River, the inoculum size decreased from bloom to fruit set in conventional orchards but then increased toward maturity and harvest. In organic orchards, the inoculum size increased throughout the season. In SO orchards, the inoculum size decreased as we moved towards commercial harvest in both organic and conventional orchards.
- Variabilities in inoculum size and dynamics throughout the season has been observed between orchards located in different districts.
- In SO trials, fungicides showed a range of effectiveness against 21 *Botrytis* isolates indicating variability in sensitivity when exposed to fungicides with different modes of action.

Methods

Objective 1. Understand the epidemiology of *Botrytis* infections and *Botrytis* causal species in orchards and their impact on gay mold development in storage

Experimental Sites: The research trials planned in this objective will be conducted at three districts in the PNW. Trials in Cashmere, WA and Hood River, OR will be led by Amiri including one conventional and one organic orchard (d'Anjou). Trials in Medford, OR will be led by KC using Comice as model cultivar because of its importance in southern Oregon.

Activity 1.1. ***Infection timing:*** Amiri (Cashmere, Hood River) and KC (Medford) (Years 1 & 2):
To investigate the impact of weather conditions and fungicide sprays on pear infection timing(s) 60 pear blossoms will be collected from two orchards at each district in the spring of 2019 and 2020. Afterward, 60 fruit will be collected from the same trees and orchards used for flowers sampling at fruit set, mid-summer, and at commercial maturity. Blossom and fruit samples will be transported in separate clean bags to the Pathology Labs at TFREC or SOREC. Thirty samples will be used for molecular quantification of *Botrytis* infections and the 30 remaining samples will be used for isolation of *Botrytis* on a semi-selective medium. Flowers will be freeze-dried and stored at -80°C. Fruit will be peeled and the peel and the flesh of the fruit will be freeze-dried separately and stored at -80°C. The separation of the peel from the flesh will help separate between infestation (spores present on the surface) from

endophyte infections (present inside the fruit). DNA will be extracted from freeze-dried samples and the presence of *Botrytis* will be detected using a quantitative polymerase chain reaction (qPCR) assay (Diguta et al. 2010). Spores of *Botrytis* will be enumerated from fresh (non-dried samples) on a *Botrytis* semi-selective artificial agar medium (Edwards and Seddon 2001). Data on *Botrytis* isolations in every stage will be quantified and compared to weather data and fungicides applications at respective stages.

Activity 1.2. Investigate the causal species of gray mold in the PNW. Amiri (Years 2 & 3):
Botrytis isolates, collected from bloom to harvest at each of the experimental orchards described above (infection timing) as well as from decayed fruit after 6-8 months of storage, will be DNA fingerprinted to determine the exact causal species of gray mold in PNW. If different species are detected in pear, the collected isolates will be tested for fungicide sensitivity to determine at what stage resistance is selected, and for their fitness that mimic pre and postharvest conditions. Isolates from Medford collected by KC will be transferred to Amiri's Lab in Wenatchee who will lead this effort including other isolates from Cashmere and Hood River.

***Weather Data**:* Wetness duration and temperatures will be collected from the Washington State University-AgWeaterNet (http://www.weather.wsu.edu/) in way to obtain data at all sampled orchards from the closet (≤ 1 mile) weather station. In Medford, the weather data will be collected from SOREC AgriMet weather station; and Bear Creek local weather station from where the samples will be collected.

Objective 2. Enhanced approaches to manage gray mold in pear

Activity 2.1. Continued testing of registered fungicides for the control of gray mold disease (KC)
Approach: The fungicides listed in table 1 will be tested in laboratory against available *Botrytis* isolates at SOREC and discriminatory doses will be identified for each fungicide.

Large scale screening of isolates based on discriminatory concentrations: To understand the population as a whole, large number of isolates are necessary to monitor the resistance status of a fungicide. Once the discriminatory concentrations for fungicides have been identified, the field isolates of *B. cinerea* collected from at least twenty orchards in southern Oregon will be screened for resistance to three fungicide groups identified earlier (M3, 14, 17, and 19).

Table 1: List of fungicides used for sensitivity assays in sub-objective 2.1.

Trade name	Active ingredient	FRAC group	Pear Disease labels	Medium	Discriminatory dose (µg/ml)
Manzate	mancozeb	M3	Scab	PDA	TBD
Ziram	ziram	M3	Scab/Storage rots	PDA	TBD
Judge	fenhexamid	17	Storage rots	PDA	10
Ph-D	polyoxin D	19	Storage rots	MEA	TBD
Botran	dicloran (DCNA)	14	None	PDA	TBD

Activity 2.2. Evaluate epidemiology-based spray programs for gray mold management

***Experimental Sites**:* The research trials planned in this sub-objective will be led by Dr. KC at research Comice block at OSU-SOREC and on d' Anjou at MCAREC. Dr. Amiri will be conducting the trials at a commercial d' Anjou orchard in Cashmere, WA.

Trials at OSU-SOREC and MCAREC (KC): Based on the results from Objective 1, the most susceptible stage for *Botrytis* infection will be identified and the trees will be inoculated with *Botrytis* inoculum at that stage. The treatment trees in respective research station will be sprayed with spore

suspension of *B. cinerea* @ 1 X 10^5 spores/ml. The control trees will receive spore sprays but not treatment sprays. The fungicides identified from previous studies and sub-objective 2.1 with promising laboratory efficacy will be tested for their field efficacy.

The fungicide management program will consist of early season application (susceptible stage of infection identified from objective 1), preharvest application, and postharvest application. Promising fungicides for each of these stages identified from laboratory tests will be tested as a program for their efficacy to manage gray mold storage rot. This program will be compared with standard grower practice (preharvest and postharvest application) for the potentially added benefit of early season applications.

For evaluation of program, at least 20 fruits from each tree will be harvested at commercial maturity and stored in normal atmosphere cold storage rooms at respective research stations facility. After six months of storage, the fruits will be evaluated for gray mold rot development. The data will be analyzed as percent disease incidence.

Trials at commercial orchard in Cashmere (Amiri): Because scab and mildew are not major concerns, most pear growers in central WA tend to limit their fungicide sprays to one application in the 3 weeks preceding harvest. We plan to test and compare spray regimes outlined in Table 2 that include a conservative (industry standard), moderate and an extensive spray program.

Table 2. Description of spray regimes to be tested at a commercial orchard in Cashmere, WA.

Treatment type	Spray timing within season	Number of sprays	Bloom	Fruit set	Summer	7 DPH	Postharvest
Control	Control	0	-	-	-	-	-
Conservative	Early	2	-	Pri	-	-	Penb
	Mid	2	-	-	Pri	-	Penb
	Late (current industry standard)	2	-	-	-	Pri	Penb
Moderate	Early-Early	3	TopM	Pri	-	-	Penb
	Early-mid	3	-	TopM	Pri	-	Penb
	Mid-Late	3	-	-	TopM	Pri	Penb
Extensive	Early-Mid-Late-No postharvest	3	-	LunaS	TopM	Pri	-
	Early-Mid-Late-Plus postharvest	4	-	LunaS	TopM	Pri	Penb

- No treatment, Pri = Pristine, TopM = Topsin M, Luna S = Luna Sensation, Penb = Penbotec

We will use Pristine® (the most widely used in the PNW) for the conservative spray, Topsin®M (FRAC 1) and Pristine (FRAC 7 + 11) for the moderate spray, and add Luna® Sensation (FRAC 7 + 11) for the extensive spray. Luna is one of the most effective fungicides in conventional orchards. Penbotec (FRAC 9) it is the most systemic fungicide among the current postharvest fungicides and is thought to be the most effective against potential latent infections. Trials will be set in a randomized complete block design with four replicate trees per treatment and fungicides will be sprayed using backpack sprayers. At commercial maturity in late August-early-September of 2020 and 2021, a total of 200 fruit/treatment (50 fruit/replicate tree) will be harvested, drenched or not with the label rate of Penbotec (**Table 2**), and stored at 1°C in a regular atmosphere for up to 8 months. Fruits will be checked for gray mold after 4 months of storage and every two months thereafter. At harvest (pre and post Penbotec application) and after 4 months of storage, 10 fruit (each time) will be removed from each treatment and subjected to qPCR analyses (Objective 1) to detect and quantify *Botrytis* inoculum. The type of fungicides and application time may be modified in Year 3 based on results from Year 2. An economic study will be conducted to estimate the costs and benefits of each spray regime in relation to the rates of gray mold after storage.

3. Conduct an outreach program to update pear growers/packers in the PNW. Outreach activities will be conducted at the end of Year 2 and 3 in WA (Dr. Amiri) and OR (Dr. KC).

RESULTS AND DISCUSSION

Objective 1. Understand the epidemiology of *Botrytis* infections and *Botrytis* causal species in orchards and their impact on gay mold development in storage

Activity 1.1. ***Infection timing*** (Year 1)
Trials at WA and Hood River

As shown on Figure 1 below, *Botrytis* was detected in Anjou orchards at almost all sampling times. There seem to be a carry-over from bloom to fruit and increases as the fruit mature. Fungicide spray programs for each orchard were obtained and are being analyzed to correlate with potential fungicide effect on reduction of *Botrytis* load on fruit as this can be explained by the slight reduction observed before harvest (Figure 1) following the preharvest spray. However, the incidence of fruit infected (not decayed) with *Botrytis* increased significantly in organic Anjou fruit to 78% in Hood River and 66% after 6 months of CA storage. The frequency of conventional Anjou fruit carrying Botrytis remained steady in CA storage compared to harvest time. It is important to note that the fruits used in this study were not treated postharvest. Correlation of *Botrytis* load on fruit with gray mold incidence in storage is awaiting the end of storage season by May-June 2020.

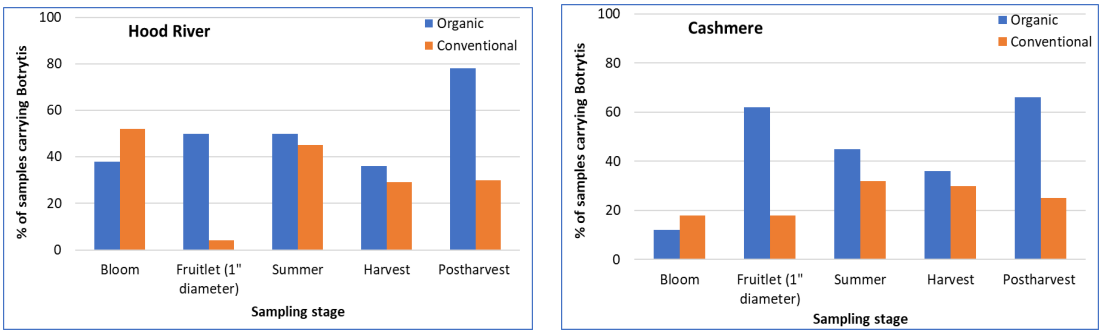

Figure 1. Evolution of *Botrytis* incidence on organic and conventional Anjou pear in Hood River and Cashmere throughout the 2019-20 preharvest growing season and after 6 months of CA storage as detected by qPCR.

Infections by *Botrytis* were observed in all organs of the fruit (cuticle, stem-bowl, calyx and inner flesh) at harvest at variable frequencies between orchards (Figure 2). This observation indicates that not only the external parts (calyx, cuticle and stem-end) of the fruit contains *Botrytis* inoculum at harvest, but also the flesh which indicates latent (dormant) infections from previous infections in the orchard. The frequency of samples carrying Botrytis remained steady or increased slightly in storage.

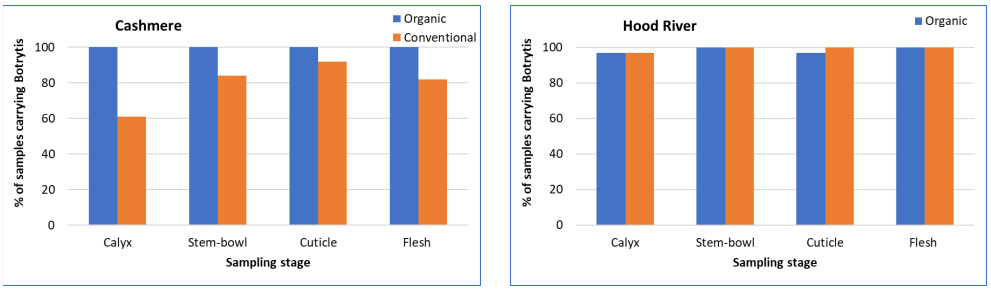

Figure 2. Incidence of *Botrytis cinerea* on different organs of the fruit at commercial maturity (harvest time) Anjou pear in organic and conventional orchards in 2019.

Trials at SO (Year 1)

Comice pears were collected in a commercial orchard in Southern Oregon starting in early April to late August of 2019. Pears were collected from conventional and organic blocks in 5 stages with stage 1 being petal fall, stage 2 being fruit set, stage 3 being fruitlet, stage 4 being mid-summer, and stage 5 being commercial maturity. Pears were then plated onto BSAM for *Botrytis* detection and isolation. Out of the collected pears that were grown conventionally, 43.33% had *B. cinerea* at stage 1, 46.67% had *B. cinerea* at stage 2, 16.67% had *B. cinerea* at stage 3, 4.17% had *B. cinerea* at stage 4, and 12.50% had *B. cinerea* at stage 5. Out of the collected pears that were grown organically, 73.33% had *B. cinerea* at stage 1, 40.0% had *B. cinerea* at stage 2, 26.67% had *B. cinerea* at stage 3, 16.67% had *B. cinerea* at the stage 4, and 12.50% had *B. cinerea* at stage 5 (Figure 3).
Interestingly the number of pears with *Botrytis* was quite high at stage 1. With a few exceptions, the number of pears with *Botrytis* decreased overtime. When analyzing weather data collected from the AgriMet weather station in Medford, the average temperature tended to increase overtime from the months of April to August (Figure 4A) while the average relative humidity tended to decrease during those same months (Figure 4B).
Daily precipitation also appeared to be more frequent during the earlier months as opposed to the latter months (Figure 4C). Relative humidity and precipitation are important factors in fungal germination. Therefore, it makes sense that the highest number of pears with *Botrytis* would occur during a period of both high average relative humidity and more frequent precipitation.Temperature also plays an important role in fungal growth with *B. cinerea* performing better at more moderate temperatures ranging from 65 °F to 78 °F. Therefore, a maximum average temperature of 84.27°F in June, 88.14°F in July, and 90.60°F in August would have been too hot and therefore not optimal for fungal growth.

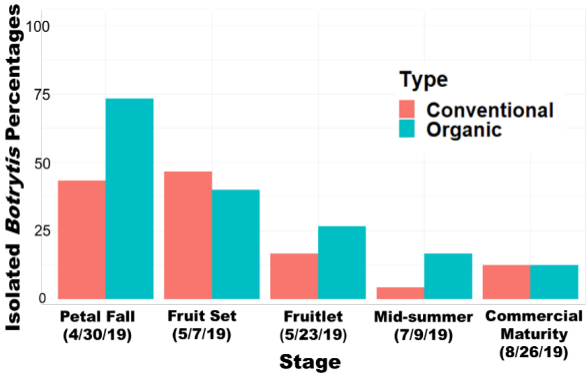

Figure 3: Percentage of *Botrytis* isolated from pears collected in Medford orchard at different stages during their development. These results are obtained from culture-based assays as opposed to qPCR based assay in WA and Hood River.

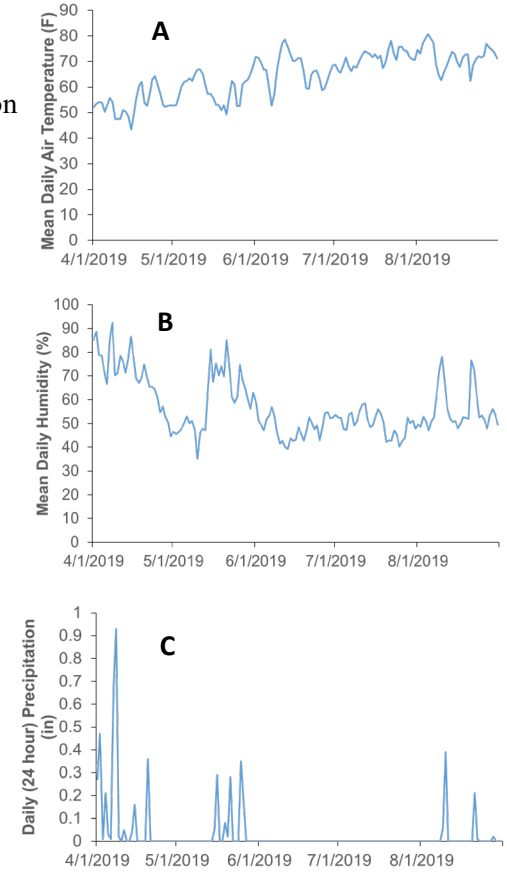

Figure 4: (A) Mean daily air temperature (°F); (B) mean daily humidity (%); and (C) total daily precipitation (in.) from April to August of 2019 in Medford, OR according to AgriMet.

Objective 2. Identify new approaches to manage gray mold in pear

Activity 2.1. ***Continued testing of registered and new fungicides***

Manzate Pro-Stick, Ziram 76DF, Ph-D, and Botran 5F which contain the active ingredients of mancozeb (FRAC M3), ziram (FRAC M3), polyoxin D (FRAC 19), and dicloran (FRAC 14) respectively were tested for their effectiveness against 21 *Botrytis* isolates in vitro. The effective concentration to reduce radial growth by 50% (EC_{50}) values for mancozeb, ziram, polyoxin D, and dicloran ranged from 21.65 µg/ml to 136.02 µg/ml, 25.33 µg/ml to 156.77 µg/ml, 4.05 µg/ml to 619.02 µg/ml, and from 4.08 µg/ml to 26.75 µg/ml respectively (Figure 5). Overall, dicloran performed the best against *Botrytis* isolates at concentrations of 10 ug/ml and above. Ph-D is labeled as being used for storage rots such as gray mold while Manzate Pro-Stick is labeled as being used for scab. Ziram is labeled as being used for both scab and storage rots, but it does not indicate if it is effective against gray mold. Botran is not labeled for any pear diseases which may explain its effectiveness against *Botrytis* isolates due to its lack of use on pear. Overall, most isolates varied in their sensitivity towards the different fungicides. It is interesting to note that when the isolates were grouped by the orchards they were collected from, some trends in sensitivity emerged. For instance, polyoxin D was effective against isolates collected from orchard 1, but not against isolates collected from orchard 3 (Figure 5). This suggests that where *Botrytis* isolates originate from may also have an effect on their resistance towards different fungicides.

In vivo studies are currently being performed to identify fungicide efficacy. Preliminary data from these fungicides being tested in pear shows that mancozeb and dicloran are the least effective against *Botrytis* followed by ziram and then polyoxin D. Ranges in fungicide efficacies were 28.99% to 91.93%, 16.79% to 48.22%, 80.54% to 100%, and 84.60% to 100% for mancozeb, dicloran, ziram, and polyoxin D respectively. This is interesting, because dicloran was the most effective against *Botrytis* in vitro. However, only 6 *Botrytis* isolates have been tested so far and some of these isolates showed some resistance to dicloran when being tested in vitro. Additional in vivo testing will need to be done to further verify the effectiveness of these fungicides in pear.

Future work:

2020-2021:

Conduct a 2nd year of field trials in the same orchards used in Year 1 for comparison.

Obtain data from cold storage facilities on samples collected from previous season

Analyze weather and fungicide usage data and impact on botrytis incidence.

Evaluate the efficacy of different preharvest spray programs

Conduct the genetic analyses of *Botrytis* collected so far.

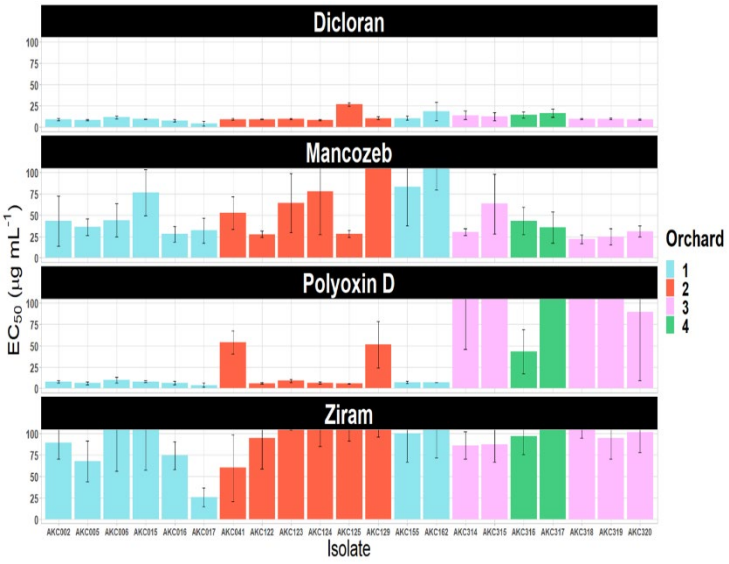

Figure 5: EC_{50} (µg/ml) values of 21 *Botrytis* isolates collected from 4 different orchards when grown on plates amended with Botran (Dicloran), Manzate (Mancozeb), Ph-D (Polyoxin D), and Ziram (Ziram)

CONTINUING PROJECT REPORT **YEAR**: No-Cost extension

Project Title: Refinement of practical fire blight control: Buffered oxytetracycline

PI: Kenneth B. Johnson
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Budget: **Year 1**: $24,202 **Year 2**: $24,686

Other funding sources: None

WTFRC Collaborative expenses: None

Budget

Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Dan Arp
Telephone: (541) 737-4066 **Email address:** dan.j.arp@oregonstate.edu

Item	2018-19	2019-20	2020-21
Salaries Faculty Res. Assist. 2 mo	9,908	10,106	No-cost
Benefits OPE 61%	6,044	6,165	
Undergraduate labor (&OPE 12%)	1,000	1,020	
Equipment	0	0	
Materials and Supplies	750	765	
Local Travel	750	765	
Plot Fees	750	765	
Medford russet trials	5,000	5,100	
Total	**$24,202**	**$24,686**	

OBJECTIVES:

1) Evaluate rate of pH-buffering on oxytetracycline-mediated suppression of fire blight pathogen populations on flowers and incidence of fire blight infection (Corvallis).

2) Evaluate effect of pH-buffering on finish quality of Comice and Bartlett pear fruit (Medford).

3) Evaluate if oxytetracycline formulation ('-hydrochloride' or '-calcium complex') influences the pH-buffering enhancement of oxytetracycline.

SIGNIFICANT FINDINGS:

- Fire blight pathogen populations on pear and apple flowers continued to increase during the post-petal fall period.

- In pear, compared to oxytetracycline alone, a citric acid-amendment enhanced oxytetracycline for fire blight suppression.

- Based on measurement of fire blight pathogen populations in flowers, a lower pH appears to prolong the inhibitory residual of oxytetracycline.

- Acidifying oxytetracycline with citric acid caused negligible effects to fruit finish (russeting).

- To date (2017-19), acidification of FireLine (applied twice) has provided 88% fire blight suppression (relative to water) compared to 79% suppression by FireLine alone. Similarly, acidified Mycoshield has been more effective (74 to 80% suppression) than Mycoshield alone (42% suppression).

METHODS:

Rationale. We observed previously that the potency of oxytetracycline for fire blight suppression was sensitive to the pH of the spray suspension. In 2019, field experiments were designed to further evaluate if a pH adjustment could enhance the effectiveness of oxytetracycline. In addition, trials in Medford pear orchards evaluated treatments of pH-reducing amendments on russet-sensitive cv. 'Comice' and on russet-tolerant cv. 'Bartlett' to assess risk to fruit finish.

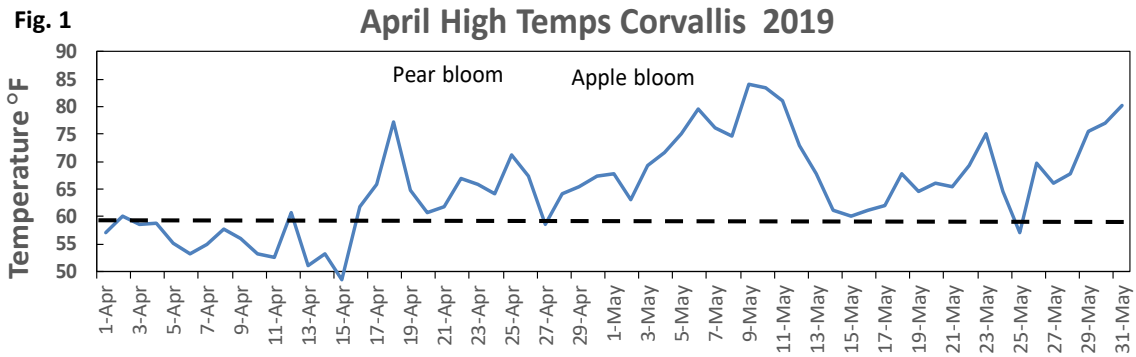
Experimental design. Experimental orchards were located at Oregon State University's Botany & Plant Pathology Field Laboratory near Corvallis (pathogen-inoculated), and at the OSU Southern Oregon Research and Extension Center near Medford, OR (fruit finish). Experiments were arranged in a randomized complete block designs with 4 replications. Treatment suspensions were sprayed to near runoff with backpack sprayers during early morning hours. To enumerate pathogen and yeast populations on flowers, five flower clusters were sampled from each replicate tree at full bloom, petal fall, and one-week post-petal fall, which was followed by washing the flowers, recording the pH of the wash, and dilution plating the wash on a selective culture media. In Corvallis trials, at 2- to 4-weeks after bloom, incidence of fire blight was determined by counting and removing the blighted flower clusters. Number of blighted clusters per tree were divided by total clusters, which were counted before bloom. In Medford, in late August the proportion of the fruit surface with symptoms of russeting was scored with a modified Horsfall-Barratt rating scale.

Data summary and analysis. Measured population sizes for *E. amylovora* were $\log_{10}$-transformed and plotted in graphical arrays. The effect of the treatments on the pH of floral surfaces were plotted similarly. The effect of sprayed treatments on incidence of fire blight and on fruit finish were subjected to analysis of variance (ANOVA).

RESULTS:

Weather in spring 2019: Fire blight risk as determined by the heat unit model, COUGARBLIGHT, was moderate through the bloom periods of pear and apple. Epiphytic pathogen populations built up quickly on inoculated control trees and remained high (> 1 million cells per flower) through bloom. Nonetheless, in pear, fire blight incidence was lower than typical for an inoculated trial because of a light bloom (following heavy bloom in 2018). In apple, weather conditions during and after petal fall were warm and dry, and therefore, all apple trees were misted with water near petal fall to promote infection. The water-controls averaged 15 infections per tree in Bartlett pear (8.5% of total clusters) and 30 infections per tree in Gala apple (15% of total clusters).

Fig. 1



Objective 1:

Infection suppression. In Corvallis, for both pathogen-inoculated trials, all treatments significantly suppressed fire blight ($P \leq 0.05$) compared to the water control except the control treatment of 'citrate (16 oz.) plus dipotassium phosphate (8 oz.)' without the addition of oxytetracycline (Table 1). **In Bartlett pear**, outstanding suppression (79 to 91% relative to the water control) was observed with FireWall (streptomycin), FireLine amended with citric acid or FireLine amended with '½ rate of Buffer Protect'. Without an acidifying amendment, FireLine provided only 58% suppression relative to water control. The addition of citric acid (16 oz.) to Mycoshield enhanced suppression of infection to 65% compared to only 26% without acidification. **In Gala apple**, all FireLine treatments, regardless of amendment, provided significant ($P \leq 0.05$) and outstanding suppression of infection (average 90% suppression) (Table 1). Mycoshield was not evaluated in the apple trial.

Pathogen populations in flowers. **In pear**, measured pathogen population sizes on flowers generally reflected the effectiveness of a treatment for disease control with acidified oxytetracycline treatments resulting in lower populations than the non-acidified oxytetracycline treatments (Fig. 2A). FireLine plus citric acid (16 oz.) had the smallest pathogen populations through the bloom period with populations being undetectable until after petal fall. **In apple**, all FireLine treatments (acidified or not) had measured pathogen population sizes that were 2 to 3 log units less than observed on flowers treated with water or citrate plus dipotassium phosphate only (Fig 2B). And, as observed with disease incidence, the addition of citric acid to FireLine did not enhance suppression of pathogen populations compared to non-acidified FireLine.

Floral pH. **In both pear and apple,** regardless of sampling date, treatments with an acidifying amendment had a floral wash pH that was 0.5 to 1.0 log units lower than the pH measured on flowers treated with water or antibiotic only (Fig. 3A&B). For the acidifying treatments, flowers sampled near petal fall (a day after the second spray application) had lower mean pH recordings (4.8 to 5.3) than flowers sampled at full bloom or at 6-7 days after petal fall.

Table 1. Evaluluation of pH-bufferd oxytetrcycline materials for fire blight control in Bartlett pear and Gala apple, Corvallis, 2019.

Treatment	Rate per 100 gallons water	Date treatment applied* Full bloom	Petal Fall	Percent blighted floral clusters***	Percent blighted floral clusters***
Water **	**-**	**X§**	**X**	**8.5 ab**	**15.0 a**
FireWall 50	2.7 oz.	X	---	0.8 e	1.4 c
Citrate K_2PO4	16 oz. 8 oz.	X	X	16.5 a	8.0 a
FireLine	16 oz.	X	X	3.6 cd	1.5 c
FireLine 1/2 Buffer Protect	16 oz. 75 oz.	X	X	1.8 de	1.8 c
FireLine Citrate	16 oz. 32 oz.	X	X	1.5 de	1.6 c
FireLine LI700	16 oz. 64 fl. oz.	X	X	2.5 cde	1.2 c
FireLine TRIFOL	16 oz. 64 fl. oz.	X	X	2.6 cde	1.6 c
Mycoshield	16 oz.	X	X	6.3 bc	-
Mycoshield 1/2 Buffer Protect	16 oz. 75 oz.	X	X	2.1 de	-
Mycoshield citrate	16 oz. 16 oz.	X	X	3.0 cde	-

* Trees inoculated on 12 (pear) and 24 April (apple) with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain).
** The water-controls averaged 15 infections per tree in Bartlett pear (8.5% of total clusters) and 30 infections per tree in Gala apple (15 % of total clusters).
*** 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 same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference

Objective 2:

Fruit russeting. Application of citric acid with buffering amendments at full bloom and petal fall resulted in negligible effects on fruit russeting for both Bartlett and Comice pear fruit grown near Medford, OR (Fig. 4). In Bartlett, no significant differences ($P > 0.05$) were observed among the treatments. Nonetheless, as in 2018, slightly elevated levels of fruit russeting were observed with treatments with higher amounts of amended buffering material(s): '½-rate of Buffer Protect', 'citrate (32 oz.) plus dipotassium phosphate (16 oz.)', and 'citrate (16 oz.) plus dipotassium phosphate (8 oz.)'. Similarly, in Comice pear, no significant differences ($P > 0.05$) in fruit rusting were observed among the treatments. In both trials, the mean russeting observed on fruit treated with citric acid only was slightly less than observed on the water-treated control.

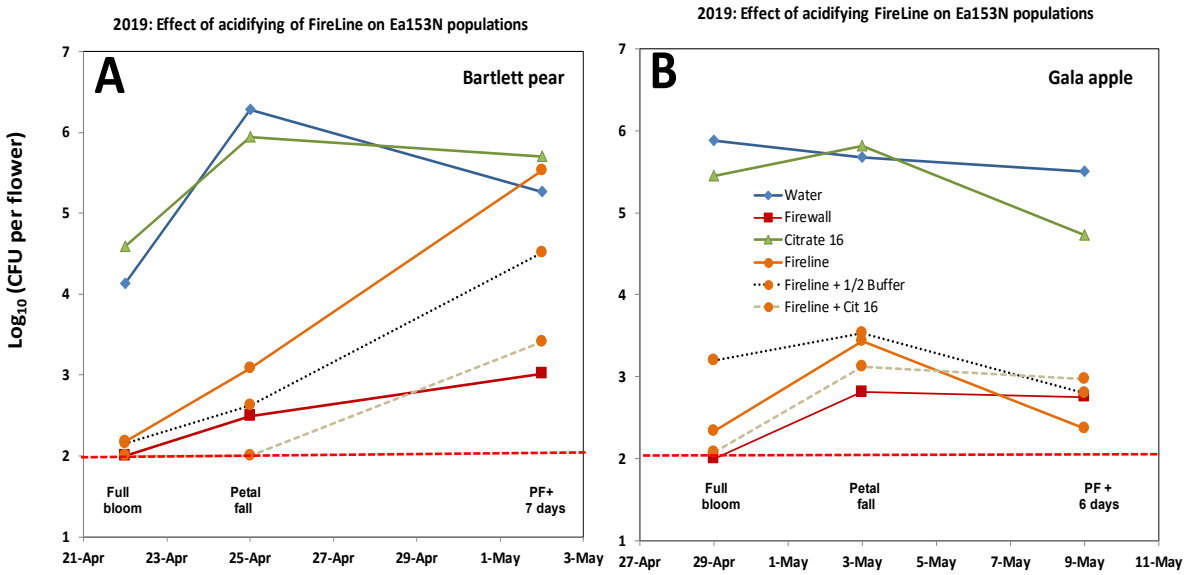


Fig 2. Effect of treatments applied to A) Bartlett pear and B) Gala apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers during April and May 2019. Pathogen populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree, and plating the wash onto a selective culture medium. Data depict mean of each treatment program on each sampling date. Note: Y-axis is log scale: a value of '2.0' is 100 pathogen cells/flower (the detection limit) and a value of '6.0' is one million cells per flower.

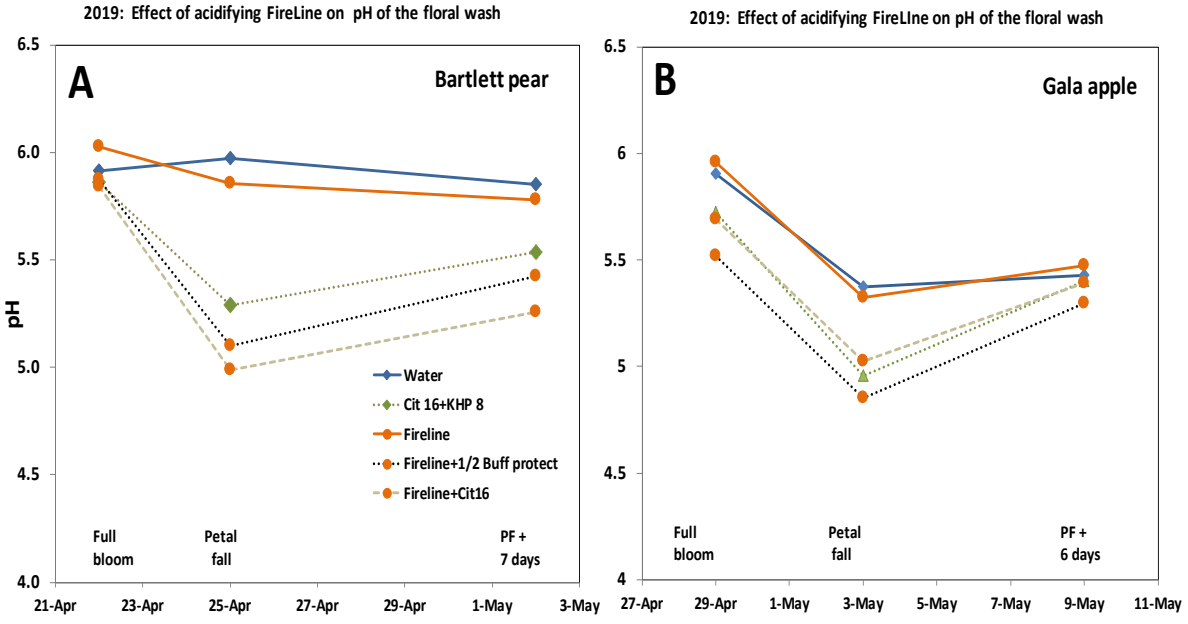


Fig. 3. Effect of treatments applied to A) Bartlett pear and B) Gala apple trees to suppress fire blight on the pH of floral surfaces during April and May 2019. A hand-held pH-probe was submerged in a deionized-water wash of five flower clusters (~25 flowers, bulked in 25 ml of water) from each replicate tree. Data depict mean of each treatment program on each sampling date.

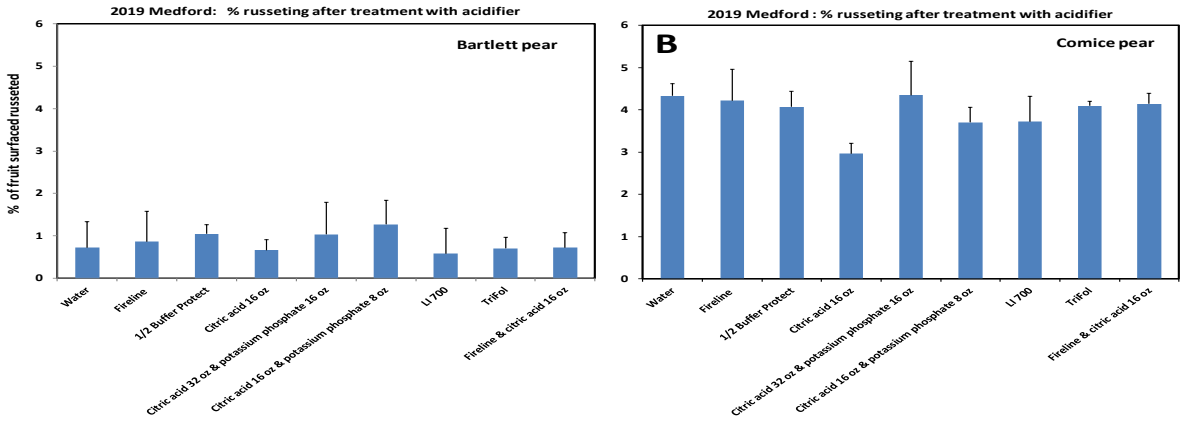


Fig. 4. Effect of citric acid and buffering amendments applied to A) Bartlett and B) Comice pear trees on severity of fruit russeting (%) in orchards located near Medford, OR, 2019. Treatments were applied at full bloom and at petal fall (April). In late August, 30 fruit from each replicate tree were rated for russeting severity. Data depict mean and standard error from four replicate trees that received each treatment. No significant differences were observed in either trial based on analysis of variance (*P*-values on F-statistics ranged from 0.57 to 0.74).

Objective 3:

Comparison of FireLine and Mycoshield. Over the first 3 years of this study, the effectiveness of both FireLine (17% oxytetracycline-hydrochloride, 17% a.i.) and Mycoshield (oxytetracycline-calcium complex, 17% a.i.) have been enhanced by lowering the pH of the spray solution (Fig. 5). Without acidification, FireLine (applied full bloom and petal fall) reduced incidence of blight by 79%; acidification enhanced suppression to 88%. Without acidification, Mycoshield has been less effective (42%) than FireLine but with an acidifying amendment, suppression was increased to 74 to 80%. Similarly, acidification of both FireLine and Mycoshield has enhanced their ability to suppress pathogen populations on floral surfaces (Fig. 6). During the critical period from full bloom to petal, suppression by Mycoshield acidified with citric acid was 1 log units (~90%) better than Mycoshield alone (Fig. 6). The same comparison for acidified FireLine shows that suppression was enhanced by 0.3 log units (~50%) relative to the non-acidified FireLine treatment.

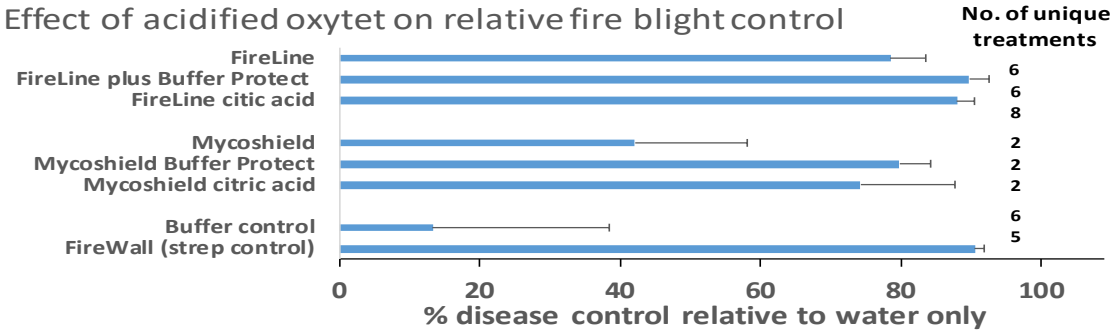
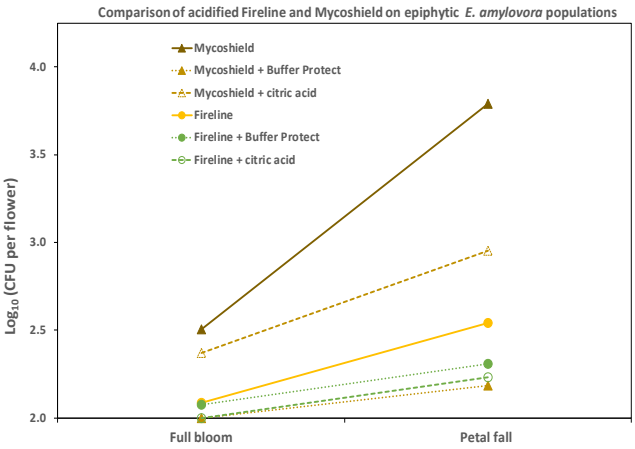


Fig. 5. Effect of acidified oxytetracycline materials for fire blight control in Bartlett pear and Gala apple expressed as % disease control relative to trees treated with water only. Data are from six orchard trials conducted from 2017 to 2019 and depict the mean and standard error from the number of unique treatments within each material subheading on the y-axis. Each unique treatment was replicated on four trees in each trial. For the 'FireLine citric acid' and Mycoshield citric acid' treatments, the amount of citric acid in the unique treatments ranged among trials from 0.9 to 2.4 g/liter and amount of Na2PO4 ranged between 0.0 and 1.2 g/liter. Rates other materials were held constant among trials.

Fig. 6. Effect of acidified oxytetracycline materials on epiphytic populations of *Erwinia amylovora* on Bartlett Pear flowers from experimental orchards near Corvallis, OR in 2018 and 2019. In each trial, each treatment was applied to four replicate trees. Pathogen populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree, and plating the wash onto a selective culture medium Y-axis is log scale: a value of '2.0' is 100 pathogen cells/flower (the detection limit) and a value of '6.0' is one



Discussion

Inhibiting growth of the fire blight pathogen on pome flowers has the immediate benefits of reducing floral infection and the longer-term benefit of reducing the amount of epiphytic inoculum carried into later phases of infection (e.g., rattail flowers and shoot blight). In enhancing these benefits, acidified oxytetracycline likely has an improved the stability in the spray tank and/or an increased effective residual period on floral surfaces. In water at pH 7 (22ºC), oxytetracycline has a half-life of 41 hours; the half-life is increased fourfold (171 hours) at pH 5. In addition to direct effects on oxytetracycline, more acidic conditions negatively affect the fire blight pathogen. The bacterium cannot grow below pH 5.0 and at pH 5.5, its potential growth rate is halved compared to pH 7.0.

With regard to fruit russeting caused by acidifying amendments, in 2 years of evaluation on moderately-sensitive Bartlett pear and highly-sensitive Comice pear, citric acid alone (in spite of its low pH) had no effect on fruit finish. In fact, in 4 of 4 trials, citric acid alone resulted in fruit finishes that were slightly better than water alone. In contrast, in most trials, treatments with citric acid plus a buffer (Buffer Protect, Na_2PO_4 K_2PO_4) to raise the pH of the spray suspension were correlated with slightly increased fruit russeting. Overall, the effects on fruit finish from any of the citric acid treatments (with or without the buffering amendment) was very small compared to other non-antibiotic materials we trialed for russeting potential (see 2020 WTFRC apple crop protection report).

Table 2.

Antibiotic	Rate of acidifying ammendment/100 gal	pH in well water
Water	-	7.78
-	1/2 Buffer Protect (75 oz.)	3.77
-	citric acid (32 oz) plus Na_2PO_4 (16 oz.)	3.48
-	citric acid (16 oz) plus Na_2PO_4 (8 oz.)	3.72
-	citric acid (12 oz) plus Na_2PO_4 (12 oz.)	4.55
-	citric acid (16 oz)	2.91
FireLine	-	6.83
FireLine	1/2 Buffer Protect (75 oz.)	3.39
FireLine	citric acid (16 oz)	3.22
Mycoshield	-	6.37
Mycoshield	1/2 Buffer Protect (75 oz.)	3.64
Mycoshield	citric acid (16 oz)	3.48

In the spring 2020 no-cost extension, we intend to address several additional concerns with using citric acid to acidify oxytetracycline without a buffering amendment. Specifically, we will evaluate different rates of citric acid mixed with the label rate of oxytetracycline. In addition, we collect data on other antibiotics (Kasumin and FireWall) amended with citric acid. In a previous trial (pear 2017), fire blight suppression by Kasumin acidified with a half rate of Buffer Protect was improved compared to Kasumin alone. If additional trees are available in the trial orchards, we will repeat treatments of FireLine amended with commercial acidifying adjuvants, LI700 and TRIFOL.

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

Project Title: Field evaluation of pear cultivars on cold hardy quince rootstocks

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Cooperators: Sara Serra, Kristal Dowell, Mike McCarthy, Dale Goldy

Total Project Request: **Year 1:** $58,110 **Year 2:** $70,585 **Year 3: $84,421**

Other funding sources
None.

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: Russell Karow**
Telephone: 541 737-4866 **Email address: Russell.Karow@oregonstate.edu**

Item	2018	2019	2020
Salaries	5,536	5,702	8,810
Benefits[1]	4,464	4,598	7,104
Wages[2]	1,300	1,300	1,300
Benefits	130	130	130
Equipment			
Supplies	0	5,500	7,500
Travel[3]	3,316	3,316	3,316
Miscellaneous			
Plot Fees[4]	5,000	5,000	5,000
Total	19,746	25,546	**33,160**

Footnotes:
[1] Benefits were calculated from actual OPE rates (OSU technician). An annual increase of 3% was applied to years 2 and 3.
[2] Wages are for part-time employee to help with general maintenance during the season; 100 hours at $13/hr. Part-time employee benefits are calculated at 10%.
[3] Travel is to cover mileage to plot for measurements and one trip per year (4 days) for Einhorn to travel to plots to perform pruning and training tasks and meet with S. Musacchi and grower collaborators (airfare was estimated at $1,000 roundtrip, four nights hotel ($150/night), car rental ($500) and per diem ($54/day).
[4] Plot fees are to compensate growers for land, resources and fruit.

Budget 2 (Musacchi)
Organization Name: WSU-TFREC **Contract Administrator: Shelli Tompkins**
Telephone: 509-293-8803 **Email address:** shelli.tompkins@wsu.edu

Item	2018	2019	2020
Salaries	21,000	21,840	22,714
Benefits[1]	8,364	8,699	9,047
Wages			
Benefits			
Equipment			
Supplies	0	5,500	10,500
Travel[2]	4,000	4,000	4,000
Plot Fees[3]	5,000	5,000	5,000
Miscellaneous			
Total	38,364	45,039	**51,261**

Footnotes:
[1] Benefits were calculated from actual WSU rates. An annual increase of 3% was applied to years 2 and 3.
[2] Travel is to cover mileage to plot for measurements to travel to plots to perform data collection
[3] Plot fees are to compensate growers for land, resources and fruit.

Objectives:
Evaluate vegetative and fruiting performance of Bartlett and d'Anjou pear trees on promising, cold-hardy quince rootstocks.

Significant Findings:

- Tree survival varied by rootstock and whether or not an interstem was used. At the OR site, only 3 of 31 combinations had mortality rates greater than 20% and 13 (approx. 1/3rd) of the combinations had 0% mortality. At the WA site, survivability was higher; only 3 of 24 combinations tested had mortality rates exceeding 20% and 66% of the tested combinations had 0% mortality. Greater mortality in OR is partly due to the site having more direct grafted Bartlett trees.
- Sources of mortality will require additional years to determine. Tree mortality was not always associated with a given scion (i.e., incompatibility).
- Several quince rootstocks had a significant portion of trees struggling to grow in 2019 based on poor shoot extension or <10% increase in trunk growth over the 2019 growing season.
- There is a wide range of variation in trunk size across genotypes (a good overall indication of canopy size and vigor). Genotype and/or combination effects are beginning to show similarly at both sites. The range in trunk size is similar from the weakest to most vigorous combinations and these generally agree between sites.
- Pruning weights were recorded at both sites in late winter 2019. Canopies are developing nicely. Tree height exceeded 8' in 80% of the combinations, with the tallest combinations reaching ~10 ft. Approximately 10% of the combinations only attained 5-7' in height, likely due to incompatibility as these were typically overly weak trees.
- At WA, average number of shoots per tree, average shoot length and distribution of shoots by length varied widely according to combination. Total annual growth per tree was between 7.5 to 29.2 m, depending on the combination. Generally, trees with more shoot growth and canopy volume had larger trunks. Average number of shoots ranged between 40 and 90 per tree with average shoot lengths between 20 and 30 cm (8-12 inches).
- Relatively insignificant flowering was observed in 2019; however, secondary bloom (~2 clusters per Bartlett and 0.2 clusters per Anjou tree) was observed. Flowering and limited fruit production (after thinning) is expected for 2020.

Methods:
Planting design: For each of the rootstock/scion combinations, trees were divided evenly between the two planting sites, irrespective of differences in the quantity of trees among rootstock/scion combinations, with the exception of a few direct-grafted combinations (mainly Bartlett) that were of insufficient number to replicate at both sites. In those cases, trees were planted in OR only. We selected three multi-tree replications per treatment. More replicates would have increased our statistical power to detect treatment differences, however, we were compelled to have a minimum of four trees per replicate to eliminate confounding effects on vigor from neighboring trees of different rootstocks and, hence, varying growth habit. We will generally only measure the center two trees when replicates are limited to four trees; in this case, the outer trees will be treated as guard trees. For the remaining 2/3rd of rootstock/scion combinations, there is a range of 6 to 15 trees per replicate. For these replicates, only the first and last tree will be excluded from data collection. For some measurements, a subset of uniform trees will be used to collect data. Both plots are equipped with trellis and supplemental irrigation. The irrigation and fertilization regime considers quince's relatively high demand for nutrients (especially N). All other inputs (pesticides, herbicides, frost protection, etc.) will be provided according to commercial standards.
Training system: Trees are being trained to a central leader, spindle architecture. Branches will be initiated using a combination of horticultural techniques (heading of leaders, girdling, and scoring

above individual buds). 2017 tree survival was high; however, variability in tree size and branch number within a given rootstock/scion combination was also high. Therefore, 2017 was regarded as an establishment year, develop root systems and determine the appropriate training strategy for 2018. We decided to head trees to encourage uniform canopy development. In cases where one or more branches had developed in 2017, limbs were removed with a bevel cut to encourage a horizontal renewal limb. Restarting trees will not eliminate tree-to-tree variability, but it will increase uniformity with respect to the number fruiting units. Newly emerged limbs considered too upright or vigorous were removed using a bevel cut in 2018 and 2019. Limb positioning (i.e., tying) will be performed if necessary, especially spreading in the case of d'Anjou.

Results and Discussion:

Table 1: Number of trees established (2017), trees alive after the 2018 and 2019 growing seasons, total percent mortality, and percentage trees struggling after the 2019 season in Parkdale, OR for Anjou and Bartlett grafted on 9 quince accessions (table sorted by cv and CYD acc. #). Type of graft was either a direct graft (no interstem between scion and roots) or with an interstem of Comice.

Cv	Quince rootstock (CYD accession)	Type of graft	Count of trees planted 2017	Count of alive trees Jan 2019	Count of alive trees Jan 2020	%Mortality	% Trees Stuggling in Jan 2020
ANJOU	22.001	Comice	22	22	22	0	0
		Direct graft	22	22	22	0	38
	23.001	Comice	12	11	11	8	8
		Direct graft	10	10	10	0	33
	57.001	Comice	17	15	14	18	0
		Direct graft	10	10	10	0	33
	65.001	Comice	20	20	20	0	10
		Direct graft	13	12	12	8	57
	67.001	Comice	12	12	12	0	0
	68.002	Comice	15	14	10	33	20
	70.001	Comice	42	42	42	0	0
		Direct graft	10	9	9	10	0
	99.002	Comice	56	55	54	4	29
		Direct graft	12	11	11	8	0
	118.001	Comice	11	10	10	9	0
		Direct graft	10	7	7	30	0
BARTLET	22.001	Comice	24	24	24	0	4
		Direct graft	15	15	15	0	12
	23.001	Comice	12	12	12	0	0
	57.001	Comice	16	16	16	0	0
		Direct graft	14	13	13	7	0
	65.001	Comice	19	19	19	0	0
		Direct graft	11	9	9	18	0
	67.001	Comice	14	13	13	7	0
	68.002	Comice	16	15	15	6	10
	70.001	Comice	43	41	41	5	15
		Direct graft	16	15	14	13	12
	99.002	Comice	57	37	34	40	14
		Direct graft	12	12	12	0	0
	118.001	Comice	48	41	39	19	33
		Direct graft	12	11	11	8	8

Table 2: Number of trees planted, trees alive or dead in the time periods (17 and 29 months) between planting (06/06/2017) in Entiat (WA) and the last assessment (11/12/2019), and percentage of trees struggling for the 2 cultivars Anjou and Bartlett grafted on 9 different quince accessions (table sorted by cv and CYD acc. #). Type of graft was or direct graft (no interstem between scion and roots) or with interstem of Comice cv.

Cv	Quince rootstock	graft type	Count of tree planted (06/06/2017)	Count of tree alive (11/12/19)	Count of tree dead (11/12/19)	Count of tree alive but struggling (11/12/19)	% failure in 17M	% failure in 29 M (Nov 2019)	% tree struggling on Nov 2019
Anjou	22.001	comice interstem	22	22			0.0	0.0	0.0
Anjou		direct graft	20	20			0.0	0.0	0.0
Anjou	23.001	comice interstem	12	12			0.0	0.0	0.0
Anjou	57.001	comice interstem	17	17			0.0	0.0	0.0
Anjou		direct graft	11	10	1		9.1	9.1	0.0
Anjou	65.001	comice interstem	17	17			0.0	0.0	0.0
Anjou	67.001	comice interstem	12	12			0.0	0.0	0.0
Anjou	68.002	comice interstem	14	9	5	1	0.0	35.7	7.1
Anjou	70.001	comice interstem	39	33	6		12.8	15.4	0.0
Anjou		direct graft	13	12	1		7.7	7.7	0.0
Anjou	99.002	comice interstem	42	42		3	0.0	0.0	7.1
Anjou		direct graft	12	2	10		66.7	83.3	0.0
Anjou	118.001	comice interstem	11	10	1		9.1	9.1	0.0
Anjou		direct graft	10	10			0.0	0.0	0.0
Bartlett	22.001	comice interstem	24	24		5	0.0	0.0	20.8
Bartlett	23.001	comice interstem	12	12			0.0	0.0	0.0
Bartlett	57.001	comice interstem	15	15			0.0	0.0	0.0
Bartlett	65.001	comice interstem	17	17			0.0	0.0	0.0
Bartlett	67.001	comice interstem	13	13			0.0	0.0	0.0
Bartlett	68.002	comice interstem	16	12	4	2	25.0	25.0	12.5
Bartlett	70.001	comice interstem	29	29		2	0.0	0.0	6.9
Bartlett	99.002	comice interstem	54	49	5	6	9.3	9.3	11.1
Bartlett		direct graft	10	10			0.0	0.0	0.0
Bartlett	118.001	comice interstem	35	35		11	0.0	0.0	31.4
		total	**477**	**444**	**33**	**30**	**5.2**	**6.9**	**6.3**

- Tables 1 and 2 illustrate the issues of some rootstocks and/or combinations as well as environmental factors on tree mortality. Observed failure for many of these combinations occurred even in the presence of an interstem, indicating that, at least for some quince rootstocks, Comice may be incompatible. Comice is generally regarded to have good compatibility with quince, along with Old Home and Beurre Hardy. Anjou/Comice 68.002 had the second highest mortality rate at both sites. However, Anjou/99.002 (direct graft) had the highest incidence of tree failure at WA (83.3%) but only 8% at OR. This illustrates differences in mortality rates for a given combination between sites. Bartlett/99.002 with Comice interstem had 40% and 9% mortality at OR and WA, respectively. However, Bartlett/99.002 direct grafts had 0% mortality at both sites. Generally, there was less mortality at WA, likely due to gopher predation during the establishment year at OR and the inclusion of a few direct-grafted accessions not at WA due to limited tree numbers. Despite these mortality rates, many accessions had 0% mortality (1/3rd and 2/3rd of all combinations at OR and WA, respectively). We also assessed trees struggling to grow during the 2019 season. In OR, most, but not all, of trees that struggled were direct grafts. These trees had <10% increase in trunk size in 2019. Most issues were observed with Anjou. Percentage of struggling trees was much lower in WA.

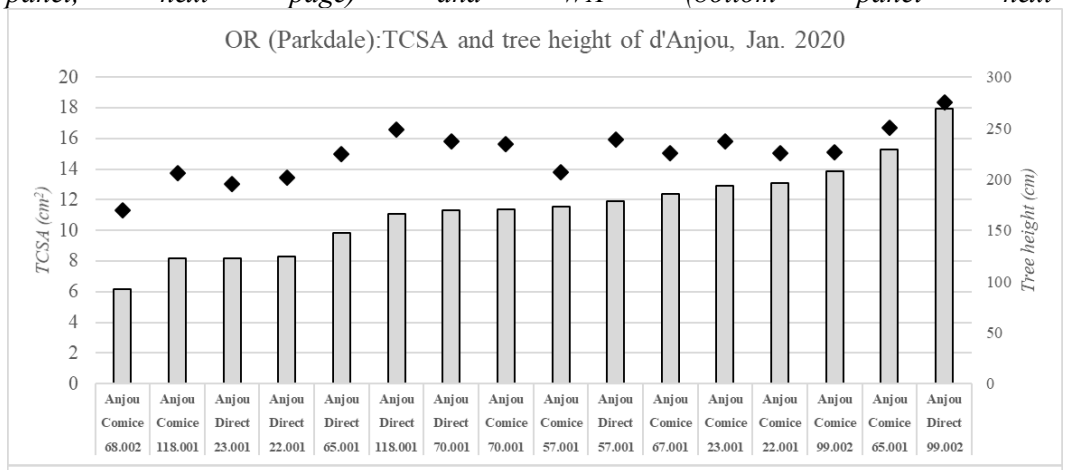
Table 3: The number of suckers per tree and weight of 2019 winter pruning material (kg/tree) prior to the 2019 growing season in Entiat (WA) and Parkdale (OR) for Anjou and Bartlett grafted on 9 different quince accessions (table sorted by cv and CYD acc. #). N/A indicates no data due to limited number of trees for establishment in WA.

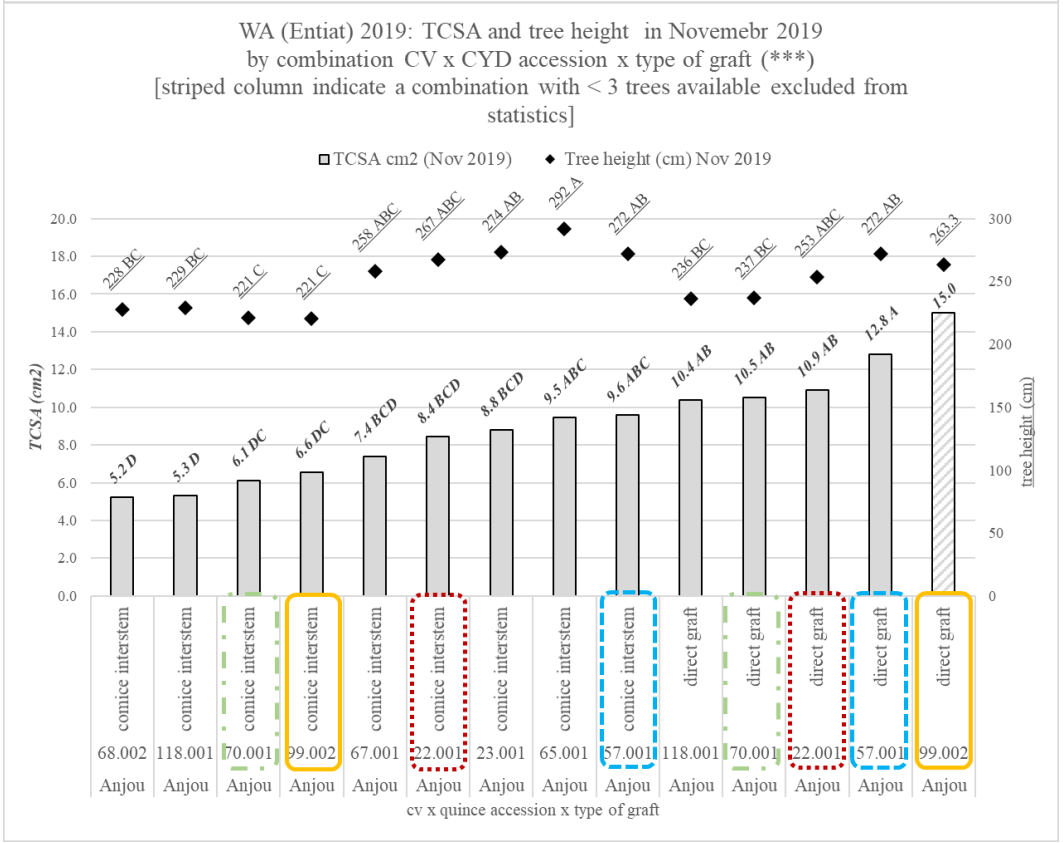
Scion	Accession	Interstem	2019 Suckers (no./tree) OR	2019 Suckers (no./tree) WA	2019 Pruning weights (kg/tree) OR	2019 Pruning weights (kg/tree) WA
Anjou	22.001	Comice	0.13 b	0 b	0.27	0.34
	22.001	None	0 b	0.1 b	0.25	0.22
	23.001	Comice	0.42 b	0 b	0.2	0.44
	23.001	None	0.33 b	N/A	0.22	N/A
	57.001	Comice	0.27 b	0 b	0.26	0.39
	57.001	None	0.08 b	0.6 b	0.16	0.42
	65.001	Comice	0.65 b	0.1 b	0.34	0.42
	65.001	None	0.27 b	N/A	0.18	N/A
	67.001	Comice	1.67 a	4.9 a	0.48	0.27
	68.002	Comice	0 b	2 b	0.29	0.11
	70.001	Comice	1.19 ab	0.9 b	0.39	0.21
	70.001	None	0.31 b	4.7 a	0.37	0.17
	99.002	Comice	0.85 ab	1.3 b	0.29	0.28
	99.002	None	0.17 b	0 b	0.19	0.24
	118.001	Comice	1.22 ab	0 b	0.28	0.19
	118.001	None	0.5 b	0.8 b	0.22	0.28
Bartlett	22.001	Comice	0.13 b	0.3 b	0.13	0.58
	22.001	None	0.27 b	N/A	0.04	N/A
	23.001	Comice	0.25 b	0 b	0.08	0.57
	57.001	Comice	0.13 b	0.2 b	0.15	0.58
	57.001	None	0.93 ab	N/A	0.2	N/A
	65.001	Comice	0.22 b	0.2 b	0.26	0.68
	65.001	None	0.78 b	N/A	0.15	N/A
	67.001	Comice	0.91 ab	4.2 b	0.26	0.32
	68.002	Comice	0.2 b	4.7 b	0.38	0.19
	70.001	Comice	0.2 b	2.7 b	0.45	0.37
	70.001	None	1.03 ab	N/A	0.63	N/A
	99.002	Comice	0.59 b	2.6 b	0.02	0.42
	99.002	None	1.58 a	10.2 a	0.48	0.51
	118.001	Comice	1.8 a	0.8 b	0.13	0.18
	118.001	None	1.83 a	N/A	0.19	N/A

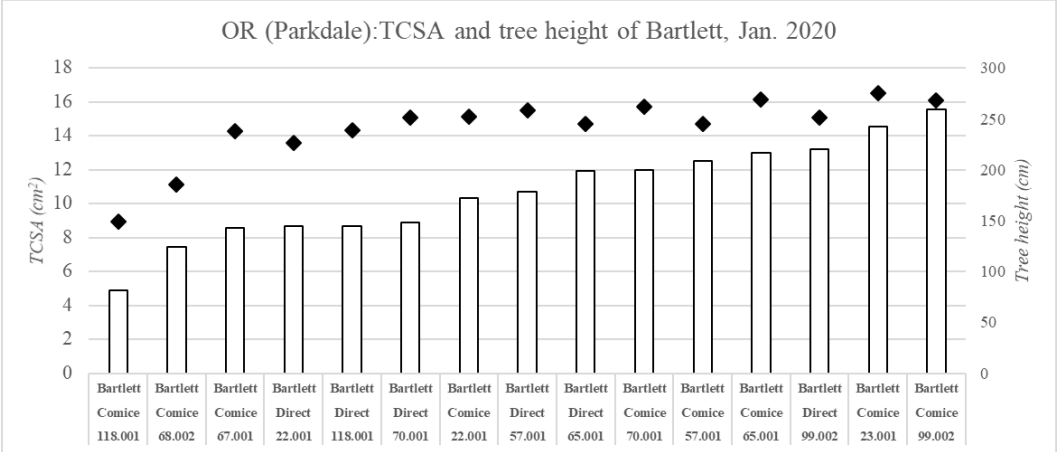
- Suckering was observed in nearly all combinations, but to a relatively low level (only ~25% of all combination in OR had between 1 and 2 suckers per tree while ~75% had <1 per tree). Higher levels of suckering occurred in WA with some accessions producing 4 to 10 suckers per tree. Still, roughly 70% of combinations had <1 sucker/tree. Pruning weights were calculated as the total weight of wood removed per replicate, then divided by the number of trees in the replicate to account for differences in tree numbers. Pruning did not remove a large amount of wood, per se, however, renewal cuts were quite successful in generating new branches, and click pruning (in the case of Barlett), produced the desired response. Differences in pruning weight followed vigor distribution of combinations (see Fig. 1). The average number of shoots per tree, average shoot length, and the distribution of shoots according to length were quantified in WA, varying considerably by combination. The average total annual growth per tree was between 7.5 to 29.2 m, depending on the combination. Generally, trees with more shoot growth and canopy volume had larger trunks. The average number of shoots per tree ranged between 40 and 90 with an average shoot length of 20 to 30 cm (8-12 inches). These

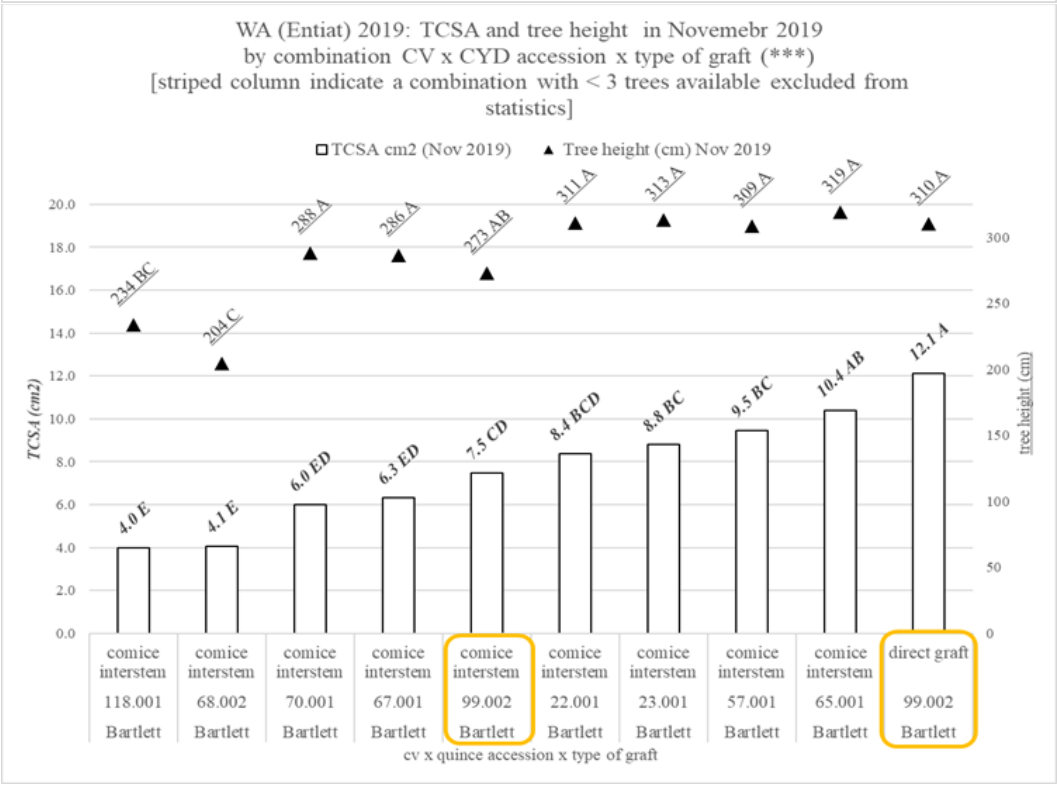
data will be collected in OR (March 2020).

Figure 1: Effect of quince rootstock selections on trunk cross sectional area and tree height of d'Anjou at the end of the 2019 growing season in OR (top) and WA (second panel) and Bartlett in OR (third panel, next page) and WA (bottom panel next page).









- A roughly 3-fold difference in tree size was observed across the combinations at both sites, irrespective of cultivar. The same rootstocks produced the weakest Anjou and Bartlett trees at both sites (a level of dwarfing deemed too strong). Similarly, the most vigorous combinations were the same. Tree height exceeded 8' for 80% of the combinations, with the tallest combinations reaching ~10 ft. 2020 will represent the first fruiting year.

Expected activities <u>Fall/Winter 2019 and 2020 season:</u>

- Limb count and distribution (OR only, WA already completed this task).
- Dormant pruning to encourage uniform branching and develop canopies.
- Assess bloom and fruit set (hand thin according to canopy size), harvest and evaluate yield and fruit quality.
- Measure trunks, tree height and qualify canopies.

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

Project Title: Evaluating dwarfing capacity of 65 diverse pear germplasm accessions

PI:	Amit Dhingra	C**o-PI**:	Kate Evans
Organization:	Washington State University	**Organization**:	Washington State University
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Address:	PO Box 616414	**Address**:	1100 N. Western Ave.
City/State/Zip:	Pullman WA 99164-6414	**City/State/Zip**:	Wenatchee WA 98801

Cooperators: David Neale, UC Davis; Joseph Postman, USDA-ARS Corvallis pear germplasm repository; Rick Sharpe, WSU Pullman and Soon Li Teh, WSU TFREC

Budget: Year 1: $40,081 **Year 2**: **$40,323** Year 3: $40,116

Agency Name: USDA SCRI Preapplication
Amount Pending: $2,800,000 (2020-2024)
Notes: "Phenotypic and Genomic Characterization of Pyrus Germplasm for Development of Dwarfing Rootstocks for Sustainable Pear Production in the USA" (PI Dhingra, Co- PI Evans). Synergistic project to characterize diverse set of Pyrus germplasm via large scale phenotyping and genotyping.

Agency Name: PNW Pear Bureau
Amount Pending: $322,003 (2019 – 2022)
Notes: "Pear Rootstock Breeding" (PI: Evans; Co-PI: Dhingra, Co-PI: Soon Li Teh)
Synergistic project to develop and establish pear rootstock seedlings to develop dwarfing rootstocks that are suited for high-density pear production.

Agency Name: PNW Pear Bureau
Amount Awarded: $34,133 (2017 – 2019)
Notes: "Greenhouse screening of 49 dwarf rootstock candidates" (PI: Dhingra; Co-PI: Evans)
Synergistic project to evaluate the dwarfing potential of aneuploid pear rootstock seedlings.

Budget
Organization Name: Washington State Univ **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2019	**2020**	2021
Salaries[1]	22,909	23,825	24,778
Benefits	8,172	8,498	8,838
Supplies[2]	5,000	4,000	2,500
Travel	1,000	1,000	1,000
Plot Fees[3]	3,000	3,000	3,000
Total	40,081	**40,323**	40,116

Footnotes:
1 – Support for technical help to multiply rootstock selections, graft with scions and manage plants
2 – Greenhouse soil and supplies, tissue culture consumables, vessels, chemicals and supplies, grafting supplies
3 – Greenhouse space usage fee per year

OBJECTIVES

1. Complete the initiation, multiplication and rooting of the remaining germplasm accessions in tissue culture and greenhouse.

At the beginning of this project, only 11 of the 65 accessions remained to be established in tissue culture. Having worked with majority of the accessions, we have various versions of pear-specific media and growing conditions identified to introduce the remaining accessions in tissue culture.

2. Graft 5 clones from each of the accession with scion wood from 'Bartlett' and 'Anjou'. Use 'OH×F 87' as a control.

'Bartlett', 'Anjou' and 'OH×F 87' plant material is already available in the program. The material that already exists in tissue culture will be multiplied and transferred to the greenhouse, grown and prepared for grafting. After the grafting, plants will be grown and maintained at the WSU greenhouse to record scion growth and habit. To assess if the dwarfing trait is transmitted to the scion, data will be recorded for trunk cross-sectional area, internode length, height, ratio between the two, crotch angle and these will be compared to the data from 'OH×F 87'. Accessions that impart dwarfing to the scions will be retained and used as parental material and if feasible also directly evaluated as rootstock in future field trials.

SIGNIFICANT FINDINGS

- Majority of the 65 accessions are well established in vitro as well as in the greenhouse.
- Of the 11 accessions that were reinitiated in vitro, only two survived. The others had too much pathogen load.
- A very small number of accessions that were already established in vitro, were overcome by endophytes and died.

METHODS

Objective 1. Complete the initiation, multiplication and rooting of the remaining germplasm accessions in tissue culture and greenhouse.

In order to enable greenhouse screening of all accessions, 11 of the 65 accessions remain to be established in tissue culture. The remaining 54 selections have been established in tissue culture as a source of developmentally and physiologically uniform, clean and genetically true to type plant material. These diverse accessions have already been genotyped using the Pear SNParray produced as part of a collaborative project with UC Davis - PI: Neale, Co-PI Dhingra, "Development of marker-based breeding technologies"; PR-14-111.

As done previously for all accessions, dormant and actively growing plant material will be collected from the pear germplasm repository at USDA Corvallis. Axially buds from dormant or actively growing plant material will be surface sterilized with bleach and washed with autoclaved water prior to being initiated on to the basic pear initiation media standardized in the Dhingra lab. Once the buds have swollen and elongated into an initial shoot, the nodes would be excised and placed onto the pear bud multiplication media. Usually, a 3-4x rate of multiplication, obtained via suckering and elongation, per 4-5 weeks is achieved routinely in the lab for standard genotypes. Given the genetic variability of the material being used, it is expected that the media may need to be standardized for some of the genotypes to achieve optimal growth and multiplication.

The goal would be to have a minimum of 50 plantlets per accession established in tissue culture. This will provide a good and constant source of plant material for subsequent steps. For this experiment, 25

plantlets will be moved from bud multiplication media to rooting media. The rooted plantlets will be moved to the greenhouse, acclimatized and grown to a height of 24-48 inches in the greenhouse to achieve a minimum caliper of 1/4th inches. Thereafter the rootstock plants will be forced into dormancy and maintained at 42-degree Fahrenheit till they are ready to be budded. Along with the 65 germplasm selections, the current industry standard rootstock 'OH×F 87' will also be processed in a similar way and will be used as a reference material in the experiment. Therefore, there will be a total of 65 selections each with 50 plants each in tissue culture which totals to 3250 plants. In the greenhouse, 1650 rootstocks (66 accessions plus control x 25 plants each) will be prepared for objective 2.

The potential limitations to the goals of this objective can be the heavy bacterial and fungal infestation in plant material derived from the germplasm repository. As an alternative approach, we will obtain plant material for the 11 accessions and establish it in greenhouse at WSU. This will serve as an alternative source of cleaner plant material for initiation in the tissue culture.

Objective 2. Graft 5 clones from each of the accessions with scion wood from 'Bartlett' and 'Anjou'. Use 'OH×F 87' as a control.

Virus and disease free, genetically true to type 'Bartlett' and 'Anjou' budwood will be used to perform chip budding of 10 clones for each of the 66 accessions (65 diverse accessions plus control 'OH×F 87'). Once the buds have callused and swollen, 5 plants of each selection per scion will be maintained in the greenhouse for phenotyping of the habit imparted to the scions. The budded plants will be screened for number of nodes produced and height of the plant achieved over a set period of time till the plants go into paradormancy. Thereafter the plants will be provided with 1200 hours of chilling and placed back in the greenhouse to initiate another spurt of growth. This aspect will be repeated for 2-3 cycles to identify the potential accessions that transmit the dwarf trait to the scion. The desirable accessions will then be selected for field-based evaluations in future projects.

The potential limitations to the goals of this objective could be issues with chip budding or due to incompatibility with the scions being used. Chip budding of 10 clones should address any issues with the final number needed. If incompatibility is observed, that will be useful information for future work with the accessions.

RESULTS AND DISCUSSION

Objective 1. Complete the initiation, multiplication and rooting of the remaining germplasm accessions in tissue culture and greenhouse.

As of the submission of this report on January 30th, majority of the accessions continue to thrive in vitro. However, as it was identified earlier the heavy bacterial and fungal infestation in plant material derived from the germplasm repository can be a potential limitation for the accomplishment of this objective. In 2019, the remaining 11 accessions were introduced into micropropagation. Only two accessions continue to grow. In addition, 6 accessions that had been previously established in the micropropagation system succumbed to endophytic infestation. Some of the source material representing these difficult accessions is available as rooted material in the greenhouse and will serve as a source of cleaner material for reintroduction into the micropropagation process in 2020. In addition, another round of plant material collection from USDA Corvallis Pear Germplasm Repository will be undertaken this spring.

A summary of status of each accession in terms of number of plants in the micropropagation process, soil or in the cold is presented in Table 1.

Table 1: Status and number of clones available for 65 accessions representing a diverse set of Pyrus spp.

	TC only (5)		All stages (40)			
	Soil only (3)	77 deg F	Plant in vitro			
	Dead (17)	39 deg F	In vitro Plants in the cold			
Location of Plant Material						
USDA Corvallis			**Dhingra Lab at WSU**			
Row	**Position**		**Sample #**	**In 77 deg F**	**In Soil**	**In 39 deg F**
23	1		1			
23	15		2	Y	17	N
23	14		3	Y	10	N
24	11		4	N	12	Y
25	8		5	N	18	Y
28	9		6	N	16	Y
30	4		7	Y	N	N
31	16		8			
32	14		9	Y	N	N
33	4		10	N	15	N
34	2		11	N	N	Y
34	7		12			
52	1		13	N	16	Y
1	17		14	Y	10	N
1	21		15	N	31	Y
2	3		16	Y	7	N
2	23		17	Y	15	N
2	27		18	N	13	Y
3	15		19	Y	4	N
3	25		20			
4	19		21	Y	N	N
4	21		22	N	15	Y
4	45		23	N	N	Y
4	49		24			
5	11		25	Y	20	N

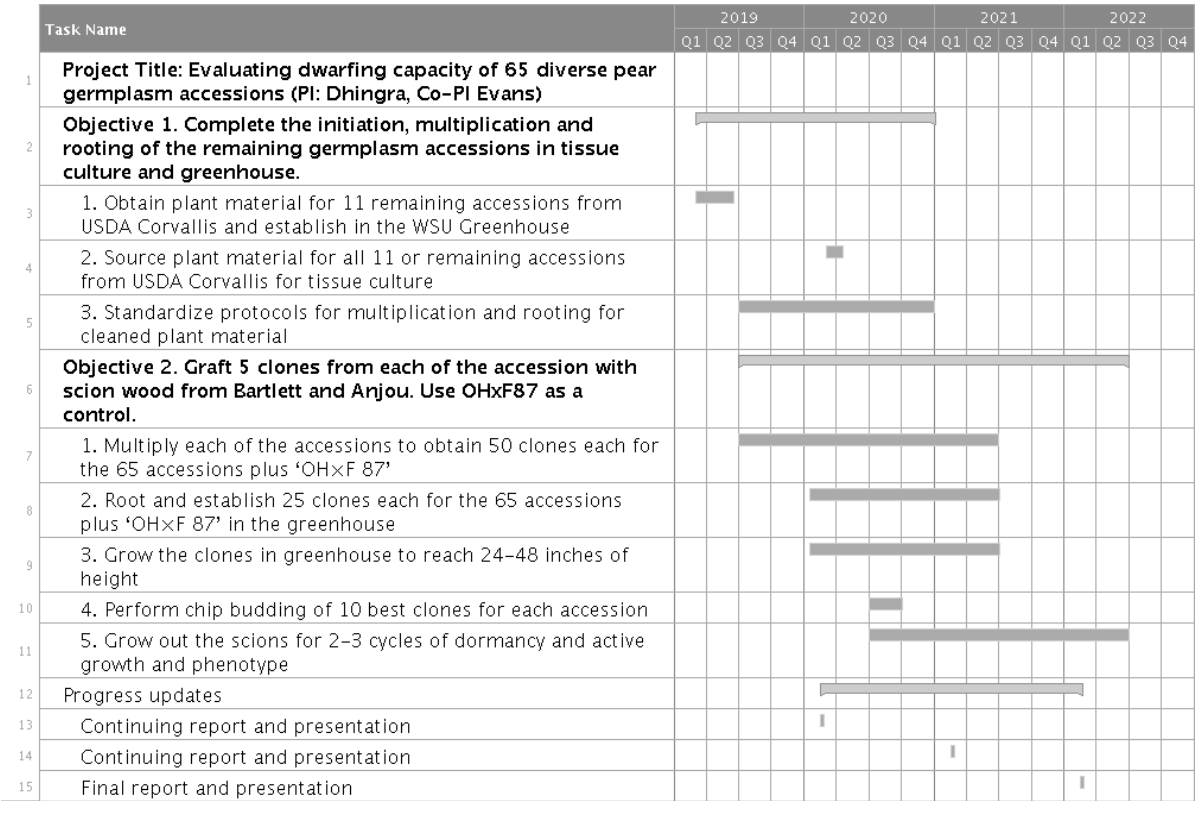
5	21		26			
6	45		27	N	10	Y
8	23		28	Y	5	N
8	25		29	N	8	Y
10	13		30	N	14	N
12	25		31	N	1	Y
12	41		32			
14	3		33	Y	2	N
14	43		34	N	2	Y
15	19		35	N	19	Y
16	29		36	N	11	Y
16	37		37	Y	35	N
16	43		38			
17	35		39	N	18	Y
17	39		40			
19	11		41	N	9	Y
19	17		42	N	18	Y
21	9		43	N	4	Y
21	23		44			
21	43		45	Y	3	N
22	7		46	N	14	N
22	41		47			
23	31		48	N	20	Y
23	47		49	Y	16	N
25	29		50	Y	18	N
25	59		51	Y	16	N
26	25		52	Y	24	N
27	1		53	N	9	Y
29	53		54			
30	5		55			
30	55		56			
31	19		57	Y	17	N
47	5		58	Y	18	N
65	17		59			
67	1		60	N	21	Y

67	7		61			
67	9		62			
67	17		63	Y	2	N
68	7		64	N	N	Y
40	1		65	N	N	Y

Objective 2. Graft 5 clones from each of the accessions with scion wood from 'Bartlett' and 'Anjou'. Use 'OH×F 87' as a control.

Several clones for 40 of the accessions have been established in the greenhouse and continue to be grown vigorously to achieve the goals of this objective. The numbers for each clone are summarized in Table 1.

Table 2: Gantt chart representing the timeline and activities underlying each objective.



As is evident from the Gantt Chart (Table 2), the project continues to progress as proposed. The difficulty of introducing some of the challenging plant material in the micropropagation process will be addressed in 2020.

This project represents the diverse germplasm – (boxed and labeled as 1 in figure 1) collaborative and multi-pronged approach to establish a foundation for the development of dwarfing pear rootstocks. It is expected that there is a natural source of dwarfing capacity available across the diverse set of accessions along with resistance to both biotic (pathogens, pests etc.) and abiotic (cold, heat, drought)

stresses.

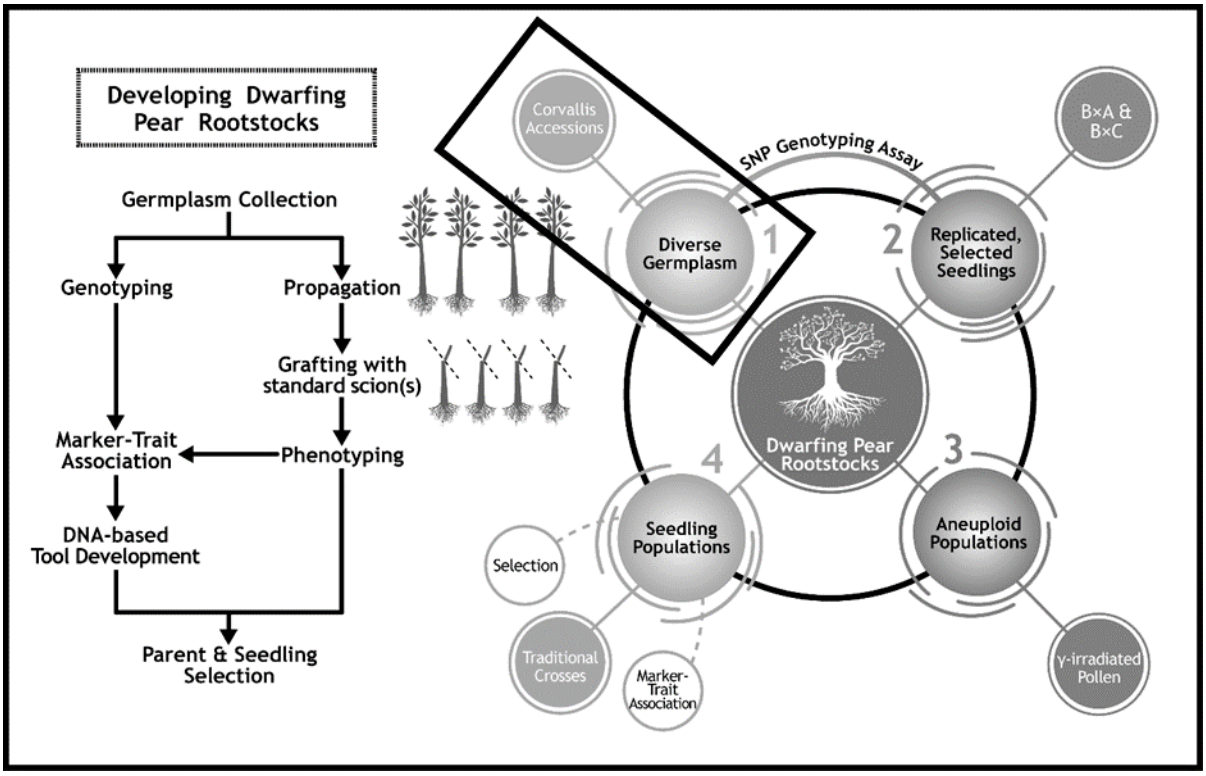


Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks.

Outreach Activities

- Soon Li Teh presented "Pear Rootstock Breeding Program" at the WSU Sunrise Research Farm Extension Field Day at Rock Island, WA on August 7, 2019.
- Soon Li Teh presented "Initiating Pear Rootstock Breeding at Washington State University" at the 2019 Annual Meeting for National Association of Plant Breeders (NAPB) at Pine Mountain, GA on August 25 – 29, 2019.
- The WSU pear rootstock breeding program was featured as a Good Fruit Grower article, "Rooting out Solutions for Pear Growers" on September 2019 Issue (https://www.goodfruit.com/rooting-out-solutions-for-pear-growers/).
- Soon Li Teh and graduate student, Zara York presented an overview of pear rootstock breeding at the WSU Tree Fruit Breeding 101 – Extension Field event at Orondo, WA on October 24, 2019.
- Amit Dhingra visited Fowler Nurseries, Sierra Gold Nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in November 2019.
- Amit Dhingra presented a seminar at Pairwise Inc. in North Carolina regarding pear genomics and rootstock breeding in September 2019.
- Zara York presented "Advancing genetic resources for pear rootstock breeding" Research News Flash talk at the Washington Horticultural Association Show, Wenatchee, WA in December 2019.
- Amit Dhingra presented on pear rootstock research in the Genomic Advances in fruit and vegetable Breeding workshop at the annual Plant and Animal Genome conference at San Diego, CA in January 2020.

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

Project Title: Optimizing irrigation frequency and timing to improve fruit quality

PI: Lee Kalcsits
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Co-PI: Tianna DuPont
Organization: Washington State University
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Email: tianna.dupont@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI: Troy Peters
Organization: Washington State University
Telephone: 509-663-8181
Email: troy_peters@wsu.edu
Address: 24106 N Bunn Rd.
City/State/Zip: Prosser/WA/99350

Cooperators: Bob Gix (Blue Star Growers); Chet Walker (S & W Irrigation), Larry and Renee Caudle, Brandon Long, Aaron Hargrove, Erica Bland

Total Project Request: Year 1: $118,792 Year 2: $84,137 64,537 **Year 3: $89,794**

Other funding sources

Agency Name: Bonneville Environmental Foundation water stewardship
Amt. awarded: $30,000
Notes: Since this was awarded, we reduced our requested budget request by $20000 in 2019 to $64,137. The remaining $10,000 in supplies will allow us to install better instrumentation at grower sites

Agency Name: Province of Murcia (Spain)
Amount awarded: $72,836
Notes: This was awarded to Dr. Victor Blanco to join Dr. Lee Kalcsits' lab for two years and will support Victor's salary and benefits and he will be able to participate in the research objectives of this project and expand on the physiology research being conducted.

Budget 1
Organization Name: WSU **Contract Administrator:** Shelli Tompkins / Katy Roberts
Telephone: 509-293-8803 / 509-335-2885
Email address: shelli.tompkins@wsu.edu / arcgrants@wsu.edu

Item	2018	2019	**2020**
Salaries[1]	45,503	47,723	49,216
Benefits[2]	18,119	18,844	19,598
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[3]	47,170[4]27,140	9,970	12,970
Travel[5]	8,000	8,000	8,000
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	118,792	84,13764,137	**89,784**

Footnotes:
[1] Salaries to support a technician at $3500/month at 75% FTE in the Kalcsits lab and a technician at $3500/month at 33.34% FTE in Tianna DuPont's program. The budget includes a 4% salary increase per year.
[2] Benefits for both technicians calculated at 39.8 %
[3] Supplies include irrigation supplies for objective 1, lab and field consumables, extension materials, analysis costs for nutrient analysis and fruit storage costs.
[4] $30,000 of supplies in year 1 is requested for irrigation supplies to retrofit commercial blocks for testing. Funding for this is also included in the grant application to Bonneville Environmental Foundation.
[5] Travel includes mileage for Kalcsits, DuPont, and Peters for regular trips to commercial orchards and the Sunrise Research Orchard and for hotel and meal per diems for overnight trips to the Wenatchee region for Dr. Peters and his M.S. student to make measurements.

OBJECTIVES

1. Test whether increasing the frequency of irrigation or changing irrigation volume applied during specific times during the season affects fruit productivity and quality.
2. The extension portion of the project will establish demonstration which showcase irrigation optimization strategies to show versus tell growers how changes to irrigation are critical to impact yield and pack out.
3. Conduct a cost-benefit analysis comparing potential increased revenue from changes to irrigation strategies with the costs of making the change.

From the completion of these objectives, we will: a) document the ability of strategies to improve yield, fruit size and fruit quality; b) document the return on investment of these strategies; c) document the changes in the water efficiency of each of these strategies. Demonstration on-farm will create advocates among stakeholders who can tell the story of adoption.

SIGNIFICANT FINDINGS

Cork spot was the highest in the research orchard when trees were watered fully. When water was withheld, cork spot % dropped for both years but variability between replicates limited confidence in these observations. One more year of consistent results will improve overall confidence.

Irrigation systems in four orchards were upgraded to solve ongoing issues to improve or optimize water delivery. For the two orchards with packout information available, significant improvements were observed.

Stem water potential based irrigation appeared to be a better approach to managing irrigation decisions but it still remains labor-intensive. In 2020, we will have access to stem water potential microlysimeters and will be installing them in pear trees to look at water responses in pear to gain a clear understanding of whether this might be a viable tool for irrigating pear trees.

In 2020, we will also monitor fruit quality in grower orchards on a spatial scale to evaluate the impact of changes to these systems.

METHODS

Objective 1

For this objective, the research was conducted at the Sunrise Research Orchard in Rock Island, WA in a semi-mature block of Anjou and Bartlett pears that was planted in 2007 at a spacing of 6’ between trees and 14’ between rows. The orchard was irrigated using microsprinklers hooked up to a variable speed drive system that allows for flexibility in water schedules. The soil in this site is a sandy loam soil with a high percentage of sand. The poor water holding capacity of the soil makes this an excellent location to manipulate soil water content and ensure that we are getting enough variation to achieve the desired effects on the trees. There were four treatments applied. The first was where soil moisture levels were maintained near field capacity for the entire irrigation season. The second was limiting irrigation to 60% field capacity from 15-60 DAFB. The third treatment was limiting soil moisture to 60% of field capacity from 60-105 DAFB. The last treatment was modified from the original proposal. We opted to implement a stem water potential based irrigation scheme where irrigation was triggered when the mean stem water potential for sampled trees was more negative than -1.0 mPa. This strategy reduced overall water use by more than 40%.

Fruit was harvested on September 2, 2019 from sample trees. Fruit was stored in regular atmosphere at 33 °F for 12 weeks. After storage, fruit quality was assessed including fruit size, weight, firmness, and soluble solids content. Cork spot incidence was also assessed in these same fruit samples. Subsamples were then taken for nutrient analysis for N, P, K, Ca, and Mg to look for changes in the ratios among these competing nutrients that may correspond to differences in cork spot, fruit size, or vegetative vigor. We are in the process of analyzing these samples. Additionally, the project team will track return bloom

in 2019 to look at the influence of irrigation frequency on return bloom in Anjou pears.

During the season, we measured plant indicators of water stress during the growing season to relate to horticultural responses such as vegetative and fruit growth. Physiological measurements were made including mid-day stem water potential and stomatal conductance. Plant water status, measured as Ψ_{md} will be assessed using a 3005 Series Plant Water Status Console (Soilmoisture Equipment Corp, Goleta, CA, USA). Leaves used for measurement of Ψ_{md} will be bagged for at least one hour in silver reflective bags to equalize the leaf and xylem water potential before readings are taken. Ψ_{md} will be measured around solar noon. Stomatal conductance (mmol m^{-2} s^{-1}) was measured on mature, sun-exposed leaves on the upper half of the canopy using a LiCor-6400XT Gas Exchange System.

Soil moisture was monitored using Decagon 5TM soil moisture and temperature sensors in each plot over the entire season to capture seasonal changes in soil moisture profiles in addition to the treatment level variations in soil moisture. In the early and late withholding treatments, volumetric soil water content was used to guide irrigation events where volumetric water content below 13% vol/vol triggered a small irrigation set to bring soil moisture levels above that threshold.

RESULTS & DISCUSSION

Objective 1. Test whether increasing the frequency of irrigation or changing irrigation volume applied during specific times during the season improves fruit productivity and quality.

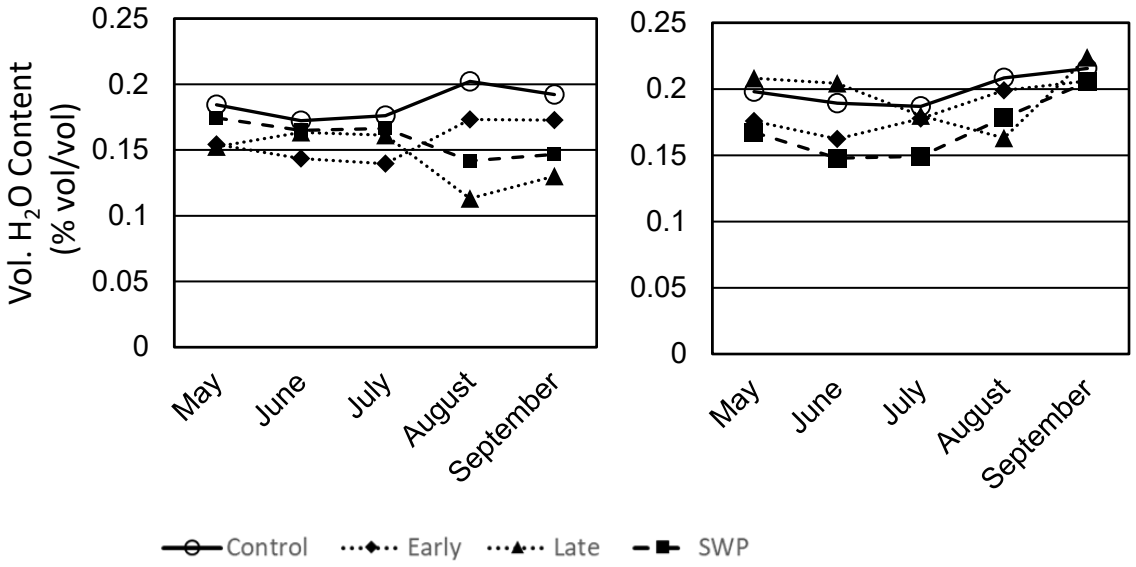
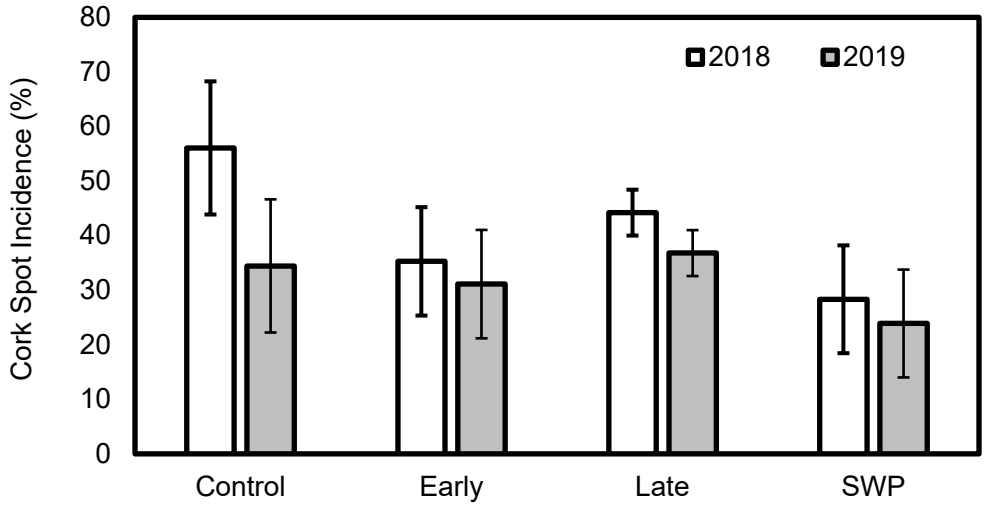


Figure 1. Volumetric soil water content (%vol/vol) for pear trees exposed to water limitations during early or late summer compared to either stem water potential (SWP) guided watering or a standard irrigated control.

Table 1. Mean fruit weight, shape, soluble solids content (°Brix), and fruit firmness (lb) of D'Anjou pears harvested in 2018 and 2019 after two months of storage at 33°F and ripened for 7 days at 68°F

	Weight (oz)	Height: Diameter	Soluble Solids Content (°Brix)	Fruit Firmness (lb)
Control	6.96 a	1.15 a	14.55 a	9.86 a
SWP	7.04 a	1.17 a	14.35 a	9.94 a
Early	6.78 a	1.18 a	14.63 a	9.80 a
Late	7.21 a	1.17 a	14.35 a	9.71 a



Objective 2. The extension portion of the project will establish demonstration which showcase irrigation optimization strategies to show versus tell growers how changes to irrigation are critical to impact yield and pack out.

Here, we will highlight changes made in two different commercial orchards with common water-related issues and the impact that this has had on fruit quality and returns to the grower.

Interviews with growers and consultants identified common challenges to irrigation efficiency in pears. These challenges include: lack of sufficient irrigation; uneven pressure and distribution due to hills; irregular water distribution across the block due to old, inappropriate or malfunctioning equipment; sandy soils with low water holding capacity; heavy soils with limited drainage; insufficient or excess watering due to inability to time water applications; and/or system inefficiencies. A call for applications was sent out in spring 2018. The selected sites include Flowery Divide Har (challenge uneven distribution due to lack of pressure and multiple nozzle sizes); Flowery Divide Low (challenge irrigation scheduling); and Williams Canyon (challenge clogging filters). Recommendation reports were created for each site and discussed with grower collaborators. For sites with irrigation design needs, Chet Walker from S&W engineered plans (Flowery Divide Har, Goat Hill). Flowery Divide Har, Flowery Divide Lo, and Williams Canyon have plans in place to install changes in early spring 2019 before irrigation water flows in the canals. An additional site at Caudle Dryden was installed in spring 2018 with non-research commission funds.

Caudle-Dryden Case Study

This change was completed by the grower in 2017-2018. The challenge at the Caudle Dryden site was run off and small fruit size. The site consists of two side by side 10-acre blocks: 'hill' and 'clover.' The existing irrigation system consisted of Rainbird impact sprinklers on a 36' x 36' spacing (34 heads/ A). The application rate was approximately 0.3 inch/ hr or 0.14 inch/ hr at 50% efficiency. The new system consists of R10 micro-sprinklers with a lower output per sprinkler (0.43 gph) compared to impact sprinklers installed at a 20' x 20' spacing (109 heads/ A). While application rate per hour was similar (0.12 inch/hr at 70% efficiency), smaller droplet size and less output per sprinkler should result in a larger percentage of water infiltrating vs running off the soil. Block 'hill' was designated as the 'Standard' treatment and not changed, block 'clover' was designated the 'new' treatment with R10s installed in June 2018.

After the first year the grower collaborator's impression of the new system was that there was *"Zero run off in the new system. Leaf color was more uniform."* He was happy that "*Before the quickest we could water was 9 days. Now if we want to, we can water the whole block in 2 days (20 lines at a time)"* This gives them more flexibility. Measurements were taken in 'Standard' versus 'New' blocks to compare tree water stress, soil moisture and fruit quality. Please note as un-replicated blocks information comparative not statistical.

Tree water stress measurements were taken in July 2018 and August 2019 measuring leaf water potential using a pressure bomb. Measurements were taken from one tree in every other row at the top of the hill. In the 'New' system trees displayed less stress with all values falling under the -1.2 mpa threshold considered to be water limited. In comparison in the 'Standard' block leaf water potential had more variation and more trees above a -1.2 mpa threshold (Figure 3).

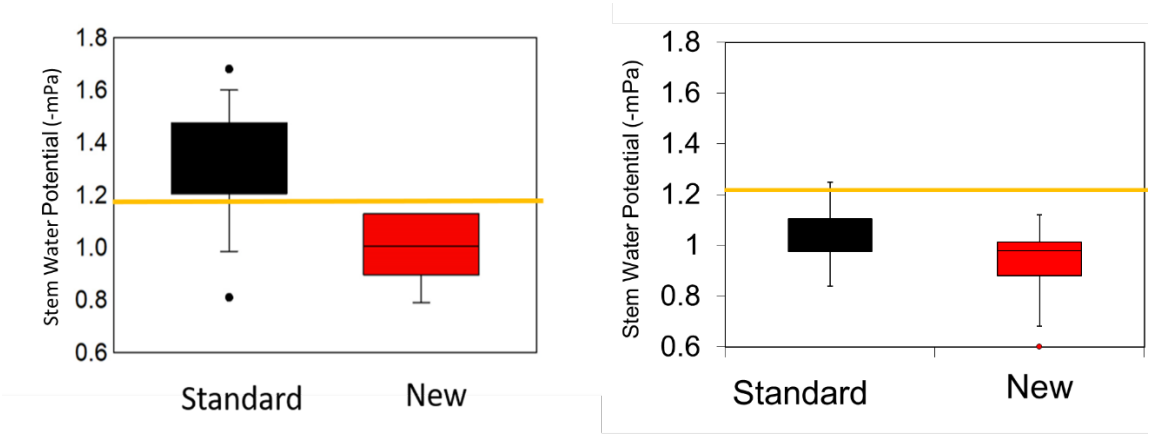


Figure 3. Box plot distribution of stem water potential for trees irrigated using the standard system compared to the modified (new) irrigation system.

For fruit quality, 20 Fruit were harvested from 8 trees in 2018 and 6 trees in 2019 on a grid pattern across the top and bottom of 'Standard' and 'New' plots. Fruit were stored for 12 weeks and then evaluated for size and quality. Fruit size was more uniform for both years in the 'New' plot compared to the 'Standard' plot (Figure 4).

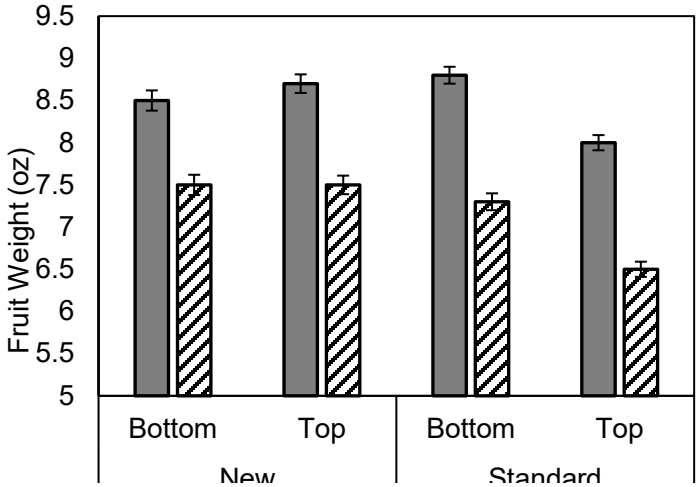


Figure 4. Fruit weight for D'Anjou pears with either the new system or old system in 2018 (solid bars) or 2019 (patterned bars).

Fifty-six bins were tagged separately, and pack-out data compared for each plot. In 2018, the percent packout was higher in the 'New' plot at 95.6% compared to 92.7% in the 'Standard' block with 22.95 packs per bin in 'New' and 22.27 in 'Standard.' **This resulted in 820 packs of US #1 per acre in the new system compared to 788 in the standard system.** The size distribution of US #1s included slightly more large fruit in the 'New' with 736 vs 734 packs of 90+ size fruit. These were primarily in the 60 and 70 class fruit with 73 vs 53 60 class and 210 vs 201 70 class. Using average FOB prices from the January 9, 2018 (Washington Tree Fruit Association Weekly Grower's Bulletin) dollar values were assigned to each size class for US #1 fruit. Based on these estimates the new system would receive approximately $700 per acre more than the standard. This would result in a reasonably quick return on the investment of $1,000 per acre.

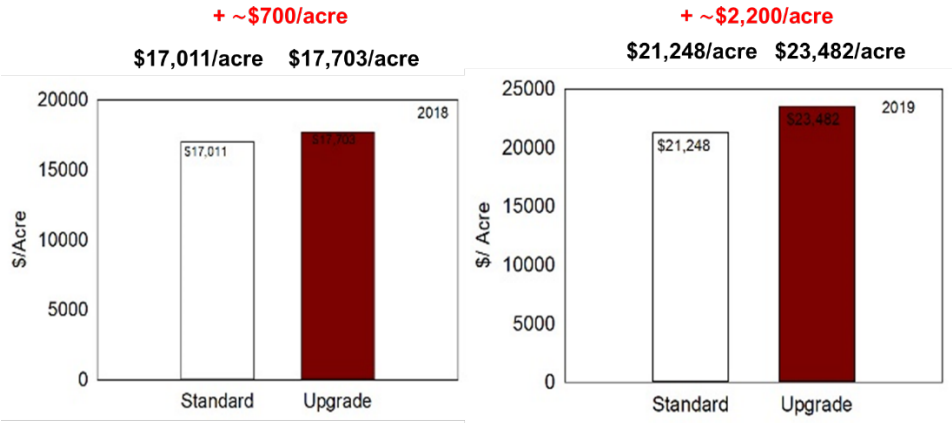


Figure 5. Estimated returns per acre in 2018 and 2019 for the Caudle upgraded orchard section compared to the standard irrigated control. Significant improvements were observed in 2019, largely due to increased yields and more consistent fruit size reported above.

Cashmere Soil Water Content Monitoring Case Study

- Severe cork.
- Did not pick block in 2017 due to 80% cork.
- In general, there was poor control over water delivery and a concern of over-irrigation
- Soil moisture sensors were installed in 2019 to inform watering decisions with the goal to meet an irrigation window.

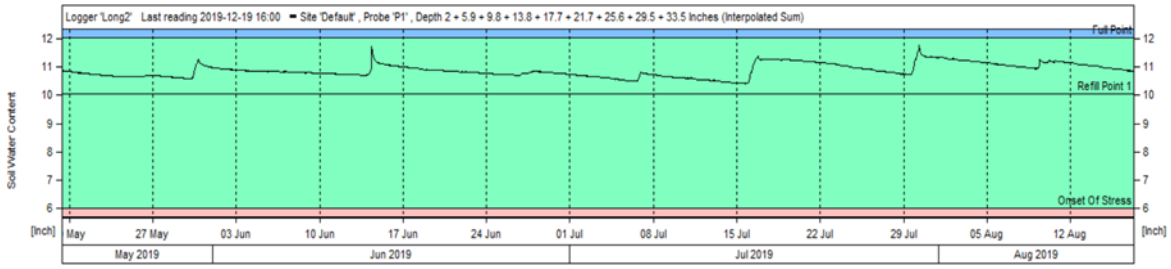


Figure 6. Output from soil water content monitoring indicating full point, refill point, and onset of stress with the goal of maintaining soil moisture between the refill and full point.

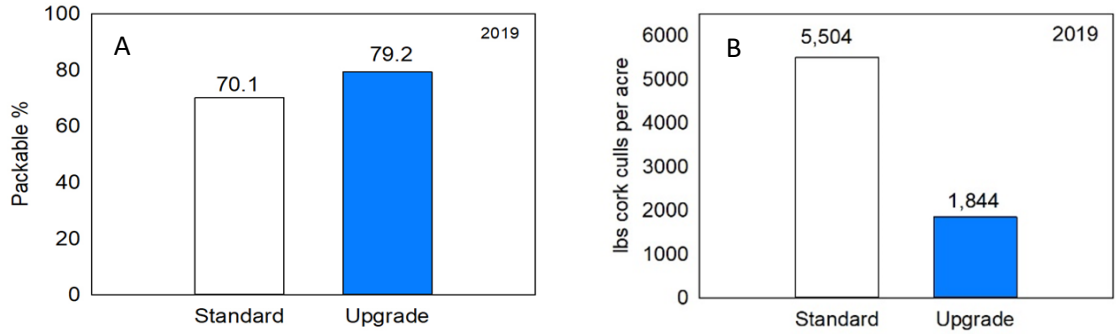


Figure 7. A. A comparison of packout percentage for the normally irrigated standard compared to the soil water content informed scheduling section. B. A comparison of the total weight of cork culls per acre in the normally irrigated standard compared to the soil water content informed scheduling section.

2020 Plans

1. Dr. Victor Blanco will be joining Lee Kalcsits' program as a postdoctoral fellow support by the province of Caledonia. He will be focused on water responses in pear and will be working with testing new instrumentation. His appointment at WSU will be for two years.
2. Repeat experiment at Sunrise in 2020 focusing on tree response to deficit water to better develop thresholds that can help growers make better irrigation decisions. We are fortunate to be acquiring stem water potential chips that allow for continuous monitoring of stem water potential. We will be installing three of these in the pear orchards to allow for us to better understand how stem water potential changes in response to soil moisture and environment to better guide irrigation decisions.
3. Seek out one additional site that requires grower transformations.
4. Conduct cost-benefit analysis of irrigation changes in case studies

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

Project Title: Fine tuning calcium application rates for cork spot

PI: Ashley Thompson
Organization: Oregon State University Extension
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Address: 400 E. Scenic Dr., Ste. 2.278
City/State/Zip: The Dalles, OR 97058

Cooperators: Gary Willis, Legacy Orchard Management

Total Project Request: **Year 1:** $22,969 **Year 2: $19,907** **Year 3:** $20,384

Budget 1

Organization Name: OSU Ag. Res. Foundation **Contract Administrator:** Russell Karow
Telephone: (541)-737-3228 **Email address:** Russell.karow@oregonstate.edu

Item	2019	2020	2021
Salaries[1]	$5,841	$6,016	$6,197
Benefits[2]	$1,986	$1,565	$2,107
Wages[3]	$7,595	$7,823	$8,058
Benefits[4]	$1,899	$1,956	$2,014
Supplies	$5,148	$2,047	$1,708
Travel[5]	$500	$500	$300
Total	$22,969	**$19,907**	$20,384

Footnotes:

[1] 0.08 FTE summer salary for 1 month for Dr. Thompson. Salary in years two and three reflect a 3% increase.
[2] OPE is calculated at 34% of 1 month summer salary. In years two and three, OPE reflects a 3% increase in salary.
[3] Wages for a Biological Technician II $15.19/h for 500 h. Wages in years two and three reflect a 3% increase.
[4]OPE is calculated at 25%, OPE reflects a 3% increase in salary.
[5]Mileage rates are $0.54/mile. This includes travel to an orchard in Hood River from the Mid-Columbia Agricultural Research and Extension Center.

OBJECTIVES:

(1) Compare the effects of six foliar calcium fertilizer rates (0, 2, 4, 6, and 8 lb Ca per acre) and two calcium fertilizers, calcium chloride and NueCal-8, on cork spot occurrence, fruit calcium, and fruit quality of 'd'Anjou'.

(2) Develop rate recommendations for calcium chloride and chelated calcium products that reduce cork spot while minimizing rusetting.

SIGNIFICANT FINDINGS:

1. Calcium application rate did not affect cork spot incidents, which ranged from 3% in the control to 10% the 2 lb Ca per acre application in the pears assessed at harvest and 1% to 12% in pears assessed after conditioning. This was likely do to variation within the orchard and a relatively small sample size.
2. Calcium Chloride and NuCal-8 applied at 4, 6, or 8 lb Ca per acre increased pear firmness after conditioning by 10%, 8%, and 11 %, respectively, compared to the untreated control.
3. After conditioning, calcium chloride treated pears were 9% firmer than pears treated with NuCal-8.
4. Calcium Chloride and NuCal-8 treated pears were smaller in circumference and mass than the untreated control.

METHODS:

Site and Experimental Design:

This experiment was established in 2019 at Willis Family Farms in Hood River, OR (45.664, -121.513) in a mature 'd'Anjou'/'Old Home x Farmingdale 97'orchard planted at a 14' x 16' spacing. The two foliar calcium formulation treatments, calcium chloride (36% Ca; Dow Chemical Co., Midland, MI) and NueCal-8 (8% Ca; BioGrow, Mabton, WA), were applied at five rates, 0, 2, 4, 6, and 8 lbs. per acre. NueCal-8 was selected since it is the grower cooperator's foliar calcium source. Each foliar calcium formulation × rate treatment was replicated on three tree-sets four times as a randomized complete block design. The end two trees in each experimental unit acted as buffer trees, and were not used for data collection. Foliar calcium was applied June 24th, July 10th, July 17th, and July 21st, 2019. We began applications later in June since this is when the grower cooperator typically applies foliar calcium. Typical recommendations for the Mid-Columbia suggest applying calcium in early June and continuing through (Castagnoli et al, 2018). Other fertilizers, such as nitrogen, potassium, and magnesium, were applied at the same rate in all treatments. Pests, diseases, and weeds were be managed uniformly following local recommendations (Thompson et al., 2019).

Measuring fruit quality:

On September 6th, 2019, 100 pears were harvested from the periphery of each treatment tree to a height of 8 ft. Immediately following harvest, a sub-sample of 20 fruit from each tree was individually weighed, and fruit quality (flesh firmness, size, acids and total soluble solids) were be measured following the procedures outlined by Sugar and Einhorn (2011). A 1 inch peel sample was collected from the circumference of the calyx end of 10 fruit to determine fruit calcium content (Baugher et al., 2017). Total peel mineral nutrient (nitrogen, phosphorous, potassium, calcium, magnesium, manganese, and zinc) concentrations will be analyzed at the Pennsylvania State Agricultural Analytical Services Laboratory. The 20 fruit sub-sample was also visually evaluated for cork spot by cutting two 0.5 inch sections from the calyx end of the pear. Remaining fruit were placed in cold storage for 90 days; then removed and held at 70 °F for 10 days to mimic typical pear storage and

conditioning. Fruit quality (flesh firmness, size, and total soluble solids) was re-assessed after conditioning. Fruit was visually evaluated for cork spot, and other calcium disorders.

Data Analysis:

Fruit quality data and cork spot data were analyzed with ANOVA using the 'GLIMMIX' procedure in SAS (v9.4, SAS Institute, Cary, NC). Calcium fertilizer, rate and calcium formulation × rate were considered fixed effects, and block was the random effect. Means separation for individual effects was determined using Tukey's honestly significant differences post hoc test at the $P \leq 0.05$ level. When interaction effects were significant, treatment effects were partitioned within each year using the LSMEANS values generated with the SLICEDIFF command and mean separation was determined using the least squares differences (LSD) test at the $P \leq 0.05$ level.

RESULTS & DISCUSSION:

Calcium formulation and application rate did not affect cork spot incidents in 2019. In samples assessed at harvest, only 3% pears from the untreated control group exhibited cork spot while pears treated with calcium chloride had an average 6% incidence and pears treated with NuCal-8 had an average 5% incidence of cork (Figs. 1 and 2). In samples assessed after conditioning, only 1% of the pears from the untreated control group exhibited cork spot and pears treated with calcium chloride had a 6% incidence and pears treated with NuCal-8 had a 5% incidence of cork (Figs. 3 and 4). I am awaiting peel data from the Pennsylvania State Agricultural Analytical Services Laboratory, which may better explain the cork spot results. Surprisingly, rate did not affect cork spot incidents in this study; however, data was highly variable. Inconsistencies in these data, such as numerically greater cork spot incidence in treated fruit and numerically greater rates of cork spot in the highest application rate, are likely due to numerous variations within the orchard, older trees, and a relatively small sample size. In addition, the grower cooperator informed me some trees in this 'd'Anjou' block was diagnosed with freckle pit, a virus suspected disorder, that leads to cork spot-like symptoms in late fall of 2019.

Calcium formulations and rate affected at harvest and after conditioned fruit quality (Table 1 and 2); however, these results were inconsistent. At harvest, calcium chloride or NuCal-8 applications did not increase fruit firmness, circumference or mass regardless of rate. At harvest, calcium formulation and rate affected fruit firmness. Calcium chloride treated pears were 9% firmer than pears treated with NuCal-8. Calcium Chloride and NuCal-8 applied at 4, 6, or 8 lb Ca per acre increased pear firmness post-conditioning by 10%, 8%, and 11 %, respectively, compared to the untreated control. When we take a closer look at calcium formulation × rate interactions, applications of calcium chloride at or above 2 lb. per acre Ca increased pear firmness. NuCal-8 application rates had inconsistent effects on fruit firmness, and 8 lb. per acre Ca were necessary to increase fruit firmness compared to the control. At harvest, circumference and mass were reduced by calcium application regardless of formulation or rate. Calcium formulation and rates did not affect soluble solids (SS) at harvest or after conditioning. Some of the significant differences observed, such as postharvest firmness and fruit circumference, are likely mathematical differences due to variability within large mature 'D'Anjou' trees and tree rows. The biological significance of these results seems questionable, as responses do not follow consistent trends.

Figure 1. The percentage of fruit with cork spot was calculated for each calcium chloride application rate at harvest. Error bars represent the standard error of the mean. There were no significant differences in cork spot presence among the different application rates.

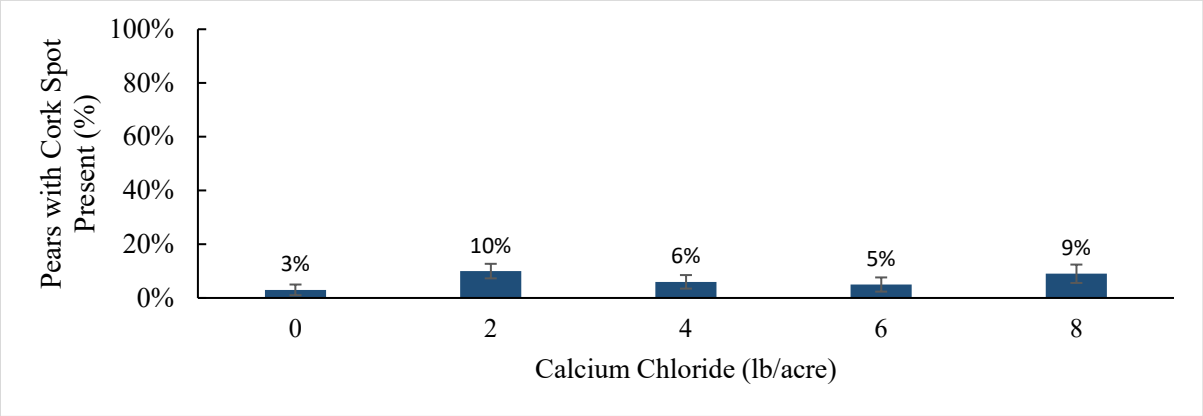

Figure 2. The percentage of fruit with cork spot was calculated for each NuCal-8 application rate at harvest Error bars represent the standard error of the mean. There were no significant differences in cork spot presence among the different application rates.

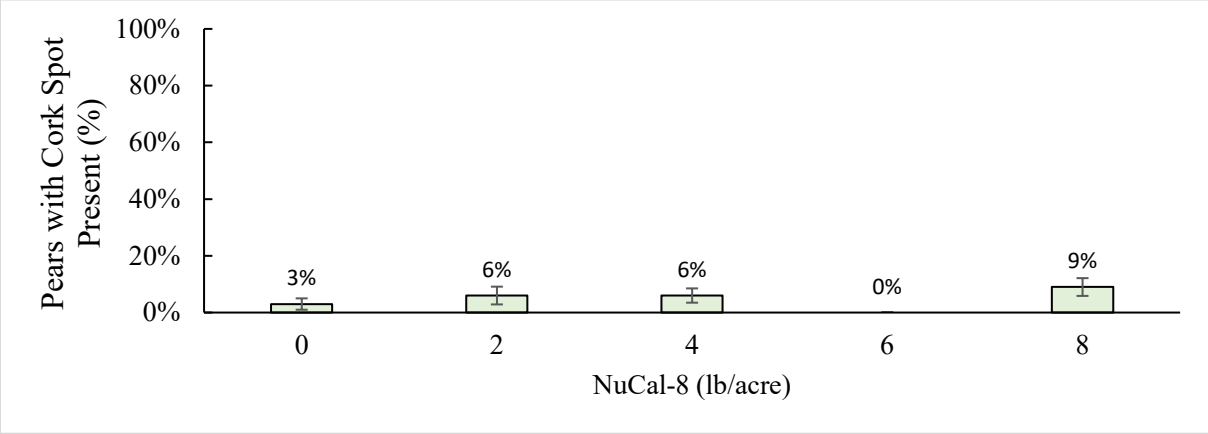

Figure 3. The percentage of fruit with cork spot was calculated for each calcium chloride application rate after conditioning. Error bars represent the standard error of the mean. There were no significant differences in cork spot presence among the different application rates.

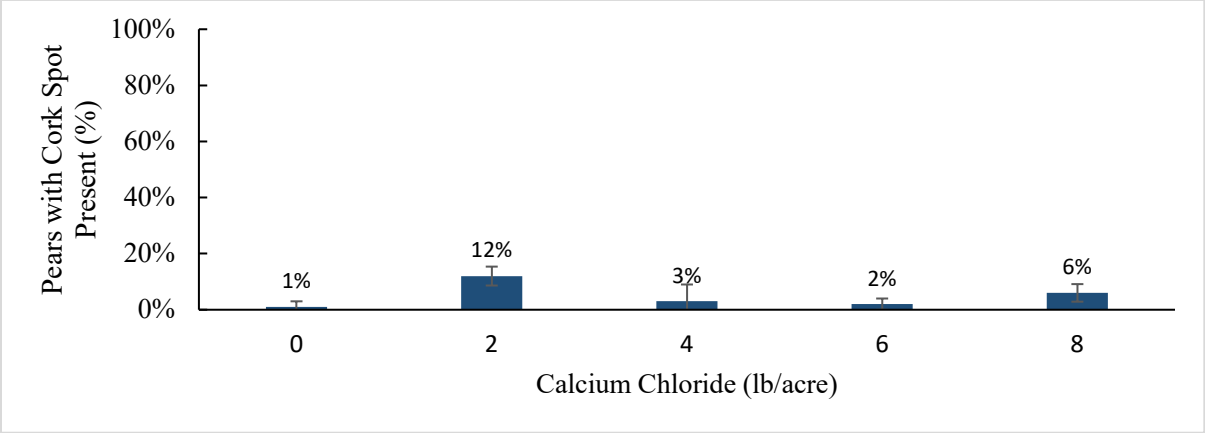

Figure 4. The percentage of fruit with cork spot was calculated for each NuCal-8 application rate after conditioning. Error bars represent the standard error of the mean. There were no significant differences in cork spot presence among the different application rates.

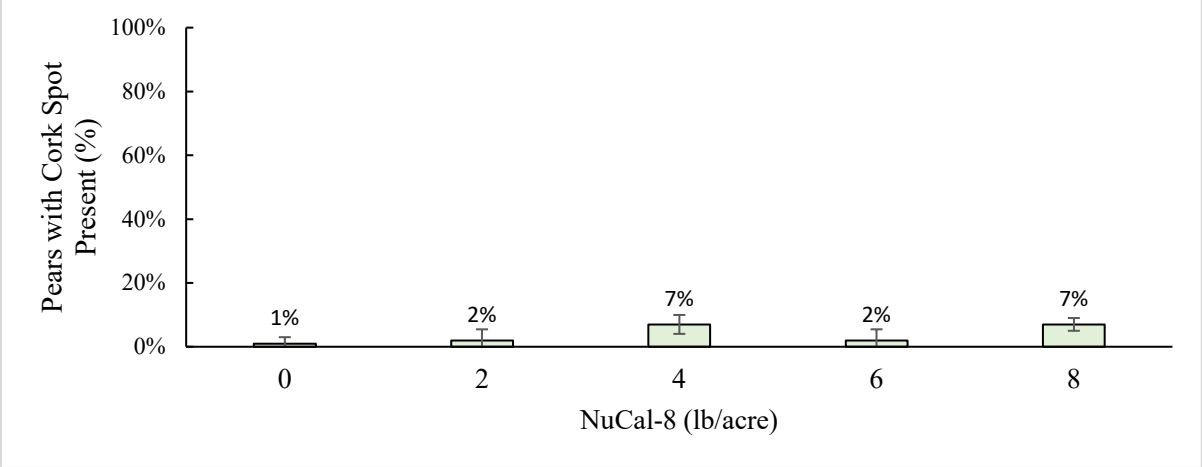

Table 1. Fruit maturity and quality was assessed for each calcium formulation and rate at harvest. Different letters within a column indicate significantly different means. NS, *, **, ***Nonsignificant or significant differences at P ≤0.05, 0.01, or 0.001, respectively.

	Harvest				
	Firmness (lb)	**Circumference (mm)**	**Mass (g)**	**Acids (g/l)**	**SS (°Brix)**
Fertilizer					
Calcium Chloride	12.3	68.6	194.79 B	3.12	12.27
NuCal-8	12.3	68.9	203.52 A	3.09	12.19
	ns	ns	*	ns	ns
Rate					
0	12.50 A	41.64 A	220.55 A	3.08	12.4
2	12.24 AB	68.92 B	198.17 BC	3.16	12.39
4	12.10 B	67.15 C	184.22 C	3.11	12.29
6	12.32 AB	67.50 C	189.42 BC	3.11	12.15
8	12.25 AB	68.41 BC	203.4 AB	3.04	11.91
	*	***	***	ns	ns
Fertilizer x Rate interaction	***	***	**	**	ns
Calcium Chloride					
0	12.49 A	71.63 A	220.55 A	3.08 AB	12.4
2	12.53 A	70.44 A	207.65 AB	3.27 A	12.7
4	11.90 B	66.45 C	178.25 BC	3.01 BC	12.35
6	12.02 B	65.77 C	173.10 C	3.29 A	12.05
8	12.37 A	68.47 B	194.40 AB	2.95 C	11.82
NuCal-8					
0	12.49 AB	71.63 A	220.55 A	3.08	12.4
2	11.95 C	69.24 B	188.70 B	3.06	12.05
4	12.29 B	68.35 BC	190.20 B	3.22	12.25
6	12.62 A	67.85 C	205.75 AB	2.96	12.25
8	12.12 C	67.01 C	212.4 A	3.12	12

Table 2. Fruit maturity and quality was assessed for each calcium formulation and rate after cold storage and conditioning. Different letters within a column indicate significantly different means. NS, *, **, ***Nonsignificant or significant differences at P ≤0.05, 0.01, or 0.001, respectively.

	After Conditioning				
	Firmness (lb)	**Circumference (mm)**	**Mass (g)**	**Acids (g/l)**	**SS (°Brix)**
Fertilizer					
Calcium Chloride	2.02 A	69.2	201.37	2.19	12.37
NuCal-8	1.85 B	69	200.94	2.19	12.3
	***	ns	ns	ns	ns
Rate					
0	1.8 B	71.31 A	219.00 A	2.29	12.20 B
2	1.93 AB	69.08 B	202.22 B	2.16	12.84 A
4	1.99 A	67.80 C	190.94 C	2.19	12.08 B
6	1.95 A	68.31 BC	193.84 BC	2.13	12.65 A
8	2.01 A	69.05 BC	199.78 BC	2.18	11.95 B
	***	***	***	ns	***
Fertilizer x Rate interaction	***	**	ns	ns	ns
Calcium Chloride					
0	1.8 C	71.31 A	219	2.29	12.2
2	2.02 B	70.1 AB	208.12	2.17	12.85
4	2.23 A	67.51 BC	192.19	2.15	12.18
6	1.98 B	67.4 C	188	2.17	12.8
8	2.07 B	69.09 B	199.56	2.16	11.85
NuCal-8					
0	1.8 BC	71.31 A	219	2.29	12.2
2	1.83 B	68.06 C	196.31	2.16	12.83
4	1.77 C	67.51 C	189.69	2.23	11.97
6	1.92 AB	69.22 B	199.69	2.09	12.45
8	1.95 A	69.09 BC	200	2.19	12.05

CONTINUING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project Number: PR-19-103

Project Title: New active ingredients for pear superficial scald control

PI: David Rudell
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Co-PI: Carolina Torres
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Co-PI: James Mattheis
Organization: USDA-ARS, TFRL
Telephone: 509-664-2280 (ext. 249)
Email: James.Mattheis@ars.usda.gov

Cooperators: Dr. Jingi Yoo

Budget: **Year 1**: $84,894 **Year 2**: **$86,893** **Year 3**: $89,036

Other funding sources

Agency Name: USDA-ARS, In-house project
Cost-sharing: $105,946/3 yrs.
Notes: In-house project with complimentary objectives. Funds for storage maintenance and costs ($8000/yr), supplies and materials ($3000/yr), travel ($5000/yr), and 0.1 FTE (PI, co-PI) and 0.05 FTE (technical).

Budget
Organization Name: WSU-TFREC **Contract Administrator:** Timothy Palacios
Telephone: (509) 768-2226 **Email address:** prosser.grants@wsu.edu

Item	2019	2020	2021
Salaries	52,196	53,679	55,290
Benefits	17,198	17,714	18,246
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous (Fruit purchase)	3000	3000	3000
Plot Fees			
Total	72,394	**74,393**	76,536

Budget

Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers

Telephone: (510) 559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2019	2020	2021
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	1000	1000	1000
Travel			
Miscellaneous*	11,500	11,500	11,500
Miscellaneous			
Plot Fees			
Total	12,500	**12,500**	12,500

Footnotes: One-third instrument service contract

OBJECTIVES:
1. Test squalane-based formulation(s) for scald control of 'd'Anjou' pear.
2. Determine mode of action of this new active ingredient.
3. Determine any quality impacts and control of other appearance-related defects.

Goals and Activities for the next year:
Project second year goals will be to test squalane formulation scald as well as CO_2 sensitivity and other conditions that impact fruit appearance a second year. We will also continue to determine how peel chemistry is impacted and how that relates to fruit appearance and scald control.

SIGNIFICANT FINDINGS:
1. No formulations were phytotoxic.
2. No formulation had any consistent influence on fruit color, ripeness, or internal quality to date.

METHODS (<u>see timeline and deliverables in Table 1</u>, final page of this proposal)

Equipment and Cooperative Summary: Fruit quality assessment, fruit chemistry analyses using analytical instrumentation (gas and liquid chromatography-mass spectrometry), and tissue cryopreservation will be performed using facilities currently in place at ARS-TFRL, Wenatchee. Storage experiments will be conducted in TFRL in-house CA chambers.

Outreach (Deliverables are summarized under "Anticipated Products" Table 1): Aside from reports to the WTFRC, new information will be disseminated through presentations at industry meetings and at professional conferences, and by publications in industry publications and peer-reviewed journals. Dr. Torres will continue to interface with crop protectant providers interested in her product.

Objective 1: Test squalane-based formulation(s) for scald control of 'd'Anjou' pear

<u>Year 1</u>
Superficial scald control using the existing formulation and other formulations containing squalane needs to be demonstrated on 'd'Anjou'. In Year 1, we are testing the previously established rate on 'd'Anjou' pears from an orchard in each of the Hood River, Yakima, Wenatchee, and Okanogan regions. We harvested 1296 fruit twice (early and late) from external canopies and double that from the Wenatchee location. Fruit were transported to TFRL, initial fruit quality evaluated, and 432 drenched 0.5% squalane formulation, 432 drenched with 1000 ppm ethoxyquin, and 432 drenched with washed with water. Additional pears (36 fruit/treatment/storage duration) from each location were drenched with 3 concentrations of another emulsion containing 0.5%, 1%, and 2% squalane, Triton X-100, and water. Pears from both harvest from every orchard as well as those treated with the Triton formulation were stored in commercial CA rooms (31°F; 1% O_2, 1.5 % CO_2) for 3, 6, or 8 months, respectively. Pears from the Wenatchee location were also stored in air (31°F) for 2, 4, or 6 months.

Scald incidence and severity as well as phytotoxicity and fruit quality are being evaluated upon removal from storage as well as after 7 and 14 at 68 °F (if intact) days of storage. Fruit quality and maturity was evaluated on all treatments at all sampling periods using fruit weight, IDA, °Hue (green to yellow), firmness, soluble solids, starch index, titratable acidity, and whole fruit ethylene production.

Objective 2: Determine mode of action of this new active ingredient

Peel from pear from the Wenatchee location that were drenched with 0, 0.5, 1, and 2 % squalane emulsion formulated with Triton X-100 and stored in CA has been or will be sampled at 3, 6, and 8 months for chemical analysis to determine the mode of action of squalane.

Objective 3: Determine any quality impacts and control of other appearance-related defects

180 pears (from each of 2 harvests) from the Yakima location were selected for an experiment looking into how antioxidant (ethoxyquin and DPA) and squalane treatments impact peel injury caused by elevated CO_2 in storage. 36 fruit (per treatment) at each harvest were left untreated or treated with 1000 ppm ethoxyquin (drench), 2000 ppm DPA (drench), 1% squalane/oleic acid emulsion (drench), or 2% squalane/Triton X-100 emulsion (drench). Pears are stored at 33 ºF, 0.5% O_2, 5% CO_2 to check for peel injury related to CO_2 sensitivity. Pear appearance will be evaluated at 6 months.

RESULTS AND DISCUSSION

Early and late harvests from all 4 locations represented a range of maturities (Tables 1 and 2). Our expectation is that this will give us the best chance of observing scald symptoms as well as phytotoxicity or other impact on fruit quality. All emulsifiable concentrate formulations, applied as drenches spread nicely on the fruit surface. Formulations with oleic acid dried more slowly than Triton X-100, but residue quickly disappeared leaving no trace. As of 4 months in air and CA storage, no scald symptoms have been detected. None of the treatments have impacted fruit quality to date (Table 1). Most importantly, none of the formulations tested developed any detectible symptoms of phytotoxicity to date.

Table 1. Maturity and quality data at harvest, following 3 months CA (31°F, 1% O_2, 1.5% CO_2) storage, and after 7 in 68°F air following CA for 'd'Anjou' pears picked on the first harvest date from each location.

	Location	Treatment	Wt (g)	IAD	Color °Hue	Firmness (lbs)	SS (°Brix)	Starch Index (1-6)
Harvest 1	Hood River	harvest	206.7	1.9	112.0	23.4	14.0	5
	Tonasket	harvest	160.2	2.0	116.2	17.1	11.8	2
	Wenatchee	harvest	176.5	1.8	114.2	14.3	11.7	2
	Yakima	harvest	184.3	2.0	111.9	24.8	13.9	5.6
3M CA–H1	Hood River	1	214.1a	2.0a	113.7a	13.7a	13.5a	6a
		2	227.9a	2.0a	114.6a	13.5a	13.5a	6a
		3	235.9a	2.0a	113.4a	14.3a	13.8a	6a
3M CA–H1	Tonasket	1	176.7b	1.8ab	112.1a	12.6a	13.7b	6a
		2	170.1ab	2.0b	107.7a	12.2a	13.1ab	6a
		3	147.7a	1.4a	114.7b	11.9a	12.3a	6a
3M CA–H1	Wenatchee	1	173.9a	1.8a	104.8a	14.6b	14.4b	6a
		2	196.8a	1.8a	106.8a	12.9a	13.6a	6a
		3	296.2a	1.8a	104.8a	13.6a	13.1a	6a
3M CA–H1	Yakima	1	192.4b	2.0a	108.3a	12.8a	13.4a	6a
		2	185.9b	2.0a	111.3a	12.6a	13.8a	6a
		3	154.4a	2.0a	113.1a	12.8a	13.4a	6a
3M CA+7d H1	Hood River	1	218.9a	1.7a	107.4a	1.0b	13.7a	6a
		2	228.7a	1.8a	110.3b	1.2b	13.6a	6a
		3	220.4a	1.7a	108.8ab	2.1a	13.8a	6a
3M CA+7d H1	Tonasket	1	144.8ab	1.4a	76.3b	2.5a	12.8a	6a
		2	157.8b	1.2a	67.4b	2.3a	12.9a	6a
		3	130.1a	1.2a	105.6a	2.8a	12.9a	6a
3M CA+7d H1	Wenatchee	1	171.0ab	1.6a	107.6a	2.3a	13.8a	6a
		2	193.8b	1.5a	106.0a	0.74a	12.7a	6a
		3	157.5a	1.6a	106.3a	1.4a	12.5a	6a
3M CA+7d H1	Yakima	1	72.9a	1.8a	108.1a	2.1a	13.3a	6a
		2	169.1a	1.6a	108a	2.5ab	13.4a	6a
		3	156.3a	1.8a	108.1a	2.9b	13.7a	6a

Table 2. Maturity and quality data at harvest, following 3 months CA (31°F, 1% O_2, 1.5% CO_2) storage, and after 7 in 68°F air following CA for 'd'Anjou' pears picked on the second harvest date from each location.

	Location	Treatment	Wt (g)	IAD	Color °Hue	Firmness (lbs)	SS (°Brix)	Starch Index (1-6)
Harvest 2	Hood River	harvest	208.8	1.9	117.3	22.4	13.2	4.4
	Tonasket	harvest	128.9	1.9	113.5	15	11.4	4.4
	Wenatchee	harvest	193.07	1.8	115.0	14.5	11.5	2.2
	Yakima	harvest	182.5	1.8	112.5	14.2	13.5	3.8
3M CA–H2	Hood River	1	208.9a	2.0a	112.1a	13.8a	13.4a	6a
		2	193.5a	1.0a	110.9a	14.3a	13.6a	6a
		3	192.5a	1.9a	110.7a	14.3a	13.6a	6a
3M CA–H2	Tonasket	1	162.5a	1.8a	112.4b	12.1a	11.8a	6a
		2	143.5a	1.9a	110.1ab	12a	12ab	6a
		3	160.7a	2.0a	106.9a	11.8a	12.5b	6a
3M CA–H2	Wenatchee	1	192.6a	1.6a	112.4a	12.5a	13.7b	6a
		2	226.3b	1.7ab	113.8ab	12.8a	13.2ab	6a
		3	217.0ab	1.8b	115.2b	12.6a	13.1a	6a
3M CA–H2	Yakima	1	192.3b	1.9a	107.8a	12.4a	14.8b	6a
		2	186.4b	1.8a	112.9b	12.5a	13.7a	6a
		3	146.8a	1.9a	110.8ab	12.4a	13.7a	6a
3M CA+7d H2	Hood River	1	195a	1.8a	110.8a	1.9a	13.6a	6a
		2	201.9a	1.8a	106.6a	2.7a	13.5a	6a
		3	227.2a	1.8a	11.1a	1.9a	13.2a	6a
3M CA+7d H2	Tonasket	1	157.3a	1.8a	107.5a	2.6b	12.3a	6a
		2	155.7a	1.8a	112.4a	1.5a	12.3a	5a
		3	151.5a	1.9a	110.7a	3.1b	11.8a	6a
3M CA+7d H2	Wenatchee	1	195.8a	1.3a	74.9a	1.4a	13.5a	6a
		2	206.0a	1.4a	66.4a	1.3a	13.6a	6a
		3	205.0a	1.5a	81.7a	1.8a	12.8a	6a
3M CA+7d H2	Yakima	1	201.9b	1.7a	110.3a	2.2a	13.8a	6a
		2	171.9a	1.8a	107.3a	2.8b	14.1a	6a
		3	200.6b	1.8a	107.3a	2.6ab	13.3a	6a

Table 1. Proposed project milestones with anticipated products of "New active ingredients for pear superficial scald control".

Objective		1: Test squalane-based formulation(s) for scald control of 'd'Anjou' pear		
Hypothesis		Existing squalene-based and new formulations will control superficial scald on 'd'Anjou'.		
Team	Months	Milestone	Anticipated Product(s)	Progress/Changes
DR,CT,JM	12	A determination of efficacy of controlling scald using existing and new formulas containing squalane.	Validation of scald control provided by formulations containing squalane on d'Anjou.	
DR,CT,JM	24	A determination of efficacy of controlling scald using existing and new formulas containing squalane.	Two years validation of scald control provided by formulations containing squalane on d'Anjou.	
DR,CT,JM	36	A determination of efficacy of controlling scald using existing and new formulas containing squalane.	Three years validation of scald control provided by formulations containing squalane on d'Anjou. Best practices (rates and timing) for controlling superficial scald on d'Anjou using squalane based products.	

Objective		2: Determine mode of action of this new active ingredient		
Hypothesis		Squalane-based superficial scald control has a different mode of action than antioxidants such as ethoxyquin.		
Team	Months	Milestone	Anticipated Product(s)	Progress/Changes
DR,CT	12	Completed initial analysis of natural chemistry as altered by squalane versus ethoxyquin.	Understanding of how squalane alters peel chemistry differently from ethoxyquin.	
DR,CT	24	Validation and refined analysis of chemical and physical changes resulting from squalane-based superficial scald control.	Understanding of squalane treatment is linked with scald.	
DR,CT	36	A model of how squalane influences metabolism.	Understanding of mode of scald control by squalane and, potentially, similar active ingredients.	

Objective		3: Determine any quality impacts and control of other appearance-related defects		
Hypothesis		Squalane formulations will provide scald control with a minimum of negative impacts on appearance including blotch-pit.		
Team	Months	Milestone	Anticipated Product(s)	Progress/Changes
JM, DR	12	Full assessment of impacts of different formulations on appearance.	Recommendations for reducing phytotoxicity of tested formulations.	
JM, DR	24	A completed experiment indicating whether squalane and/or ethoxyquin controls disorders related to CO_2 sensitivity. Full assessment of impacts of different formulations on appearance.	A protocol for reducing disorders related to CO_2 sensitivity. Recommendations for reducing phytotoxicity of tested formulations.	
JM, DR	36	Full assessment of impacts of different formulations on appearance.	Recommendations for reducing phytotoxicity of tested formulations.	

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

Project Title: Pear Rootstock Breeding

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Cooperators: Sara Montanari (UC Davis), Stefano Musacchi (WSU-TFREC), Joseph Postman (USDA-ARS Corvallis, OR), Nahla Bassil (USDA-ARS Corvallis, OR)

Total Project Request: Year 1: $104,731 **Year 2: $108,371** Year 3: $108,541

Other Funding Sources

Agency Name: USDA-SCRI Pre-application
Amount Pending: $2,800,000 (2020 – 2024)
Notes: "Phenotypic and genomic characterization of *Pyrus* germplasm for development of dwarfing rootstocks for sustainable pear production in the USA" (PI: Dhingra; Co-PI: Evans)
Synergistic project to characterize diverse set of *Pyrus* germplasm via large-scale phenotyping and genotyping.

Agency Name: PNW Pear Bureau
Amount Awarded: $120,000 (2019 – 2021)
Notes: "Evaluating dwarfing capacity of 65 diverse pear germplasm accessions" (PI: Dhingra; Co-PI: Evans)
Synergistic project to evaluate the dwarfing capacity of diverse germplasm to be used as parental material in pear rootstock breeding.

Agency Name: PNW Pear Bureau
Amount Awarded: $34,133 (2017 – 2019)
Notes: "Greenhouse screening of 49 dwarf rootstock candidates" (PI: Dhingra; Co-PI: Evans)
Synergistic project to evaluate the dwarfing potential of aneuploid pear rootstock seedlings.

WTFRC Collaborative Expenses: None

Budget

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Organization Name: WSU-TFREC
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Item	2019	**2020**	2021
Salaries[1]	$52,358	$54,452	$56,630
Benefits[1]	$17,011	$17,691	$18,399
Wages[2]	$6,240	$6,490	$6,750
Benefits[2]	$4,412	$4,588	$4,772
Equipment & Supplies (TFREC)	$19,600	$19,200	$15,200
Travel[3]	$3,190	$3,190	$3,190
Plot Fees	$1,920	$2,760	$3,600
Total	$104,731	**$108,371**	$108,541

[1]Salaries for postdoctoral research associate (Evans lab) who is the point person for pear rootstock;
[2]Wages for time-slip labor for orchard management and trait phenotyping;
[3]In-state travel between TFREC and orchards for orchard management and trait phenotyping.

OBJECTIVES

1. Develop seedling populations to produce new rootstocks
2. Validate published markers for parent and seedling selection
3. Conduct marker-trait association for dwarfing-related traits in seedling populations
4. Expand the pear rootstock parent germplasm
5. Evaluate B × A and B × C selections

This project aims to build on a previous project (PI: Evans "Pear rootstock breeding"; PR-15-105) to develop a long-term, dedicated pear rootstock breeding program at the Tree Fruit Research and Extension Center, Wenatchee. Diverse germplasm that was previously collected from USDA-ARS, Corvallis is being used as crossing parents. New germplasm will be produced using traditional breeding of crossing and selection. DNA genotyping/sequencing using previously developed pear genomic resources (PI: Neale "Development of marker-based breeding technologies"; PR-14-111) is currently underway. In the upcoming year, genetic maps will be built using these DNA sequences. These genetic maps can then be associated with phenotypic data of rootstock-related traits to identify genomic regions associated for dwarfing (and precocity, if available), which can be developed into a DNA-based tool to enable selection of dwarfing individuals (parents or seedlings). However, this DNA-based tool development is beyond the timeframe of this proposal.

SIGNIFICANT FINDINGS

- Six new crosses were made in 2019 with over 5,500 seeds produced in the WSU pear rootstock breeding program. 1,200 seeds are being stratified and will be sown in the greenhouse in winter 2020. The remaining seeds will be held in reserve for future years.
- Seedlings from over 1,000 seeds from 2018 crosses were germinated in winter 2019 and are being maintained in the greenhouse.
- Approximately 100 rootstock seedlings at the WSU Columbia View orchard were rebudded with 'd'Anjou' scions by Dan Whitney.
- Over 600 seedlings at the WSU Columbia View orchard were phenotyped for rootstock branch angle.
- Approximately 700 individuals, comprised of seedlings and diversity parents, were extracted for DNA. Of these, DNA from 190 seedlings were genotyped with the Pear SNP Array (PI: Neale; PR-14-111).

METHODS

Objective 1: Develop seedling populations to produce new rootstocks

The long-term continuity of the pear rootstock breeding program relies on: (1) using parents to provide pollen for crossing, (2) harvesting seeds to be germinated in the greenhouse, (3) planting seedlings in the orchard, (4) routine phenotyping of rootstock-conferred scion traits, (5) selecting seedlings to be advanced to the next phase, and (6) propagating selections at various sites for further evaluations.

Within the timeframe of this proposal, the trees will remain in the field where shoot length, trunk diameter, branch angle, and precocity will be evaluated as a measure of vigor. The seedlings will also be monitored for scion-rootstock compatibility. In the event of incompatibility, an alternative scion (e.g., 'Bartlett') would be considered.

Seedlings with superior dwarfing potential will be advanced to 'Phase 2'. However, 'Phase 2' is beyond the timeframe of this proposal. These selections will be propagated and further tested in the

orchards in replicated planting. A further round of selection is envisaged before final decisions are taken for wide-scale propagation.

Objective 2: Validate published markers for parent and seedling selection
Several DNA-based markers have been reported to be linked to dwarf or dwarfing traits, each in only one bi-parental population. The effect of each of these loci/markers is quite limited, however it is still worthwhile to validate them in our germplasm (pear [dwarf] – *PcDw* locus [Wang et al., 2011; Wang et al., 2016]; apple [dwarfing] – *Dw1*, *Dw2* and *Dw3* loci [Rusholme Pilcher et al., 2008; Celton et al., 2009; Fazio et al., 2014; Harrison et al., 2016]).

These DNA-based markers will be tested initially on parents to determine allelic polymorphism/differences. If polymorphic, markers will also be tested on the seedling populations which will enable validation of the markers once dwarfing information is collected. The seedling populations in the orchard have already been grafted with a standard scion variety to determine their dwarfing potential.

Research efforts in *Objective 3* may result in the identification and development of new DNA-based markers (depending on the time frame), which will also be incorporated for testing.

There have also been DNA-based markers reported to be involved in precocity (ss475878191 AB allelotype [Knäbel et al., 2015] and GD142 [Ntladi et al., 2018]). Similarly, these markers will be tested on both the parent and seedling populations to ascertain their predictability in the germplasm.

Objective 3: Conduct marker-trait association for dwarfing-related traits in seedling populations
This objective goes in tandem with *Objective 1*. In spring 2019, fresh young leaves of the rootstock (not the scion 'd'Anjou') seedling populations will be collected and freeze-dried. DNA extraction will be conducted to meet the quality and quantity needed for genotyping/sequencing.

Final SNP filtering and selection will be carried out in-house and used to build genetic maps. Construction of genetic maps will be conducted using JoinMap® 5 software. Subsequently, marker-trait association will be conducted on statistical software (e.g., FlexQTL™, R/qtl) to identify genetic determinants for dwarfing-related traits (e.g., shoot length, trunk diameter and precocity). This process combines the genetic maps with the trait information described in *Objective 1*.

Objective 4: Expand the pear rootstock parent germplasm
The existing rootstock breeding program consists of crossing parents that will continue to be evaluated for principal breeding traits, such as growth habit, ease of propagation and resistance to fire blight.

In addition to the current *Pyrus* rootstocks, a subset of serviceberry (genus *Amelanchier*) and quince (*Cydonia oblonga* L.) accessions will be collected from the current field trials in OR and WA (PR-12-103 and PR-18-102) to expand the pear rootstock germplasm. Hardwood cuttings will be collected after leaf fall, and the number of accessions will depend on the availability of sufficient propagating wood. The long-term priority is to develop ideal pear rootstocks conferring dwarfing with desirable horticultural traits (e.g., cold hardy, fire blight resistant, excellent root anchorage) that are suited for high-density pear production.

Objective 5: Evaluate B × A and B × C selections

There are 14 unique selections grown in triplicate at the WSU Columbia View orchard. These selections originated from crosses of 'Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice' that were selected by Dr. Dhingra due to their compactness and reduced vigor at WSU Pullman greenhouse. These populations were developed as part of a collaboration between Dhingra and Evans programs in 2012. These trees were budded with 'd'Anjou'. Field measurements of their shoot length, trunk diameter, precocity and yield will be assessed to characterize their growth habit and dwarfing potential with the view of selecting the best individuals to further trial.

RESULTS AND DISCUSSION

Objective 1: Develop seedling populations to produce new rootstocks

Previously, four *Pyrus* seedling populations segregating for vigor, precocity and other horticultural traits were established at the WSU Columbia View orchard, Orondo, WA. These seedlings were budded with a standard scion variety, 'd'Anjou' in 2018. Approximately 100 seedlings that failed to bud were rebudded in fall 2019. In the event of incompatibility, an alternative scion (e.g., 'Bartlett') would be considered.

In spring 2019, rootstock branch angle measurements of over 600 seedlings (five branch angles per seedling) were collected. For seedlings with scion bud initiation and growth, the rootstock portion above the graft union was pruned. However, there was some variability in the timing of scion bud initiation, resulting in some rootstocks being cut back at different times.

All seedlings will be maintained in the field for the remainder of the project, where annual shoot length, trunk diameter and scion branch angles (and precocity, if relevant) will be evaluated as a measure of vigor. Given the variable timing of rootstocks being pruned in spring 2019, an additional of pruning will be made in next spring as a correction factor to standardize future phenotyping of scions.

In addition to the existing seedling populations in the field, six new crosses were generated in 2019, resulting in over 5,500 seeds. 1,200 seeds produced from the crosses are currently being vernalized and will be germinated in the TFREC greenhouse in winter/spring 2020. The remaining seeds are held in reserve for future use. Additionally, seedlings (from 2017 crosses) that were germinated in winter 2019 will be planted at the WSU Columbia View orchard in summer 2020.

Objective 2: Validate published markers for parent and seedling selection

In spring/summer 2019, fresh young leaves from the rootstock parent germplasm were collected. DNA extraction of the parent set was carried out at WSU Pullman – Dhingra lab. The DNA quality and quantity were verified to meet the threshold needed for future genotyping. These DNAs will be tested with genetic markers associated with dwarf (pear – *PcDw* locus) and dwarfing (apple – *Dw1*, *Dw2* and *Dw3* loci) to determine if there are difference in the genetic copies (i.e., alleles) of these parents.

Objective 3: Conduct marker-trait association for dwarfing-related traits in seedling populations

Similar to the accomplishment in *Objective 2*, fresh young leaves from over 600 seedlings (of the four *Pyrus* seedlings populations) were collected. DNA extraction was conducted at WSU Pullman – Dhingra lab. The DNA quality and quantity were verified to meet the threshold needed for future DNA genotyping/sequencing. Of the > 600 seedling DNAs, 190 were submitted for high-resolution pear genotyping/sequencing array, which was a pear genomic tool previously developed by Dr. David Neale's group ("Development of marker-based breeding technologies"; PR-14-111).

Objective 4: Expand the pear rootstock parent germplasm
The existing rootstock breeding program was supplemented with several diverse *Pyrus* seedlings collected from USDA-ARS, Corvallis to replace a few *Pyrus* parents that died due to fire blight disease.

Objective 5: Evaluate B × A and B × C selections
The 14 unique selections grown in triplicate are being maintained at the WSU Columbia View orchard. Growth was good this year and phenotyping will commence this winter.

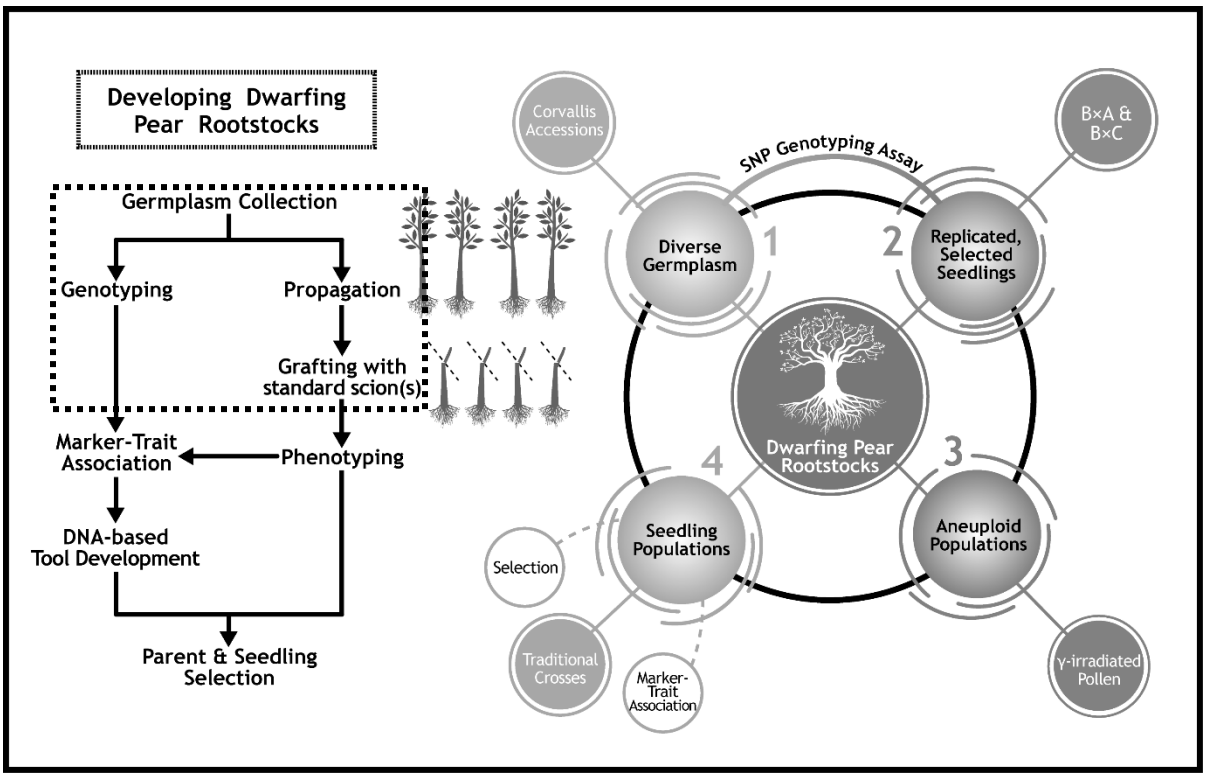

Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. Current accomplishments, highlighted within the dotted box, include (a) initial DNA genotyping/sequencing, (b) propagation of rootstock seedlings with a standard commercial scion variety, and (c) supplementing the existing rootstock parent germplasm with additional *Pyrus* seedlings from USDA-ARS, Corvallis.

OUTREACH

- Soon Li Teh presented "Pear Rootstock Breeding Program" at the WSU Sunrise Research Farm Extension Field Day at Rock Island, WA on August 7, 2019.
- Soon Li Teh presented "Initiating Pear Rootstock Breeding at Washington State University" at the 2019 Annual Meeting for National Association of Plant Breeders (NAPB) at Pine Mountain, GA on August 25 – 29, 2019.
- The WSU pear rootstock breeding program was featured as a Good Fruit Grower article, "Rooting out Solutions for Pear Growers" on September 2019 Issue (https://www.goodfruit.com/rooting-out-solutions-for-pear-growers/).
- Soon Li Teh and graduate student, Zara York presented an overview of pear rootstock breeding at the WSU Tree Fruit Breeding 101 – Extension Field event at Orondo, WA on October 24, 2019.
- Amit Dhingra visited Fowler Nurseries, Sierra Gold Nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in November 2019.
- Amit Dhingra presented a seminar at Pairwise Inc. in North Carolina regarding pear genomics and rootstock breeding in September 2019.
- Zara York presented "Advancing genetic resources for pear rootstock breeding" Research News Flash talk at the Washington Horticultural Association Show, Wenatchee, WA in December 2019.
- Amit Dhingra presented on pear rootstock research in the Genomic Advances in fruit and vegetable Breeding workshop at the annual Plant and Animal Genome conference at San Diego, CA in January 2020.

FINAL PROJECT REPORT

Project Title: Integrated fruit production for pears

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Cooperators: None

Other Funding Sources: None

Total Project Funding: $352,709

Item	2016	2017	2018	2019
WTFRC expenses	0	0	0	0
Salaries[1]	63,597	75,054	78,056	0
Benefits[2]	21,932	26,250	27,300	0
Wages[3]	6,240	6,490	6,749	0
Benefits[4]	626	651	677	0
Equipment	0	0	0	0
Supplies[5]	4,000	4,000	4,000	0
Travel[6]	3,529	3,529	3,529	0
Miscellaneous	0	0	0	0
Plot Fees[7]	5,500	5,500	5,500	0
Total	105,424	121,474	125,811	0

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.*
5. *Evaluate pesticide efficacy for specific pesticide and pest issues.*
6. *Communicate project results as they become available.*

Significant Findings:

- Two applications of Surround CF applied at 50lb/acre, one at delayed dormant and one at popcorn stage, controlled psylla colonization equally or slightly better than a conventional program (Surround + Malathion at delayed dormant and a broad-spectrum tank mix without Surround at popcorn stage).
- Reflective plastic mulch laid in weed strips showed impressive control of psylla pre-bloom, equal or better than conventional programs. This is the first account of this method for control of pear psylla.
- Surround WP suppressed psylla adults and eggs more than other particle films and olfactory repellents.
- Surround WP applied at 100 lb/acre in fall provided acceptable control of psylla the following spring, similar to a March spray at the conventional rate of 50 lb/acre. Fall application worked best when applied after leaf drop (late October or early November).
- A transitional soft program using only particle films, organic materials, and selective IGRs provided acceptable suppression of psylla, but psylla densities and were higher than in conventional blocks that used broad spectrum insecticides. Tree-washing via overhead sprinklers used in the second year of this test reduced psylla injury dramatically. Soft blocks had 50–60% greater nymph densities than conventional blocks, but there was similar (low) fruit russet with just three washes during the summer: late-July, mid- and late August; 6 hours/wash; and 70 gallons/minute/acre.
- FujiMite and Agri-Mek were acutely toxic to larvae and adult females of the predatory mite *Galendromus occidentalis*. FujiMite also completely prevented egg hatch, while Agri-Mek did not affect egg hatch. Acramite was not harmful to any life stage.
- Delegate, Bexar, and Assail were the most toxic materials to psylla overall, resulting in greater than 80% mortality to all life-stage in most bioassays. Softer products such as Cinnerate, Aza-Direct, Neemix, Esteem, Ultor, Centaur, and Dimilin suppressed psylla in the field without harming natural enemies.
- Experiments including bioassays and field trials are available to the public on WSU Tree Fruit website under the Pear IPM section http://treefruit.wsu.edu/crop-protection/pear-ipm/. Updates are also reported in the Fruit Matters Newsletter and via the Pear IPM email listserv (40 members).

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

1A. IPM Demonstration Blocks. Methods. In 2016 and 2017, an unreplicated demonstration experiment was conducted to examine soft conventional (herein called 'soft') vs. grower standard programs (herein called 'conventional') at Sunrise Orchard in Rock Island, WA. A 4-acre plot of 12-year-old trees (Bartlett and Anjou) was divided half, and each side followed a program written by a group of crop advisors. One side received a standard conventional program without restrictions while

the other was restricted to selective materials including oil, Surround, neem products, synthetic insect growth regulators (IGRs) (excluding Rimon), lime sulfur, Vendex, Envidor, Cyd-X, Intrepid, and Altacor. In 2017, an overhead honeydew washing system (separate from the under-tree irrigation system) was installed and used to dissolve and remove psylla honeydew only in the soft plot (explained more in Obj. 3). All insects and mites were counted weekly for both years using various techniques: tray taps, bud collections, leaf collection, earwig traps, and sticky traps with plant volatile lures. Fruit were rated at the end of the season for various injuries, although psylla-induced russet was the primary measurement.

Results and Discussion. In 2016, psylla adults remained more abundant in soft plots than conventional plots for most of the season, then evened by mid-August. Psylla nymph counts were similar for most of the season, but a large spike occurred in July in the soft plot, and fruit injury was greater in the soft plot than the conventional plot. Approximately 50% of fruit in the soft plot had minor honeydew marking (1-10% of surface marked, potentially resulting in a downgrade) and ca. 10% had significant marking (10-50%of surface marked, likely resulting in a downgrade or cull), whereas in the conventional plot 20% of fruit had minor marking and 5% had significant marking. Most natural enemies were less abundant in the conventional plot with the exception of lacewings and syrphids, which were similarly abundant between plots. In 2017, similar trends were observed for densities of each psylla life stage and of natural enemies (i.e., higher in the soft plot), however honeydew marking was similar between plots year. In both plots, less than 5% of fruit had any honeydew marking. In addition to both spray programs being better attended, the honeydew washes in the soft plot likely helped even out the injury levels between soft and conventional blocks. Soft plots were washed three times: late-July, early August, and late August.

Fig. 1. Reflective mulch plot, Sunrise Orchard

In summary, soft programs require diligence to keep psylla populations low. Additionally, it can take multiple years to build up natural enemies to levels that will provide effective biological control. This can make growers wary of IPM approaches or less likely to continue them after a difficult transition year. The addition of tree washing allows growers to tolerate higher psylla densities while biological control is rebuilding and could therefore be a very effective strategy to help ease this transition to softer programs.

1B. ***Pre-bloom Surround and Reflective Mulch.*** Methods. Four psylla management programs were compared in 2018 and 2019: two soft programs, one conventional program, and one untreated check (Table 1). Each program was executed on four replicate plots (16 plots total). Each treatment plot consisted of 40 trees across four rows (two Anjou and

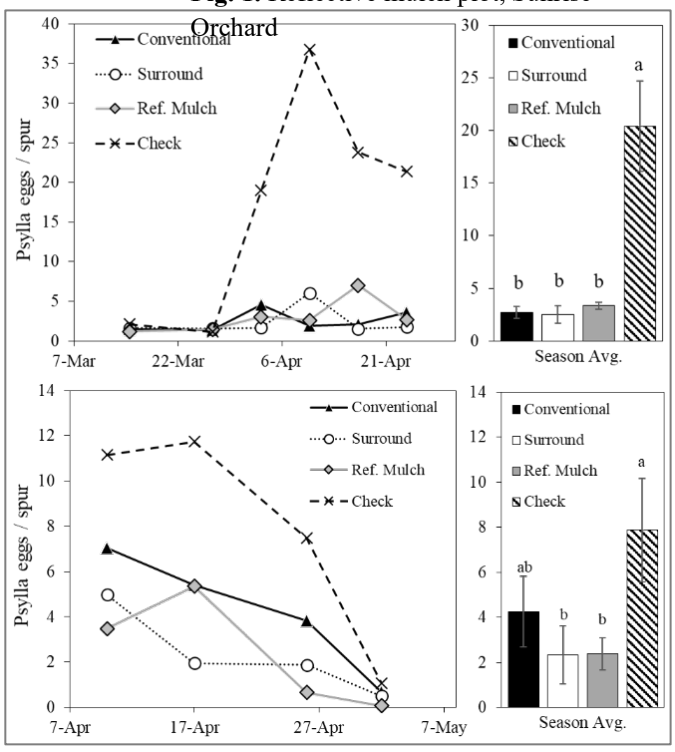

Fig. 2. Psylla egg counts prior to bloom in conventional, reflective mulch, Surround only, and check plots. Counts from six to ten spurs per plot were averaged weekly (left) and cumulative averages calculated (right) in 2018 (top) and 2019 (bottom).

two Bartlett) (Fig. 1);. The conventional program was developed with local fieldmen. The two soft programs only differed from each other in pre-bloom management (one used Surround, the other reflective mulch), and were followed with regular sprays of IGRs and/or organic insecticides (Table 1). Measurements were taken weekly in these blocks on psylla life stages, spider mites, natural enemies, fruit-set, honeydew residues on leaves, and fruit injury.

Results and Discussion. Psylla pressure in the orchard was high in both 2018 and 2019, as shown by high egg counts in the check plots (Fig. 2). The Surround and reflective mulch early season programs provided equal control of the first generation of psylla eggs and nymphs as the conventional program in both years (Fig. 2). Following these early season management strategies with soft spray programs through the summer resulted in similar suppression of psylla eggs and nymphs throughout the summer. Injury levels were significantly lower in both soft treatments than in the conventional treatment in 2018, but were similar in 2019 (data not shown). Natural enemies were present at moderate densities for all treatments including the check. Lack of differences in natural enemies may be due to smaller plot sizes and untreated boarders, allowing natural enemies to redistribute quickly.

Table 1. Products, rates and timings for pear psylla management programs (mite and codling moth management was the same among all treatments and is not shown) implemented at Sunrise Orchard in 2018 and 2019.

	Conventional (2018/2019)*	**Surround (2018/2019)**	**Reflective Mulch (2018/2019)**	**Check**
Delayed Dormant	Malathion[1, 2] Surround CF 50lb 440 IAP oil 4%	Surround CF 50 lb 440 IAP oil 4%	(reflective mulch installed)	–
Popcorn	Assail / Bexar Rimon / Rimon – / Ultor	Surround CF 50 lb	(reflective mulch)	–
Petal fall	Actara / Assail Rimon / Rimon – / Ultor	– / Surround CF – / Cinnerate 50 fl oz	(mulch removed) – / Surround CF – / Cinnerate 50 fl oz	–
Petal fall + 14	Ultor 1.25L / Delegate Rimon / FujiMite Exirel / Microna	Surround WP / Celite – / Cinnerate – / Neemix	Surround WP / Celite – / Cinnerate – / Neemix	–
Early June	Delegate / Assail	Surround WP / Celite Aza–Direct / Cinnerate Esteem / Neemix	Surround WP / Celite Aza–Direct / Cinnerate Esteem / Neemix	–
Mid June	–	Aza–Direct / – Centaur / –	Aza–Direct / – Centaur / –	–
Late June	Actara	Aza–Direct / – Centaur / –	Aza–Direct / – Centaur / –	–
Mid July	Delegate / Delegate	Aza–Direct / –	Aza–Direct / –	–

*Slashes (/) indicate that a treatment changed from year to year. If there is no slash, no change occurred. Dashes (–) mean that no spray happened. For example: "– / Ultor" means Ultor was sprayed at that timing in 2019 only.

[1] If rate is not listed, the product was sprayed at the highest labeled rate for that pest and crop.

[2] All sprays included 0.5% IAP 440 oil unless otherwise listed (i.e. oil 4%)

In summary, these experiments suggest that two Surround sprays pre-bloom or the use of reflective mulch can provide equal control of pear psylla as standard conventional programs in Central Washington, which use only one Surround spray and many broad spectrum materials. This experiment demonstrated an alternative approach to pre-bloom psylla management that has been used in the past: going soft early. The results show that suppressive techniques involving repellents can sufficiently manage the first generation of psylla and provide a foundation for a soft summer program. This can save growers money in pesticide costs and lower risk of biological control disruption (although we did not see major differences in natural enemies in our experiments). This

also provides an alternative approach for the future, since broad-spectrum materials will likely continue to be slowly phased out of use. Finally, reflective ground covers have been shown to increase flowering and yield in the lower canopy of pear trees (and in the tree overall), adding a potentially significant bonus to using this method, considering declining pear yields seen over the last decade.

1C. ***Repellent Sprays-Greenhouse***. Methods. In late winter of 2017, various materials were evaluated for their ability to repel pear psylla adults when sprayed on pear trees. The experiment used potted Anjou trees on OHxF rootstocks in a greenhouse cage. Materials were applied to individual trees, ca. 2.5 ft tall about 2 weeks prior to bud break. Trees were sprayed with hand-held spray bottles until completely wet, ca. 50 ml (1.7 fl oz) per tree. After treatments dried, trees were placed in a 4×4×16 ft mesh cage in a greenhouse. Adult psylla were collected from pear trees at the TFREC in Wenatchee, and 1,200 were released into the cage. Six days after release, the trees were visually inspected for adults and eggs.

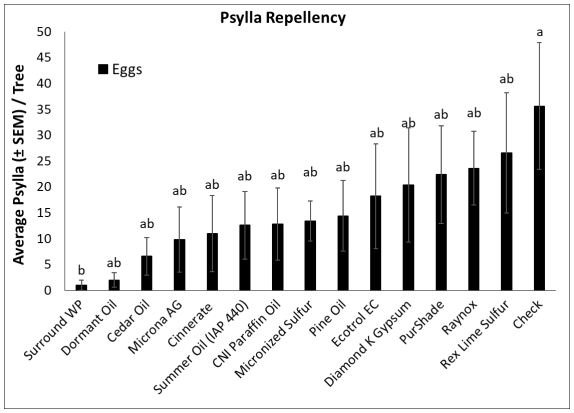

Fig. 3. Average psylla eggs on potted trees treated with various repellents in a greenhouse cage choice test.

Results and Discussion. Due to high variability, there were few statistically significant differences between treatments. The Surround WP treatment had the clearest effect, resulting in the lowest number of eggs (Fig. 3) and adults (not shown). Dormant oil, Microna and cedar oil caused notable but not statistically significant reductions in both eggs and adults.

1D. ***Fall Surround sprays***. Methods**.** Experiments were conducted in the field and near-field to examine the potential for Surround WP applied to pear trees in the fall to control psylla the following spring. This experiment was conducted to address the common issue that higher elevation pear orchards on slopes are difficult to access by tractor sprayer due to snow or wet ground in the early spring, when the first sprays for psylla are necessary. Because Surround WP is formulated with a spreader sticker and is known to be very rainfast, we hypothesized that Surround applied in fall could last through the winter and provide repellency of pear psylla. In the fall of 2017, two-acre blocks on three orchards in Cashmere and Peshastin WA were sprayed with 100 lb of Surround in late October. In 2018, six orchards were studied and another treatment was added: Surround applied before leaf drop in late September. The following spring, a separate block was sprayed with Surround CF at 50b lb/a (standard commercial rate) in March, and another block was left unsprayed (check). Psylla adults were counted via tap counts in the February, March and April of 2018 and 2019, and eggs were counted on spurs in April of 2019 only. At all sampled dates, and immediately before and after sprays, branches were collected from trees to quantify Surround residue decline over the season. Branches were photographed (Fig. 4) and processed in ImageJ software to quantify surface "whiteness" according to mean grey values.

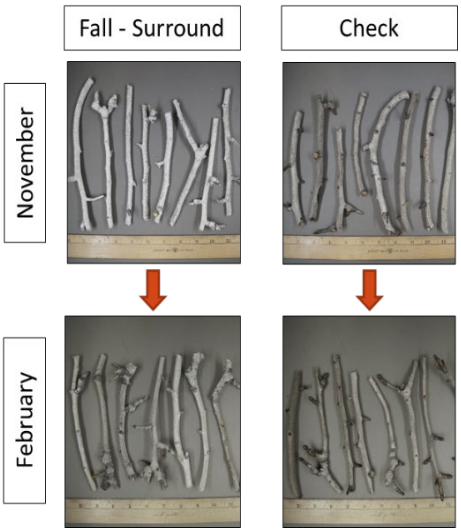

Fig. 4. Photos of cut branches from commercial plots in the November and February; analyzed for Surround residues using Image J.

A corresponding near-field experiment on potted Anjou trees was conducted both years. The trees received the same Surround treatments and timings and were left outside for the winter. In March, additional trees were treated, then all were brought into the greenhouse for a pear psylla choice experiment. One tree from each treatment was placed into a cage (6 cages, 6 replicates). Fifty pear psylla adults were then introduced into each cage. Adults and eggs were counted on each tree about 10 days after introduction.

Results and Discussion. In both years, adult densities were lower in orchard blocks treated with Surround in October than in check blocks. Adult densities were lowest, numerically, in treatments where Surround was applied in March, however the difference was not significant. Surround applied after leaf drop (late October) provided significant reductions in eggs the following spring compared to the check, and similar reductions compared to Surround applied in March (Fig. 5A). Image J analysis of residue decline showed that coverage was better for late fall sprays (October) than early fall sprays (September), and residues in spring from late fall sprays were whiter than those from early fall sprays. The corresponding potted plant bioassay mostly confirmed these findings, except that early fall sprays achieved better control compared with the check (Fig 5B), likely because we were still able to gain good coverage on these small trees despite leaves being present. Surprisingly, the late fall Surround sprays resulted in better control than dormant oil applied just 24 hours prior to adult introduction in the spring. In summary, applying Surround in the fall is a good option for problematic orchard blocks that are hard to access in the spring. The following approaches seem to improve this method: apply after leaf drop, use higher rates of Surround (100 lb/a), use Surround WP because it has a spreader-sticker in the formulation, consider adding an additional spreader-sticker such as NuFilm.

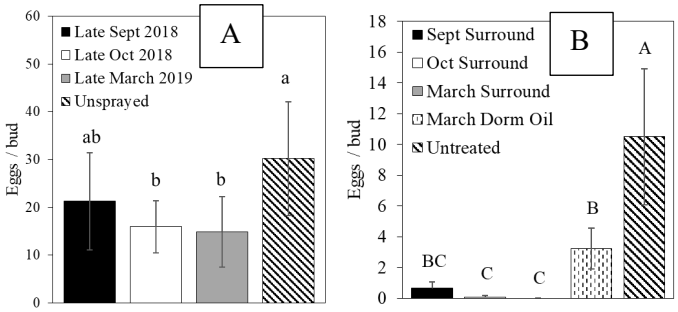

Fig. 5. Average eggs per bud among trees with Surround applied in the fall or spring, and check plots in commercial orchards (A) and a potted tree choice test (B)

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. An experiment to examine the effects of the IGR Esteem (pyriproxyfen) applied after harvest was initiated in fall of 2016. The experiment was planned to involve spraying orchard pear trees under field cages, followed by measurement of mortality and/or fecundity of adult psylla the following spring. Unfortunately, a windstorm broke many of the cages, terminating the experiment. Additional attempts to measure the potential for postharvest IGR sprays were not pursued, and instead data on this topic was collected with literature reviews and discussions with researchers (D. Horton, USDA) who studied this concept in the past. Based on this, we concluded that this method is unlikely to provide meaningful control. A primary indicator is that the product, fenoxycarb, which has a similar mode of action to Esteem and was the basis of this work due to its and ability to break adults psylla's reproductive diapause, did

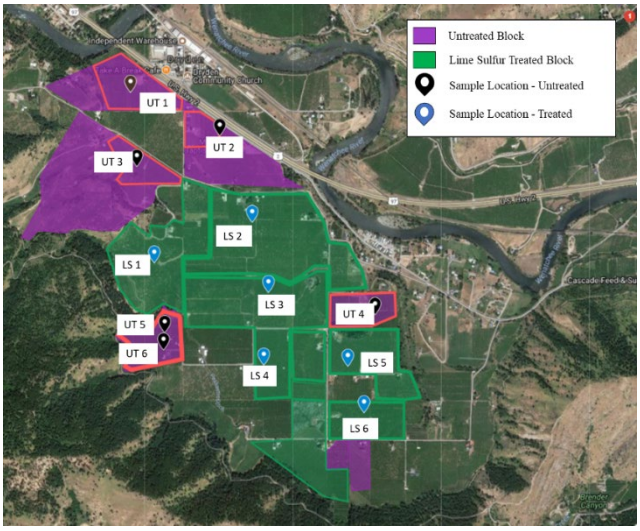

Fig. 6. Pine Flats, WA area-wide lime sulfur map.

not provide control when applied to adults post-harvest despite disrupting the psyllids' reproductive diapause (Krysan 1990).

An attempt at areawide post-harvest sprays was attempted in the fall of 2016 using lime sulfur. About 60% of the orchard acres in the Pine Flats region of Dryden, WA (area south of Hwy. 2, Fig. 6.) received lime sulfur applications following harvest. Adults were sampled in both sprayed and unsprayed locations the following spring. Six treated and untreated sites (12 total) for were sampled via tray taps (10/site) in March 2017.

Results and Discussion. No differences were seen among treated and untreated plots in the Pine Flats sites the following spring (2017). Plots had high psylla adult densities throughout, approximately 35–60 per tray in both treatments. While this does not mean postharvest sprays cannot be made effective, it does suggest that area-wide control requires true area-wide coverage. Because psylla undergo their largest redistribution event in the late mid to late fall, post-harvest sprays must cover enough acreage to reduce the area-wide populations.

Obj. 3 Evaluate tree washing techniques for control of pear psylla and mites.

Methods. In 2017, An overhead sprinkler tree washing system was established in half of the 4-acre pear block at Sunrise Orchard. This system is separate from the under-tree micro-sprinkler irrigation system, and it was not used for irrigation at any point. The system delivers 70 gallons of water per minute per acre using 50 Rain Bird sprinkler heads (R2000) on PVC risers per acre. The system was used in Obj. 1's 2017 demonstration block experiment. The system was run for six hours during the day three times during the season (27 July, 9 August and 29 August). For each wash, a non-ionic surfactant, Regulaid, was injected into the system mid-way through the cycle at 1 pint per acre. The NIS passes through the system in about 5 minutes.

Results and Discussion. In 2016 and 2017, the soft spray programs resulted in higher psylla densities than the conventional program. In 2016, injury resulting from psylla was also greater in soft plots, commensurate with psylla densities. In 2017, following the integration of tree washing, injury was not different among the programs (Fig. 6), suggesting that tree washing removed injurious honeydew. These results also demonstrate how tree-washing systems could be the missing link to help growers adopt soft or organic programs. Transitioning to softer programs has been historically difficult for pear growers due to slow recolonization of natural enemies, often leaving growers with little help from biological control in initial years. Because tree washing increases the tolerance threshold for psylla, higher psylla densities that occur in transition years can be mitigated by simply removing their honeydew. While the ability for honeydew washing to reduce injury has been shown by past research using handgun methods (Brunner and Burts 1981), this is to our knowledge the first demonstration of successful washing via overhead sprinklers.

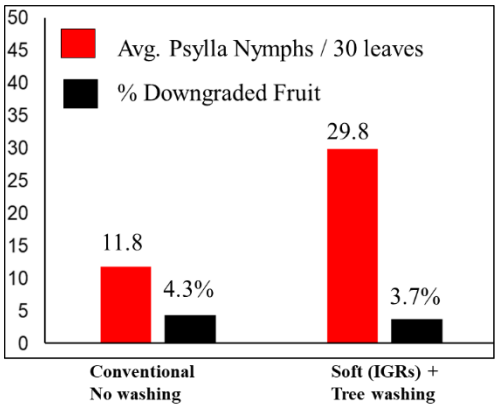

Fig. 7. Psylla nymphs and injury in a demonstration conventional plot without tree washing vs. soft plot with washing.

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 diameter with ample prey in the form of twospotted spider mite eggs and larvae. Fifty arenas per acaricide 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 there was poor survival (15%) in the Agri-Mek treatment (Fig. 8). Net fecundity and production of live larvae were greatly reduced in these two treatments. 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. Ovicidal miticides (Zeal, Envidor, Onager) will be tested in a separate test in the future.

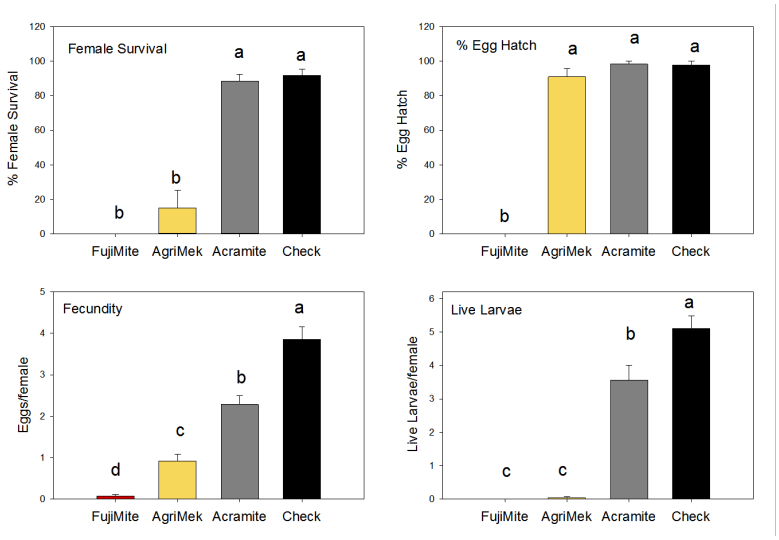

Fig. 8. Various Nontarget effects from miticide/insecticides on the predatory mite *G. occidentalis.*

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

Methods. Many field spray trials and laboratory bioassays for chemical control of pear psylla and mites were completed over the course of this project. Slide dip and Potter spray tower methods were used to measure acute mortality and potted plants or excised shoots were used for longer-term studies. Spray trials were conducted in small, replicated field plots (3–4 trees/plot; four replicates) with either single sprays or multiple, program integrated, sprays. All bioassays with reliable outcomes (low check mortality and, if included, high positive control mortality) have been made available to the public on the WSU Tree Fruit Pear IPM webpage: http://treefruit.wsu.edu/crop-protection/pear-ipm/.

Results and Discussion. Seven field trials examining efficacy of specific insecticides and particle films were completed. For conventional products targeting psylla adults, such as Bexar and Malathion, our primary finding was that adulticide sprays should be applied at or before delayed dormant. Adulticides sprayed from tight cluster to petal fall were usually past the adult peak, so adults were naturally declining, and many eggs were already laid. For post-blooms sprays, our trials suggest that insecticides with high psylla toxicity, such as Bexar, Assail, Minecto Pro, and Delegate, can be sprayed less frequently if timed with appropriate life stages. However, these materials do have measurable impacts on natural enemies, which can lead to increased populations of psylla in following generations. Softer materials such as Cinnerate, neem (Aza-Direct and Neemix), IGRs, and particle films, require more frequent applications, especially from petal fall into June; but this allows for development of natural enemies which eventually take over, usually by early July. For small plot experiments, it can be difficult to obtain clear results due to migration potential of psylla; however, some of the most clear reductions in psylla we observed came from short interval sprays of soft materials, and these reductions occurred without commensurate reductions in natural enemies (Fig. 9). Fig. 9 shows two trials testing soft materials, and all treatments resulted in significant suppression of pear psylla nymphs (left graphs). The only treatment in both trials that resulted in a significant

natural enemy reduction was the conventional standard positive control in spray trial A (red arrow in right graph, Fig. 9A).

Lab bioassay results are shown in Table 2. Due to limited space, only the product names are shown and rates are only displayed if multiple rates were used within the assay, otherwise, either the high field rate or recommended field rate was used. Differences in percentage mortality were determined using either Tukey HSD or Fisher's LSD tests. Treatments not sharing a letter represent significant differences in percentage mortality.

Objective 6. *Communicate project results as they become available.*

Experiments, including bioassays and field trials, were posted to the WSU Tree Fruit website under the Pear IPM section http://treefruit.wsu.edu/crop-protection/pear-ipm/, usually within one month of completion. Updates were also reported in the Fruit Matters Newsletter and via the Pear IPM email listserv (40 members). Over the course of the project the postdoc, Louis Nottingham, delivered 49 extension presentations, attended numerous grower meetings, field days, research and reviews in addition to other forms of industry engagement.

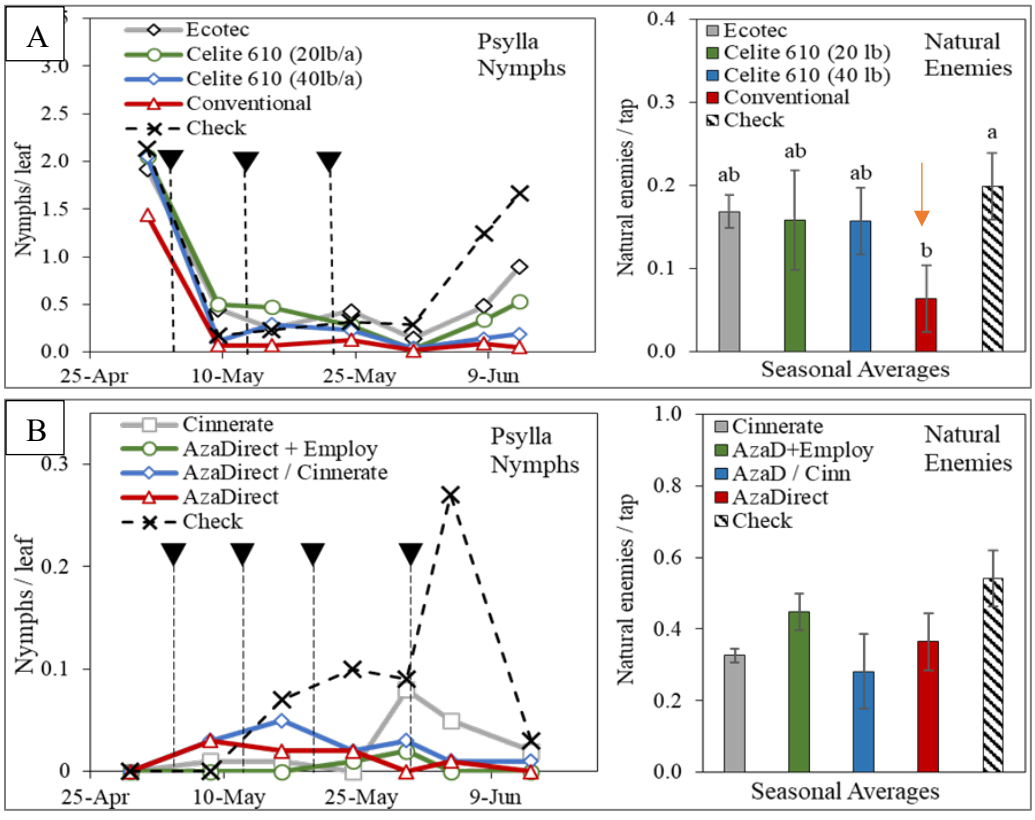

Fig. 9. Two spray trials (A and B) examining soft material programs. Left graphs show resulting psylla nymphs by date and sprays (vertical lines). Right graphs display seasonal natural enemy averages.

Table 2. Percentage mortality for pear psylla life stages (A–C) and pear rust mite (D) from lab bioassays.

A. Nymphs, Pear Psylla					
Instars 1-3, Leaf Dip, 2017		**Instars 3-5,** Leaf Dip, 2017		**Instars 1- 3,** Leaf Dip, 2018	
Pesticide	**% Mortality**	**Pesticide**	**% Mortality**	**Pesticide**	**% Mortality**
Delegate 25WG	100.0 a	Bexar 1.31SC	93.3 a	Bexar 1.31SC	86.67 a
Assail 70WP	100.0 a	Assail 70WP	90.0 a	Nexter 75WP	66.93 ab
Admire Pro 4.6	100.0 a	Agri-Mek 0.7SC	90.0 a	Actara 25WG	59.79 ab
Nexter 75WSP	94.0 ab	Delegate 25WG	86.2 a	FujiMite 0.4SC	28.33 bc
Actara 25WDG	92.0 ab	Actara 25WDG	85.0 a	Vendex 50WP	13.33 c
Bexar 1.31SC	86.3 ab	Exirel 0.83SE	69.3 abs	Nealta 1.67L	0.00 c
Exirel 0.83SE	77.0 abc	Nexter 75WSP	67.6 ab	Check	0.00 c
FujiMite SC	75.3 abc	Admire Pro 4.6	56.0 abcd		
Agri-Mek 0.7SC	62.8 bcd	Altacor 35WDG	17.5 bcd		
Altacor 35WDG	41.8 cd	FujiMite SC	14.0 d		
Check	16.5 d	Check	15.0 cd		

B. Adults, Pear Psylla					
Potter Spray Tower, 2017		**Slide Dip, 2017**		**Potter Spray Tower, 2018**	
Pesticide	**% Mortality**	**Pesticide**	**% Mortality**	**Pesticide**	**% Mortality**
Bexar 1.31SC	100.0 a	Bexar 1.31SC	100.0 a	IAP 440 Oil 4%	100.0
Delegate 25WG	99.0 a	Delegate 25WG	99.0 a	Malathion 5EC	100.0 a
Malathion 5EC	97.0 ab	Malathion 5EC	97.0 ab	Dimethoate 4EC	100.0 a
Lorsban 4EC	94.1 abc	Lorsban 4EC	94.1 abc	Assail 70WP	92.0 ab
Cobalt Adv + PBO	84.0 bcd	Cobalt Adv + PBO	84.0 bcd	Lime Sulfur	78.0 b
Dimilin 2L	79.2 dc	Dimilin 2L	79.2 dc	Wet. Sulfur 15lb	20.0 c
Danitol 2.4EC+ PBO	65.0 d	Danitol 2.4EC+ PBO	65.0 d	Check	8.0 c
Warrior II + PBO	34.0 e	Warrior II + PBO	34.0 e		
Exirel 0.83SE	32.0 e	Exirel 0.83SE	32.0 e		
Check	30.0 e	Check	30.0 e		

C. Eggs, Pear Psylla			
Leaf Dip, 2017		**Potted tree spray, 2017**	
Pesticide	**% Mort.**	**Pesticide**	**% Mort.**
Assail 30SG	87.0 a	Bexar 1.31SC	89.6 b
Bexar 1.31SC	55.6 ab	Assail 70WP	89.9 b
Agri-Mek 0.7SC	18.5 bc	Envidor 2SC	25.1 a
Microna AG	16.7 bc	Ultor 1.25L	25.4 a
Rimon .83EC	15.3 bc	Exirel 0.83SE	23.2 a
FujiMite 0.42SC	14.2 bc	Neemix 4.5	0.9 a
Exirel SE	10.3 c	Centaur 70WDG	11.3 a
Celite 610	9.3 c	Esteem 35WP	32.8 a
Centaur 70WDG	9.2 c	Rimon 0.83EC	0.0 a
Dimilin 2L	7.9 c	Dimilin 2L	9.1 a
Manzate 75DF	4.5 c	Intrepid 2F	38.2 a
Esteem 35WP	3.5 c	Check	13.8 a

D. Pear Rust Mite	
Excised shoots, 2017	
Pesticide	**% Mort.**
Nexter 75WP	98.67 a
Cinnerate 60 floz	95.56 a
Cinnerate 25 fl oz	85.26 ab
Neemix 4.5	67.26 abc
Entrust SC	32.51 cd
Pyganic	53.68 bcd
Azera	51.38 bcd
TetraCURB	88.89 ab
SucraShield	67.84 abc
Summer Oil	84.93 ab
Check	12.33 d

Cinnerate	2.9 c
Ultor 1.25L	2.2 c
Envidor 2SC	1.7 c
Check	4.8 c

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Brunner, J. F., and E. C. Burts. 1981. Potential of Tree Washes as a Management Tactic Against the Pear Psylla. J. Econ. Entomol. 74: 71-74.

Krysan, J. L. 1990. Fenoxycarb and Diapause: A Possible Method of Control for Pear Psylla (Homoptera: Psyllidae). J. Econ. Entomol. 83: 293-299.

Executive summary

Title: Integrated fruit production for pears

Keywords: Pear Psylla, *Cacopsylla pyricola*, cultural control, biological control, chemical control, reflective mulch, particle films, insect growth regulators

Abstract. The goal of this project was to test multiple strategies and contributing factors to improve IPM programs for pear pests, mainly pear psylla. This project examined cultural controls such as reflective mulches and particle films, chemical insecticide efficacy, non-target effects of insecticides on natural enemies and full season programs. Additionally, a key element of this project was to provide research findings to industry stakeholders in real-time. This was accomplished through the development and updating of the WSU Tree Fruit Pear IPM website, writing newsletter articles in the WSU Extension newsletter Fruit Matters, and by delivering presentations at over 45 stakeholder events.

Summary. Pear psylla is a secondary pest, meaning it can be controlled by natural enemies when undisrupted. However, psylla emerge and colonize orchards in late winter, long before natural enemies. Therefore, it is important to strike a balance between active management and conservation to prevent early season injury without causing late season outbreaks. This is the fundamental principal behind IPM, or 'soft' management. In this project, we developed IPM techniques, and eventually programs, that integrate cultural controls, selective IGRs and organic materials to provide similar control of pear psylla as conventional programs, with fewer side effects. Although soft programs are likely to have elevated psylla populations in initial years due to the time it takes for natural enemies to recolonize, we worked to develop economical strategies to ease this transition, such as using repellents pre-bloom followed by shorter interval sprays of soft materials. Additionally, high water volume tree washing techniques resulted in major improvements to transitioning soft programs by simply washing away excess honeydew.

The use of reflective plastic mulch to repel colonizing psylla was a key novel finding of this project. Early season psylla management has historically been done with multiple broad spectrum sprays which contribute to the lack of natural enemies in conventional orchards. Reflective mulch alone provided equal control to broad spectrum sprays, and past research has shown that it significantly increases pear yields. More work is necessary to optimize this strategy for commercial use and examine if it is economically practical.

Kaolin clay (Surround) has been a common dormant or delayed dormant spray for pear psylla for nearly twenty years. Our examinations of this product provide practical use strategies which have helped to reestablish its importance in commercial programs. A key finding of this project was the importance of multiple pre-bloom kaolin applications. In doing so, we eliminated the need for any additional insecticides prior to bloom. This finding has had a major impact on the industry, taking kaolin from a program additive to a mainstay.

Going forward, a logical direction is to develop management programs that time sprays and cultural strategies to psylla life-stages. Degree-day models have made incredible improvements to management of other tree fruit pests and diseases, and the same is possible for pear psylla. Currently, psylla sprays occur on a 12-18 day schedule, which leads to high costs and wasted sprays. With the gained knowledge from this project about control techniques and the recently developed psylla degree-day model, it is now possible to put these pieces together.

FINAL PROJECT REPORT **YEAR: 3 (of 2+1yr NCE)**
WTFRC Project Number: PR-17-104

Project Title: Functional genomics of 'd'Anjou' pear fruit quality and maturity

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Cooperators: Stefano Musacchi & Sara Serra (WSU-TFREC), David Rudell & Jim Mattheis (USDA-ARS), Claude dePamphilis (PennState)

Total Project Request: Year 1: $52,707 **Year 2:** $33,488 Year 3: NA

Other funding sources: USDA-ARS technician salary and benefits - $31,734

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

Item	2017	2018
Wages[1]	$12,500	$12,500
Equipment[2]	$1,980	NA
Supplies	$8,407	$5,483
Miscellaneous[3]	$29,820	$15,505
Total	$52,707	$33,488

Footnotes:
[1] Cooperative Agreement to Penn State for data processing and data analysis
[2] Service contract for CLC genomics workbench support
[3] Illumina sequencing & library prep at Penn State Genomics Core via Cooperative Agreement

OBJECTIVES

Long term objective: Develop a detailed understanding of the genetics of pear fruit maturation, ripening and quality towards enhanced diagnostics, therapeutics, and production practices, focusing on postharvest technology.

Specific objectives:
1) **Identify gene activity** correlated with fruit quality and maturity as it relates to on-tree fruit position
2) **Discover genes** in 'd'Anjou' pear for comparative genomics with 'Bartlettt' pear
3) **Generate a list of potential biomarkers** for use in research and fruit production

SIGNIFICANT FINDINGS

Progress on specific objectives:

- **Objective 1) Complete –** A full analysis of gene activity that relates to on tree fruit position is complete, including analyses leveraging the new 'd'Anjou' genome
- **Objective 2) Exceeded –** Instead of building fruit specific models for genes using 'd'Anjou' pear fruit gene activity data, we sequenced the entire genome of 'd'Anjou' pear capturing virtually all of the genes in this cultivar
- **Objective 3) Complete – We have a candidate list of genes for additional maturity work in European pear, and the 'd'Anjou' genome provides the opportunity to discover additional genes and gene forms that are related to cultivar differences between 'Bartlett' and 'd'Anjou'**

Other important findings:

- **Synergy with "**Enhancing reference genomes for cross-cultivar functional genomics" provides a foundation to fully explore genetic differences between 'Bartlett' and 'd'Anjou' pear with fully annotated *de novo* 'd'Anjou' pear genome
- **Massive gene activity changes occur during storage**
- **Relatively few gene activity signatures distinguish fruit from internal canopy positions vs. external canopy positions**
- **Genes do not act alone – co-expression and differential expression implicate groups of genes**

RESULTS & DISCUSSION

Recap of project proposal justification

In European pear, tens of thousands of genes are active when fruit are harvested. The activity of these genes changes as fruit matures, in response to postharvest conditions, and is different in various fruit tissues. By examining these gene activity signatures we gain insight into poorly understood biological processes that influence pear fruit ripening and quality. Additionally, gene activity that is correlated with fruit maturation and ripening may provide a tool to finely monitor fruit. In the context of research, this could facilitate experiments that target manipulation of fruit maturity and ripening by monitoring gene signals in responses to various postharvest conditions. Also, because changes in gene activity often precede otherwise undetectable physiological changes in plant tissues these changes can be potentially used to predict future fruit quality, thereby providing tools to enhance fruit quality

management for the industry. The approach for this project included functional genomics which aims to use very large gene activity data sets (100s of millions of measurements) to learn about complex biological processes in plants. The specific method, called **RNA-Seq**, is the method of choice because it can be used to monitor the activity of *all* genes simultaneously.

A critical requirement for RNA-Seq is the availability of a reference genome. The reference genome contains the genes that are needed to interpret the massive gene activity data sets generated by RNA-Seq. When this work was proposed, the only available reference genome for European pear was for 'Bartlett.' In the past 3 years, genomes for various *Pyrus* species/cultivars (including a dwarfing rootstock and 'Bartlett' v2.0) have been published (https://www.rosaceae.org/species/pyrus/all) reflecting the increased accessibility of genomes for applied plant research. Towards exploring the effect of using genetically mismatched genomes for RNA-Seq, this project was synergistic with Honaas' Tech project "Enhancing reference genomes for cross-cultivar functional genomics (TR-17-100)." That project:

> **1)** Aimed to improve the reference genomes for cross-cultivar RNA-Seq – for instance interpreting 'd'Anjou' gene activity data with the genetically mismatched 'Bartlett' genome
> **2)** Contributed data that enhanced gene discovery in 'd'Anjou' to facilitate comparative genomics in European pear.

The synergy with Honaas' tech project directly impacted each objective in this project – the 'd'Anjou genome enhances gene activity analyses, facilitates gene discovery, and expands our potential gene candidate list in our search for genes that influence European pear fruit quality in the postharvest period.

This project also had significant cooperation with the WTFRC project "Improving Quality and Maturity Consistency of 'd'Anjou'" that was led by Stefano Musacchi. Primarily, we were given access to cryopreserved fruit samples (for gene activity analysis) and associated fruit quality data that could be used to guide the gene activity analysis. We also used data from that project to guide sample selection for gene activity analysis - **Musacchi's project showed that 'd'Anjou' pear fruit from external canopy positions ripened differently compared to fruit from internal canopy positions.** We reasoned that targeting a very fine contrast – pear fruit harvested from the outer canopy vs. the inner canopy that also fell into the same DA meter class – would provide the best opportunity to find gene activity signatures that could differentiate the fruit. This project, by drawing on other WTFRC funded work, has provided a foundation for functional genomics in 'd'Anjou' pear towards development of biomarkers to predict future fruit quality, especially with regard to ripening capacity.

RNA-Seq: the first look at global gene activity measurements in 'd'Anjou' pear

Our first step was to extract the cryopreserved samples from Musacchi's project (see above) using the protocol developed in Honaas' lab (https://doi.org/10.1186/s13104-017-2564-2) specifically for the recalcitrant tissues of long-stored pears. We chose to analyze all 5 biological replicates from Musacchi's banked tissues to maximize our statistical power for identification of significantly differential gene activity signatures between fruit from the inner vs. outer canopy. However, the 'Bartlett' v1.0 genome was not an ideal reference due to the low rate of data inclusion, most likely due to the genetic differences between the 'Bartlett' genome and 'd'Anjou' gene activity data. Our initial RNA-Seq experiment brought about 56% of the RNA-Seq data into the analysis, similar to published work (https://doi.org/10.3389/fpls.2017.00455). While we did not expect all of the data to be brought into the analysis (for a host of reasons that are beyond the scope of this report), failing to bring nearly half of the data into the analysis certainly results in many false negatives, providing at best an incomplete picture of the biology the experiment attempts to describe.

The story that can be told from the parts of the biological picture we can see must be validated. There are no hard and fast standards for validation, though the research community generally agrees that congruent gene activity estimates from another technology, like quantitative PCR, are acceptable.

However, how to choose genes, and what threshold for congruence is acceptable varies widely. The data from this project was used to refine our published RNA-Seq validation protocol (https://doi.org/10.21273/JASHS04424-18), and results in improved validation results (Figure 1) specifically by targeting genes, and regions of genes, that are highly similar between 'Bartlett' and 'd'Anjou.' This suggests that the genetic differences between the two cultivars are problematic when attempting to estimate gene activity from one using the genome of the other.

Without the 'd'Anjou' genome, we did not have genetically matched reference genes to interpret our RNA-Seq data – but the next best option was to build gene models directly from our RNA-Seq data – an approach called *de novo* transcriptome assembly (for more info see - https://doi.org/10.1371/journal.pone.0146062). This approach has limitations that are inherent to the process that result in fragmented and incomplete gene models – but the models will exactly match the gene activity data and should therefore allow more of it to be assigned than when we use the 'Bartlett' genome. Indeed this was the case as we were able to increase the data inclusion rate by about 15% when using our *de novo* gene models. While this confirmed that genetic differences may be interfering with gene activity measurements, the limitations of using these gene models do not make it a better choice than the 'Barlett' v1.0 genome, albeit for different reasons.

Concurrent with the aforementioned experiments, Honaas' WTFRC Tech project shifted from improving the 'Bartlett' v1.0 reference to generating data to build a 'd'Anjou' genome from scratch. Because the gene activity data we had showed structure that was sufficient to distinguish fruit that ripened differently in the postharvest period (inner canopy vs. outer canopy positions – Figure 2) the additional sequencing budget was used to generate several types of genome data for 'd'Anjou' pear rather than to generate more transcriptome data – this additional data substantially improved the 'd'Anjou' genome assembly (roughly 7 fold).

Gene discovery in 'd'Anjou' pear for comparative genomics

We met objective 2 by gathering a massive gene activity data set (~1.5 billion reads) from pear fruit that was then used to build gene models via *de novo* transcriptome assembly. This approach provided models for genes were active in our pear fruit experimental samples but exclude roughly 1/3 of pear genes that were not active in our samples. By pivoting to whole genome sequencing for gene discovery, we cast a broader net that included a near complete compliment of genes. The annotation, or survey of genes across the genome, showed that the number of genes in our 'd'Anjou' draft genome (45,981) was similar to the draft genome of Bartlett (45,217).

During the last months of this project a second, higher quality 'Bartlett' genome became available and was officially published in Dec 2019 (https://doi.org/10.1093/gigascience/giz138). The gene count in this genome was lower than the draft genomes for 'Bartlett' and 'd'Anjou' at 37,445 due in part to the purge of alleles from the genome - alleles can cause considerable redundancy in gene predictions. If this process were error-free, the gene complement of 'Bartlett' v2.0 would be a perfect subset of 'Bartlett' v1.0, thus we explored the gene content differences between the 3 genomes (Figure 3). We found that the genes in the 'Bartlett' v2.0 genome were not a perfect subset of 'Bartlett' v1.0, and that the cultivars 'Bartlett' and 'd'Anjou' do potentially contain a small number of genes that are cultivar specific. The reasons for the gene content differences might include real genetic differences in the artificially induced double haploid line used for 'Bartlett' v2.0 as well as the bioinformatic filtering of alleles during annotation of the new genome.

Importantly, in addition to finding cultivar specific genes we now have the evidence to survey the genome to find genes that are shared between the cultivars, but have small differences that may help explain cultivar specific traits. In our apple experiments, the analysis of 'Granny Smith' vs. 'Golden Delicious' showed that of the ~5.2 million polymorphisms, nearly 0.5 million occurred in genes. Others (Zhang et al. 2019 - https://doi.org/10.1038/s41467-019-09518-x) found, in apple as well, that >1/3 of apple genes encode altered proteins when comparing cultivars, specifically 'Hanfu' vs. 'Golden Delicious.' Recall that we also surveyed the 'Bartlett' genome for differences with 'd'Anjou'

and found roughly 5.6 million small polymorphisms. We expect the pattern to be similar in pear, which would result in hundreds of thousands of polymorphisms in several thousand genes.

The gene discovery phase of this project is important for two main reasons. First, as the results of Honaas' WTFRC Tech project show, a genetically matched genome is preferred for RNA-Seq data because it can recover gene activity signal that is lost when using a genetically mismatched genome. Second, the ability to survey genomes for even small differences between cultivars of European pear offer the possibility to discover the genetic basis for cultivar differences. For 'd'Anjou' pear, of particular interest are the genetic factors that explain the different chilling requirement for proper ripening compared to 'Bartlett.'

A preliminary list of candidate genes for biomarker development

The primary deliverable of this project is a foundation to discover biomarkers that can be used to predict future fruit quality. Towards that end, we have leveraged the resources from this project, and those from the WTFRC projects "Improving Quality and Maturity Consistency of 'd'Anjou'" led by Stefano Musacchi, and Honaas' Tech project "Enhancing reference genomes for cross-cultivar functional genomics (TR-17-100)," to generate a list of candidate genes for biomarker development. This effort began with RNA-Seq using the 'Bartlett' v1.0 genome, but the low data inclusion rate indicated a need for a better reference. During the last year of the project, while we were finishing 'd'Anjou' v1.0 the next 'Bartlett' genome, v 2.0, was made publicly available prior to publication via the Genome Database for Rosaceae (https://www.rosaceae.org/). We repeated the full RNA-Seq analysis using both 'Bartlett' v2.0 and our 'd'Anjou' v1.0. The data inclusion rate improved substantially with each of the new genomes, increasing ~10% (Figure 4 – from TR-17-100 final report).

This result was reassuring, but not without surprises. The amount of data that matched to genes was similar for each new genome, 63.8% for 'Bartlett' v2.0 and 65.8% for 'd'Anjou' v1.0. However, the fraction of RNA-Seq reads that matched uniquely was lower in 'd'Anjou, and the fraction of reads that mapped in between genes was also lower for 'd'Anjou. These are likely artifacts of annotation and will be improved as these genome resources are improved. The annotation (a highly iterative exercise) for the new 'Bartlett' genome was more mature than our first pass annotation of 'd'Anjou.' Furthermore, because the data inclusion rate in both experiments was similar, we used the RNA-Seq analysis with 'Bartlett' v2.0 in subsequent steps.

Biomarker candidates - differential expression analysis and functional enrichment analysis

Perhaps the most striking shifts in gene activity were from harvest through the end of the storage period (8 months). The differences between fruit from internal canopy positions vs. external positions was dwarfed by changes during storage, often by a factor of >10 (Figure 5a). The peak for differential gene activity signatures between treatments was after 3 months of storage (Figure 5b), which is also the time point for the most divergent ripening between the internal vs. external canopy fruit. At this time point (3 months of storage) the gene activity signatures included enhanced cellular transport processes in fruit from the external canopy positions, while internal canopy positions had signatures of osmotic stress and wound response.

Biomarker candidates - Gene Co-expression Network (GCN) analysis

Using the same gene activity data set, John Hadish, a student in the lab of Dr. Stephen Ficklin (Honaas' collaborator from the WSU Horticulture department) constructed a GCN that considered gene activity in all internal tissues vs. all external tissues, we did not distinguish between peel and cortex. We expected that the fine contrast in fruit quality characteristics and differential gene activity would be reflected in a comparative network analysis, showing a relatively small number of genes. Indeed this analysis showed a small number of co-expressed genes, and several of these genes show significant co-

expression in both treatments (Figure 6). The lists of differentially active genes and co-expressed genes serve as a starting point for biomarker candidate selection.

Biomarker candidates – preliminary selection

We manually searched the gene activity signatures for a subset of genes that are both differential and co-expressed to find signatures that can distinguish pear fruit that we know have different ripening characteristics in the postharvest period (see examples in Figure 7). We found a variety of patterns that were able to distinguish the fruit, including differences at harvest that persist throughout storage (Figure 7a), differences that emerge during storage (Figure 7b), and more complex changes that may require integration to predict ripening characteristics (Figure 7c & 7d).

Perspectives

This project has provided the foundation to take the next steps for biomarker discovery in European pear using functional genomics combined with experimental horticulture. In this initial search we explored a very fine contrast – fruit from the same tree that had similar, at-harvest fruit quality characteristics, but had divergent ripening characteristics in the postharvest period. We used very aggressive statistical methods to select the only the most prominent signatures in this fine contrast as a proof of concept. We readily found candidate genes that could distinguish fruit at harvest and during storage by manually curating the data. These results show biological signatures exist in the data that, with sufficient development, may be used to create postharvest tools for both research and industry. Next steps include leveraging cutting-edge bioinformatic approaches to mine the data for additional candidates, and then deploying these preliminary biomarkers for validation studies.

FIGURES

Figure 1. RNA-Seq validation with qPCR improves when validation genes are genetically identical. This indicates that genetic polymorphisms influence gene activity estimates.

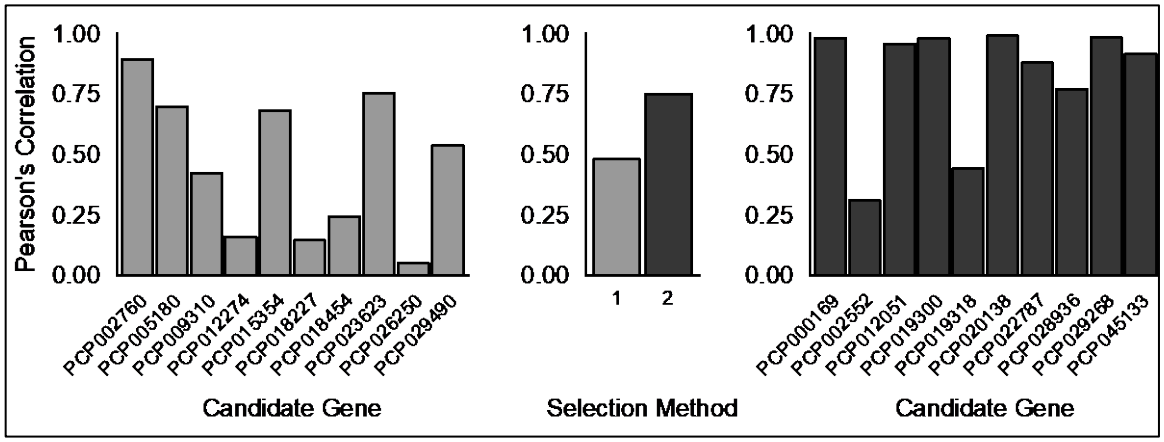


Figure 2. **A Principle Component Analysis of gene activity data shows structure that separates fruit from internal vs. external canopy positions.** This indicates that the gene activity data we gathered for the project might contain individual signatures that may be useful to distinguish fruit that have different ripening characteristics in the postharvest period.

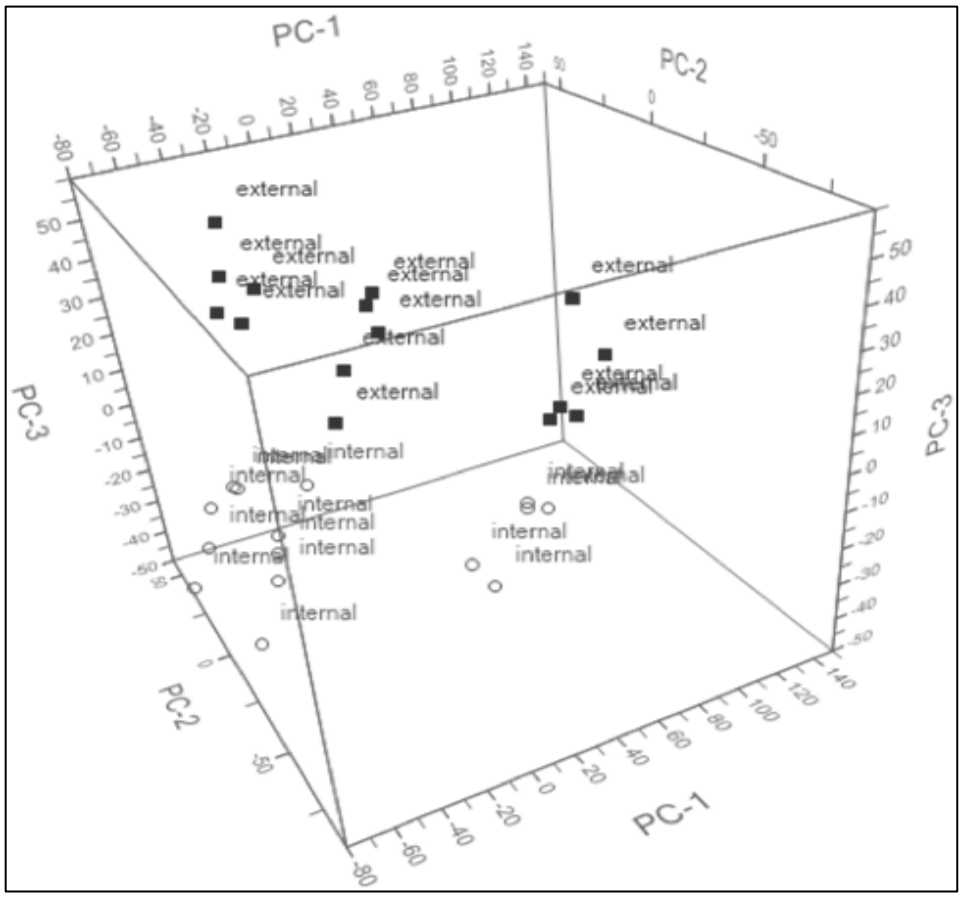


Figure 3. **Genome wide analysis of known plant gene families shows a majority of pear gene predictions from 3 different genomes overlap, but there are potentially cultivar specific gene families (plant gene families typically contain a small number of genes).** These differences in gene family predictions are probably attributable to real biological differences and also methodological differences between the approaches used to build and annotate each of the genomes.

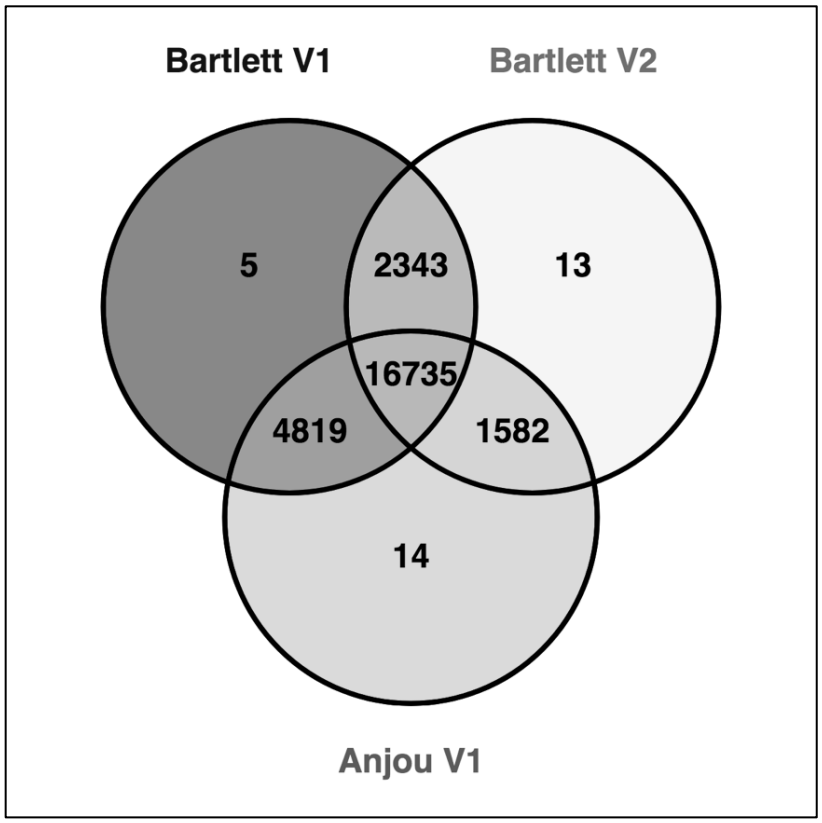


Figure 4. **The amount of 'd'Anjou' gene activity data included in RNA-Seq analyses increases in superior genomes (Bartlet v2.0) and genetically matched genomes (d'Anjou v1.0).** The 'Bartlett' v2.0 genome is superior because the genome assembly is less fragmented than 'Bartlett' v1.0. Even though the 'd'Anjou' v1.0 genome is more fragmented, the pieces are genetically matched to the RNA-Seq data, allowing higher data inclusion.

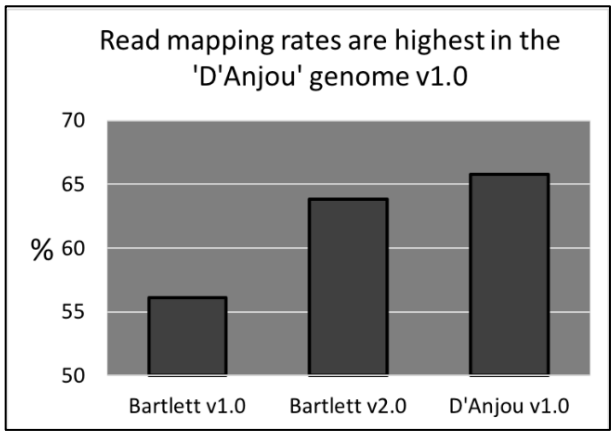


Figure 5.
A: Statistically significant (Bonferroni p<0.05) gene activity *changes* during storage dwarf gene activity *differences* between fruit that have different ripening characteristics in the postharvest period. IC = cortical tissue/internal canopy fruit, **EC** = cortical tissue/external canopy fruit, **down IC & EC =** less gene activity over time, **up IC & EC** = more gene activity over time. In external canopy (**E**) fruit cortical tissue vs internal canopy (**I**) fruit cortical tissue after 8 months of storage, **"down E vs I" =** less gene activity and **"up E vs I"** = more gene activity.
B: The maximum gene activity difference between fruit at 3 months of storage correspond to the largest differences in ripening characteristics, further suggesting the potential to identify gene activity signatures that can distinguish the fruit.

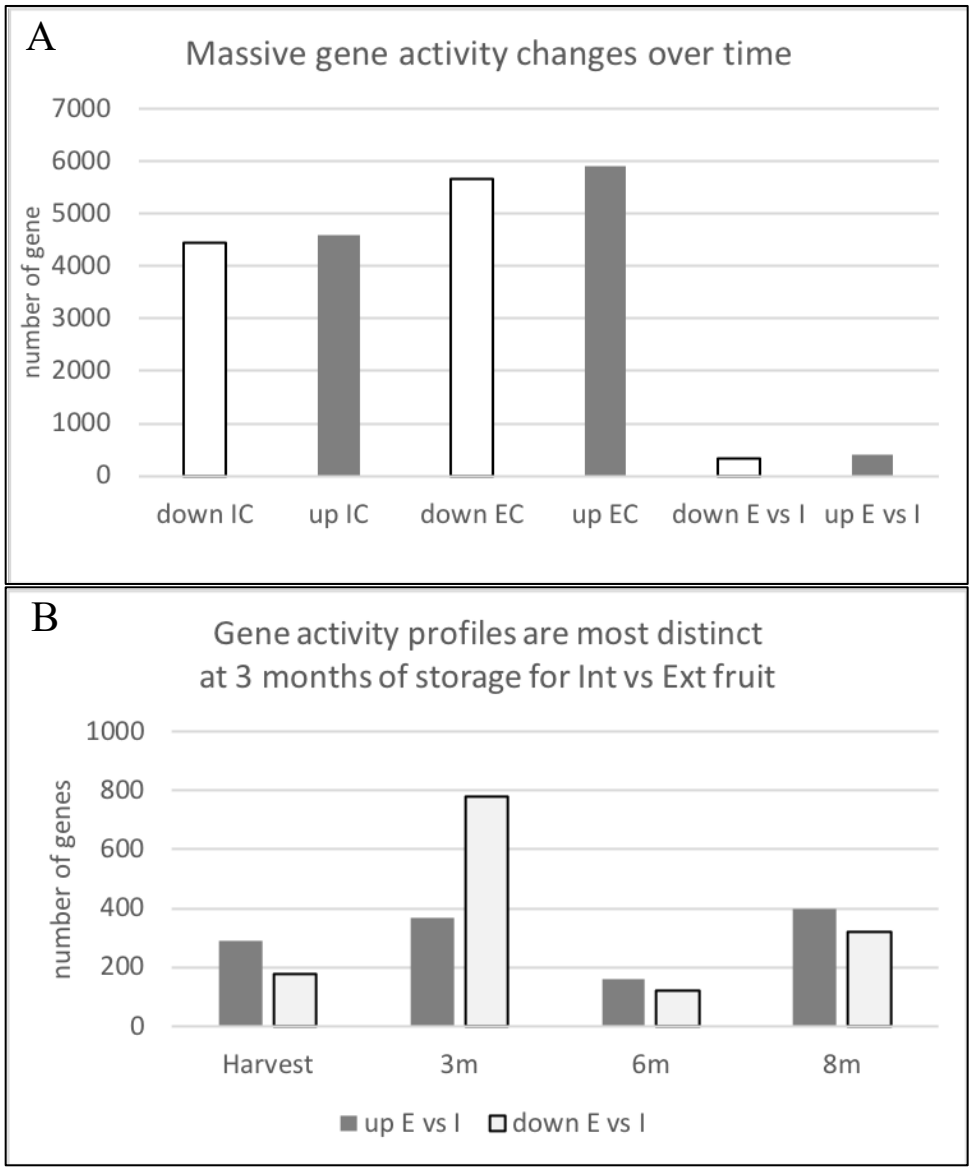


Figure 6. **The Gene Co-expression Network highlights genes with correlated expression during the postharvest period.** Dark gray edges indicate a significant relationship in fruit from internal canopy positions, light gray edges indicate a significant relationship in fruit from external canopy positions. The fruit from internal vs. external canopy positions have different ripening characteristics, and some gene activity signatures are shared (double lines) while others are distinct.

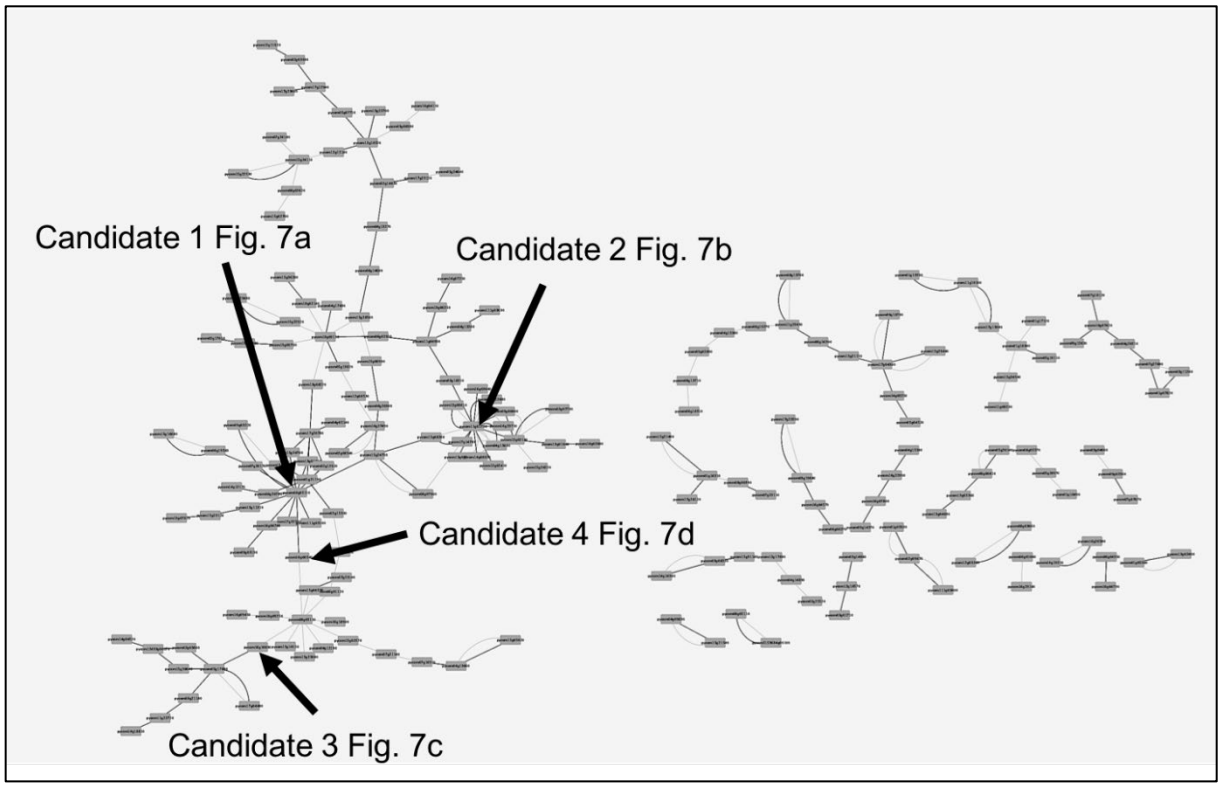
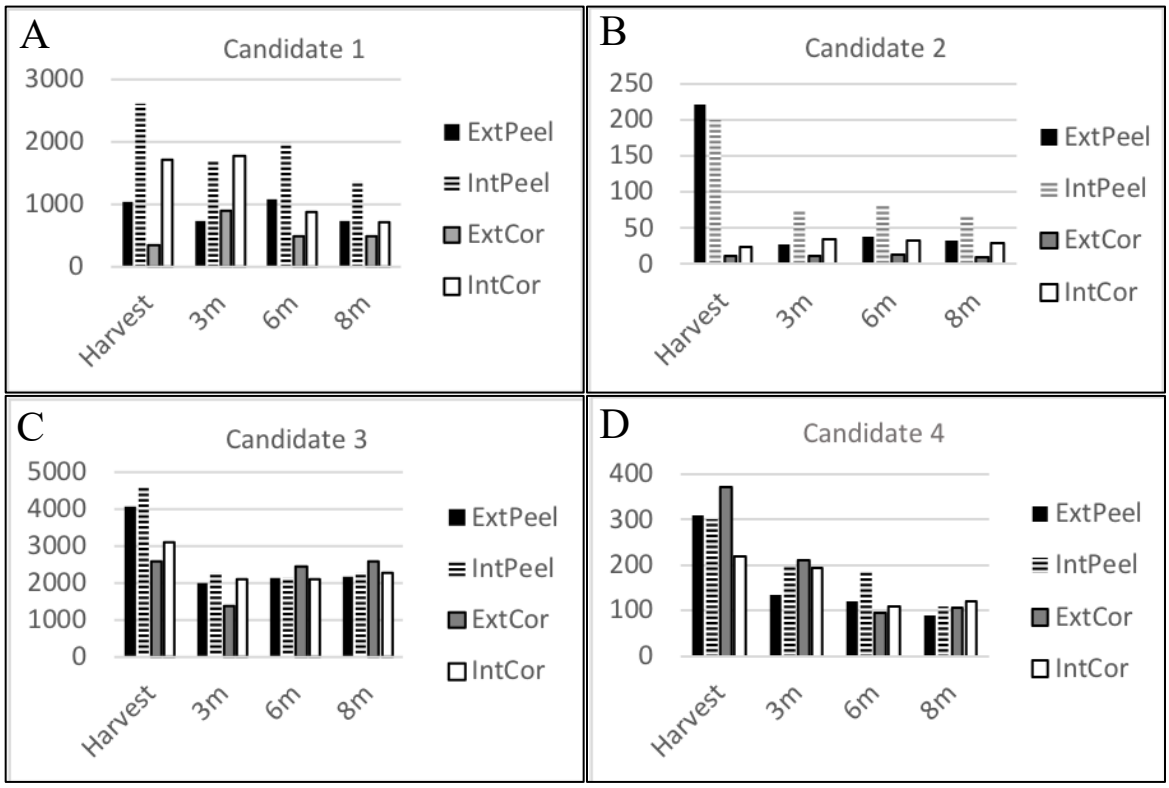


Figure 7. Gene activity signatures can be used to distinguish fruit that have different ripening characteristics – in this experiment the contrast is *internal* canopy vs. *external* canopy 'd'Anjou' pear fruit. Normalized gene activity is on the **Y-axis**, sample times are on the **X-axis**. The examples below of hand selected biomarker candidates are indicated above in Figure 6.



EXECUTIVE SUMMARY

Project title: Functional genomics of 'd'Anjou' pear fruit quality and maturity

Key words: d'Anjou, European pear, maturity, RNA-Seq, genome, gene, TR-17-100, PR14-108A

Abstract: We scanned *Pyrus communis* 'd'Anjou' fruit gene activity profiles to find genes related to postharvest quality and capacity to ripen. We now have a list of candidate biomarkers for future work aimed at understanding the genetic basis of pear fruit quality traits. We will emphasize development of tools to predict future fruit quality, especially as it relates to ripening capacity.

Summary: The aim of this project was to generate genomic resources for 'd'Anjou' pear towards the development of postharvest tools for enhanced pear fruit quality. We leveraged existing WTFRC funded research from Stefano Musacchi ("Improving Quality and Maturity Consistency of 'd'Anjou'') by using samples from their cryopreserved biobank of pear fruit tissue and the associated fruit quality data. From that project we selected fruit that ripened differently in the postharvest period – fruit from internal vs. external canopy positions. We proceeded to gather massive gene activity data sets from these samples, and began to mine the data for signatures, or hints, about future fruit quality especially with regard to estimating maturity and the capacity to ripen.

Concurrently, Honaas' WTFRC project "Enhancing reference genomes for cross-cultivar functional genomics" helped to meet our gene discovery goal for 'd'Anjou' pear by sequencing the genome of this variety. Thus we discovered virtually all of the genes in this variety, rather than just genes that were active in our fruit samples as initially proposed. This allowed us to exceed our goal for gene discovery and to build a stronger foundation for comparative genomics in European pear. During this same time period, several *Pyrus* genomes became available, including a new version of the 'Bartlett' genome. We repeated our full gene activity analysis using both versions of the 'Bartlett' genome (v1.0 and v2.0) plus our new 'd'Anjou' genome. We found that our 'd'Anjou' genome and the new 'Bartlett' genome were better than the first 'Bartlett' genome for analyzing our gene activity data sets. Because the finishing steps for building a genome were more mature for 'Bartlett' v2.0, we continued our search for potential biomarkers using that genome as a reference.

We found that the changes in gene activity (i.e. number of genes that were different) from harvest to 8 months of storage dwarfed the differences between fruit from equivalent storage time points, often by more than a factor of 10. Yet it was clear that our gene activity data set had structure that distinguished fruit with different ripening characteristics. When we combined the analysis that showed which genes were different between samples, with one that identified genes that had highly similar activity signatures within samples, we had a starting list of candidate biomarkers. By manually digging through the data we identified genes that had interesting signatures. This included patterns that allowed us to differentiate fruit from internal vs. external canopy positions at harvest and also during storage. The next steps include leveraging our new genomics resources and cutting-edge bioinformatics tools to mine the data for additional candidates, and then deploying these preliminary biomarkers in validation studies.

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

Project Title: Interstem grafts to evaluate pear germplasm for dwarfing potential

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Cooperators: Kate Evans, Washington State University

Total Project Request: **Year 1**: $18,000 **Year 2**: $9,000 **Year 3**: no cost extension

Budget:

Organization Name: USDA-ARS **Contract Administrator: Richard Kimball**
Telephone: 510-559-6019 **Email address: Richard.Kimball@ars.usda.gov**

Item	2017	2018	2019
Salaries			
Benefits			
Wages	$12,000	$9,000	
Benefits			
Equipment			
Supplies	$6,000		
Travel			
Miscellaneous			
Plot Fees			
Total	**$18,000**	**$9,000**	**$0**

OBJECTIVES

Clonal propagation of pear selections as self-rooted trees for rootstock trials is challenging, and a procedure to pre-screen selections for dwarfing ability will help focus resources. The USDA living pear germplasm collection in Corvallis, OR has numerous potential pear rootstock selections, and also includes a very large and diverse assortment of pear selections and species that have never been evaluated for rootstock potential. The objective of this project was to investigate whether interstem grafts can be used to identify pear selections that have dwarfing potential, and provide a relatively rapid assay for screening pear germplasm to be included in future rootstock trials. Interstem pieces of 30 potential rootstock selections were grafted onto seedling rootstocks, and then each interstem was top-worked with a bud of either 'Bartlett' or 'Bosc'. Trees were grown and evaluated in a greenhouse for the first year, and then in a field planting for two additional years.

SIGNIFICANT FINDINGS 2017 to 2019

- **2017**: The goal of generating interstem trees in one season was accomplished. 194 'Bosc' trees and 204 'Bartlett' trees on 29 different interstem candidates were produced. Following a full season of growth in the greenhouse there was inadequate growth on the potted trees to detect any interstem effect. A single growing season under greenhouse conditions was not adequate for evaluating dwarfing potential (January 2018 Progress Report).
- **2018**: 174 'Bosc' trees and 184 'Bartlett' trees on 28 different interstems were field planted in early July (Figure 1). Rootstock, interstem, and cultivar stem diameters; cultivar stem heights; and number of side branches was recorded at the end of the 2018 growing season to evaluate possible dwarfing. No significant differences were detected between treatments (January 2019 Progress Report).
- **2019**: 154 'Bosc' trees and 192 'Bartlett' trees on 33 different interstems (Table 1) are well established in a field plot (Figure 2). Most grafted trees were generated during early 2017, however 26 trees from a 2016 preliminary greenhouse trial had also been field planted and were evaluated along with the 2017 grafts. The 2016 grafts were not included in the 2018 report, but are included here. Vegetative growth data was recorded in late October 2019:
    - Diameters of the 'Bartlett' and 'Bosc' scions measured approximately 1 cm above the graft unions did not differ significantly from the controls ('Bartlett' and 'Bosc' interstems). Trees with '**Granatnaya'** and '**Passe Crassane'** interstems had mean stem diameters that differed only from the two most vigorous interstem treatments at a very low level of significance (Table 2).
    - Total tree height was not a useful measure of dwarfing. Some trees had single leaders, some had double leaders, and trees differed in number and length of side branches. Tree height was therefore not a reliable measurement of vegetative vigor.
    - 'Bartlett' grafts produced twice as many side branches (mean = 15.4) as 'Bosc' (mean = 6.9), but when average branch length was calculated, there was no difference (44.8 cm vs. 45.7 cm respectively). Number or length of side branches was not a useful measure of dwarfing, however some side branches appear to have many flower buds and differences in precocity and fruit production may be apparent in 2020 and beyond.
    - When the sum of all branch lengths (main stem and all side branches) was calculated (Table 3), 'Bartlett' had nearly twice the vegetative growth (mean = 847 cm) as 'Bosc' (482 cm). Two potentially dwarfing interstems produced significantly less vegetative growth than the most vigorous interstems ($p < .05$) although there was no significant difference from the 'Bartlett' and 'Bosc' interstem controls. The two most dwarfing interstems were '**Granatnaya'** and '**P. calleryana D6'**.

- A "vegetative growth efficiency" was calculated based on total shoot growth (sum of all stem and side branch lengths) per mm of stem diameter (Table 4). The same interstems as above (**'Granatnaya'** and '**P. calleryana D6'**) differed significantly from the 4 most vigorous interstems ($p < .05$) but not from the control treatments.
- Pear selections known to be either dwarfing or vigorous as rootstocks did not control vigor in a similar fashion when used as interstems during the 3 years of this study. More than 3 years, including the transition from vegetative growth to fruit production, are required to properly evaluate rootstock potential.

METHODS

Interstem Grafts. Scions were collected in January 2017 from interstem candidates and from virus-free mother trees of cultivars Bartlett and Bosc (for top-working) and stored at 4 °C (40 °F). In April 2017 pear seedling rootstocks were planted in 2" x 10" deepots. In May, 15 cm (6 in) long interstems were grafted onto seedling rootstocks in a cool greenhouse. Twenty grafts were made with each of 31 interstem candidates. 'Bartlett' and 'Bosc' were also used as two of the interstem treatments and can be considered controls. A number of pear selections known to be either dwarfing or vigorous as rootstocks were among the interstem candidates (Table 1). Approximately 2-3 weeks after interstem grafts were made, 10 of each were chip-budded with 'Bartlett' and 10 with 'Bosc' at the top of the interstem. Grafted trees were maintained in pots and flood irrigated during the growing season. Rootstock and interstem shoots were regularly removed to force the cultivar buds. Interstem graft survival and cultivar top-graft survival was assessed. Length of cultivar bud growth was measured in mid-October. Results from 2017 were reported at the pear research review in February, 2018.

A small number of preliminary interstem grafts were made in late summer 2016 as proof of concept using the dwarfing rootstock 'Pyrodwarf' and the genetic dwarf clones 'Le Nain Vert' and 'P. nivalis compact hybrid'. 'Bartlett' and 'Bosc' interstems were controls. Survival and replication of these preliminary grafts was not sufficient to evaluate statistical significance, but the data may be of interest and is included in the tables below.

Field plot established in 2018. In early July 2018, 'Bosc' and 'Bartlett' trees on 28 different interstems from 2017 grafts, and 5 interstems from 2016 grafts were field planted in a randomized block design with two blocks per treatment. A narrow irrigation pipe trencher was used to prepare planting furrows for easily lining out the trees on 24 inch centers (Figure 1). Trees were fertilized, regularly irrigated, and weeds were controlled for the remainder of the growing season. The total height of 'Bartlett' and 'Bosc' shoots was measured from the bud union and the number of side branches was counted after leaf-fall in late October. Stem diameters were measured at three points using a digital caliper: below the interstem graft union (rootstock), at mid-interstem (interstem), and above the interstem (cultivar). Analysis of variance was conducted and Tukey's HSD was used to compare means from the 2017 grafts; no statistically significant differences were detected. A no-cost extension was requested for this project to assess tree growth for another year in the field.

Field plot growth in 2019. Drip irrigation was installed and weeds were controlled during the 2019 growing season. No tree pruning or training was done, other than removing rootstock and interstem suckers and staking trees that were badly leaning. In mid-October stem diameters were measured for the 'Bartlett' and 'Bosc' top-grafts approximately 1 cm above the graft union (Table 1), and tree heights were measured at the tallest point (data not shown). In early-November the length of every shoot and side-shoot was measured and the values were added to obtain the number of side branches and the total vegetative growth of each tree (Table 2). As an alternative measure of vigor, total vegetative growth (cm of length) was divided by the scion stem diameter (mm) to obtain a "vegetative growth efficiency" similar to the "yield efficiency" used in fruit productivity studies. The statistical package ASRemi-R was used to account for treatment and replication effects for a mixed linear model, and Tukey's HSD was used for making all pairwise comparisons.

RESULTS & DISCUSSION

At the end of the 2018 growing season, 358 trees with 28 different interstems had survived in the field. 'Bosc' on 'Bosc' interstems and 'Bartlett' on 'Bartlett' interstems were reference controls. While some cultivar/interstem combinations resulted in trees that were generally smaller or larger than controls none of the differences were statistically significant (January 2018 Progress Report).

At the end of the 2019 growing season, 346 interstem trees (154 with 'Bosc' scions and 192 with 'Bartlett') with 33 different interstems are well established in a field plot (Table 1). A number of very weak trees evaluated in 2018 did not survive, thus reducing some of the variance that impeded pervious year statistical analysis. Both the total length of vegetative growth (Table 3) and the "vegetative efficiency" calculation (Table 4) suggest that the intergeneric hybrid '**Granatnaya'** (Crataegus x Sorbus) and the Australian rootstock selection '**P. calleryana D6'** deserve further evaluation as size-controlling stocks.

A number of interstem candidates were included that are known to be either dwarfing or non-dwarfing as rootstocks. This should allow the validation of whether the dwarfing effect as an interstem is similar to the expected influence as a self-rooted stock. For example for the two South African rootstock clones, 'BP-1' is considered to be semidwarfing and 'BP-2' is considered to induce more vigor. Likewise, among the several 'Old Home x Farmingdale' clones included, 'OHxF 97' is considered to be more vigorous than 'OHxF 69' and 'OHxF 333'. The rootstock clones 'Pyrodwarf' and 'BU 2/33' (or 'Pyro II') should induce dwarfing.

Unfortunately, after 1 year in pots and 2 years in the field, the dwarfing results from this interstem study were not consistent with the expected size control for many of the rootstock clones. While the differences were not statistically significant, 'BP-2' was more dwarfing than 'BP-1' with 'Bartlett' scions, and 'BP-1' was more dwarfing when 'Bosc' was the scion (Table 3, Table 4). Also, while not statistically significant, 'OHxF 69' tended to be more dwarfing than 'OHxF 97' as expected, but 'OHxF 333' was more vigorous. 'Pyrodwarf' had an inadequate number of surviving replicates to evaluate (none for 'Bartlett') but the few surviving trees were more dwarf than other interstem treatments. 'BU 2/33' had no surviving 'Bosc' replicates, and the 'Bartlett' grafts did not differ at all from control interstems.

It is not entirely unexpected that cultivars grafted onto size-control rootstocks do not exhibit dwarfing in the nursery or as young orchard trees, and only exhibit dwarfing relative to other rootstocks as a result of precocious fruit production. Shifting tree resources to producing fruit rather than producing shoots and leaves is known to be an important component in vigor control. While 2 or 3 years may be inadequate for assessing the ability of a rootstock candidate to induce dwarfing and precocity, the use of interstems, rather than self-rooted rootstocks, may still have great potential as an efficient method to screen germplasm for rootstock potential. An interstem assay will avoid the expensive and time-consuming effort to generate self-rooted rootstocks using tissue culture or conventional cutting propagation.

2020 and beyond. This interstem field plot is now well established on the USDA germplasm farm. While no flowers or fruit have yet been produced, there is evidence of abundant flower buds on some trees that should produce a fruit yield in 2020. We anticipate much more useful information to result from this planting in future years and hope to continue to monitor this planting and collect production data for several additional seasons.

Acknowledgements: Jaimie Green and Laura Duncan assisted with tree maintenance and data collection. Jason Zurn conducted ASRemi statistical analyses. Jill Bushakra reviewed this report and suggested useful improvements.

Figure 1 – Interstem trees field planted in early July 2018.

Figure 2 – Interstem trees at end of 2019 growing season.

Table 1. Pear interstem candidates with USDA accession number and National Clonal Germplasm Repository inventory number.

Interstem	Accession	Inventory	Taxon	Name
1	CIGC 5	CIGC 5.001	*×Crataegosorbus miczurinii*	Granatnaya (Crataegus x Sorbus)
2	PI 689459	CIGC 9.001	*×Pyronia veitchii*	Pyrus communis x Cydonia IRP 82-1
3	PI 300693	CPYR 38.001	*Pyrus communis*	Bartlett (2017)
4	PI 300693	CPYR 38.001	*Pyrus communis*	Bartlett (2016)
5	PI 436538	CPYR 101.001	*Pyrus communis*	BP-1 (Bien Donne 1)
6	PI 420810	CPYR 102.001	*Pyrus communis*	BP-2 (Bien Donne 2)
7	PI 322035	CPYR 344.001	*Pyrus communis*	Le Nain Vert (2017)
8	PI 322035	CPYR 344.001	*Pyrus communis*	Le Nain Vert (2016)
9	PI 324134	CPYR 406.001	*Pyrus communis*	Mustafabey
10	PI 131662	CPYR 441.001	*Pyrus communis*	Passe Crassane
11	PI 214185	CPYR 665.001	*Pyrus calleryana*	P. calleryana D6
12	PI 541370	CPYR 726.001	*Pyrus communis*	OHxF 97
13	PI 541745	CPYR 866.001	*Pyrus nivalis hybrid*	P. nivalis compact hybrid (2017)
14	PI 541745	CPYR 866.001	*Pyrus nivalis hybrid*	P. nivalis compact hybrid (2016)
15	PI 541945	CPYR 890.001	*Pyrus regelii*	P. regelii
16	PI 312149	CPYR 920.001	*Pyrus syriaca*	P. syriaca - Armenia No. 1087/62
18	PI 541387	CPYR 1165.001	*Pyrus communis*	Bosc - OP-5 (2017)
19	PI 541387	CPYR 1165.001	*Pyrus communis*	Bosc - OP-5 (2016)
21	PI 541405	CPYR 1329.001	*Pyrus communis*	OHxF 333
22	PI 665738	CPYR 1343.001	*Pyrus communis*	OHxF 69
23	PI 541415	CPYR 1345.001	*Pyrus communis*	OHxF 87
24	PI 541652	CPYR 1496.001	*Pyrus fauriei*	P. fauriei Selection 12-14
26	PI 502179	CPYR 1639.001	*Pyrus elaeagrifolia hybrid*	Sbkta
27	PI 541953	CPYR 1697.001	*Pyrus salicifolia hybrid*	P. salicifolia Russia sdlg. 1
28	PI 541953	CPYR 1697.005	*Pyrus salicifolia hybrid*	P. salicifolia Russia sdlg. 5
29	PI 541007	CPYR 2291.002	*Pyrus betulifolia*	P. betulifolia - Shaanxi
30	PI 617598	CPYR 2522.004	*Pyrus korshinskyi*	P. korshinskyi 94011 - Kyrgyzstan
31	PI 617654	CPYR 2598.002	*Pyrus communis*	Pyrodwarf (2016)
32	PI 617679	CPYR 2699.001	*Pyrus communis*	BU 2/33 - Pyro II
33	CPYR 2704	CPYR 2704.002	*Pyrus communis*	QR 708-12
34	PI 638009	CPYR 2817.001	*Pyrus elaeagrifolia*	Gasparian 38, Kotayk sdlg. 1
35	PI 657923	CPYR 2882.001	*Pyrus sachokiana*	P. sachokiana Georgia-2006-115
36	PI 665763	CPYR 2968.001	*Pyrus spinosa*	P. spinosa Albania-2011-038

Table 2. Mean stem diameter (mm) above interstem/scion graft.
n = number of replicate plants, x = no data)

		Bartlett		Bosc	
interstem	**N**	**Diam (mm)**	**n**	**Diam (mm)**	**p < 0.1**
Pyrodwarf 2016	0	x	2	15.0	
Granatnaya (×Crataegosorbus)	3	14.3	5	16.2	**a**
Le Nain Vert 2017	2	18.0	2	20.0	
BP-2	8	18.4	7	20.0	
QR 708-12	8	18.4	6	19.0	
Passe Crassane	8	18.6	7	13.7	**a**
P. calleryana D6	5	19.0	3	18.7	
P. betulifolia - Shaanxi	6	19.2	4	13.0	
P. sachokiana	6	19.2	5	17.8	
P. regelii	6	19.5	0	x	
Bosc - 2016	7	19.6	0	x	
Pyronia (Pyrus x Cydonia)	7	19.7	8	18.4	
OHxF 87	8	19.8	8	18.1	
Bartlett 2017	8	20.5	5	17.6	
BU 2/33 - Pyro II	7	20.7	0	x	
P. fauriei 12-14	8	20.9	2	15.5	
OHxF 97	7	20.9	2	20.0	
P. syriaca - Armenia	6	21.0	5	15.6	
P. elaeagrifolia Armenia-38	4	21.0	3	17.0	
P. salicifolia (Russia hybrid 5)	8	21.1	6	18.5	
OHxF 69	8	21.3	4	13.8	
Bosc - 2017	7	21.3	8	19.9	
BP-1	6	21.3	7	17.6	
P. nivalis compact 2017	3	21.7	4	19.3	
Mustafabey	7	21.7	7	20.6	**b**
Bartlett 2016	1	22.0	5	16.8	
P. korshinskyi sdlg 4	7	22.1	7	18.0	
P. salicifolia (Russia hybrid 1)	8	22.4	5	20.4	**b**
OHxF 333	8	22.6	7	18.4	
Sbkta (P. elaeagrifolia)	8	22.6	7	18.6	
P. spinosa Albania-38	3	24.0	6	18.3	
P. nivalis compact 2016	2	24.5	4	17.8	
Le Nain Vert 2016	2	25.5	3	16.3	
Mean Stem Diameter		**20.7**		**17.7**	

Table 3. Total vegetative growth: Average sum of all branch lengths (cm).
(n = number of replicate plants, x = no data

	Bartlett		Bosc		
interstem	**n**	**Length (cm)**	**n**	**Length (cm)**	**p < .05**
Pyrodwarf 2016	0	x	2	291.5	
Granatnaya (×Crataegosorbus)	3	399.7	5	346.0	**a**
Bosc - 2016	7	501.3	0	x	
P. calleryana D6	5	599.2	3	457.3	**a**
QR 708-12	8	647.1	6	527.8	
BP-2	8	667.6	7	662.9	
P. nivalis compact 2016	2	674.0	4	382.5	
P. syriaca - Armenia	6	729.7	5	490.6	
Le Nain Vert 2017	2	733.0	2	417.0	
Bartlett 2016	1	750.0	5	379.4	
P. betulifolia - Shaanxi	6	750.3	4	245.5	
BU 2/33 - Pyro II	7	764.0	0	x	
P. regelii	6	769.7	0	x	
Passe Crassane	8	814.3	7	397.4	
Bartlett 2017	8	829.4	5	558.0	
P. fauriei 12-14	8	866.5	2	399.0	
OHxF 69	8	874.1	4	276.5	
Sbkta (P. elaeagrifolia)	8	885.6	7	548.0	
P. spinosa Albania-38	3	891.0	6	468.7	
OHxF 87	8	891.5	8	501.3	
P. salicifolia (Russia hybrid 5)	8	897.8	6	522.2	
P. elaeagrifolia Armenia-38	4	898.0	3	285.3	
BP-1	6	898.7	7	465.9	
Pyronia (Pyrus x Cydonia)	7	919.6	8	489.9	
Bosc - 2017	7	957.1	8	586.0	
Mustafabey	7	998.6	7	699.0	**b**
OHxF 97	7	1006.6	2	547.5	
P. nivalis compact 2017	3	1018.0	4	610.0	
OHxF 333	8	1028.8	7	545.3	
P. sachokiana	6	1057.0	5	496.0	
P. salicifolia (Russia hybrid 1)	8	1114.9	5	720.0	**b**
P. korshinskyi sdlg 4	7	1115.0	7	550.7	**b**
Le Nain Vert 2016	2	1139.5	3	589.3	
Mean Sum of Branch Lengths		**846.5**		**481.9**	

Table 4. “Vegetative Efficiency”: Total branch length (cm)/stem diameter (mm).
(n = number of replicate plants, x = no data)

	Bartlett		Bosc		
interstem	**n**	**length/diam (cm/mm)**	**n**	**length/diam (cm/mm)**	**p < .05**
Pyrodwarf 2016	0	x	2	17.6	
Granatnaya (×Crataegosorbus)	3	24.8	5	19.8	**a**
Bosc - 2016	7	25.6	0	x	
P. nivalis compact 2016	2	27.6	4	18.6	
P. calleryana D6	5	30.4	3	24.3	**a**
Bartlett 2016	1	34.1	5	21.9	
P. syriaca - Armenia	6	34.6	5	32.2	
BP-2	8	34.9	7	32.3	
QR 708-12	8	35.3	6	28.0	
BU 2/33 - Pyro II	7	36.3	0	x	
P. spinosa Albania-38	3	37.1	6	25.8	
P. betulifolia - Shaanxi	6	38.0	4	18.3	
P. regelii	6	38.4	0	x	
Sbkta (P. elaeag.)	8	39.2	7	28.5	
Bartlett 2017	8	39.8	5	31.5	
Le Nain Vert 2017	2	40.0	2	20.7	
OHxF 69	8	40.4	4	20.6	
P. elaeagrifolia Armenia-38	4	40.9	3	16.9	
P. salicifolia (Russia hybrid 5)	8	41.1	6	27.2	
BP-1	6	41.3	7	25.4	
P. fauriei 12-14	8	41.4	2	23.9	
Passe Crassane	8	41.8	7	30.7	
OHxF 87	8	43.2	8	27.8	
Le Nain Vert 2016	2	43.7	3	36.5	
Bosc - 2017	7	44.1	8	28.5	
Pyronia	7	45.1	8	25.7	
OHxF 333	8	45.4	7	29.6	
Mustafabey	7	46.0	7	33.8	**b**
OHxF 97	7	46.3	2	26.5	
P. nivalis compact 2017	3	46.8	4	30.6	
P. salicifolia (Russia hybrid 1)	8	49.9	5	35.3	**b**
P. korshinskyi sdlg 4	7	50.6	7	29.1	**b**
P. sachokiana	6	51.8	5	27.5	**b**
Mean Total Length/Diameter		**39.9**		**26.5**	

EXECUTIVE SUMMARY

Project title: Interstem grafts to evaluate pear germplasm for dwarfing potential
Key words: Pear, Pyrus, Rootstock, Dwarfing, Germplasm, Interstem
Abstract: Interstem grafts on seedling rootstocks were evaluated as way to screen pear selections for dwarfing potential with Bosc and Bartlett scions. After 3 years two selections were identified as possibly dwarfing. However known dwarfing or vigorous rootstocks did not perform as expected, indicating 3 years are insufficient to properly evaluate rootstock potential.

The use of interstems as an alternative to grafting onto self-rooted rootstocks was investigated as a more rapid way to screen pear germplasm for dwarfing potential. Interstem pieces of 30 candidate pear selections were grafted onto seedling rootstocks, and then top-worked with buds of either 'Bartlett' or 'Bosc'. Trees were grown and evaluated in a greenhouse for the first year, and in a field planting for two additional years. Based on both total vegetative growth (sum of all stem and side branch lengths), and also on "vegetative growth efficiency" (total shoot growth per mm of stem diameter) following the third growing season, selections 'Granatnaya' and 'P. calleryana D6' were identified as worthy of further evaluation. Several pear selections known to be either dwarfing or vigorous as rootstocks did not control scion vigor as anticipated during the 3 years of this study. Additional years, including the transition from vegetative growth to fruit production, are required to properly evaluate rootstock potential. While a short-term assay may not be adequate for identifying dwarfing, the use of interstems rather than self-rooted rootstocks may still have great potential as an efficient method to screen germplasm for rootstock potential. The use of interstems avoids the expensive and time-consuming effort to generate self-rooted rootstocks by tissue culture or cutting propagation. This interstem field plot is now well established on the USDA germplasm farm, and there is evidence of abundant flower buds on some trees. We anticipate more useful information to result from this planting in future years.

FINAL PROJECT REPORT **YEAR**: 2019
WTFRC Project Number: PR-17-100

Project Title: Fire blight management: new products and effective rates

PI: S. Tianna DuPont
Organization: Washington State University
Telephone: (509) 663-8181
Email: tianna.dupont@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: None

Total Project Request: Year 1: 14,134 **Year 2**: 13,812 **Year 3**: 14,256

Other funding sources

Agency Name: Industry Gift Grants, IR4
Amt. : $1,500 per product/rate screened. $78,000 total.
Notes: For screening of individual new products. Does not include multiple rates or individual products proposed here.

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Kim Rains/Katy Roberts
Telephone: 509.663.8181/509.335.2885 **Email:** kim.rains@wsu.edu/arcgrants@wsu.edu

Item	2017	2018	2019
Salaries[1]	7,800	8,112	8,436
Benefits[2]	2,884	3,000	3,120
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[3]	950	200	200
Travel[4]	500	500	500
Miscellaneous	0	0	0
Plot Fees[5]	$2,000	$2,000	$2,000
Total	**14,134**	**13,812**	**14,256**

Footnotes:
[1]Salary for one technician at $3,900 per month for two months.
[2] Benefits at 37% for one technician.
[3]Supplies include a new power misting backpack sprayer in year one ($750), and safety and application materials in all years.
[4]925 miles per year for travel to research plots, to organize project and present results.
[5]Plot fees included here are for a pear block at CV Research Orchard for russet trials.

OBJECTIVES

1. Test the efficacy of three commercially available copper and biological products (Cueva, Previsto, Blossom Protect) and one experimental product (Alum) at five rates in order to determine at which rates products are effective. Treatments will be assigned randomly to plots within a randomized complete block and compared to untreated inoculated and untreated non-inoculated controls.
2. Investigate russet potential in order to determine when products are effective with little or no russet risk. Four products will be applied at four rates in a randomized complete block and assessed for russet.
3. Provide research-based recommendations to pear producers on appropriate rates for new products.

SIGNIFICANT FINDINGS

- The optimum range of metallic copper application for fire blight control was between 0.16 and 0.25 lbs per 100 gal per acre of metallic copper equivalent.
- Alum was most effective at 8 lbs per 100 gal.
- Fruit marking was low overall but with statistically significant levels for Previsto at 5 qrt/100gal.
- New product trials found that Alum (Potassium aluminum sulfate) at 8 to 10 lbs/ 100 gal has provided consistent positive results with an average control of 75% statistically similar to the oxytetracycline check (82% control). Copper product Instill provided 75% relative control and Mastercop (2.5 pt per 100 gal) 57% control comparable to copper standards across multiple years.

METHODS

Site: A 0.42 acre mature Bartlett & Anjou pear block at WSU Columbia View Orchard Orondo, WA was used for russet evaluations. A two-acre research block of mature Red Delicious & Golden Delicious apples at WSU Columbia View Orchard 48 Longview Rd. East Wenatchee, WA 98802-8283 was used for the inoculated trial. Soils are a Cashmont Gravely Sandy Loam with a 3-8% slope. The site has good air drainage and some wind protection.

Plots: Four blocks of 40 trees (apples) and three blocks of 21 trees (pears) were designated (1-2 tree rows each). Individual trees were marked as plots in a randomized complete block where suitable trees were selected based on sufficient bloom (100+ flowers on lower branches).

Inoculum: Freeze-preserved cultures (4°C) of the *Erwinia amylovora* 153 (streptomycin sensitive fireblight strain) were grown for 72 hours 28°C in NYDA agar to propagate dormant colonies. Subsequent inoculations were made transferring cultures to fresh NYDA plates every 24 hours to ensure fresh (<48 hrs old) plates.

Cluster Inoculation: Fresh cultures were diluted to $1x10^7$ CFU ml^{-1} and verified using an optical density spectrometer. A 1:9 dilution of the $1x10^7$ CFU ml^{-1} solution was used to obtain $1x10^6$ CFU ml^{-1} solution used in field inoculation. A one-liter sprayer was used to lightly wet each cluster. 100 clusters per plot were inoculated when the king blooms were at an average of 100% bloom on the branch.

Treatments: Products were applied by tree to the area of the tree to be inoculated according to manufacturer recommendations using a Stihl SR420 blower mister backpack sprayer with a wetting agent (Biolink, organic; Regulaid, conventional). Products were applied to wet, near dripping

previously calibrated to equal 100 gal/A. Included in this trial as "treated checks" were FireLine (oxytetracycline 17%) at 1.5 lbs. / 100 gal. / A and FireWall (streptomycin sulfate 17%), at 1.5 lbs. / 100 gal. / A, both antibiotics from AgroSource, Inc., as standards). An untreated-inoculated check was included.

Evaluations & Statistics: Trees were visually evaluated for flower cluster infection for four weeks following inoculation. Cluster infection counts were summed across all dates. Fruit was evaluated for russet fruit skin marking during July. Data were analyzed for treatment differences using analysis of variance (SAS 9.4). Incidence data were also subjected to linear and polynomial regression analysis.

RESULTS & DISCUSSION
Note: 2018 trials had unexpectedly low levels of infection and data is not reported here.

Rate trials Analysis based on metallic copper content of copper products combined over multiple years and products showed an optimum range of metallic copper application for fire blight control between 0.16 and 0.25 lbs per 100 gal per acre of metallic copper equivalent. The regression for relative control to lbs of metallic copper per acre applied was significant (Figure 1; $p<0.001$; $R2=0.46$).

Analysis of individual products in individual years showed a trend where higher rates of copper for Cueva and Previsto were more effective. Rate for Previsto had a significant linear regression in 2019 ($p=0.03$; R2 0.21) and non- significant in 2017 (Table 1: $p=0.96$; $R2=0.001$). The regression for rate for Cueva was not significant in 2017 ($p=0.35$; $R2=0.048$) or 2019 ($p=0.29$; $R2=0.05$) (Table 2).

Alum was most effective at 8 lbs per 100 gal with no significant benefit to 10 lb per 100 gal. Regression analysis for Alum was not significant in 2017 (2017 $p=0.73$; $R2= 0.009$) but significant in 2019 ($p= -.01$; $R2=0.40$). Alum treatments at 8 and 10 lbs per 100 gal were effective comparable to antibiotic controls and not significantly different than one another (Table 3).

Russet marking from product applications was low across all rates in all three years (Table 4). However there was a rate effect where higher rates affected marking for Cueva (2018: $p <0.001$; $R2=0.75$, 2019: NS) and Previsto (2018: $p=0.004$; $R2=0.4$, 2019: $p=0.03$; $R2=0.255$) but not alum (2018, 2019=NS). Previsto at 5 qt per 100 gal had significantly higher russet than other treatments in 2017 (Table 4; $p<0.05$).

Figure 1. Relative control from copper products.

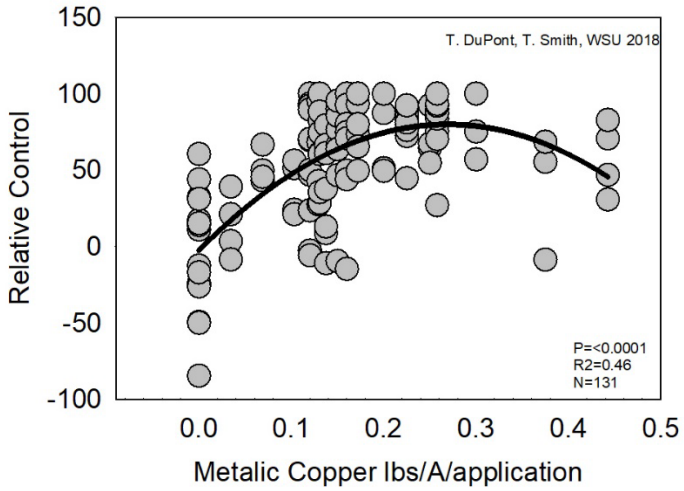


Table 1. Effect of Copper Hydroxide (Previsto) rates on incidence of apple clusters diseased with fire blight in pathogen-inoculated trials conducted in Wenatchee, WA

	2017				2019			
Untreated, Inoculated Check	22.6	±	5.0	a	20.9	±	11.1	a
Previsto (1 qt)	11.7	±	7.2	ab	16.9	±	8.6	abc
Previsto (2 qt)	4.7	±	2.6	b	18.2	±	11.8	ab
Previsto (3 qt)	6.1	±	2.3	b	7.8	±	3.7	bc
Previsto (4 qt)	3.9	±	2.4	b	14.5	±	3.9	abc
Previsto (5 qt)	10.6	±	5.3	ab	8.5	±	3.7	abc
Streptomycin (Firewall)	0.3	±	0.6	b	4.8	±	2.8	c
Oxytetracycline (Fireline)	3.8	±	3.4	b	5.7	±	3.1	bc

Table 2. Effect of Copper Octanoate (Cueva) rates on incidence of apple clusters diseased with fire blight in pathogen-inoculated trials conducted in Wenatchee, WA.

	2017				2019			
Untreated, Inoculated Check	22.8	±	5.1	a	20.9	±	11.1	ab
Cueva (1 qt)	12.5	±	2.9	ab	29.6	±	14.4	a
Cueva (2 qt)	11.8	±	5.0	ab	14.1	±	7.2	ab
Cueva (3 qt)	11.0	±	4.7	bc	16.0	±	5.1	ab
Cueva (4 qt)	13.0	±	4.6	ab	11.5	±	4.1	b
Cueva (5 qt)	6.5	±	3.0	bc	15.6	±	10.1	ab
Streptomycin (Firewall)	0.3	±	0.3	bc	4.8	±	2.8	b
Oxytetracycline (Fireline)	3.8	±	1.7	c	5.7	±	3.1	b

Table 3. Effect of Aluminum Potassium Sulfate on incidence of apple clusters diseased with fire blight in pathogen-inoculated trials conducted in Wenatchee, WA.

	2017				2019			
Untreated, Inoculated Check	22.6	±	10.0	b	21.0	±	11.0	b
Alum (4 lb)	5.8	±	6.9	a	8.3	±	4.7	ab
Alum (6 lb)	6.6	±	2.6	a	9.0	±	3.5	ab
Alum (8 lb)	7.6	±	6.2	a	4.3	±	2.7	a
Alum (10 lb)	4.3	±	1.6	a	4.5	±	2.4	a
Streptomycin (Firewall)	0.3	±	0.6	a	4.8	±	2.8	a
Oxytetracycline (Fireline)	3.8	±	3.4	a	5.7	±	3.1	ab

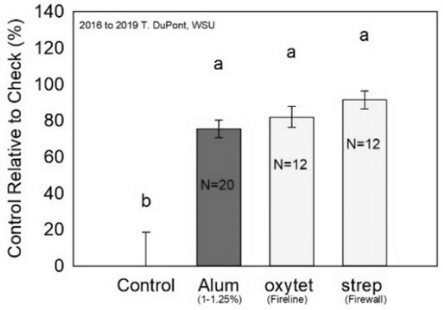
Table 4. Fruit marking on a 0-15 scale for Copper Hydroxide (Previsto), Copper Octanoate (Cueva) and Aluminum Potassium Sulfate (Alum).

Rate per 100 gal per A	2017			2018			2019		
Untreated Check	1.56	±	0.38	0.87	±	0.15	0.1	±	0.03
Previsto (1 qt)	0.66	±	0.17	1.18	±	0.17	0.1	±	0.03
Previsto (2 qt)	0.87	±	0.24	1.50	±	0.41	0.13	±	0.11
Previsto (3 qt)	1.39	±	0.43	1.39	±	0.46	0.08	±	0.06
Previsto (4 qt)	0.99	±	0.20	1.60	±	0.42	0.43	±	0.22
Previsto (5 qt)	4.34	±	3.53	2.53	±	0.62	0.37	±	0.14
Untreated Check	1.56	±	0.38	0.02	±	0.02	0.1	±	0.03
Cueva (1 qt)	1.28	±	0.18	0.05	±	0.00	0.1	±	0.06
Cueva (2 qt)	0.99	±	0.16	0.17	±	0.14	0.22	±	0.07
Cueva (3 qt)	1.14	±	0.55	0.28	±	0.10	0.17	±	0.06
Cueva (4 qt)	0.64	±	0.13	0.45	±	0.06	0.15	±	0.06
Cueva (5 qt)	1.08	±	0.19	0.57	±	0.03	0.12	±	0.07
Untreated Check	1.56	±	0.38	0.02	±	0.02	0.1	±	0.03
Alum (4 lb)	0.74	±	0.24	0.17	±	0.07	0.15	±	0.10
Alum (6 lb)	1.11	±	0.28	0.05	±	0.05	0.4	±	0.13
Alum (8 lb)	0.77	±	0.07	0.25	±	0.10	0.25	±	0.08
Alum (10 lb)	0.32	±	0.07	0.18	±	0.12	0.65	±	0.19

New product trials: New product trials included approximately 20 products per year. A number of new products have had consistent positive results. (Note industry gift grants support new product trials).

Alum (Potassium aluminum sulfate) has been tested for three years in Washington. It has had consistent positive results with an average of 75% control relative to the untreated check in 2016, 2017 and 2019 when the product was applied at an 8 to 10 lb per 100 gal rate (Figure 2). This control was lower than but not significantly different than the oxytetracycline check (82% control) and the streptomycin check (91% relative control). Marking from chemical russet was negligible in all three years (< 1 on a 0 to 15 scale).

Figure 2. Relative control of *Erwinia Amylovora* by Alum in Washington 2016 to 2019[1].

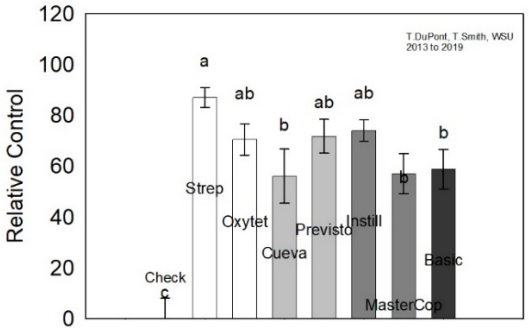


[1]Alum applied at full bloom (approx.12 hr before inoculation) and petal fall at a rate of 8-10 lb/100 gal. Antibiotics applied at 50%, 100% bloom and petal fall.

Two copper products performed well comparable to the soluble copper standards (Cueva 4 qt/100 gal, Previsto 3 qt/100 gal). Instill provided an average of 74% relative control (N=28) comparable to the

soluble copper standards (Figure 3: Cueva 56%, N=12; Previsto 72%, N=12). Mastercop at 2.5 pint/100 gal had an average control compared to the non-treated check of 57%, also providing comparable control to soluble copper standards (N=16).

Figure 3. Control of *Erwinia Amylovora* compared to water-treated check of copper compounds[1].



[1]Firewall (streptomycin standard) 28oz/100 gal; Fireline (oxytet standard) 24 oz/100 gal; Cueva (copper octanoate) 4 qt/A; Previsto (copper hydroxide) 3 qt/A; Instill (copper sulfate pentahydrate) 30 oz/A; Mastercop (copper sulfate pentahydrate) 2.5 pint/A; NuCop (Copper Hydroxide basic) 1lb/A. Antibiotics applied at 50% bloom, 100% bloom and petal fall. Coppers applied day before and day after 100% bloom and petal fall. Inoculation at 100% bloom and petal fall.

Every year multiple new products are tested (Table 5 & 6). When multiple years of data provide consistent results, data for individual products are summarized. The following tables report individual year data for new products where numbered compounds are products without a commercial label and products where a category is given (e.g. Essential oil, *Bacillus subtilis*) are products were insufficient years of data exist to provide reliable conclusions. Product Y1 in 2017, Oxidizers A and B, as well as Essential oil A in 2019 had promising results and will be included in subsequent trials. Essential oil A and Oxidizers A and B had significant marking in 2019 and will be used at lower rates and earlier timings. The SAR product did not perform well. It will be trialed in younger trees at a higher rate which has shown promise in trials in other states. Prohexodione alcium functions by thickening cell walls which is thought to reduce *Erwinia*'s ability to infect. In the 2019 Washington trial it produced no positive effect where it did in other states. We hypothesize that a higher rate and application approximately two weeks before inoculation could lead to better efficacy.

Table 5. Effect of new products on incidence of apple clusters diseased with fire blight in pathogen-inoculated trials conducted in Wenatchee, WA in 2017.

Treatment	Strikes per 100 clusters[1]				Rate per 100 gallons water	Application timings[2]
Untreated, Inoculated Check	23	±	5	ab	water	FB
Firewall 17 standard strep w Tech Mg	0.3	±	0	j	28.8 oz	50% bloom, FB, PF
Fireline 17 (standard oxytet) w Tech Mg	3.8	±	2	fghij	24 oz	50% bloom, FB, PF
F45	10	±	2	cdefghij	9.6 oz	50% bloom, FB, PF
F50	2	±	1	ghij	9 oz	50% bloom, FB PF
Blossom Protect + Buffer Pro., Kasumin	6.5	±	4	defghij	1.25 + 8.75 lb, 64 oz	20% bloom, 50%, 80% bloom
Blossom Protect + Buffer Pro.	10	±	4	cdefghij	1.25 lb + 8.75 lb	20% bloom, 80% bloom
VP20	9.3	±	4	cdefghij	9 lb	100% bloom, PF
Blossom Protect + Buffer Pro. Followed by VP20 then Cueva+ Serenade Opt.	8.8	±	4	cdefghij	1.25 lb + 8.75 lb, 9 lb, 2 qrt, 20 oz	BP+buff 20% bloom, 80% bloom; VP20 FB, Cueva PF
BW165N	13	±	4	bcdef	3 lbs	100%, +7 day

MXMCMBK11	6	±	5	defghij	1.5 pt	day before and day after FB
Master Cop	3.8	±	2	fghij	2.5 pt	day before and day after FB
CX-10250	16	±	8	abcd	4.5 oz	TC & 50% bloom
CX-10250 & Double nickel	9.8	±	5	cdefghij	4.5 oz, 2 qrt	50% bloom; Double nickel day before and day after FB
Double nickel	15	±	6	abcde	2 qrt	day before and day after FB
Instill	6.3	±	3	defghij	30 oz	day before and day after FB
Spectrum	9.3	±	3	cdefghij	30 oz	day before and day after FB
Regalia	13	±	4	bcdef	2 qt.	20% bloom, 80% bloom, PF
Regalia + Blossom Protect + Buffer	11	±	3	cdefghi	2 qt, 1.25 lb, 8.75 lb	20% bloom, 80% bloom, PF
Regalia + Cueva	25	±	8	a	2 qt, 3 qt	Regalia at 20% bloom, Regalia + Cueva at 80% bloom & FB+1
Y1	1.8	±	1	hij	10 ppt	day before and day after FB

[1] Inoculated with *Erwinia amylovora* 153 (streptomycin sensitive fireblight strain) at 100% bloom (FB) $1x10^6$ CFU ml^{-1} solution.
[2]FB = full bloom (100% bloom of king bloom); PF= petal fall.

Table 6. Effect of new products on incidence of apple clusters diseased with fire blight in pathogen-inoculated trials conducted in Wenatchee, WA in 2019.

Treatment	Strikes per 100 clusters[1]			Rate per 100 gal	Application timings[2]
Streptomycin (Firewall 17)	4.6	± 2.7	a	28 oz	50% bloom, FB, PF
Oxytetracycline (Fireline 17)	5.8	± 3.2	a	24 oz	50% bloom, FB, PF
Organic Standard	7.8	± 3.1	ab	6 gal, 1.24 lb, 8.75 lb, 4 qrt	LS: 70%, BP 20%, 80%; PR FB, PF
Oxytetracycline + oxidizer a	3.9	± 2.5	a	24 oz, 128 oz	Oxytet at 50% bloom, FB, PF; oxidizer at FB + 5, 7, 10, 14 days
Alum	4.4	± 2.7	a	8 lb	FB, PF
Oxytetracycline + oxidizer b	4.7	± 1.6	a	24 oz, 128 oz	Oxytet at 50% bloom, FB, PF; oxidizer at 100% + 5, 7, 10, 14 days
Previsto	7.8	± 3.7	ab	3 qt	day before and day after FB, PF
Essential oil A	9.2	± 5.3	abc	2 qt	50%, FB, PF, PF + 3, 5,10 days
Mastercop	9.9	± 2.6	abc	2.5 pt	day before and day after FB, PF
Instill	10.5	± 4.6	abcd	30 oz	day before and day after FB, PF
Basic Copper (50% metallic)	11.4	± 4.0	abcd	1 lb	day before and day after FB, PF
Cueva	11.5	± 4.1	abcd	4 qt	day before and day after FB, PF
phage	17.3	± 3.6	bcde	…	50% bloom, FB, PF
Bacillus Subtillus 1	18.2	± 4.2	bcde	40 oz	50% bloom, FB, PF
SAR1	20.6	± 5.4	de	2 oz	day before and day after FB, PF
Bacillus Subtillus 2	22.5	± 7.1	e	30 oz	day before and day after FB, PF
PhCa[3]	24.1	± 7.0	e	6 oz	pink
Water, Inoculated Check	19.0	± 9.9	cde	NA	FB, PF

[1] Inoculated with *Erwinia amylovora* 153 (streptomycin sensitive fireblight strain) at 100% bloom (FB) $1x10^6$ CFU ml^{-1} solution.
[2]FB = full bloom (100% bloom of king bloom); PF= petal fall.
[3]PhCa – prohexodione calcium

Extension Multiple presentations and articles were shared with tree fruit producers between 2017 and 2019.

For a summary of Fire Blight information visit http://treefruit.wsu.edu/crop-protection/disease-management/fire-blight/

Articles:

Johnson, K., and DuPont, T. 2020. Fire blight in the plant nursery. Digger 64(1):33-36.
DuPont, S.T. Getting Ready for Fire Blight Prevention. Fruit Matters. April 5, 2019.
DuPont, S.T. Fall Fire Blight Considerations. Fruit Matters, October 10, 2018.
DuPont, S.T. Cutting Fire Blight Strikes. Fruit Matters, May 28, 2018.
DuPont, S. T. Dealing with Fire Blight Once it is in the Orchard. Fruit Matters, July 22, 2017.
DuPont, S. T. Fire Blight Season Approaches. Fruit Matters, April 24, 2017.
DuPont, S.T. Fire Blight Management. Tips for Using Blossom Protect. Fruit Matters, April 10, 2017.
DuPont, S.T. Canker Removal Now is Critical for Fire Blight Management. Fruit Matters, February 27, 2017.

Presentations:

2019. Fire Blight Outbreaks and Controls in Washington State, United States. *International Symposium* on *Fire Blight* of Rosaceous Plants. Traverse City, MI.

2019. Fire Blight Outbreaks and Controls in Washington State, United States. Michigan Grower Meeting. Traverse City, MI. *(invited)*

December 2019. Mancha de Fuego Preguntas and Respuestas (Fire Blight). Annual Meeting Washington State Tree Fruit Association. Wenatchee, WA. *(invited)*

December 2019. Fire Blight Status Management and New Research. Annual Meeting Washington State Tree Fruit Association. Wenatchee, WA. *(invited)*

December 2018. Management of Fire Blight *(Spanish)* Washington State Tree Fruit Association Annual Conference. Yakima, WA *(invited)*

March 6, 2019. Fire Blight Control Strategies. Blue Bird Grower Meeting. Wenatchee, WA. *(invited)*

February 20, 2019. Fire Blight an Interactive Discussion. POME Club. Yakima, WA. *(invited)*

February 14, 2019. Fire Blight Management for 2019 – Plan Now, Integrated Control, Cut Hard. Northwest Wholesale. Royal City, WA. *(invited)*

February 5, 2019. Fire Blight Common Questions and Answers. Okanogan Horticultural Association Annual Meeting. Okanogan, WA.

February 5, 2019. Mancha de Fuego. Okanogan Horticultural Association Annual Meeting. Okanogan, WA.

January 29, 2019. Mancha de Fuego (Fire Blight Management). Wilbur Ellis. Yakima, WA. *(invited)*

January 28, 2019. Fire Blight Control Strategies. Wilbur Ellis Grower Meeting. Wenatchee, WA. (*invited*)

April 26, 2018. A Discussion of Fire Blight Management. Fruit Club, Pasco, WA. *(invited)*

April 18, 2018. A Discussion of Fire Blight Management. POM Club, Yakima, WA. *(invited)*

February 15, 2018. Fire Blight New Products and Effective Rates. Pear Research Review. Wenatchee, WA.

February 13, 2018. Fire Blight Common Questions and Answers. Northwest Wholesale. Royal City, WA. *(invited)*

February 8, 2018. Fire Blight Common Questions and Answers. Manson Growers. Manson, WA.

February 6, 2018. Fire Blight Common Questions and Answers. Okanogan Horticultural Society. Omak, WA.

February 1, 2018. Fire Blight Controls In Apples and Pears: New Products and Rates. Northwest Wholesale. Okanogan, WA. *(invited)*

February 23, 2017. Fire Blight Management. Manson Growers. Manson, WA.

February 9, 2017. Managing Fire Blight After a Bad Year. North West Wholesale Grower Meeting. Royal City, WA. *(invited)*

February 2, 2017. Fire Blight (Spanish). G.S. Long Grower Meeting. Wenatchee, WA. *(invited)*

January 23, 2017. Managing Fire Blight in your orchard after a problem year, the use of antibiotics Kausmin, Mycoshield, and Actigard. Northwest Wholesale. Orroville, WA. *(invited)*

January 19, 2017. Fire Blight Management in Apples. North Central Washington Apple Day. Wenatchee, WA.

January 19, 2017. Prevencion y Control de Fire Blight. *(Spanish)* Manejo de Frutales. Wenatchee, WA.

January 18, 2017. Fire Blight Management. North Central Washington Pear Day. Wenatchee, WA.

KEYWORDS, ABSTRACT AND EXECUTIVE SUMMARY

Keywords: Fire blight; *Erwinia Amylovora,* copper, Previsto, Cueva, Alum

Fire blight prevention trials were conducted in Washington State between 2017 and 2019 to test new products and effective rates. Trials found that the optimum range of metallic copper application for fire blight control was between 0.16 and 0.25 lbs per 100 gal per acre of metallic copper equivalent. Alum (Potassium aluminum sulfate) was most effective at 8 lbs per 100 gal. Fruit marking was low overall but with statistically significant levels for Previsto at 5 qrt/ 100gal. New product trials found that Alum at 8 to 10 lbs/ 100 gal has provided consistent positive results with an average control of 75% statistically similar to the oxytetracycline check (82% control). Copper product Instill provided 75% relative control and Mastercop (2.5 pt per 100 gal) 57% control comparable to copper standards across multiple years.

FINAL PROJECT REPORT

Project Title: Greenhouse screening of 49 dwarf rootstock candidates

PI: Amit Dhingra
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Telephone: 509-663-8181
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Cooperators: UC Davis project funded by Pear Bureau NW and Cal Pears.

Total Project Request: Year 1: 34,133 **Year 2**: 19,289 **Year 3**: 20,037

Other funding sources
Agency Name: USDA SCRI Preapplication
Amount Pending: $2,800,000 (2020-2024)
Notes: "Phenotypic and Genomic Characterization of Pyrus Germplasm for Development of Dwarfing Rootstocks for Sustainable Pear Production in the USA" (PI Dhingra, Co- PI Evans). Synergistic project to characterize diverse set of Pyrus germplasm via large scale phenotyping and genotyping.

Agency Name: PNW Pear Bureau
Amount awarded: $322,003 (2019 – 2022)
Notes: "Pear Rootstock Breeding" (PI: Evans; Co-PI: Dhingra, Co-PI: Soon Li Teh)
Synergistic project to develop and establish pear rootstock seedlings to develop dwarfing rootstocks that are suited for high-density pear production.

Agency Name: Washington State University Graduate school
Amt. awarded: $34,000 (2017)
Notes: Support for Danielle Guzman, Graduate student in the Dhingra lab.

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 Funding

Item	2017	2018	2019
Salaries[1]	21000	10920	11357
Benefits	10133	5269	5480
Supplies[2]	1000	1000	1000
Travel	500	500	500
Plot Fees[3]	1500	1600	1700
Total	34133	19289	20037

Footnotes:
1 – Support for technical help to multiply rootstock selections, graft with scions and manage plants
2 – Greenhouse soil and supplies
3 – Greenhouse space usage fee per year

OBJECTIVES

1. Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically, and physiologically uniform rooted material for subsequent grafting experiments.
2. Graft 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as control.

The project plan to introduce all selections into tissue culture and establish enough clones for each selection in the greenhouse has been completed. These will then be grafted over with budwood from Bartlett and Anjou. OHxF 87 rootstock will be used as a control. The grafted plants will be grown and maintained in the WSU greenhouse to assess if the dwarfing trait is transmitted to the scion. Data on internode length, height, and ratio between the two; crotch angle will be recorded. Seedlings that impart dwarfing to the scions will be evaluated as rootstock candidates in field trials to be performed after the completion of this project.

SIGNIFICANT FINDINGS

- Budwood from each of the seedling has been successfully established in the micropropagation system.

- The seedlings being cycled through rapid growth process in the greenhouse have achieved a height of 24-40 inches depending on the seedling they were derived from. Besides the variability in height, the caliper is highly variable and at present not suitable for budding.

RESULTS AND DISCUSSION

Objective 1: Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform material for subsequent grafting experiments.

The 49 dwarf seedlings were obtained from crosses made in 2013. The growth of these seedlings has been fast tracked using horticultural rapid cycling process which includes providing ecodormancy (cold requirement) treatments in a cold room. The plant material was incubated in the cold for 4 months and has been recently moved to the green house, where the plant material is undergoing active growth (Figure 1). We initiated nearly 50 buds per selection. All of the selections have been successfully introduced and established into the micropropagation system.

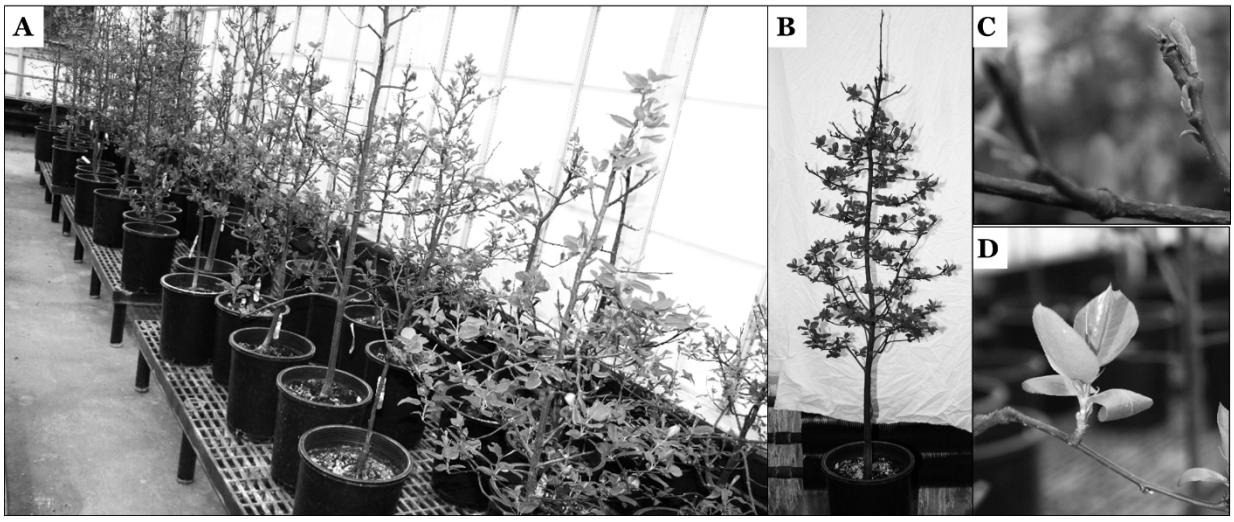

Figure 1: 49 dwarf seedlings in the greenhouse. A. An overview of all the seedlings. B. One of the selections exhibiting a compact growth habit. C and D – actively growing shoots that are being processed to be initiated in the micropropagation system.

Objective 2. Graft or bud 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

All of the plant material has been cloned and the plants are currently growing in the greenhouse. The plants have reached a height of 24-40 inches depending on the seedling they have been cloned from.

Figure 2: Twenty clones representing each of the 49 seedlings growing actively in the greenhouse.

This project represents one of the four distinct but complementary approaches to establish a foundation for developing dwarfing pear rootstocks (Figure 1). The aim of this project was to evaluate if the dwarf habit of the potentially aneuploid seedlings will transmit to the scion. Promising selections out of the 49 dwarf seedlings could be used as a rootstock selection, or a parent for the pear rootstock breeding program.

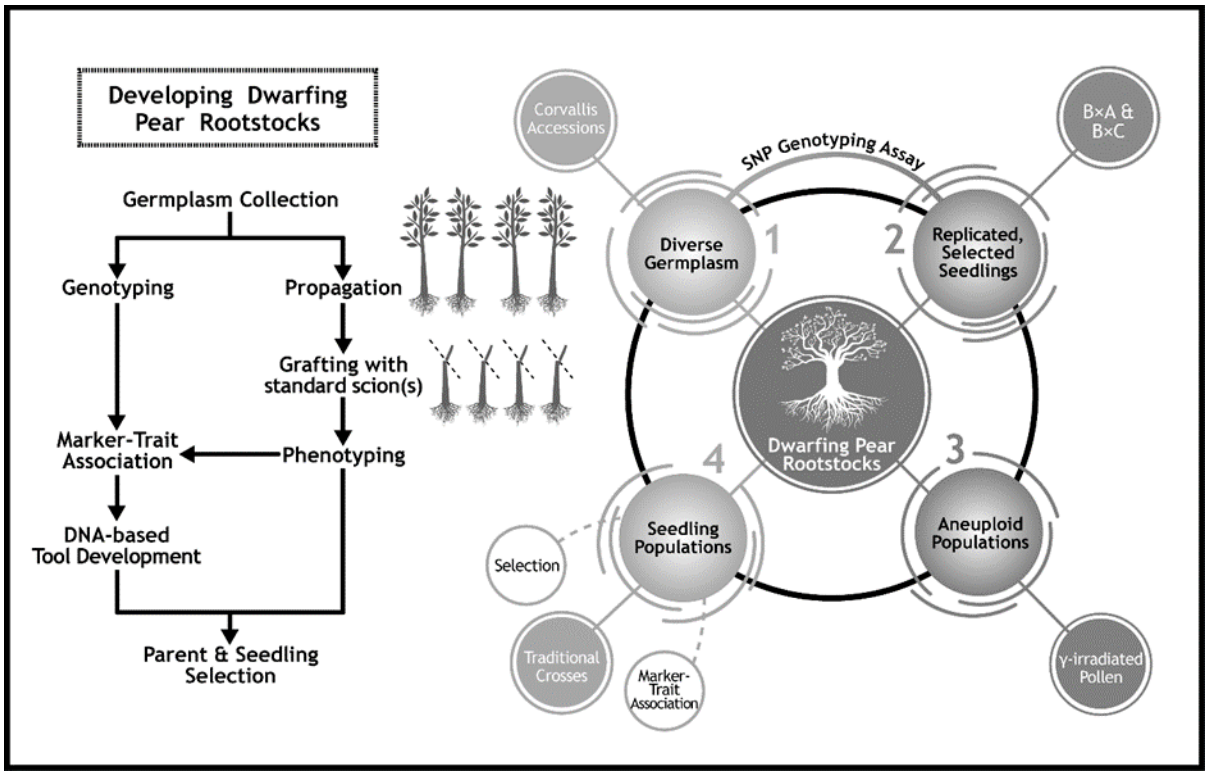

Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. This project focuses on germplasm developed using gamma (γ) irradiated pollen and labeled as number 3.

Outreach

- Presentation by Amit Dhingra - 'The foundation for the future of pear production in the PNW' Research News Flash talk at the Washington State Tree Fruit Association Meeting, December 2017.
- Soon Li Teh presented "Pear Rootstock Breeding Program" at the WSU Sunrise Research Farm Extension Field Day at Rock Island, WA on August 7, 2019.
- Soon Li Teh presented "Initiating Pear Rootstock Breeding at Washington State University" at the 2019 Annual Meeting for National Association of Plant Breeders (NAPB) at Pine Mountain, GA on August 25 – 29, 2019.
- The WSU pear rootstock breeding program was featured as a Good Fruit Grower article, "Rooting out Solutions for Pear Growers" on September 2019 Issue (https://www.goodfruit.com/rooting-out-solutions-for-pear-growers/).
- Soon Li Teh and graduate student, Zara York presented an overview of pear rootstock breeding at the WSU Tree Fruit Breeding 101 – Extension Field event at Orondo, WA on October 24, 2019.
- Amit Dhingra visited Fowler Nurseries, Sierra Gold Nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in November 2019.
- Amit Dhingra presented a seminar at Pairwise Inc. in North Carolina regarding pear genomics and rootstock breeding in September 2019.

- Zara York presented “Advancing genetic resources for pear rootstock breeding” Research News Flash talk at the Washington Horticultural Association Show, Wenatchee, WA December 2019.
- Amit Dhingra presented on pear rootstock research in the Genomic Advances in fruit and vegetable Breeding workshop at the annual Plant and Animal Genome conference at San Diego, CA January 2020.

EXECUTIVE SUMMARY

Project title: Greenhouse screening of 49 dwarf rootstock candidates
Keywords: Dwarfing rootstocks, aneuploidy, micropropagation, genetic diversity, germplasm, seedling population
Abstract: All the seedlings representing 49 dwarfing rootsock candidate were successfully introduced and established into the micropropagation process as a means to preserve the precious genetic material. In addition, a minimum of 20 clones each were established and rooted in the greenhouse. The clones achieved the necessary height, however, the caliper has remained inadequate for budding. The clones will be grown for another season in the greenhouse prior to being budded.

Dwarfing rootstocks have transformed the production and training systems of various tree fruit and nut crops. For instance, in the last 20 years, apple production has increased over 50%, with much of this increase attributed to the adoption of dwarfing rootstocks. The economic impact of dwarfing rootstocks on the U.S. apple industry is estimated to be between $500 million and $1.2 billion per year when comparing low-density and high-density orchards. In the case of pear, dwarfing rootstocks have been used for several decades in Europe, however, in the U.S., 97% of the pear orchards still represent low density plantings with large 3-dimensional pear trees that can reach up to 15 feet in height.

As a foundation for developing dwarfing rootstocks, in 2013, a seedling population was developed from gamma-irradiated pollen to induce aneuploidy in the resulting seedlings. Aneuploidy is a condition where the chromosomal regions of an organism are deleted or duplicated. Utilizing this approach, phenotypes such as dwarfing can be rapidly generated. The resulting seedlings demonstrate a great range of diversity in terms of vigor and branch angle. The next key step is to identify seedlings that induce dwarfing onto the grafted scion

The first aim of this 3-year project was to establish all the aneuploid seedlings in the micropropagation process both to preserve and multiply the material as needed. The second aim was to graft ‘Bartlett’ and ‘D’Anjou’ scions to observe if the compactness of the individual used as a rootstock dwarfed the scion. All of the seedlings have been successfully established in the micropropagation process and continue to be maintained. From this micropropagation repository, 20 clones of each of the seedling have also been established and continue to be grown in the greenhouse. These seedlings are less vigorous and the growth rate is slower than a typical pear seedling. While the clones have reached an average height of 24-40 inches, the caliper needed for successful budding remains to be achieved. The plants will continue to be maintained in the greenhouse and budded in the fall.

The aneuploid population will be used to not only meet the objectives of this project but also to understand which chromosal regions contribute to dwarfing through future project proposals. This material is also being used to support two federal grant proposals - USDA SCRI due March 13th and USDA AFRI – due summer 2020.

FINAL PROJECT REPORT

Project Title: Mechanisms and practical solutions to control scald of pears

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Cooperators: Steve Castagnoli, Paul Chen, Craig Mallon, Grady Leiblein, Allison Walston, Arden Reed

Other funding sources
none

Total Project Funding: 117,062

Budge History:

Item	2017	2018	2019
Salaries	20,222[1]	20,829	21,454
Benefits	1,950[2]	2,009	2,069
Wages	10,744[3]	11,066	11,398
Benefits	1,074[4]	1,107	1,140
Equipment			
Supplies	3,500[5]	3,500	3,500
Travel	500[6]	500	500
Miscellaneous			
Total	37,990	39,011	40,061

Footnotes:
[1]Postdoctoral Research Associate: 1/2 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: 800hr 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 storage and CA storage 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. Understand completely the physiological mechanisms of scald development; understand growing season conditions and harvest maturity effects on the natural antioxidant capacity associated with the oxidation of α-farnesene into conjugated trienols (CTols) and therefore scald susceptibility of Anjou pear.
2. Study commercially-feasible methods for controlling scald of susceptible Anjou pear; the potential of the combination treatments of Harvista/ReTain + ethoxyquin + low-O_2.
3. Study the potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coatings as alternatives to ethoxyquin for controlling scald of Anjou pear.
4. Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.

SIGNIFICANT FINDINGS

- Physiological mechanism of scald development. The reduction of α-farnesene and increase in CTols during storage are associated with superficial scald development.
- Higher ACU in Anjou pears reduced superficial scald by increasing antioxidant metabolites, antioxidant enzymes, and total antioxidant capacity.
- Pre-harvest Ca spray increased fruit calcium content, antioxidant metabolites, antioxidant enzymes, and total antioxidant capacity of Anjou pears and resulted in a slight reduction in superficial scald after 4 and 5 months in storage.
- Sunlight exposure prior to harvest delayed the accumulation of α-farnesene and CTols, and inhibited the development of superficial scald development.
- Harvest maturity affected scald development. More mature fruit developed more scald.
- Regardless of rate and timing, NAA application did not affect superficial scald. After 4 months of storage, all NAA treatments had relatively high incidence of superficial scald, with no significant differences between treatments and the untreated control.
- O_2 concentrations of 0.5 and 1% inhibited the development of superficial scald, with 0.5% O_2 stored fruit remaining free of scald over the entire storage period, whereas 2% O_2 stored fruit developed 68% scald at 6 months and increased to 90% after 10 months.
- AVG applied 1 WBH at 60 or 120 ppm resulted in reductions in scald compared to the untreated control after 3 and 5 months of RA storage. Incidence of superficial scald of all treatments increased through the storage period and reached 100% by month 7.
- Harvista at 120 g/acre applied 10 d before harvest to 'Anjou' pears reduced scald development compared to the control after 4 and 6 months of RA storage.
- Pears treated with AVG at 60 and 120 ppm and stored at 0.8 % O_2 could were scald free following 10 months of storage.
- AsA did not affect scald development, while Lovastatin at rates of 2 and 5 % provided greater effects on reducing scald incidence than ethoxyquin treatment.

RESULTS

1. Understand completely the physiological mechanisms of scald development

α-Farnesene metabolism

For Anjou pear harvested at 16-15 lbs. and stored in regular-air (RA) at 30 °F, the incidence of superficial scald after 7 d at 68 °F remained relatively low through three months of storage but increased thereafter (Fig 1). α-Farnesene showed marked increase from one month of storage, peaking at three months and then declining. CTols concentration showed a similar pattern but peaked one month later than α-farnesene and preceded the large increase in scald.

Fruit with varied accumulated cold units (ACU) on fruit scald susceptibility
Fruit with ACU ranging from 12-316 at commercial harvest maturity was collected from 5 orchards located at elevations ranging from 500 to 2,000 ft. and placed in RA for up to 6 months. After 2-6 months in storage, fruit with ACU at 227 and 316 had lower incidence of superficial scald than fruit with ACU at 15 or 118 (Fig. 2). Higher ACU significantly reduced the production of α-farnesene and CTols and maintained relatively high levels of TP, TFO, SOD, CAT, APX, GR, DPPH, and FRAP after 5 months of storage (Table 1).

Fruit with varied Ca concentration on fruit scald susceptibility
$CaCl_2$ was applied at 3.47 pound/acre (100 gal/acre, Ca rate at 0.15%) 6 times from 1 month after full bloom to 1 week before harvest. Fruit were collected at commercial harvest maturity, then placed in RA for up to 5 months. Superficial scald and fruit physiological and biochemical responses were evaluated after 2, 3, 4, and 5 months of storage. Pre-harvest Ca sprays resulted in higher calcium content of fruit flesh at harvest, but no significant difference in fruit skin Ca content (Table 2). Ca sprays resulted in a slight reduction in superficial scald after 4 and 5 months in storage (Fig. 3). Additionally, Ca treated fruit showed trends towards reduced α-farnesene, and CTols as the storage duration increased. Ca sprays resulted in an increase in the antioxidant metabolite TP but no difference in TFO, or the antioxidant enzymes SOD and CAT or APX (Table 3). GR was higher in Ca treated fruit as was FRAP. DPPH showed no response to Ca treatment.

Different sunlight exposure on fruit scald susceptibility
Bagged and unbagged fruit were collected at commercial harvest maturity and placed in RA storage. Fruit were evaluated at harvest, and 2, 3, 4, and 5 months after storage. Scald was evaluated on the blushed (sun-exposed) and shaded portions of the fruit peel. The blushed peel of unbagged fruit had no incidence of scald throughout the 5-month storage period, while the shaded peel developed 0, 8, 89 and 100% scald incidence at 2, 3, 4, and 5 months, respectively. Bagged fruit developed 0, 39, 95, and 100% scald incidences at 2, 3, 4, and 5 months storage, respectively. There were no significant differences in α-farnesene concentration of blushed and shaded peels of unbagged fruit from harvest through 5-month storage periods (Fig. 4). Both had the highest level of α-farnesene at 3 to 4 months of storage. In bagged fruit, the concentration of α-farnesene was similar to that of unbagged fruit at harvest but was higher by 2 months of storage, and peaked earlier and at a much higher level. The blushed peel of unbagged fruit had the lowest concentration of CTols throughout the experimental period. The blushed and shaded peels of unbagged fruit accumulated the highest CTols concentrations at 4 months, while the bagged fruit reached the highest level of CTols 1 month earlier. The shaded peels of unbagged fruit and peels of bagged fruit had similar CTol concentrations at 4 and 5 months of storage. Compared to the shaded peels and bagged fruit, the blushed peel remained higher TP, TFO, SOD, APX, GE, DPPH, and FRAP after 5 months of storage (Table 4).

Influence of harvest maturity on fruit scald susceptibility
Anjou pears harvested at 15.9-13.9 lbs. developed less superficial scald than fruit harvested at 13.9-11.8 lbs. after 3-4 months of RA storage and 3-7 months in CA at 30 °F following 7 days at 68 °F (Fig. 5). Anjou pears harvested at 13.9-11.8 lbs. had relatively high incidence of superficial scald development after 3-4 months in RA storage and 5-6 months in CA storage following 7 d at 68 °F. For fruit stored in RA, all harvest maturities developed nearly 100% incidence of scald by 5 months of storage. Scald incidence of all harvest maturities of CA stored fruit increased up to 9 months of storage.

Influence of NAA on fruit scald susceptibility
NAA was applied at 20 ppm 1 and 2 WBH and 40 ppm 1, 2, and 3 WBH. At harvest, fruit were placed in RA storage. Non-treated and NAA-treated fruit were evaluated after 2, 3, and 4 months of RA storage plus 7 d at 68 °F. After 4 months of storage, all NAA treatments had relatively high incidence of superficial scald, with no significant differences between treatments and the untreated control (Fig. 6). α-

Farnesene content and CTols content of NAA-treated fruit and the untreated control increased during storage, with no significant differences between treatments and untreated control.

2. Commercially-feasible methods for controlling scald of susceptible Anjou pear.

Effects of low-O_2 CA on fruit scald susceptibility

Anjou pears were harvested at commercial harvest maturity from MCAREC, and after 2 d of cold storage, fruit were loaded into gas-tight cabinets. The cabinets were flushed with purified nitrogen and then CO_2 concentration was adjusted to < 0.5% by adding hydrated lime. O_2 concentration was adjusted to 2.0, 1.0, or 0.5% within 6 d of sealing the cabinets. Air-stored fruit was placed in storage at 30 °F. After 6, 8, and 10 months of storage, fruit were removed and held at 68 °F for 7 days. Superficial scald incidence of air stored fruit was 100% following 6 months of cold storage (Fig. 7). The 2% O_2 stored fruit developed 68% scald at 6 months and increased to 90% after 10 months. The lower O_2 concentrations of 0.5 and 1% inhibited the development of scald, with 0.5% O_2 stored fruit remaining free of superficial scald over the entire storage period. Ethylene production in air-stored fruit decreased from 5 to 1 ng kg^{-1} s^{-1}, while respiration rate increased from 11 to 13 μg CO_2 kg^{-1} s^{-1} from month 6 to 10. Low O_2 treatments had lower ethylene production and respiration rates at month six, then increased thereafter. The ethylene production rate of 2% O_2 stored fruit increased, reaching a maximum level of 7 ng kg^{-1} s^{-1} after 8 months of storage, before decreasing to 4 ng kg^{-1} s^{-1}. The ethylene production rate of 0.5 and 1% O_2 stored fruit increased between 6 and 10 months of storage, reaching 3 and 5 ng kg^{-1} s^{-1}, respectively. Total antioxidant capacity of air and CA stored fruit decreased during ripening. Reducing O_2 concentration from 21% to 0.5% inhibited the reduction of total antioxidant capacity, but no significant differences were observed among 2, 1 and 0.5% O_2 treatments. The effect of low O_2 treatments on α-farnesene content were similar to those on ethylene production. CTols of air and CA stored fruit gradually increased, and the 0.5 and 1% O_2 stored fruit had lower CTols contents than other treatments. These results indicate that reducing O_2 concentration can reduce superficial scald of Anjou pears with 1 and 0.5% O_2 providing nearly complete and complete control.

Effect of pre-harvest Retain on fruit scald susceptibility

AVG (Retain) was applied at 60 ppm 1 WBH and 120 ppm applied 1 and 2 WBH. After harvest, fruit were placed in RA storage. Fruit were evaluated after 1, 3, 5, and 7 months of RA storage plus 7 d at 68 °F. Incidence of superficial scald of all treatments increased through the storage period and reached 100% by month 7 (Fig. 8). After 3 (Fig. 8, right picture) and 5 months of RA storage AVG applied 1 WBH at 60 or 120 ppm provided reductions in scald compared to the untreated control.

Effects of pre-harvest Harvista on fruit scald susceptibility

Harvista (1-MCP) was applied at 120 g/acre 10 d before harvest. Fruit was collected at commercial harvest maturity and 4 days later and placed in RA storage. Fruit was evaluated after 3, 4, 6, and 8 months of storage. Based on reductions of FF and green color (data not shown), Harvista extended the harvest window by 3-4 days. During 4-6 months of storage, both Harvista and delay-harvest pears had significantly lower superficial scald incidence (Fig. 9).

Effects of the combination of pre-harvest AVG + ethoxyquin + 0.8 % O_2 CA on fruit scald susceptibility

AVG was applied at 60 and 120 ppm 1 WBH. Fruit was harvested at commercial harvest maturity from MCAREC, and after 2 d of cold storage fruit from each treatment was treated with 1000 ppm ethoxyquin, and then loaded into gas-tight cabinets. The cabinets were flushed with purified nitrogen and then O_2 concentration was adjusted to 0.8 % within 6 d. Fruit were evaluated after 6 and 10 months of storage. Regardless of AVG treatment, decreasing O_2 level from 2.3 to 0.8 % prevented development of superficial scald following 10 months of storage (Fig. 10).

3. The potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coating as alternative to ethoxyquin for controlling scald of Anjou pear

Anjou pears were harvested at 14.23 lbs and treated with Lovastatin at rates of 1, 2, and 5% or ascorbic acid (AsA) at rates of 1, 2, and 5% for 10 min, and then stored in RA storage for 4 and 6 months. Compared to the untreated fruit, AsA did not affect superficial scald after 4 and 6 months of storage (Fig. 11). Conversely, Lovastatin at rates of 2 and 5 % significantly reduced superficial scald, with better control than ethoxyquin. We intended to test the combination of 5% Lovastatin with Semperfresh and Chitosan, but Lovastatin failed to dissolve in Semperfresh or Chitosan and no results of these combinations are available.

4. Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.

In this study, we investigated both preharvest and postharvest practices that may provide reductions in scald development. The effects of environmental factors such as sunlight exposure and ACU may be set at harvest, and therefore outside the control of growers, but they may be managed to some degree through inventory control at the packing house (e.g. ship low ACU fruit first). We determined that there are benefits of high antioxidant capacity in fruit to reducing scald development. This finding suggests that practices that increase this capacity should be optimized. Pre-harvest Ca application and harvest at optimum maturity both contribute to fruit with high antioxidant capacity and low scald during storage. Preharvest application of Retain and Harvista helped reduce scald at optimal RA storage time. Excessive storage time or extending the harvest window resulted in reduced efficacy of ReTain and Harvista for controlling scald. Decreasing the O_2 level in CA storage from 2.0 to 1.0 % provided better scald control. Decreasing the O_2 level to < 0.8 % when combined with pre-harvest ReTain treatment provided an additional reduction in scald incidence, but pears failed to ripen.

Our results with Lovastatin indicate that it may be an alternative to ethoxyquin for controlling scald. Additional study is needed, however, to optimize its use in combination with other coating formulations.

Table 1. Total polyphenols (TP), total flavonoids (TFO), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, and ferric reducing antioxidant power (FRAP) of flesh tissue in pears with varied ACU after 5 months of storage plus 7 d at 68 °F.

	TP (mg g^{-1})	TFO (mg g^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	APX (U g^{-1})	GR (U g^{-1})	DPPH (mM g^{-1})	FRAP (mM g^{-1})
ACU 12	0.5 b	0.5 c	0.9 cd	0.9 b	81 c	5 b	1.9 b	0.3 c
ACU 15	0.6 b	0.6 c	1.0 c	0.8 b	65 e	6 b	1.7 c	0.5 b
ACU 118	0.6 b	0.6 c	0.8 d	0.6 c	77 d	5 b	1.3 d	0.2 c
ACU 227	0.8 a	0.7 b	1.4 a	1.3 a	93 a	8 a	2.2 a	0.6 a
ACU 316	0.8 a	0.9 a	1.2 b	1.2 a	89 b	8 a	2.3 a	0.7 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

Table 2. Effects of Ca treatment on calcium content of skin and flesh tissue in pears at harvest.

	Calcium content (ppm)	
	Skin tissue	Flesh tissue
Control	377.63 a	1258.53 b
Ca treatment	396.60 a	1401.00 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

Table 3. TP, TFO, SOD, CAT, APX, GR, DPPH radical scavenging capacity, and FRAP of flesh tissue in pears with and without Ca treatment after 5 months of storage plus 7 d at 68 °F.

	TP (mg g^{-1})	TFO (mg g^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	APX (U g^{-1})	GR (U g^{-1})	DPPH (mM g^{-1})	FRAP (mM g^{-1})
Control	0.6 b	1.2 a	1.4 a	0.9 a	78.0 a	9 b	2.5 a	1.4 b
Ca treatment	0.8 a	1.1 a	1.5 a	1.1 a	68.0 a	14 a	2.8 a	2.2 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

Table 4. TP, TFO, SOD, CAT, APX, GR, DPPH radical scavenging capacity, and FRAP of flesh tissue in blushed and shaded of unbagged pears and whole peel of bagged fruit after 5 months of storage plus 7 d at 68 °F.

	TP (mg g^{-1})	TFO (mg g^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	APX (U g^{-1})	GR (U g^{-1})	DPPH (mM g^{-1})	FRAP (mM g^{-1})
Blushed peel	1.1 a	1.3 a	1.9 a	0.6 a	124 a	14 a	3.4 a	1.2 a
Shaded peel	0.6 b	0.8 b	1.0 b	0.6 a	48 b	6 b	1.8 b	0.8 b
Bagged pears	0.5 b	0.7 b	0.7 c	0.5 a	46 b	5 b	1.7 b	0.7 b

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

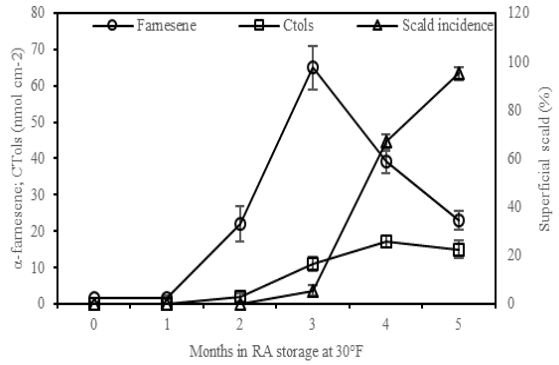

Fig. 1. α-Farnesene, conjugated trienols (CTols), and superficial scald incidence of Anjou pear following 7 d at 68 °F during 5 months storage in regular-air (RA) at 30 °F. Values are means ± standard deviation.

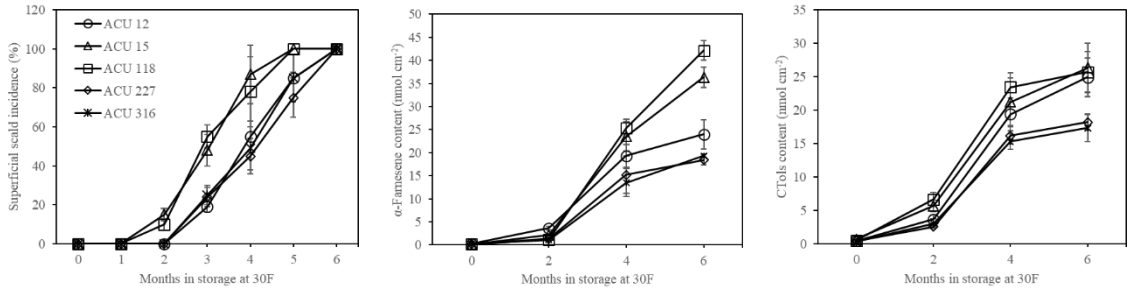

Fig. 2. Effects of varied ACU on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 6 months of RA storage at 30 °F.

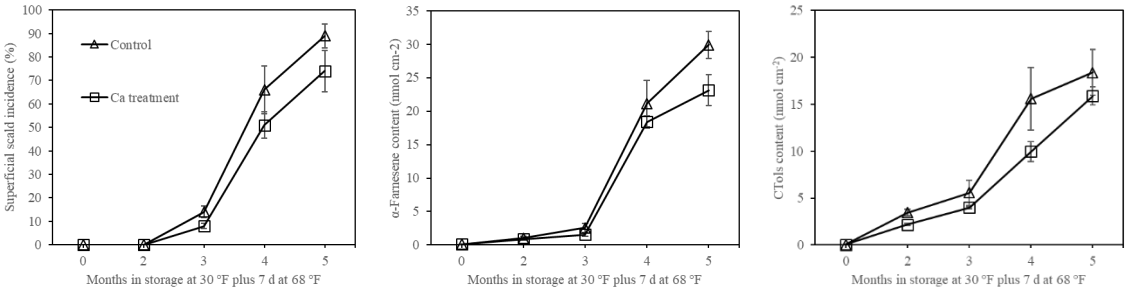

Fig. 3. Effects of Ca treatment on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 5 months of RA storage at 30 °F.

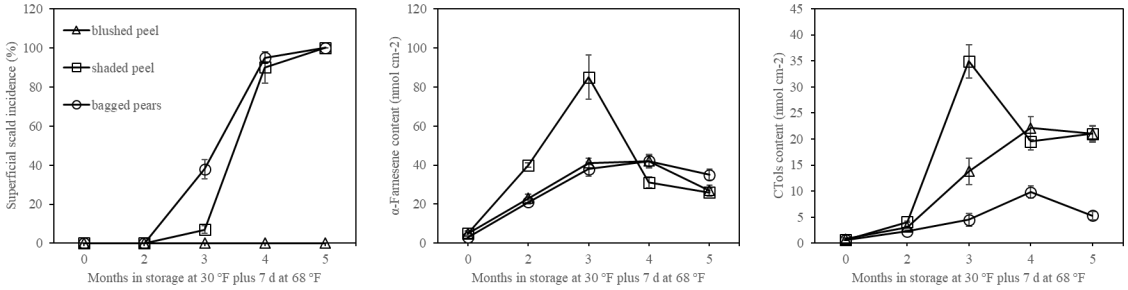

Fig. 4. Effects of sunlight exposure on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 5 months of RA storage at 30 °F.

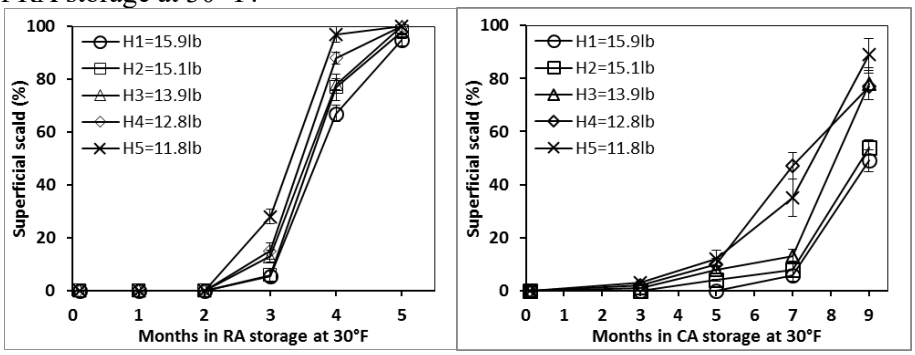

Fig. 5. Effects of harvest maturity on superficial scald incidence of Anjou pear following 5 months of RA storage at 30 °F or 9 months of CA storage at 30 °F, both plus 7 d at 68 °F.

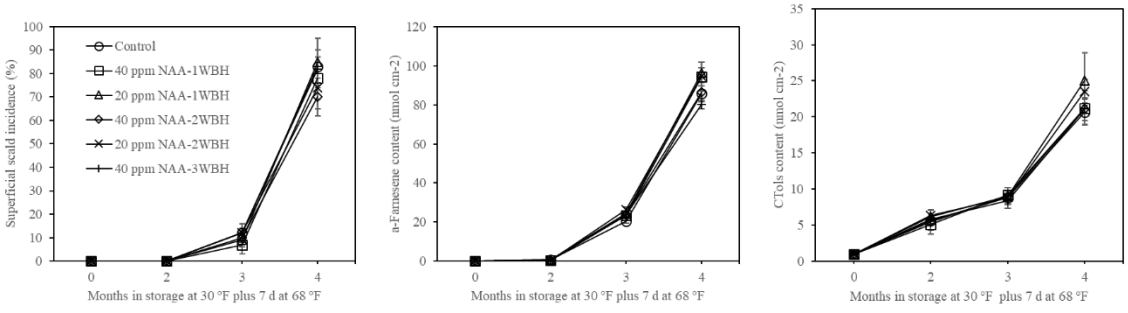

Fig. 6. Effects of pre-harvest NAA applied at 20 or 40 ppm 1, 2, or 3 weeks before commercial harvest on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 4 months of RA storage at 30 °F.

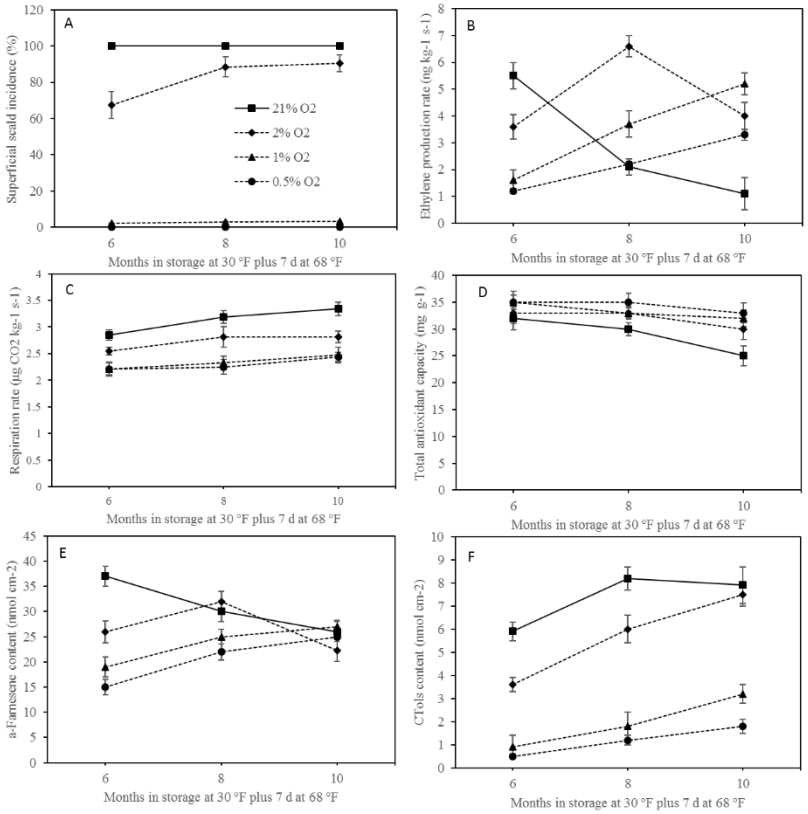

Fig. 7. Effects of different O_2 regimes on superficial scald incidence, EPR, RR, total antioxidant capacity, α-farnesene content, and CTols content of Anjou pears during 10 months of storage at 30 °F. O_2 concentrations were 21 % (air), 2 %, 1 %, and 0.5 % with CO_2 < 0.05 %.

Fig. 8. Effects of preharvest treatments of AVG at rate of 60 and 120 ppm applied at 1 and 2 weeks before commercial harvest on superficial scald of Anjou pears during 7 months of RA storage at 30 °F.

Fig. 9. Effects of preharvest Harvista applied 10 d before commercial harvest on superficial scald incidence of Anjou pears during 8 months of RA storage at 30 °F.

Fig. 10. Effects of preharvest 60 and 120 ppm AVG applied 1 w before commercial harvest on superficial scald incidence of Anjou pears during 10 months of CA storage (2.3 and 0.8% O_2) at 30 °F.

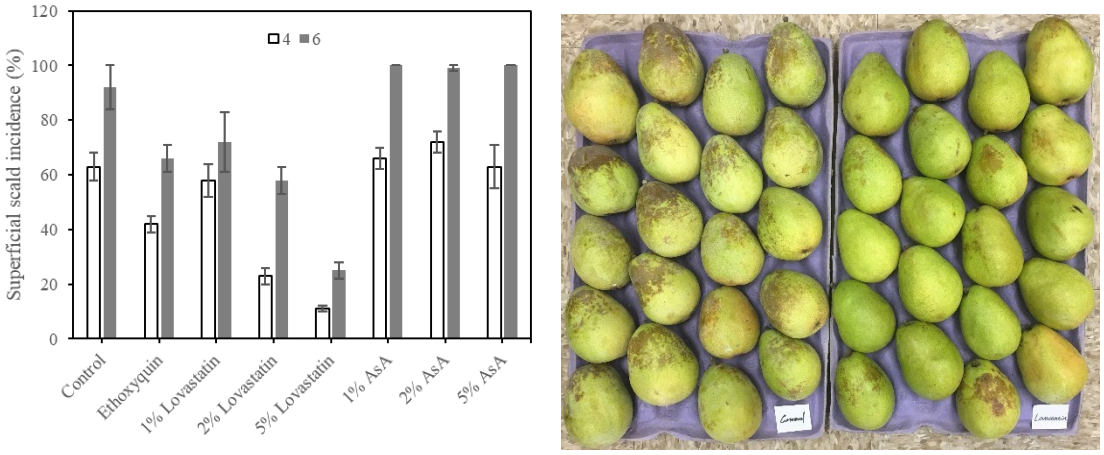

Fig. 11. Effects of 1-5 % Lovastatin and 1-5% ascorbic acid on superficial scald incidence of of Anjou pears after 4 and 6 months of RA storage at 30 °F.

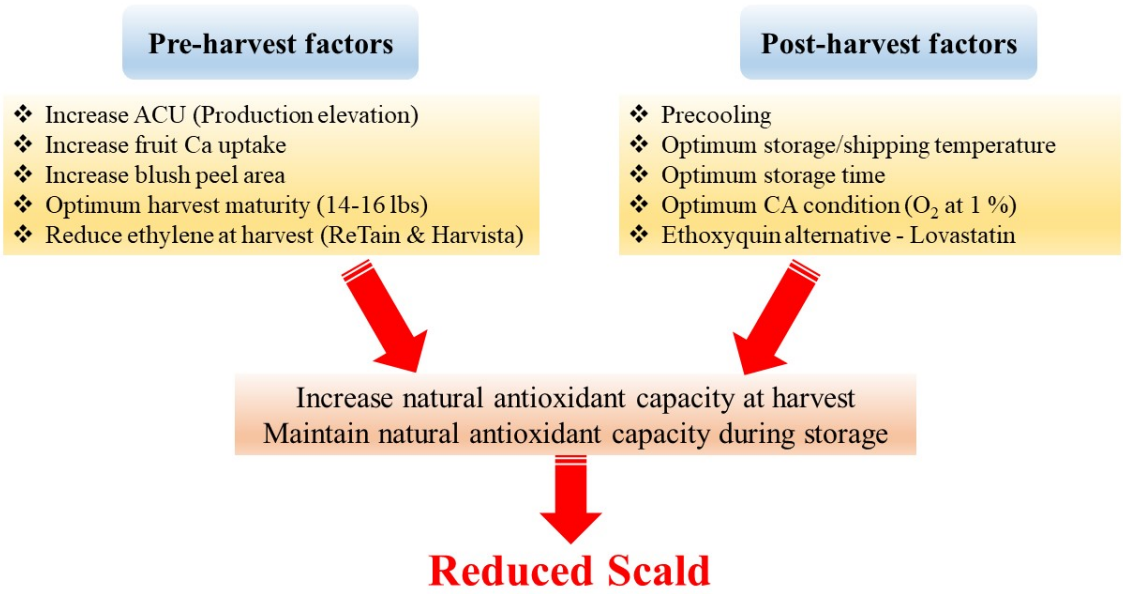

Fig. 12. Summary of pre- and postharvest practices for reducing scald susceptibility by increasing the natural antioxidant capacity in fruit.

EXECUTIVE SUMMARY

Project Title: Mechanisms and practical solutions to control scald of pears
Keywords: Anjou Pears; Scald control; Antioxidant; Practice
Abstract: Understanding of the mechanisms and pre- and postharvest factors affecting scald development are necessary for developing procedures to reduce scald loss. This project found that the reduction of α-farnesene and increase in CTols are associated with the development of scald. Antioxidant metabolites played important roles in controlling scald. High ACU, high fruit Ca content, sunlight exposure, and optimum harvest maturity resulted in high antioxidants at harvest and reduced scald. Optimizing practices in ReTain, Harvista, O_2 regimes, and Lovastatin coating provided a great scald control than ethoxyquin application alone.

Controlling superficial scald of Anjou pears relies on the pre-storage application of the antioxidant ethoxyquin. This practice, however, does not provide adequate control because significant storage losses due to scald continue to occur in some years. Additionally, the European Union has withdrawn authorization for plant protection products containing ethoxyquin. Therefore, alternatives to ethoxyquin application for scald control are needed. Ethylene biosynthesis triggers and enhances the synthesis of α-farnesene in the fruit peel with subsequent induction of scald development. Greater understanding of the mechanisms and pre- and postharvest factors affecting scald development are necessary for developing procedures to reduce losses due to scald.

In this project, we confirmed that the reduction of α-farnesene and increase in CTols during storage are associated with the development of superficial scald. We also showed that antioxidant metabolites in storage played important roles in controlling scald. Furthermore, we found that preharvest environmental conditions such as ACU, fruit Ca content, sunlight exposure, and harvest maturity impacted antioxidant metabolites at harvest. Significant findings include:

- Pears with low ACU had higher scald incidence due to the lower antioxidant metabolites.
- Pre-harvest Ca application increased fruit Ca content, antioxidant metabolites, antioxidant enzymes, and total antioxidant capacity and reduced scald in storage.
- Sunlight exposure delayed the accumulation of α-farnesene and CTols, and inhibited the development of scald by increasing antioxidants and related enzymes at harvest.
- More mature fruit developed more scald.

Additionally, we found that scald development was influenced by pre- and post-harvest practices. Significant findings include:

- Pre-harvest NAA application did not affect scald in storage.
- AVG applied at 60 and 120 ppm 1 week before harvest significantly reduced scald incidence during 3-5 months of RA storage, but not beyond 5 months.
- AVG applied 2 weeks before harvest did not affect scald.
- Harvista application at 120 g/acre 10 d before harvest extended harvest window for 4 d and controlled scald for 4 months.
- Reducing storage O_2 level from 2 to 1 or 0.5% resulted in complete control of scald over 10-month storage period.
- Storage O_2 at 0.8 % plus AVG at 60 and 120 ppm prevented scald in ethoxyquin treated fruit for 10 months.
- AsA did not affect scald development.
- Lovastatin applied at 2 and 5 % provided significant reductions in scald incidence.

Optimizing these practices and conditions should provide a greater degree of scald control than ethoxyquin application alone.

FINAL PROJECT REPORT

Project Title: Erythritol: An artificial sweetener with insecticidal properties

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Cooperators: None

Other funding sources: None

Total Project Funding: $16,310

Budget History:

Item	Year 1: 2019
WTFRC expenses	
Salaries	$14,690, USDA; $1560, WSU
Benefits	$1,175, USDA; $145, WSU
Wages	
Benefits	
Equipment	
Supplies	$445
Travel	$1917, WSU
Plot Fees	
Miscellaneous	
Total	$19,932

ORIGINAL OBJECTIVES

The project directly addressed several research priorities identified by the Pear Research Sub-committee, namely integrated control of pear psylla, mites, and codling moth. Specific objectives included:

1. Examine insecticidal and deterrent properties of erythritol against arthropod pests of pear under controlled laboratory conditions.

Subobjective 1a: Determine the lowest dose of erythritol needed to cause pear psylla mortality and determine whether erythritol has antibiotic (toxicity) or antixenotic (repellency) effects against psylla.

Subobjective 1b: Determine whether erythritol is lethal to pear rust mite and spider mite.

Subobjective 1c: Determine whether erythritol is lethal to codling moth larvae.

2. Determine whether foliar applications suppress arthropod pest populations in a pear orchard.

SIGNIFICANT FINDINGS

Objective 1:

Subobjective 1a: Erythritol was psyllicidal when applied to pear at mixtures of 20% w/v and killed nearly all psylla nymphs and adults within three days. The psyllicidal effects were both antibiotic and antienotic.

Subobjective 1b: Erythritol was lethal to both pear rust mite and spider mite.

Subobjective 1c: Erythritol was lethal to codling moth larvae when added to artificial diet.

Objective 2:

Foliar applications of erythritol significantly reduced pear psylla nymph populations under field conditions, but only when erythritol was completely dissolved into solution by aid of heat. Reductions in blister mite damage was also noted.

RESULTS & DISCUSSION

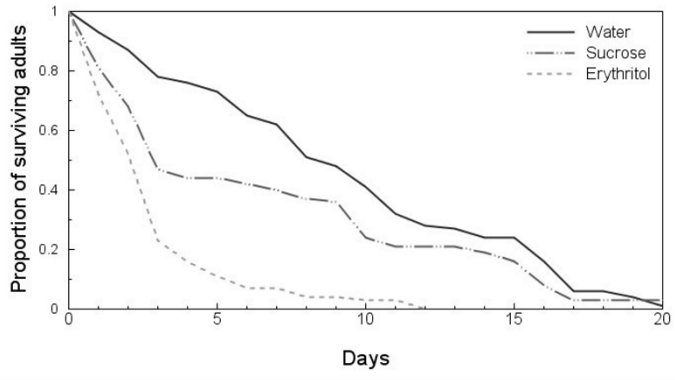
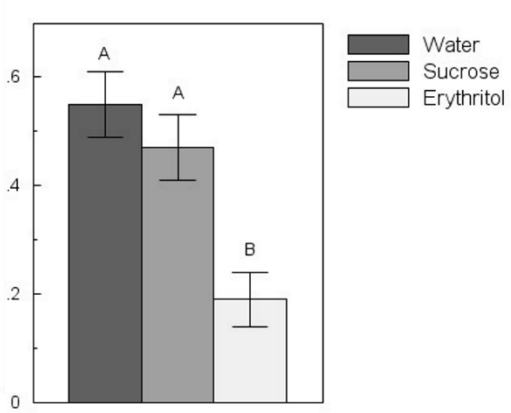


Figure 2. Survival rates of pear psylla adults on liquid diets consisting of water, 30% sucrose, or 30% erythritol. Although the x-axis is limited to 20 days to allow visual comparisons, survival on water and sucrose exceeded 30 days.



Proportion (±S.E.) of living psylla adults that were eding after a 24-h settling time on liquid diets of water only, 30% erythritol, or 30% sucrose. letters denote significant differences among treatment means.

Subobjective 1a. There were significant differences among treatments in pear psylla feeding activity following a 24-h settling time (Fig. 1; $F=9.1$; d.f.=2, 39; $P=0.006$). Fewer pear psylla were observed feeding on erythritol diets compared with those consisting of sucrose or water only (Fig. 1). There were also significant differences among treatments in adult longevity (Fig. 2; $\chi 2=49.6$; d.f.=2; $P<0.001$). Survival time was significantly reduced for pear psylla that were fed erythritol compared with those that were fed pure water or 30% sucrose. After demonstrating that 30% erythritol was lethal to pear psylla adults when ingested from liquid diets, we examined the lethality of various erythritol concentrations ranging from 0 to 30%. Analysis of the 3-d mortality rates of pear psylla

indicated significant differences among treatments (F=16.0; d.f.=4, 38; P<0.001) where morality rates generally increased with increasing concentrations of erythritol (Fig. 3).

Results of these assays demonstrate that erythritol has dose-dependent insecticidal properties when ingested from liquid solutions by pear psylla adults. We did not specifically compare the effects of erythritol on winterform and summerform psylla, but our assays included either morphotype depending upon availability and demonstrated that erythritol is lethal to both seasonal morphotypes. Results of these assays are consistent with reports that erythritol is insecticidal (Baudier et al. 2014, O'Donnell et al. 2016, Choi et al. 2017, Burgess and King 2018, Sampson et al. 2018, Gilkey et al. 2018, Caponera et al. 2019), but our study is the first to demonstrate that erythritol is lethal to a phloem-feeding insect.

Observations after the 24-h settling time revealed altered behavior or impaired motor skills by pear psylla that ingested erythritol. Pear psylla that were fed erythritol appeared to spend more time grooming their stylets compared with psylla that were fed sucrose or water. In addition, many of the erythritol-fed psylla appeared to have an impaired ability to climb on the lid of the petri dish. While these observations should be interpreted cautiously because our experiment was not specifically designed to examine the effects of erythritol on the behavior of pear psylla, these observations are indeed consistent with a report of impaired motor functions by fruit flies after ingesting erythritol. In that experiment, *Drosophila melanogaster* adults that were fed erythritol exhibited a decreased ability to climb compared with adults that were fed water or other artificial sweeteners (Baudier et al. 2014).

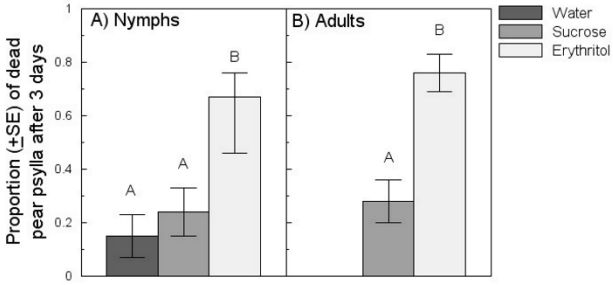


Figure 5. Three-day mortality (±S.E.) of pear psylla adults (A) and nymphs (B) confined to pear leaves treated with water, 30% sucrose, or 30% erythritol. Different letters denote significant differences among treatment means.

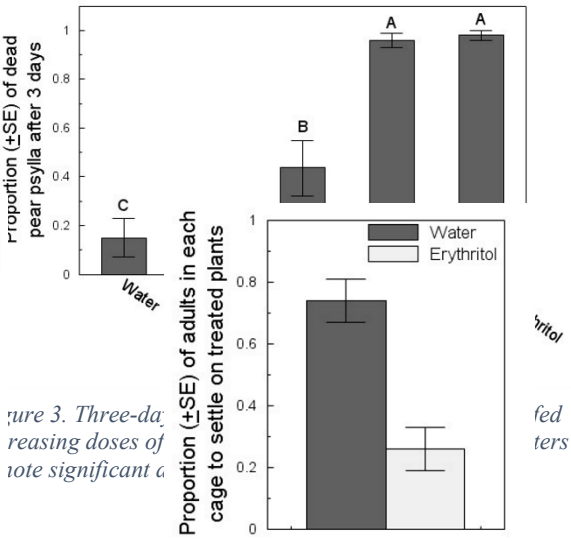


Figure 4. Mean proportion (±S.E.) of the total number of pear psylla adults in each cage to settle on pear leaves treated with water (control) or 30% erythritol in preference assays.

Laboratory assays with excised leaves were used to examine the effects of foliar treatment of erythritol on mortality of pear psylla adults and nymphs. Mortality rates of both nymphs (F=6.7; d.f.=2, 11; P=0.013) and adults (F=6.0; d.f.=2, 23; P=0.008) were significantly different among treatments (water, 30% sucrose, and 30% erythritol). Mortality rates of nymphs confined to leaves treated with water or sucrose were similar, but mortality was significantly higher for nymphs that were confined to leaves treated with erythritol (Fig. 4A). All pear psylla adults that were confined to leaves treated with water survived, so this treatment was removed from the analysis. As observed for nymphs, significantly more adults died after three days when confined to erythritol-treated leaves compared with those confined to sucrose-treated leaves (Fig. 4B). Nearly all nymphs, regardless of treatment were still living three days after being briefly submersed in erythritol, sucrose, or water, suggesting that the psyllicidal properties are due to ingestion of erythritol, not residual activity. Results of our choice-preference assays showed a clear preference by adults for untreated versus erythritol-treated leaves (Fig. 5; F=16.9; d.f.=1, 12; P=0.002).

Subobjective 1b. Erythritol was moderately toxic to twospotted spider mite on contact, but only at the highest (30%) rate; the lower rates were not different from the check (Fig. 6). Runoff was low throughout the test, indicating the materials tested were not repellent. The low mortality in the check (8%) and the high mortality in the FujiMite standard (98%) indicate a valid bioassay response.

Mortality following exposure to residues was low to negligible in all treatments, although significantly higher in the FujiMite treatment; the erythritol treatments were not significantly different from the checks. Egg numbers were significantly lower in the FujiMite and 30% erythritol treatments in comparison to the check (Fig. 7).

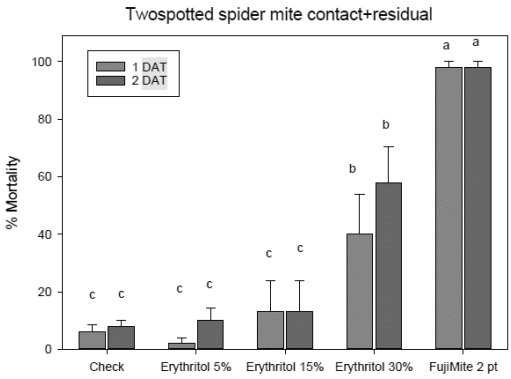


Figure 6. Mortality of twospotted spider mite following contact and residual exxposure to erythritol and FujiMite.

In the contact+residual bioassay at 2 DAT, mortality of female *G. occidentalis* was 100% in the FujiMite standard, but moderate to high in the erythritol treatments (Fig. 8). Inexplicably, the highest rate of the erythritol produced mortality that was lower (20%) than the two lower rates, which averaged about 58% mortality. Egg numbers at 2 DAT were lower in the FujiMite and erythritol 15 and 30% treatments.

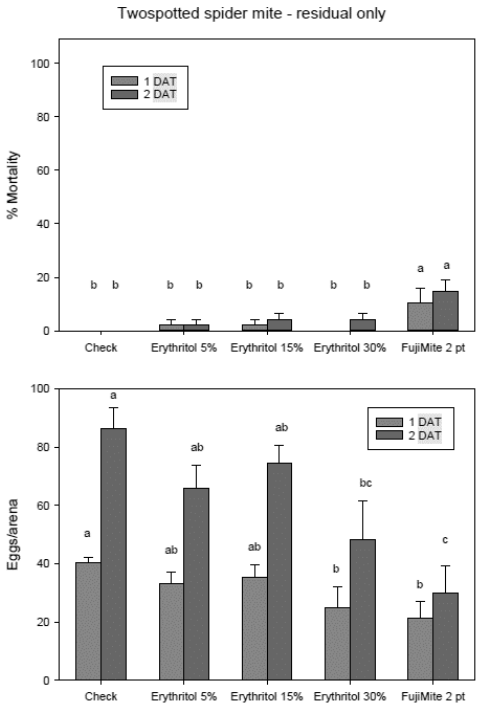


Figure 7. Mortality (upper) and fecundity (lower) of twospotted spider mites exposed to residues of erythritol and FujiMite.

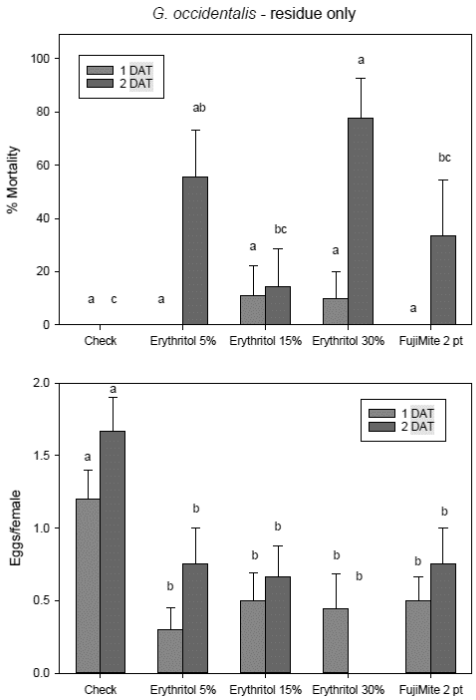


Figure 8. Mortality (upper) and fecundity (lower of western predatory mites exposed to residues of erythritol and FijiMite.

In the residual-only bioassay, there was very little mortality in any of the treatments, including the FujiMite standard, after 24 h. Mortality increased considerably at 48 h, with the highest levels in two of the erythritol treatments (5 and 30%). There was no rate effect due to the low levels of mortality in the 15% treatment; however, there was a trend for the erythritol treatments to cause more mortality than FujiMite. Erythritol appears to have only slightly lower toxicity to *G. occidentalis* by exposure to residues as it is by direct contact. Egg numbers were reduced by all treatments relative to the check.

Pear rust mite mortality was low in the check and high (100%) in the standard (Nexter) treatment at 2 DAT, bracketing the erythritol treatments (Fig. 9). The three rates tested ranged from 33 to 74%, indicating a moderate degree of activity. While several applications at the high rate may be necessary, this material shows promise of an organic rust mite material.

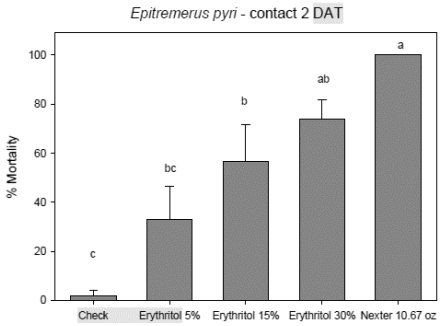


Figure 9. Mortality of pear rust mites exposed by contact of erythritol and Nexter.

Subobjective 1c. Because of uncertainties about when codling moth larvae enter fruit (Garczynski, personal communication), the effects of erythritol on codling moth was tested by mixing 30% erythritol into standard codling moth rearing diet and examining for mortality. Within a week, all codling moth larvae confined to diet mixed with erythritol had died and were still located on the surface of the diet. In contrast, all larvae confined to control diets were still living and had burrowed into the diet.

Objective 2, Field trials. A previous report indicated that higher concentrations of erythritol is harmful to both corn and tomato seedlings (Scanga et al. 2018), but erythritol did not appear to damage the foliage of pear. Repeated measures analysis of the numbers of pear psylla nymphs collected in an orchard near Moxee, WA did not indicate a significant treatment by week interaction indicating that the effects of treatment were similar among weeks (F=2.3; d.f.=4, 12; P=0.118). Numbers of nymphs on all treatments declined from week 1 to week 3 (Fig. 10A; F=16.6; d.f.=2, 6; P=0.004), and were lower on trees treated with erythritol or erythritol plus Regulaid compared with controls (Fig. 10B; F=6.2; d.f.=2, 6; P=0.035). Numbers of adults did not differ among treatments (F=0.22; d.f.=2, 36; P=0.805) and there was no treatment by week interaction (F=0.9; d.f.=10,36; P=0.547), but numbers of adults generally declined each week regardless of treatment (F=22.0; d.f.=5, 18; P<0.001).

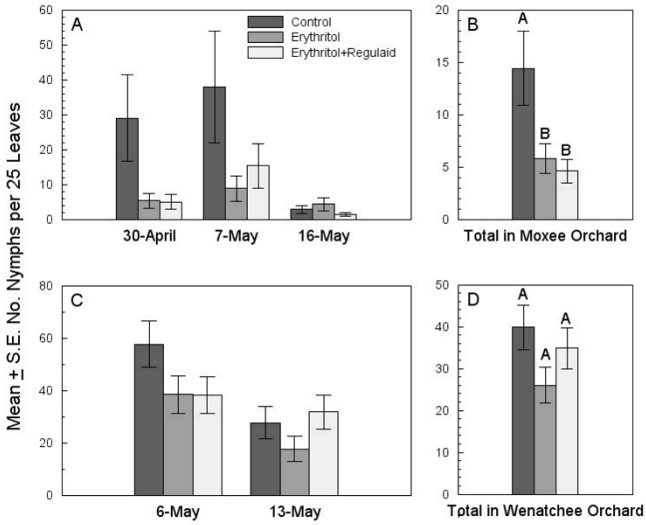


Figure 10. Mean number (±S.E.) of pear psylla nymphs per 25 leaves on trees treated with control (water mixed with surfactant), erythritol, or erythritol mixed with a surfactant in orchards near Moxee, WA (A and B) or Wenatchee, WA (C and D). Different letters within dates denote significant differences among treatment means.

Repeated measures analysis of the numbers of pear psylla collected in an orchard near Wenatchee, WA did not indicate a significant treatment by week interaction for nymphs (F=1.4; d.f.=2, 8; P=0.312) or adults (F=0.7; d.f.=12, 48; P=0.760). As observed in Moxee, populations of nymphs (Fig. 10C; F=11.1; d.f.=1, 4; P=0.029) and adults (F=50.2; d.f.=6, 24; P<0.001) declined from the initial sampling date. Although there were numerically fewer nymphs on erythritol-treated trees than on control trees on week 1 (Fig. 10D), the analysis did not indicate significant differences among treatments for nymphs (F=2.0; d.f.=2, 8; P=0.195) or adults (F=0.5 d.f.=2, 8; P=0.608).

The experiment conducted at the Moxee location was consistent with the results obtained from laboratory assays but results of field experiments from the two locations conflicted. The major difference in methods used at the two locations is the method in which the erythritol treatments were prepared. Solutions were heated to dissolve the erythritol before treatment of trees at the Moxee location, but not before treatment at the Wenatchee location. We conducted a follow-up laboratory experiment using excised leaves to compare pear psylla survival on leaves treated with erythritol that was dissolved into solution by heating versus erythritol that was suspended in water without heat. That experiment indicated that heat-dissolving is required for erythritol to be effective as an insecticide against pear psylla when applied to pear leaves (Fig. 11; F=9.4; d.f.=2, 11; P=0.004). Overall, results or our field experiments are consistent with other reports that demonstrated insecticidal properties of erythritol under field conditions (Sampson et al. 2017, 2018).

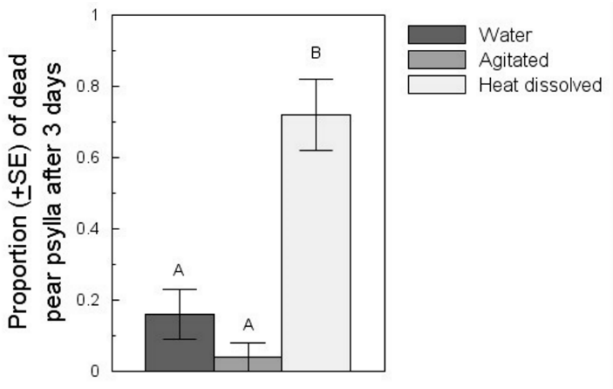


Figure 11. Three-day mortality (±S.E.) of pear psylla adults confined to pear leaves treated with water, 30% erythritol that was agitated into solution, or 30% erythritol that was dissolved into solution by heat treatment. Different letters denote significant differences among treatment means.

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EXECUTIVE SUMMARY
Project Title: Erythritol: An artificial sweetener with insecticidal properties

Key words: pear psylla, twospotted spider mite, rust mite, codling moth

Abstract:
Although safe for human consumption, the artificial sweetener, erythritol, has been shown to be insecticidal against fruit flies. We found that 20-30% erythritol is also insecticidal to pear psylla, twospotted spider mites, rust mite, and codling moth. Erythritol could be developed into an organic insecticide to manage key pear pests.

CONTINUING REPORT **Year: 2019**

Project Title: WTFRC Internal Program – Food Safety Efforts

PI: Ines Hanrahan
Organization: WTFRC
Telephone: 509 669 0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S.18th St., Suite 100

City/State/Zip: Union Gap, WA, 98903

Cooperators: Jacqui Gordon (WSTFA), Faith Critzer, Meijun Zhu & Girish Ganjyal (WSU), Manoella Mendoza and Mackenzie Perrault (WTFRC), Rob Atwill & Ronny Bond (UC Davis), Bonnie Fernandez-Fenaroli (CPS)

Other funding sources

Agency Name: WA SCBGP
Amt. awarded: $ 216,682 Title: Enhanced food safety education and training for tree fruit producers (awarded to WSTFA with Jacqui Gordon as PI)
Agency Name: FDA
Amt. awarded: $243,651 for FY19 awarded to WCFSS (Atwill et al.) Title: Facilitating implementation of FSMA regulations for agricultural water quality
Agency Name: CPS
Amt. awarded: $290,000 to Zhu and Suslow; Title: Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention
Agency Name: WA SCBGP
Amt. /awarded: $ 248,227 to Zhu; Title: *E. faecium* as a surrogate for *L. monocytogenes* intervention in apple dump tank systems (additional $80,000 of matching funds)

WTFRC internal program budget:

Item	2019
Salaries[1]	3,100
Benefits	1,325
Wages[2]	7,500
Benefits	3,230
Supplies[3]	350
Travel[4]	3,100
Total	**18,605**

Footnotes:
[1]Salaries: 5% of Mendoza (with 41% benefits), not included in salaries: 15% of Hanrahan time, 1% Schmidt
[2]Wages: 53% benefit rate
[3]Supplies include 1 poster for ASHS and misc.
[4]Travel includes: CPS annual meeting, 3 trips to WSU in Pullman, in state day travel to attend trainings, Annual NW Food Safety and Sanitation Conference in Portland

OBJECTIVES

1. Enhance collaboration in all areas of food safety (research, policy, FSMA implementation)
    a. Participate in development of training for industry
    b. Develop an effective food safety outreach program

SIGNIFICANT ACCOMPLISHMENTS IN 2019

Food safety remains one of the highest priority items within the industry. As some compliance dates of FSMA have been effective, it is of utmost importance to continue to provide the Washington growers with timely assistance. Further, in order to interest and engage microbiologists to work on problems related to food safety for tree fruit, a strong collaboration from scientists with a horticulture background is of great advantage to ensure that project goals and outcomes reflect immediately actionable items. Lastly, translating research into layman's terms and providing a bridge between science, politics and farming is another important goal of this project.

Research:

We participated in several on-going and new collaborative projects, funded by WTFRC, CPS, SCBG and FDA (see Table 1). Notably, WTFRC is increasingly sought as collaborator in national grant applications to NIFA or SCRI.

The WTFRC, under leadership of Ines Hanrahan, continued to serve as a partner in research for the Center for Produce Safety (CPS) and she attended the annual meeting in Austin, TX. Tree fruit specific research priorities were developed and integrated into the annual CPS call for proposals, with the help of the NHC Food Safety Committee. During the proposal process Dr. Hanrahan frequently serves as subject matter specialist to answer questions asked by scientists preparing to propose new research projects. In 2019, one project involving local scientists has been brought to completion: 'Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention' (Zhu/Suslow; $290,000). WTFRC has developed and executed a packing line survey for this project in 2017 to determine the current industry practices related to spray manifold interventions and has been assisting Dr. Zhu's team to set-up packingline validation studies with industry collaborators in 2018-19. The team has also been sourcing fruit for the experiments.

Our team collaborated with Dr. Zhu on another project in 2019: "*E. faecium* as a surrogate for L. monocytogenes intervention in apple dump tank systems", funded through the SCBGP. WTFRC developed and conducted a dump tank survey to assist the project team in developing industry relevant experimental methods.

In 2018 and 2019 Hanrahan has participated in SCRI industry relevance reviews of proposals submitted to USDA-NIFA in the area of food safety.

Table 1: Summary of WTFRC collaborations* in food safety research in 2019 and pending research for 2020

Keyword	PI's	Affiliation(s)	Funding Source	Amount
Continuing/finishing/new in 2019				
Listeria storage*	Zhu/Amiri/Hanrahan	WSU, WTFRC	WTFRC	195,414
Imp. Dryer	Ganjyal/Zhu	WSU	WTFRC	57,000
Water safety	Atwill/Bond	UC Davis, WTFRC	FDA	243,651

Food Safety Training	Gordon	WSTFA	SCBG	216,682
List. Cleaning*	Zhu/Hanrahan	WSU, WTFRC	WTFRC	306,285
FMSA PCHF	Ganjyal	WSU, WTFRC	WTFRC	98,971
Brush bed sanitation*	Critzer et al.	WSU, WTFRC	WTFRC	51,967
Listeria monitoring	Kovacevic et al.	OSU	ODA SCBG	174,540
Packing sanitation*	Critzer et al.	WSU, WTFRC	WTFRC	203,000
Rapid detection tools*	Critzer	WSU, WTFRC	WTFRC	112,000
Ozone in storage*	Zhu	WSU, WTFRC	WTFRC	300,000
E.Faecium as surrogate	Zhu et al.	WSU	WA-SCBG	250,000
Water treatment	Critzer	WSU	WA-SCBG	194,000
Listeria growth/survival	Strawn	Virginia Tech	CPS	185,052
Pending for 2020				
Lm apple waxing*	Zhu	WSU	WTFRC	TBD
Microbiome*	Zhu	WSU	WTFRC	TBD
Sensing platforms	Critzer et.al.	WSU	USDA	TBD
Effective mgt. for FSMA	Danyluk	U. of Florida	USDA-CAP	TBD
Listeria HUB	Kovacevic	OSU	USDA-NIFA	TBD
Wax supplements	Wang	UC Davis	USDA-NIFA	TBD

*collaborations involve a separate WTFRC internal budget

FSMA implementation: In order to best serve the needs for timely information distribution related to new laws pertaining to food safety, WTFRC staff (Hanrahan, Mendoza) leads efforts to coordinate outreach activities by the various industry organizations. Specifically, NHC (policy), WSTFA (education) and WTFRC (research) efforts have continued to be combined and talking points were coordinated to assure clarity of messaging, when stakeholders are learning how to implement the already complicated laws. Further, the WSTFA continued to host numerous PSA training sessions in 2019. WTFRC staff assisted in meeting logistics and Hanrahan frequently serves as expert to help field questions.

Development of industry training modules: Under leadership of the WSTFA and in collaboration with UC Davis, a workshop named: "FSMA water quality testing" was held in two locations in 2019. This workshop built on a curriculum originally developed in 2016. At the time it was the first of its kind in the nation to address practical considerations for water testing under FSMA. Workshops are designed to give participants theoretical background knowledge of water testing, in combination with outdoor activities geared towards learning based on examples, coupled with hands on training. The entire workshop was video taped and is available at no cost to industry members through the WSTFA portal.

Food safety outreach: For the Annual WSTFA meeting in Wenatchee, Drs. Critzer (WSU) and Hanrahan lead a session named: "Fruit Maturity & Precision Harvest Management". This session included several food safety related items such as: "Risk management within the harvest environment" (review of OFRR's, panel discussion) and "Produce Safety Rule On-farm Inspections" (panel discussion on implementation of on farm inspections).

In addition, Ines Hanrahan is serving as an adjunct faculty member for the WSU/UI School of Food Science. She is currently participating as a committee member on two Ph.D. and two MSc. committees in the Food Science Department. All students work in the general area of food safety on very relevant tree fruit industry topics and are interested in a career in tree fruit upon graduation.

Further, Dr. Hanrahan is serving on the PNW Food Safety Committee, which is housed by NHC. She contributes to the annual in-person meeting program development and serves as presenter. Other outreach

activities covered by WTFRC staff, may include, but are not limited to posters at national/international meetings, invited talks, lectures for WSU classes. The following is a list of the most important invited talks/posters/lectures given in 2019:

Ines Hanrahan, 2019, Food Safety Research Priorities: Looking into the Future, talk at PNW Food Safety Committee annual meeting

Ines Hanrahan, 2019, Food Safety Research Roundup, talk at PNW Food Safety Committee annual meeting

Ines Hanrahan, 2019, Food Safety for the Tree Fruit Industry, 75-minute lecture for FS 220 students at WSU/UI

Manoella Mendoza, Ines Hanrahan, Lina Sheng, Xiaoye Shen, Meijun Zhu, 2019, Fate of Listeria on Granny Smith Apples Treated with Continuous Ozone During Storage, talk at ASHS presented by Mendoza

Alexis Hamilton, Ines Hanrahan, Marcela Galeni, Victor Villegas, Martin Blackburn, Monique Aguilar Borba, Cecilia Yiu, Daniel Gleason and Faith Critzer, 2019, Assessment of the Efficacy of Rapid Tests on Predicting Bacterial Growth on Apple Packinghouse Equipment Surfaces, poster at IAFP presented by Hamilton

Ronald F. Bond, Melissa L Partyka, Jennifer A. Chase, Ines Hanrahan, Justin Harter and Edward R. Atwill, 2019, The Whole Is Greater Than the Sum of Its Parts: Building Cooperative Monitoring Programs Among Farms, talk presented by Bond at IAFP

FINAL PROJECT REPORT

Project Title: Epidemiology and management of postharvest decay on pears
PI: Achala N. KC
Organization: Oregon State University
Telephone: 541-772-5165 Ext 222
Email: achala.kc@oregonstate.edu
Address: 569 Hanley Rd.
City/State/Zip: Central Point, OR-97502

Cooperators: Mike Naumes (Naumes Inc, Medford, OR), Matt Borman (Harry & David, Medford, OR),

Other funding sources: None

Total Project Funding: $90,737

Budget History:

Item	2017-18	2018-19
WTFRC expenses	0	0
Salaries Faculty Research Assistant	22,500	23,175
Benefits OPE 63%	14,198	14,624
Wages	0	0
Benefits	0	0
Equipment	0	0
Supplies	6,000	6,180
Travel	2,000	2,060
Miscellaneous	0	0
Plot Fees	0	0
Total	$44,698	$46,039

Footnotes: Annually: FRA 6 mo + fringe, 6K supplies and consumables, 2K local and in-state travel, 3% inflation

ORIGINAL OBJECTIVES

1. Monitoring prevalence of major fungal pathogens throughout the pear growing season towards understanding postharvest disease epidemiology

   Major goal of this objective is to identify the time period in growing season where the prevalence of major fungal pathogens is highest. Monitoring at preharvest conditions allows us to determine the sources of inoculum, patterns of spread, and modes of entry to the fruit tissues. The information generated from this objective will help in developing preharvest spray schedules and/or assessing the risk of postharvest infection and thus following subsequent disease management strategy.

2. In vitro sensitivity of postharvest decay pathogens to currently available fungicides and efficacy of new fungicides toward resistance management

   Major goal of this objective is to quickly assess the efficacy of currently available fungicides as well as the population dynamics of major postharvest pathogens in terms of fungicide resistance. Since fungicides have been the major control strategy for most of the postharvest pathogens, the tendency of developing resistance is equally high. In vitro screening of the available fungicides to major fungal pathogens would allow us to determine the resistance and/or efficacy of certain group of fungicides and thus direct the identification of new fungicides and management of fungicide resistance.

3. Manipulating postharvest storage conditions to reduce the susceptibility of fruit infection

   The major goal of this objective is to identify the best storage conditions to minimize the postharvest decay and thus enhance the longevity of pear storage. Different storage conditions and treatments that minimize the infection by major fungal diseases will be identified. The information generated from this will be combined to the pre/postharvest fungicide application to develop a sustainable disease management strategy to combat postharvest decay in pears.

SIGNIFICANT FINDINGS

- Postharvest rot pathogens, *Botrytis cinerea, Cladosporium herbarum, Penicillium expansum,* and *Alternaria* spp. were prevalent in orchards at the initial stages of fruit development
- Three new pathogens, *Dothiorella omnivora*, *Diaporthe rudis,* and *Fusarium* sp. were isolated from bloom time samples and occasionally isolated from culled samples in packing houses (not included in this study)
- *Botrytis cinerea* causing gray mold on postharvest fruits were isolated most frequently during bloom time and in lower frequencies after petal fall and field bins.
- Some isolates of *B. cinerea* tested in this study are resistant to the fungicides used for scab management; triflumizole, cyprodinil, and dodine that may result in indirect selection of resistant *B. cinerea* population to these groups of fungicides
- One hundred percent of isolates tested in this study were sensitive to a group of fungicide, iprodione (FRAC 2) that is not registered for pear in PNW
- Preharvest application of 1-MCP (Harvista) did not result in fungicidal properties against *Botrytis cinerea.* However, the storage capability of fruits under normal atmosphere increased by reducing senescence damage on Harvista applied fruits two weeks prior to harvest.

- The negative effects of 1-MCP on fruits ripening after storage may be mitigated by hanging fruits longer after 1-MCP application pre-harvest.

RESULTS & DISCUSSION

Objective 1: Monitoring prevalence of major fungal pathogens throughout the pear growing season towards understanding postharvest disease epidemiology

Based on culture and spores morphology, four pathogens previously reported as postharvest rot pathogens, *Botrytis cinerea, Penicliilum expansum, Caldosporium herbarum,* and *Alternaria* spp. were identified. These pathogens were consistently isolated from full white, full bloom, petal fall, fruitlets, and field bins at harvest. Several fungal species were isolated along with the pathogenic species, the identity of which could not be established based on culture and spore morphology. They were grouped into thirteen unique species based on cultural characteristic on PDA media. The molecular diagnostic methods using the ITS and elongation factor 1-alpha (EF1-α) sequencing, thirteen unique fungi were identified. The thirteen species were tested in surface sterilized wound inoculated fruits for their ability to cause disease. Out of thirteen, three species were identified as pathogenic on wound inoculated fruits (Figure 1, continuing project report 2019, page 101). The three new species were identified as *Dothiorella omnivora, Diaporthe rudis, and Fusarium* sp. A plant disease note on first report of *D. rudis* causing fruit rot of European pears in the United States is published in Plant Disease journal (https://doi.org/10.1094/PDIS-12-18-2184-PDN). Interestingly, the two species *D. omnivora* and *D. rudis,* also cause trunk disease in grapes (a complex of many fungal pathogens). With the increasing wine grape acreage in the area, we might not only be sharing the acreage but also the pathogens to some extent. It will be important to keep monitoring for newer pathogens as the area's commercial agriculture change over time.

This study shows that these pathogens can colonize the floral organs at early stages of fruit development, can follow through picking bins, and possibly end up to the storage facilities (Figure 2, continuing project report 2019, page 101). We recovered highest percentage of *B. cinerea* isolates from full bloom and petal fall stages and lowest from the fruits at picking bins during commercial harvest. *Botrytis cinerea* can cause both calyx and stem end gray mold in storage. Calyx end gray mold is generally initiated by infection of calyxes in the orchard close to full bloom, remaining latent throughout the growing season and resulting calyx end gray mold in storage. The stem end gray mold can initiate from cracks or damage at stem end during harvest or postharvest handling during transport and storage. Once the infection starts, the decay spreads in the storage within a container to the nearby fruits. These results suggest for integrating management approaches of gray mold storage rot between seasonal management in orchard and post-harvest management at packinghouses.

Several *Alternaria* spp. were recovered from early stages of fruit development to picking bins. The species were collectively recorded as *Alternaria* spp. in this study as the sequencing of ITS only did not delineate the species complex. The genus *Alternaria* includes more than 280 species that can be both pathogenic and saprophytic. On pears and apples, *Alternaria alternata*, *A. tenuissima, A. arborescens*, *A. ventricosa, and A. yaliinficiens* are reported as pathogenic species causing Alternaria rot, pear black spot, core browning, and moldy core. The isolated *Alternaria* spp. from this study will be further characterized for their species diversity and pathogenicity on four cultivars of European pears including Bartlett, Green Anjou, Comice, and Bosc.

Penicillium expansum and *Cladosporium herbarum* were recovered in similar frequencies until petal fall stages. At fruitlet and picking bins the recovery percentage of *P. expansum* stayed similar as earlier stages, however the recovery percentage of *C. herbarum* increased significantly at these stages with *C. herbarun* being the frequently recovered species at picking bins. At fruitlet and picking bins stages, the tissues used for isolation included calyx end and stem end tissues. At these stages, *Alternaria* spp., *B. cinerea, C. herbarum, and P. expansum* were the only pathogenic species recovered from the samples. Both *P. expansum* and *B. cinerea* are the most common pathogens causing storage rot and can initiate in calyx

end, stem end, or wounded sites on fruit surface. Species like *Alternaria* spp., and *C. herbarum* on the other hand are weak pathogens and infect compromised fruits such as wounds or senescing fruits. Recovery of these species at two major infection sites during harvest suggests the possibility of inoculum being carried to the storage for both calyx end, and stem end rot, as well as for the undiscovered wound sites during postharvest handling of fruits.

The three new pathogens, *Diaporthe rudis, Dothiorella omnivora,* and *Fusarium* sp. were recovered at lower percentages until petal fall. At fruitlets and picking bins, they were not recovered. However, these pathogens were occasionally isolated from post-harvest fruits with storage rot symptoms in packinghouses. Given the type of tissues used at these two stages in this study, there may exist other conduits for pathogen inoculum to initiate storage rots in packinghouse.

Objective 2: In vitro sensitivity of postharvest decay pathogens to currently available fungicides and efficacy of new fungicides toward resistance management

The effective concentration to reduce radial growth by 50% (EC_{50}) was calculated for each pathogen-fungicide combination. The EC_{50} for isolates against cyprodinil ranged from 1.35 to 26 μg ml^{-1}. No discriminatory dose for use on pears was found in the literature, but a 1 μg ml^{-1} was considered a discriminatory dose for *B. cinerea* on New Zealand wine grapes (Beresford et al 2017), and all isolates except one exceeded this limit by at least threefold. The range of EC_{50} values against dodine was 73.4 to 195.7 μg ml^{-1}. No baseline or discriminatory dose information for *B. cinerea* was found in the literature. While the EC_{50} values seem high, the labeled rate for the product used to supply this active ingredient is also high, with a maximum of 1,485 μg active ingredient ml^{-1}. The range of EC_{50} values against iprodione was 0.53 to 1.32 μg ml^{-1} No discriminatory dose for use on pears was found in the literature, but all isolates were below the 10 μg ml^{-1} concentration determined in a study of *B. cinerea* on Southern US strawberries (Fernández-Ortuño et al 2014). The range of EC_{50} values against triflumizole was 0.38 to 1.53 μg ml^{-1}. Except for cyprodinil, the tested isolates were sensitive within labeled field rates. Two isolates (10%) were not sensitive against cyprodinil at the maximum labeled field rates and EC_{50} for 85% isolates were greater than 5 μg/ ml.

Among, the isolates tested for fungicide efficacy, 100% of the tested isolates were sensitive to iprodione, a fungicide group that is not registered for pear in PNW (Figure 3 and 4, continuing project report 2019, page 102). Only 30% of the isolates were sensitive to triflumizole and all the sensitive isolates were collected from SOREC research orchards. The remaining of other isolates (70% of the tested isolates) with reduced sensitivity to triflumizole were collected from conventionally managed commercial orchards. Triflumizole (FRAC group 3) includes a common group of fungicides, including Inspire Super for managing pear scab diseases and powdery mildew. Among the tested isolates, 65% and 75% of the isolates were sensitive to dodine and cyprodinil respectively. Even though dodine is currently not used for commercial application, it was a fungicide of choice for scab management until the resistance became an issue. Similarly, cyprodinil (FRAC group 9) is also an important component of pear scab management. While the resistance frequencies to these groups among the tested isolates are relatively low precautionary measures should be taken for fungicide rotations. Identification of a group of fungicide with high efficacy, iprodione (FRAC group 2) is one of the major accomplishments of this project. However, the results need to be verified with larger number of isolates and further steps needs to be taken toward registration and labeling.

Objective 3: Manipulating postharvest storage conditions to reduce the susceptibility of fruit infection

2017-Preharvest 1-MCP application and wound inoculated fruits: Preharvest application of foliar 1-MCP alone did not significantly reduce the wound initiated *B. cinerea* infection in cold storage for both bosc and comice pears. The disease progress over time was lower on Bosc fruits treated with 1-MCP a week prior to harvest at minimum rate; however, it was not statistically significant. Disease progress on other treatments were significantly higher than water control treatments. Similar result was observed on comice fruits treated with 1-MCP. Application of 1-MCP at preharvest is not fungicidal enough to control the disease caused by wound initiated *B. cinerea.*

2017-Preharvest 1-MCP application and normal atmosphere stored fruits: The fruits stored in normal atmosphere cold storage from 2017 pre harvest 1-MCP application resulted comparable firmness relative to control treatments. At two months after storage, Harvista applied comice fruits two weeks prior to harvest were firmer than fruits with Harvista treatments one week prior to harvest and control treatments with no Harvista application. However, all fruits were below 2.5 lbs after five days of ripening. At four months after storage, no significant differences were observed on any of the Harvista treated fruits. All fruits were below 1.5 lbs after five days of ripening (Fig. 5). Similar results were observed on bosc fruits (Fig. 6). At two months after storage, Harvista treated fruits were firmer compared to no harvista treated control fruits. At four months after storage, no significant differences were observed among the treatments. At six months after storage, fruit texture data were difficult to interpret due to hardening of outer layer. That could be due to loss of moisture resulting rubbery texture of fruit periderm and subsequent difficulties for the probe to puncture fruits for texture data. Differences in percent senescence were observed among the treatments. In bosc after six months of storage, the percent senescence on the fruits treated with Harvista two weeks prior to harvest resulted in zero senesced fruits with minimum rate and 35% senesced fruits with maximum. However, on fruits treated a week before harvest resulted in 50 and 55% senesced fruits with minimum and maximum rate. The fruits with no Harvista treatment resulted 80% senesced fruits (Fig. 7)

The results were promising from this study as the firmness/ripeness of the fruits were not affected by pre harvest application of 1-MCP and that the fruits can be stored longer with less loss to senescence. However, these data was generated from only one years of study. We repeated the two weeks prior to harvest application with two rates in 2018 and other objectives were added for the treatments effect.

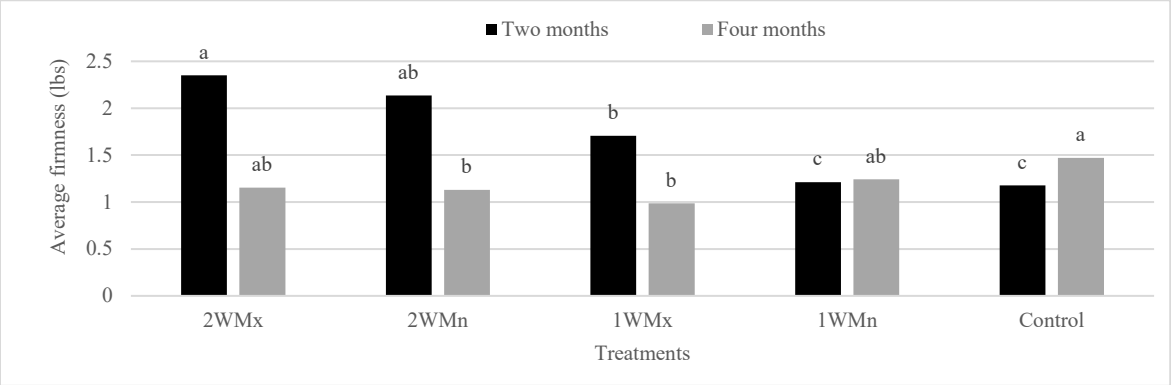

Figure 5: Post-storage fruit texture on Harvista applied **comice**. The bars with same letters are not significantly different ($P<0.05$*)*

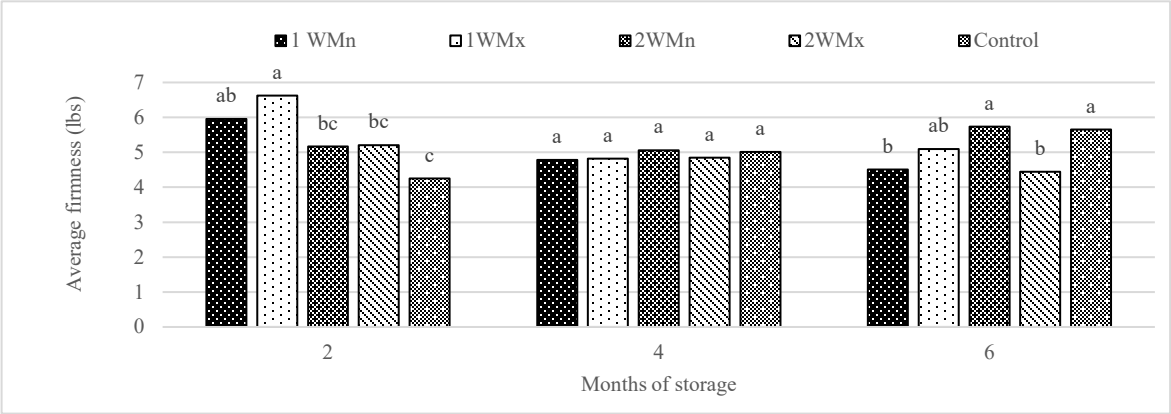

Figure 6: Post-storage fruit texture on Harvista applied **bosc**. The bars with same letters are not significantly different (*P*<0.05*)*

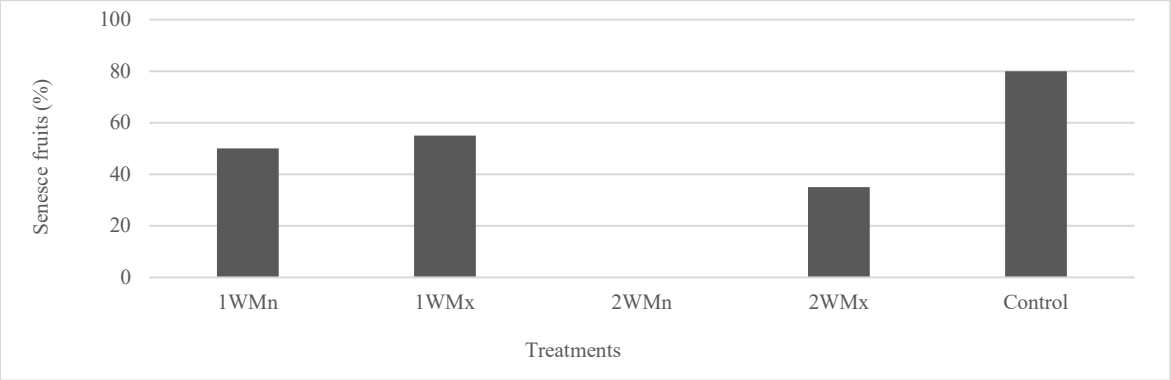

Figure 7: Percent senescence on **bosc** stored in normal atmosphere cold storage for six months.

2018-Preharvest 1-MCP application and normal atmosphere stored fruits: We picked fruits at three different time points (commercial harvest, a week, and two weeks after commercial harvest) and analyzed the efficacy of 1-MCP to maintain fruit texture in tree for better harvest planning. Bosc fruits at commercial harvest were picked two weeks after Harvista application, however due to the sudden drop in fruit texture in comice fruits in 2018, the commercial harvest fruits were picked three days after Harvista application. The average fruit texture on Bosc at the day of Harvista application was 16 lbf. The texture ranged from 14 to 15 lbf two weeks after application; and 13 to 14 lbf three and four weeks after application. The fruit texture on Bosc treated with maximum rate of 1-MCP were consistently one unit firmer compared to minimum rate and control fruits in all harvest time points. No significant differences on fruit texture were observed between fruits treated with minimum rate of 1-MCP and the non–treated control fruits. The fruits treated with maximum rate of 1-MCP dropped two units from the day of application to 21 days later, whereas the ones with minimum rate of 1-MCP and control dropped three units (Fig. 8a). This effect was not significant in comice pears. Due to early harvest in comice, the final harvest commenced 17 days after Harvista application. Although no significant differences on fruit texture were observed 17 days after application among the treatments, the fruits treated with 1-MCP tended to stay firmer than control fruits in all harvest time points. The average fruit texture on comice at the day of Harvista application was 11.9 lbf. The texture ranged from 10.7 to 11.8 lbf three days after application; 10 to 10.9 ten days after application; and 9.9 to 10.3 lbf seventeen days after application (Fig. 8b). Besides maintaining fruit texture, one of the benefits of leaving fruits longer on trees would be fruit

size. However, leaving fruits on trees two weeks longer than commercial harvest did not make significant differences on fruit weight in Bosc and increased by less than an ounce in comice (Fig. 8c, Fig. 8d).

The another set of harvested fruits were stored in both normal atmosphere cold storage and controlled atmosphere cold storage. Fruits were stored in cardboard boxes in 2018 compared to plastic bins in 2017 to protect fruits from moisture loss during long-term storage. Bosc and comice fruits were stored for six and four months respectively in normal atmosphere cold storage and five and four months respectively in controlled atmosphere cold storage. After storage, the fruits were allowed to ripen at room temperature for seven days. The fruits firmness were measured and the data on fruit texture were analyzed. Similar to the preharvest fruit texture on Bosc, the fruits treated with maximum rate of 1-MCP were approximately one unit firmer than control fruits. Within the maximum rate of 1-MCP treated fruits, the fruits got softer when picked a week and two weeks after commercial harvest. The average post-storage fruit texture on maximum 1-MCP treated fruits picked at commercial harvest was 9.1 lbf that reduced to 7.7 lbf on fruits picked one and two weeks after commercial harvest (Fig. 9a). On comice, no significant differences were observed between the treatments. However, the fruit texture decreased significantly on fruits harvested one week and two weeks after commercial harvest including maximum 1-MCP treated fruits (Fig. 9b). Similar results were observed on fruits stored at controlled atmosphere cold storage (Fig. 9c, Fig. 9d). These results suggest the negative effects of 1-MCP on fruits ripening after storage may be mitigated by hanging the fruits longer after 1-MCP application pre-harvest. However, this study needs to be validated with more years of data and with additional cultivars.

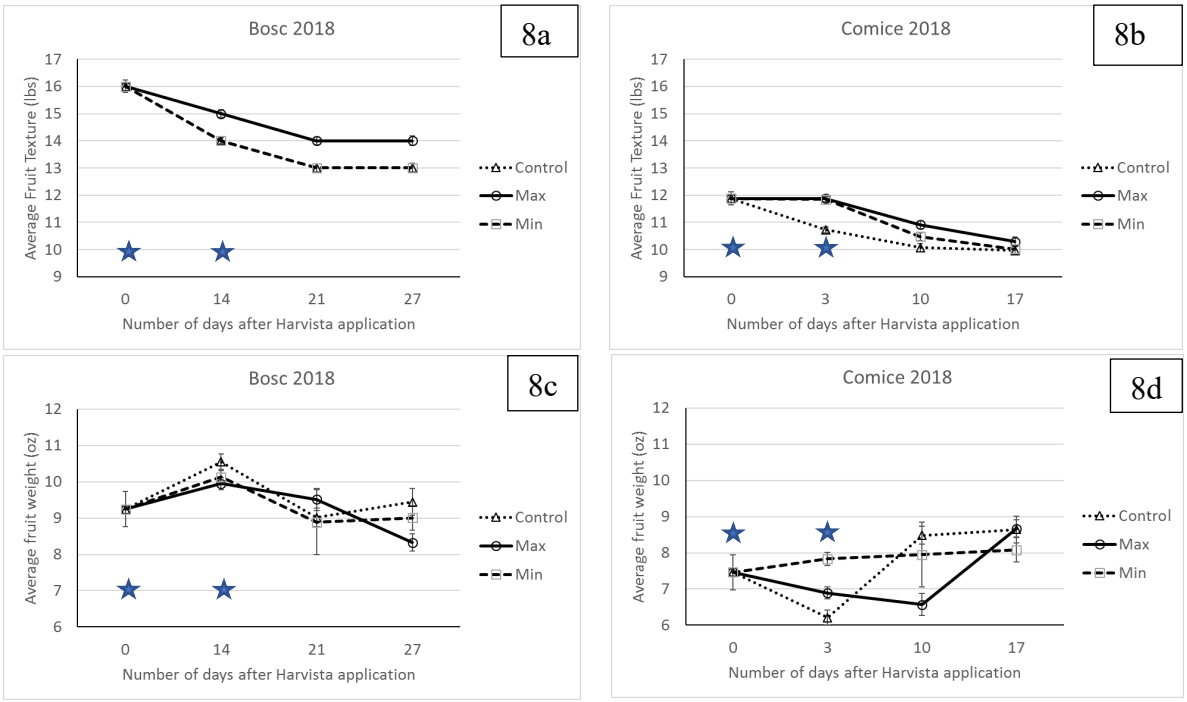

Figure 8: Pre-storage fruit texture (a & b) and fruit weight (c & d) on Bosc and Comice. The data represent an average of 40 fruits. The star symbols represent day of Harvista application and commercial harvest days on respective cultivars.

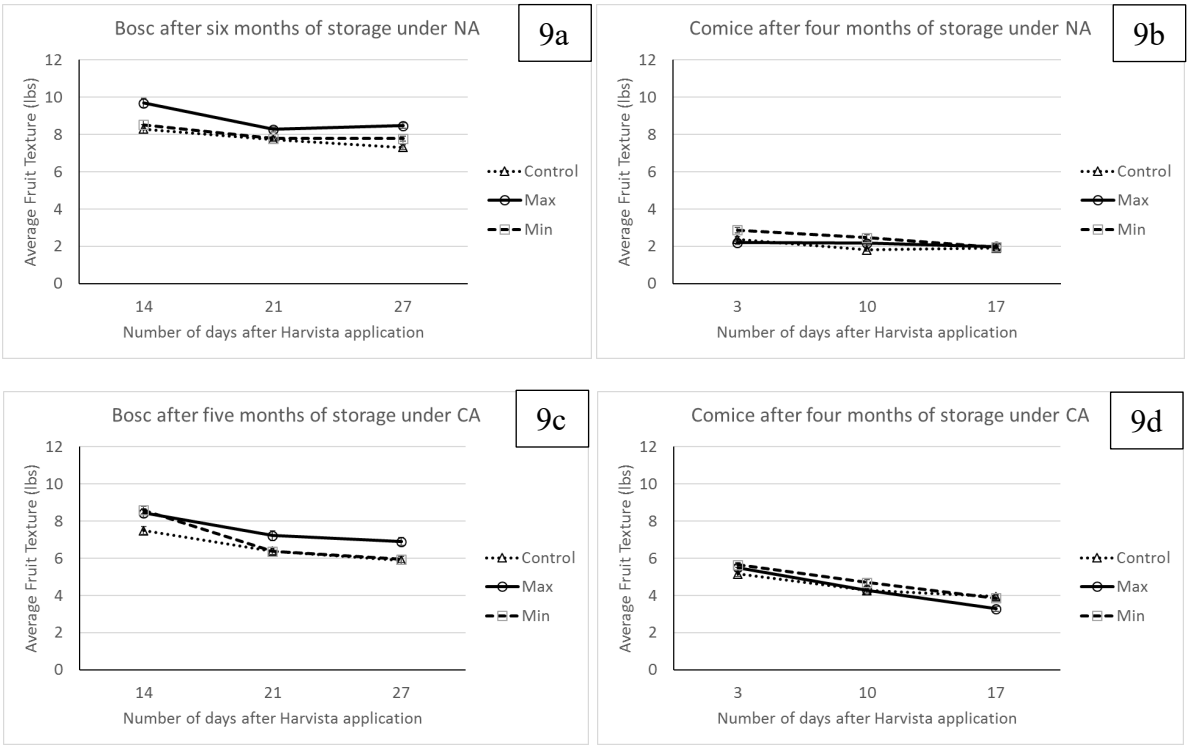

Figure 9: Post-storage fruit texture on Harvista applied bosc and comice stored under natural atmosphere (a & b) and controlled atmosphere (c & d). The data represent an average of 40 fruits per treatment. The fruits were picked 14, 21, and 27 days after Harvista application, where 14 days is the commercial harvest date.

EXECUTIVE SUMMARY

Project title: Epidemiology and management of postharvest decay on pears

Key words: postharvest decay, fungicide sensitivity, 1-MCP

Abstract: Postharvest decay in pears is caused by several fungal pathogens. This study identified several of these pathogens colonizing tissues from initial stages of fruit development, and fungicides and other products that can be applied at bloom time and integrated into existing pre-harvest and postharvest fungicide management programs.

Summary of findings:

- Postharvest rot pathogens, *Botrytis cinerea, Cladosporium herbarum, Penicillium expansum,* and *Alternaria* spp. were prevalent in orchards at the initial stages of fruit development
- Three new pathogens, *Dothiorella omnivora*, *Diaporthe rudis,* and *Fusarium* sp. were isolated from bloom time samples and occasionally isolated from culled samples in packing houses (not included in this study)
- *Botrytis cinerea* causing gray mold on postharvest fruits were isolated most frequently during bloom time and in lower frequencies after petal fall and field bins.
- Some isolates of *B. cinerea* tested in this study are resistant to the fungicides used for scab management; triflumizole, cyprodinil, and dodine that may result in indirect selection of resistant *B. cinerea* population to these groups of fungicides
- One hundred percent of isolates tested in this study were sensitive to a group of fungicide, iprodione (FRAC 2) that is not registered for pear in PNW
- Preharvest application of 1-MCP (Harvista) did not result in fungicidal properties against *Botrytis cinerea.* However, the storage capability of fruits under normal atmosphere increased by reducing senescence damage on Harvista applied fruits.
- The negative effects of 1-MCP on fruits ripening after storage may be mitigated by hanging the fruits longer after 1-MCP application pre-harvest.

Future directions:

Continuing monitoring of newer pathogens as the commercial agriculture dynamics of Southern Oregon changes: Most pathogens share several hosts and it is possible that either the newer pathogens for storage rots are introduced or the existing pathogens get severe due to the availability of alternate hosts.

Developing an integrated fungicide management programs that will include bloom time, pre-harvest, and postharvest applications: Given the frequencies of pathogens isolated at different stages, an integrated approach to target stage specific pathogens appears necessary for managing different kinds of storage rot. At the same time fungicides with different modes of action needs to be evaluated for their efficacy against these pathogens as well as continued monitoring of individual pathogen for their shift in sensitivity to commonly used fungicides.

Continued testing of 1-MCP application as pre-harvest treatment: As we were narrowing the optimum treatments (rate and timing of application, timing of harvest) for pre-harvest 1-MCP applications, the results are produced from one year of field applications. The results look promising but the repeatability of the treatments and results need to be validated with additional sites, cultivars, and years.