Northwest Pear Research Review

Virtual Format

Wednesday, February 17, 2021

Time	Page	Presenter	Title	Yrs
8:40		Gix	Welcome	
8:45		Schmidt	Meeting etiquette, houskeeping, pear web resources, current funding from other sources	
			Continuing Projects & NCE Final Reports (10 min each)	
9:00	1	Nottingham	Developing a phenology-based management program for pear psylla	21-23
9:10	2	Nottingham	Improving pear pest management with integrated approaches	20-22
9:20	3	Serrano	Identification of pear tree volatiiles atractive to winterform psylla	21-23
9:30	9	Schmidt-Jeffris	Incorporating trechnites into a psylla biocontrol program	20-22
9:40	20	Northfield	Enhancing pear psylla biological control through predator recruitment: ***NCE***	20
9:50	24	Cooper	Using transcriptomics to target key behaviors of pear psylla: ***NCE***	19-21
10:00	31	Crowder	Acoustically based mating disruption of winterform psylla	18-20
10:10	41	DuPont	Fire blight product testing for effective recommendations	21-23
10:20	48	Johnson	Refinement of fire blight control strategies: buffered oxytetracycline	19-20
10:30			**BREAK**	
10:40	60	Murray/Hedstrom	IPM strategic planning for pears in WA and OR: ***NCE***	20
10:50	64	KC	Epidemiology and management of pear gray mold in the PNW	20-22
11:00	72	Rudell	New active ingredients for pear superficial scald control	20-22
11:10	81	Dhingra	Evaluating the dwarfing capacity of 65 diverse pear accessions	20-22
11:20	88	Evans	Pear rootstock breeding	20-22
			Final reports (15 Minutes)	
11:30	96	Einhorn	Field evaluation of pear cultivars on cold hardy quince rootstocks	19-21
11:45	110	Kalcsits	Optimizing irrigation frequency and timing to improve fruit quality	19-21

CONTINUING PROJECT REPORT (No Report Submitted)

Project Title: Developing a phenology-based management program for pear psylla

PI: Louis Nottingham

CONTINUING PROJECT REPORT (No Report Submitted)

Project Title: Improving pear pest management with integrated approaches

PI: Louis Nottingham

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

Project Title: Identification of pear tree volatiles attractive to winterform psylla

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Cooperator: David Horton, USDA-ARS in Wapato, WA.

Total Project Request: **Year 1:** $30,000 **Year 2: $30,000** **Year 3:** $30,000

Other funding sources

None

Budget 1

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

Item	2021	2022	2023
Salaries	$8650	$8866	$9088
Benefits	$2768	$2837	$2908
Wages			
Benefits			
Equipment			
Supplies	$17582	$16,797	$16,504
Travel		$500	$500
Miscellaneous			
Plot Fees	$1000	$1000	$1000
Total	$30,000	**$30,000**	$30,000

Footnotes:

OBJECTIVES: Goals, Year 2 Activities, and expected results

1) Determine if volatiles emitted by post-dormant (bud-swell) pear trees are attractive to post-diapause winterform pear psylla.

Prior to Year 1, the laboratory did not possess enough equipment and supplies to allow the volatile sampling from more than one tree at a time. Therefore, all preliminary results (from 2019-2020) represent samples taken from one tree at any given time. No volatile collections were conducted during Year 1 of funding, due to the timing of the project (February-March) and when research funds were received (late summer 2020). We have purchased enough volatile collecting materials, including powerful air and vacuum pumps, that will allow us to perform simultaneous collections from multiple trees. Collections will begin in Year 2, using methods described below.

Preliminary results from caged bioassays were promising and suggest that pear tree volatiles may be attractive to winterform psylla. However, the results were not significantly different, likely due to flaws in the bioassay methods. Therefore, we will use different bioassay methods in years 2-3, which will allow us to individually compare responses of psylla to a volatile stimulus.

Expected results. Preliminary results indicate that winterform pear psylla may be attracted to pear tree volatiles. We will better determine the extent of this in Years 2-3, using Y-tube bioassays and GC-EAD analyses.

2) Identify pear tree volatiles that are responsible for attraction of post-diapause winterform pear psylla.

We will continue volatile compound identifications in Years 2-3. Attempts to collect and identify volatiles prior to Year 1, were conducted by former WSU graduate student in winter 2019. Differences in volatiles were found when comparison were made between pear tree samples and the blank control. During winter 2020 the methods were replicated, however we did not obtain the same results. This was due to issues with the GC-MS instrument that was available in the laboratory for analyses. During Year 1, additional funding was secured to purchase a new GC-MS instrument. As a result, all future analyses will be conducted using the brand-new instrument, which will be more reliable and sensitive than the older instrument. In addition, the new instrument is equipped with an autosampler, which will allow us to process samples faster and more accurately.

Expected results. Volatiles will be analyzed and identified from extracts of volatiles sampled from trees during the proposed time. This will include analyses of any phenological differences in tree volatiles and pear psylla.

3) Develop a synthetic lure, based on attractive pear tree volatiles, that can be used in a trap to detect, monitor, or manage migrating post-diapause winterform pear psylla.

We will begin conducting this work in Year 3.

Expected results. If lures are attractive to winterform psylla, then this information will also help us develop new tools that can be used in pear psylla integrated pest management programs.

SIGNIFICANT FINDINGS

- Method for collecting volatiles established, and there was a difference in volatiles sampled from a pear tree compared to the blank control. However, old GC-MS instrument not reliable enough for future analyses due to sensitivity issues.

- Preliminary caged bioassays suggest that pear tree volatiles are attractive to winterform psylla.

METHODS (Updates included)

Insect collection

Diapausing and post-diapause winterform psylla will be collected Years 2-3 from pear trees (non-dispersing) and from various shelter hosts including Juniper, Pine, *Salix*, and apple in January–February. Collections will be made from plants located at the ARS facility in Wapato and the USDA experimental farm near Moxee (Figure 1). Winterform psylla have been collected from these shelter hosts in previous years by Cooper and Horton, however additional sites will be sought out if sufficient numbers of psyllids are unable to be collected. The insects will be confined to cut shoots of plants from which they were collected, and kept in growth chambers maintained at 35°F with an 8:16 (L:D) hour photoperiod until they are used in the bioassays or GC-EAD analyses.

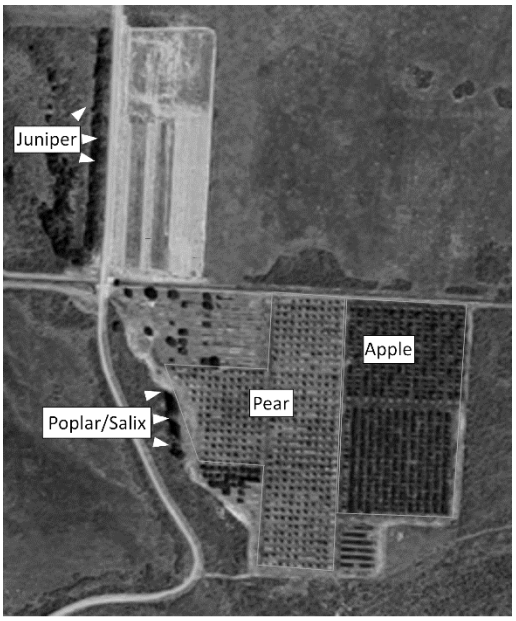

Figure 1. Layout of pear orchard at the USDA experimental farm in Moxee, where winterform psylla will be collected and where volatile collections will take place.

Collection of volatiles

We will collect volatiles from two cultivars of Bartlett pear trees during the dormant phase through the bud-swell phase when psylla re-entry is known to occur. Collecting volatiles from trees in the dormant phase until they experience bud-swell will allow us to determine specific tree volatiles that may play an important role in attracting migrating psylla, as they colonize pear trees during this period. These collections will take place semiweekly from February through late March. The environmental conditions (i.e. temperature, relative humidity, and light humidity) will be recorded when collections take place. Phenological growth stage of the tree will also be recorded, following the BBCH identification keys of pome fruit trees (BBCH Monograph 2018).

Volatiles will be collected from 10 trees in orchards in Moxee, WA (Figure 1). Methods similar to Giacomuzzi et al. (2017) will be used to collect volatiles from pear trees. Briefly, branches will be wrapped in polyethylene bags that will be fitted with an inlet and outlet for filtered air flow to be introduced using vacuum and air pumps. A charcoal filter will by attached to one end of the bag, and a volatile collector will be connected on the other side and to the vacuum line (Figure 3). Each collection will be conducted over four hours during peak daylight hours (approximately 10:00-14:00). Once the volatile collections are complete, the collectors will be removed, transported back to the laboratory, then extracted with high purity methylene chloride ($MeCl_2$) into glass vials, which will be stored in a freezer until analyses.

Figure 2. Example of volatile collection set up with a Bartlett pear tree at the USDA experimental farm in Moxee.

Analyses of Volatiles

The extracts are will be analyzed by coupled gas chromatography-mass spectrometry (GC-MS) to tentatively identify compounds present in the volatile profile of the trees (via mass spectra

interpretation). The identification of the compounds will be confirmed, where possible, by comparisons or retention times and mass spectra with those of authentic standards. Prior to GC-MS analyses, extracts will also be spiked with a small aliquot of $MeCl_2$ that contains a known amount of internal standard (e.g. undecane), which will aid with the quantification of compounds. Quantification of emitted volatiles will allow us to develop lures that better represent the natural release rates and ratios of compounds emitted by the trees. Volatile components will be quantified by comparing integrated peak data from the GC-MS response to increasing quantities of the internal standard used using a calibration curve. The analyses of the extracts of volatiles will be conducted for approximately one to three months after samples are collected. The major limitation of this portion of the project will be availability of compounds, whether they can be purchased commercially or synthesized in the laboratory.

Qualitative and quantitative comparisons will be made between extracts of volatiles from pear trees present throughout the duration of the collections. These comparisons will be made within and between varieties, across difference phenological growth stages. A software program (i.e. MassHunter) will be used to conduct a subtraction analysis of the GC-MS data of extracts of volatiles from dormant and post-dormant trees, to determine putative attractants that consistently are present only in the odor of post-dormant trees.

In parallel, coupled GC-electroantennogram detection (GC-EAD) will be used to determine if any compounds in the extracts of volatiles elicit antennal responses from adult psylla. Antennae from male and female winterform adult psyllids (pre- and post-diapause) will be used for GC-EAD analyses (see below) of extracts, which will be conducted on an instrument that is located at the ARS laboratory in Wapato. Compounds determined to be antennally active to adult psylla and also emitted by post-dormant pear trees will be selected for further evaluation as potential attractants.

Bioassays

Psylla attraction to pear tree odor will be tested using several methods. In the laboratory, extracts of volatiles, plant material, and synthetic lures that contain antennally active components for psylla will be tested in the laboratory for orientation to the extracted plant odor. First, a Y-tube olfactometer will be used with filtered and humidified airflow through holding chambers holding a chemical stimulus or with a control treatment and then into the arms of the olfactometer. The Y-tube bioassay methods and system that will be used are similar to that described and used in previous psylla attraction studies that were conducted at the ARS facility in Wapato (Horton and Landolt 2007; Horton et al. 2007, 2008; Guédot et al. 2009a, 2009b).

For GC-EAD analyses and Y-tube bioassays, we will attempt to examine variation in responses of winterform males and females between field collected diapausing winterform and field collected post-diapause winterform.

The field bioassays will be conducted from February through March at the same locations where volatile collections will be conducted. There will be at least three treatments tested: 1) traps with no lure; 2) traps with solvent control; and 3) traps with lures. The number of lure treatments will be dependent on the number of candidate attractants that we identify, as we will likely test various blends if we identify three or more putative attractant compounds. Lures will be attached to clear sticky traps, and each trap will be suspended from shepherds' hooks and placed in habitats surrounding orchards. Treatments will be deployed in a randomized complete block design with 30 m between each block and 10 m between each treatment. The number of blocks at each location will be dependent on the amount of space available. Traps will be checked and replaced semiweekly, and psylla on traps will be sexed and counted in the laboratory. Lures will be replaced weekly, at which time the position of each treatment will be rerandomized to prevent location effects. Lures will be made in-house using technologies appropriate to the desired release rates, ratios, and lure longevities (sachets, vials, septa, etc). Chemicals for lures used in lab and field bioassays will either be synthesized in-house or purchased from scientific supply companies where available.

RESULTS AND DISCUSSION

Preliminary analyses of volatiles

In March 2019, preliminary volatile collections were conducted with a Bartlett pear tree at the USDA-ARS farm in Moxee, using methods described above. As a control, volatiles were sampled from a collection bag that did not contain a pear tree. Collected volatiles were then extracted and analyzed via GC-MS. Results from this analysis showed that there were differences in volatile profiles between the pear tree and the control, especially during the earlier minutes of the analysis (Figure 3). Additional samples were collected semi-weekly during March 2020, and analyzed via GC-MS. Compounds detected in 2019 analyses, were not detected in any of the samples taken in March 2020. During the analyses, there appeared to be no characteristic plant compounds, which suggested issues with GC-MS instrument (results not shown). We will be using a new instrument in Years 2 and 3, which will result in better analyses of volatiles sampled in the field.

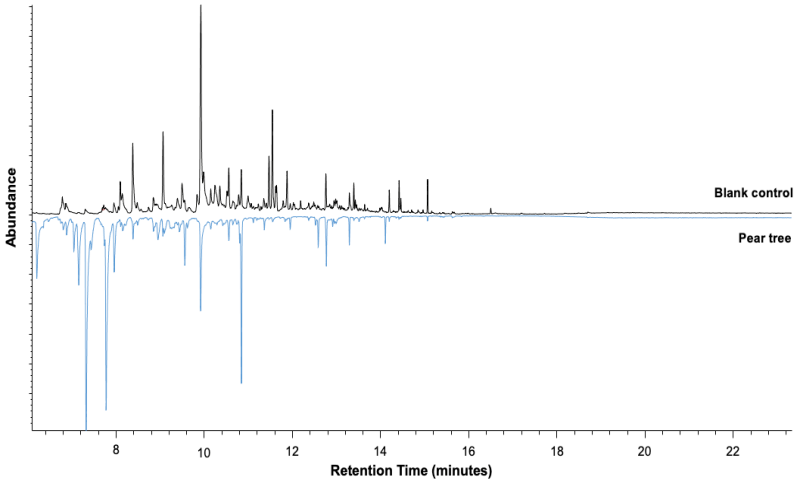

Figure 3. Representative GC analysis of volatiles sampled from an empty sampling bag (control), and a sampling bag that contained portions of a pear tree. Top trace: GC chromatogram of blank control. Inverted trace: GC chromatogram of sampled pear tree.

Preliminary bioassays

Results from caged bioassays were promising and suggest that pear tree volatiles may be attractive to winterform psylla (Figure 4). However, the results were not significantly different, likely due to flaws in the bioassay methods. In short, a dual choice assay was conducted in a small cage, where 40 psylla were introduced and presented with two traps, one containing an untreated piece of filter paper, and the other containing filter paper treated with volatiles collected from pear trees. Although the results, were not significantly different, they do suggest that the pear psylla may be attracted to pear volatiles. We believe that with more replication, and different bioassays methods, that we will be able to demonstrate attraction at a significant level.

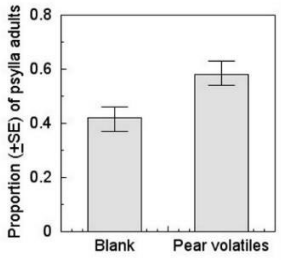

Figure 4. Mean (±SE) number of pear psylla caught in traps baited with a nontreated piece of filter paper ("Blank") and pear volatiles.

Significance to the industry. The development of an attractant lure for post-diapause winterform psylla has the potential to reduce the number of fertile and/or gravid females that reestablish on pear after overwintering on a non-host plant, which will reduce the number of eggs laid on pear trees. An attractant lure will also improve pear integrated control for multiple reasons. By having the ability to detect and monitor migration of post-diapause winterform psylla, growers can make better decisions on when to release natural enemies and/or spray. If a highly potent attractant is developed, it can used in traps to help manage populations of post-diapause psylla through mass trapping and/or attract-and-kill strategies. This is very likely due to the fact that lures will be made from volatiles emitted by host plants and should be attractive to both sexes, unlike a sex-specific pheromone. A lower number of establishing winterforms can ultimately lead to lower populations of summerform psylla. Due to the potential use in detection, monitoring, and management, a highly effective attractant can lead to fewer spray applications and can provide growers (both conventional and organic) with a new tool to manage psylla populations.

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

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

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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	2020	2021	2022
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]
Wages			
Benefits			
Equipment			
Supplies[5]	$8,500	$7,500	$7,000
Travel[6]	$500	$500	$500
Miscellaneous			
Plot Fees			
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	2020	2021	2022
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]
Wages			
Benefits			
Equipment[3]			
Supplies			
Travel[4]	$200	$200	$200
Miscellaneous			
Plot Fees			
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	2020	2021	2022
Salaries[1]	$1,560[2]	$1,599[2]	$1,639[2]
Benefits[3]	$145	$149	$152
Wages			
Benefits			
Equipment			
Supplies			
Travel[4]	$2,445	$2,445	$2,445
Miscellaneous			
Plot Fees			
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 3 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 3. Percent parasitism will now be estimated using only PCR of pear psylla nymphs, which we have determined to be the most efficient method. We have obtained WSDA SCBG funding to hire a postdoc to work on model development and additional data collection for the next ~3 years.

   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 two peer-reviewed publications, 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.**

   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. At the end of Year 1, we determined that we could collect adequate numbers of *Trechnites* mummies by placing overwintering bands in orchards and quantified the percent emergence from those mummies. After confirming the efficacy of the bands in Year 1, we deployed much higher numbers of bands in Year 2. The bands were retrieved from the field in January 2021 and we will conduct our assays with adults emerging from the bands this spring. We will deploy the bands again in Winter 2021 to conduct assays on the mummies in early spring 2022, which will complete the non-target effects testing work.

   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.

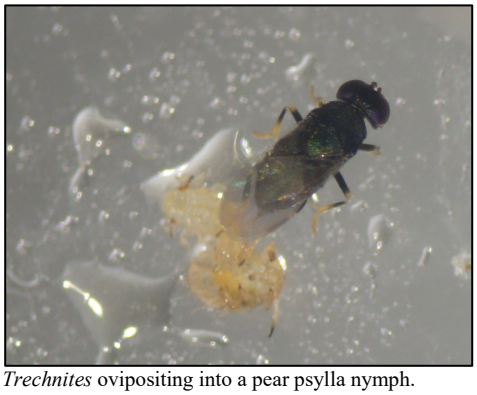
4. **Examine native psyllids from multiple locations for *Trechnites*.**

   We will continue examining native psyllids for *Trechnites* parasitism. We have found *Trechnites insidiosus* attacking native, non-pest *Cacopsylla* spp. We have also identified another *Trechnites* species, *T. sadkai*, in the Tieton area near bitterbrush, but it is unclear what hosts these wasps were using. We placed overwintering bands in pear blocks in Tieton and will examine the wasps emerging from the mummies to determine if *T. sadkai* also attacks pear psylla. We successfully obtained 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 1-2 results indicate that *Trechnites insidiosus* does parasitize native psyllids. The new grant funding from the WSDA will allow us to better determine if *Trechnites* regularly

parasitizes native psyllids. If so, planting native plants that host these psyllids near pears may improve biological control of pear psylla.

SIGNIFICANT FINDINGS


Trechnites ovipositing into a pear psylla nymph.

- 3D-printed tube traps and screened sticky cards continue to be successful at capturing adult *T. insidiosus*
- Rearing pear psylla nymphs proved to be ineffective at determining *T. insidiosus* parasitism levels, as survival in laboratory cages was poor
- PCR was determined to be the most effective way of assessing parasitism levels
- Overwintering bands were effective at obtaining large numbers of *T. insidiosus* for bioassay work and at assessing hyperparasitism levels. From the Wenatchee research orchard, 50% of bands contained at least one mummy. 73% of mummies had a wasp emerge, the vast majority of which were *T. insidiosus*.
- 48 *Trechnites sadkai* were found in beat tray samples from Tieton bitterbrush (Jun-Oct), potentially attacking *Purshia* psyllids. Tube traps collected both *T. sadkai* and *T. insidiosus* in Tieton bitterbrush. Several other parasitoid species were collected, including *Tamarixia* spp. from *Purshia* psyllids.
- We successfully obtained WSDA SCBG funding ($245,974) to support a postdoc who will expand on this work.

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. We will discontinue this sampling in the two Oregon research orchards, as *Trechnites* populations remain low. We will expand the use of traps in Oregon, but remove the random leaf/targeted nymph samples described below.

   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. 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 attempted to use emergence cages to monitor percent parasitism instead of dissection. Ten psylla from each plot at a location were placed inside a cage on a detached

pear leaf and monitored for emergence of parasitoids. Survival was poor using this method and was discontinued. For the rest of the project, percent parasitism will be determined solely by PCR detection of *Trechnites.*

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. The postdoctoral researcher hired on the new WSDA grant has substantial experience with model development.

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.

   In February 2020, we determined that adequate numbers of adults and mummies for these assays could be obtained by placing overwintering bands in pear orchards (Oct 2019) and using collected individuals. Emerged adults from the initial banding were used to conduct preliminary bioassays to test our experimental methods. Emerged adults from this year (Feb-Mar 2021) will be used to conduct the first set of bioassays.

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 1-2, but will expand in Year 3 and as part of the new WSDA grant (2021-2023). 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. We continued to sample orchards in four locations. Analysis of collected nymphs for parasitism by PCR is in progress. Fig. 1 shows abundance of *T. insidiosus* and number of psylla collected at each date in 2020. Both 3D-printed tube traps and sticky cards continue to collect high numbers of *T. insidiosus* when they are present. PCR of 2020 samples will be complete by April 2021. In 2021, we will focus percent parasitism sampling efforts on the Washington orchards, as abundance of *T. insidiosus* in Hood River and Medford remains low. Sticky cards and 3D traps will continue to be deployed in the Oregon locations to better understand *Trechnites* phenology.

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 collected random leaf samples to determine pear psylla age distribution. We successfully obtained funding from the WSDA to expand this work to hire a postdoc with expertise in modelling. By April 2021, we will create preliminary models which will determine the types of sampling conducted in Year 3.

Obj. 3. We were unable to rear *Trechnites* in sufficient numbers to begin this objective in Year 1. In Year 1 (Oct 2019), we placed cardboard bands in the research orchards in Moxee and Wenatchee. We determined that parasitized psylla nymphs used these bands as overwintering sites and form mummies within the bands. In Feb 2020, we assessed emergence from these bands. At the Wenatchee site, we placed 115 bands in Bartlett trees and 99 bands in Anjou trees. There were 1.1 mummies per band in Bartlett and 0.5 mummies per band in Anjou. Differences may be due to bark texture – there are more alternative locations for shelter in Anjou trees. From the 186 mummies we collected, 73% had a wasp emerge, most of which were *T. insidiosus*. Other wasps (n=5) were *Dilyta* spp., a hyperparasitoid. Nearly all emergence occurred within 13-14 days of removing the mummies from the cold. Given the success of this method for obtaining large numbers of *Trechnites*, we dramatically increased the number of bands placed and spread them throughout three minimally sprayed orchards with high *Trechnites* populations in October 2020: Wenatchee, Moxee, and Naches. In Jan 2021, we recollected the bands. Mummies will be removed from the bands in February and emerging adults will be used in assays shortly thereafter.

In February 2020, we used *T. insidiosus* emerging from bands to conduct a pilot bioassay to test a method using treated vials. Bexar, Rimon, or water was added to 20 glass vials each at field rate. Solutions were then poured out and allowed to dry overnight. One *T. insidiosus* was added to each vial with a small amount of honey and monitored once daily for two days. This method resulted in low mortality in the check and will be used for future assays (Fig. 2).

Obj. 4. In 2019, 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. In both years, *Trechnites* were also collected by tube traps placed near native willows and bitterbrush, demonstrated that the tube traps are also effective in native habitats outside of pear orchards. 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**.** Funding from the WSDA will allow us to expand the number of locations monitored for *Trechnites* in native habitat.

In 2020, we also found *T. sadkai* in beat samples and tube traps in bitterbrush in Tieton. However, none of the mummies collected from bitterbrush psyllids had a *T. sadkai* adult emerge (other species only). Old samples from the Tieton area (2002-2003) from both bitterbrush and a neighboring soft pear orchard were consulted. While the bitterbrush samples contained *T. sadkai*, the pear orchard samples were only *T. insidiosus*. At this point, it is unclear which psyllid species *T.*

sadkai is using as a host. We placed overwintering bands in three orchard blocks in Tieton (Oct 2020) to see if any adults emerging from mummies in this location are *T. sadkai*. Observations of emergence will begin Feb-Mar 2021. Based on collected samples of both *T. sadkai* and *T. insidosus*, Gene Milicsky has created guidelines to distinguish the two species (Table 1).

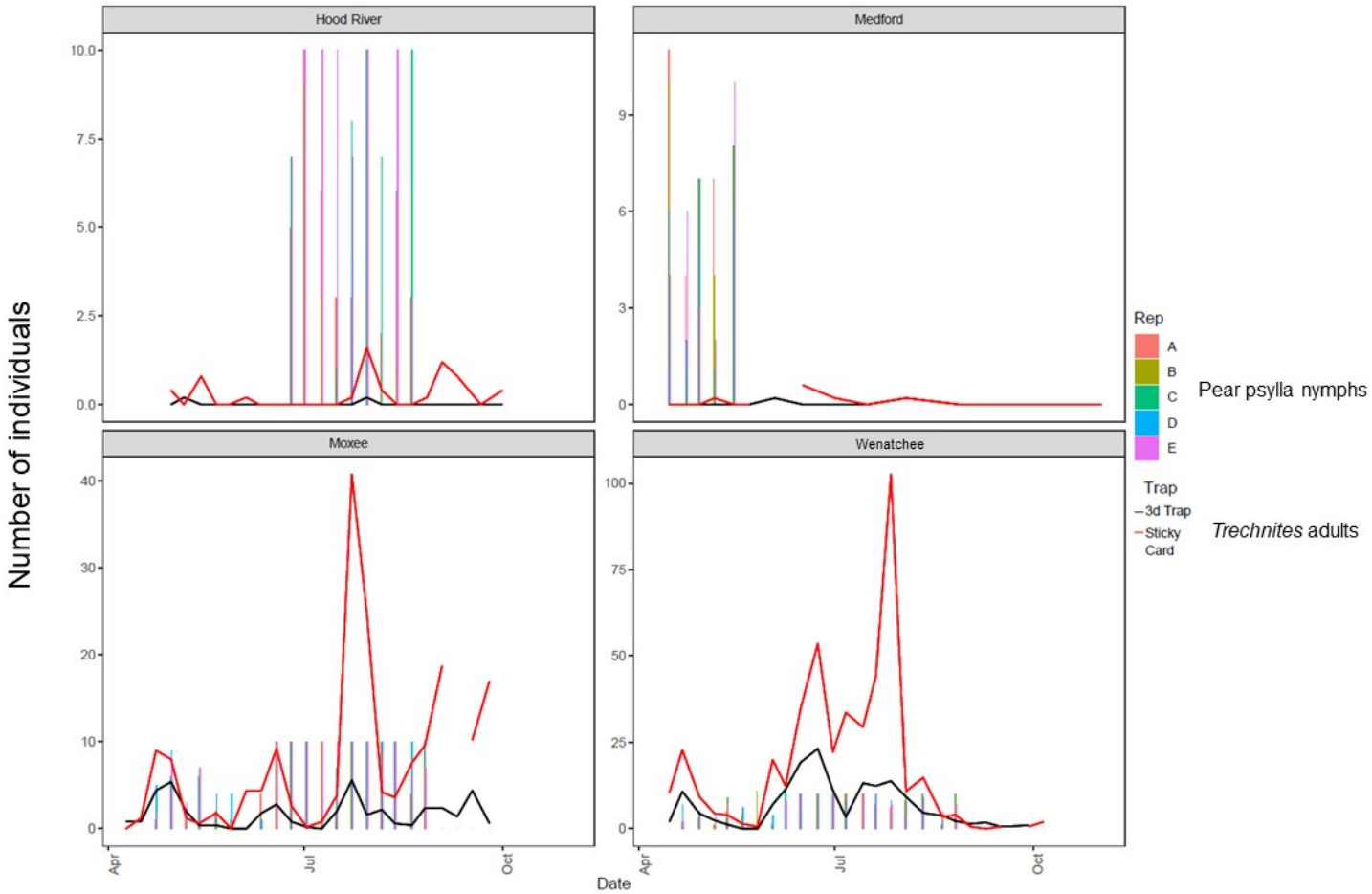


Fig. 1. Trechnites abundance measured by 3D-printed tube trap (black line) and screened sticky cards (red line) measured at four orchards. Number of psylla nymphs collected for PCR indicated by multicolored lines, five replicates per orchard. These samples will be used to conduct PCR (in progress).

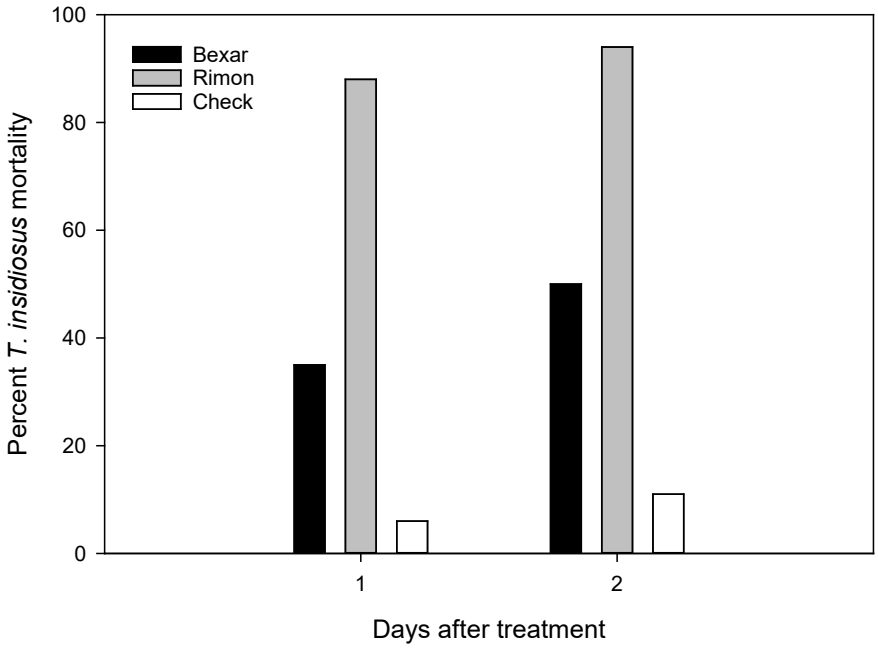


Fig. 2. Adult *T. insidiosus* mortality in vials treated with pesticide or water.

Table 1. *Trechnites insidiosus* vs (presumed) *Trechnites sadkai* from Washington State.

	T. insidiosus	*T. sadkai*
Size	Slightly larger	Slightly smaller
Notauli	Incomplete/absent	Complete; may be difficult to see in fresh specimens
Mesoscutum	Dark with vague purplish reflections	Distinct metallic green reflections
Vertex	Dark; similar to mesoscutum	Green; similar to mesoscutum
Scutellum	Distinct metallic blueish reflections; contrasts strongly with mesoscutum	Metallic green reflection; very similar to mesoscutum, although sculpture is different
Apex of abdomen	Upturned slightly	Upturned more strongly
Males	Rare	Common
Antennae	5-segmented funicle*; same as *sadkai*	5-segmented funicle; same as *insidiosus*
Leg coloring	Similar to *sadkai*	Similar to *insidiosus*
Sculpture of scutellum	Similar to *sadkai*	Similar to *insidiosus*

*5-segmented funicle separates *Trechnites* from all other Encyrtidae

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:** Shelli Tompkins / Katy Roberts
Telephone: 509-665-8271, ext **Email address:** shelli.tompkins@wsu.edu / arcgrants@wsu.edu
Station Manager/Supervisor: Chad Kruger **Email Address:** cekruger@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 occurred in 2020 when we were able to initiate the first early-season field experiments.

To get a jump start on the project and simulate spring conditions during late summer months, in 2019 we attempted 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 have since remedied the problem and used these growth rooms for maintaining pear psylla colonies in 2020. In 2019, we also conducted a small field experiment focused on psylla predation late in the summer as a pilot experiment to develop methods.

In 2020 experiments were being initiated but were disrupted by COVID restrictions in the spring, and the loss of the key employee that went on family medical leave for the summer that could not be replaced mid-summer. We plan to restart the experiments in spring 2021 to look at direct and indirect interactions between thrips and pear psylla.

METHODS

2020

On March 1st 2020 prior to leaf growth, we set up 40 exclusion sleeve cages on pear trees at the Wenatchee WSU Tree Fruit Research and Extension center in the pear orchard. (Fig. 1). To set up the cages, we first removed any overwintering pear psylla from the trees and put the sleave cages on branches to ensure that all branches were free from psylla and thrips. This would allow us to introduce to the cages four treatments: 1) pear psylla only, 2) thrips and pear psylla, 3) anthocorids and pear psylla, and 4) thrips, anthocorids, and pear psylla. Our plan was to collect anthocorids from surrounding vegetation, and use the most commonly collected anthocorid species for experiment (likely an *Anthocoris* sp.). However, COVID restrictions occurred in March before the trial could be initiated, shutting down the experiment before it could begin. Later, in early

Figure 1. Sleeve cages on pear trees March 1, 2020 waiting for insect addition.

Figure 2. Pear trees growing in the greenhouse in November 2020 for winter experiments.

summer we were able to develop lab protocols that allowed for methods to conduct research but reduce potential for COVID transmission and began planting pear trees for a similar experiment in growth rooms. However, the employee funded by the project needed to go on family medical leave, and we were not able to hire a new employee. To account for this, we kept 20 trees in a cold room so that we could plant them and grow them in a greenhouse with supplemental light when we were able to restart the experiments. In the fall we established pear psylla colonies, seeded from a colony at USDA Wapato that we kept on potted pear trees, and in November, we planted the pear trees from the cold room to prepare for an experiment (Fig. 2). However, the trees never sprouted, potentially due to either an issue in the cold room, or from the shock of being transplanted to a warmer environment. Therefore, we plan to restart the experiment in Spring 2021.

2019

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 observe apparent overlapping psylla generations, such that there was very 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

In our 2019 experiment, 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: No-Cost Extension**

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**: $11,750 **Year 4:** $0

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

Total Project Funding: $33,750

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

Item	2019	2020	2021	2022
WTFRC expenses				
Salaries			$8,045	
Benefits			$2,955	
Wages				
Benefits				
Equipment				
Supplies	$11,000	$9,000	$750	
Travel				
Plot Fees	$1000	$1000		
Miscellaneous				
Total	$12,000	$10,000	$11,750	0

Budget 2

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

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

Item	2019	2020	2021	2022
Salaries			$8,045	
Benefits			$2,955	
Wages				
Benefits				
Equipment				
Supplies				
Travel				
Miscellaneous				
Plot Fees				
Total	0	0	$10,000	0

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.

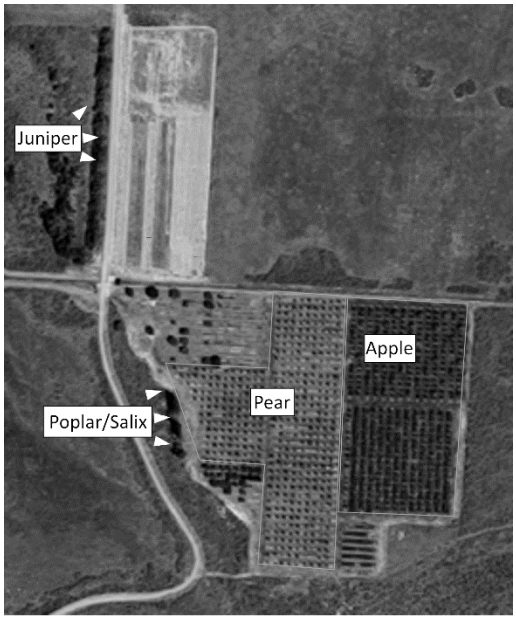

Figure 2. Winterform pear psylla will be collected from a pear orchard at the USDA experimental farm, and from surrounding shelter hosts including apple, Juniper, Poplar, and Salix.

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

Pear psylla occurs as two distinct seasonal morphotypes - summerform and winterform - that differ with respect to diapause, feeding behavior, plant attraction, and association with bacterial endosymbionts (Figure 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). Summerforms undergo several overlapping generations each year. The nymphs develop exclusively on pear, and summerform adults are rarely found on other plants (McMullen and Jong 1976). Nymphs develop into winterform in response to shortening photoperiods of early autumn. Winterform are larger and darker compared with summerform, and occur as a single overwintering generation (Mustafa and Hodgson 1984, Horton et al. 1998). The winterforms begin autumn and winter in reproductive diapause characterized by a lack of mating and ovarian development. Reproductive diapause seems to be associated with reduced tolerance to certain insecticides (Unruh and Krysan 1994), and winterforms are more likely than summerforms to harbor certain bacterial endosymbionts (Cooper et al. 2017). Autumn leaf drop displaces winterforms from pear trees prompting many psylla to disperse from orchards (Horton et al. 1994). Diapausing winterforms remain attracted to the color of foliage, and often visit or settle upon evergreen trees and shrubs, or deciduous trees with leaf drop occurring later than in pear (Fye 1982, Kaloostian 1970). Winterforms break diapause in late December, but reproductive development remains slow due to cold temperatures (McMullen and Jong 1976). As temperatures warm in February and March, post-diapause winterforms return to pear and begin laying eggs destined to become the first summerform generation.

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. Comparative transcriptomics has proven highly useful to examine the seasonal or other life cycle shifts in behavior or physiology by other insect pests. The goal of our study was to use complete transcriptomes to compare gene expression among summerform, diapausing winterform, and post diapause winterform, and will also allow us to pinpoint the exact timing for these changes (Figure 1).

Pear psylla were collected in July (Summerform), December (diapausing winterform), and February (post-diapausing winterform) from a Bartlett pear orchard located at the USDA-ARS research farm near Moxee, WA. RNA was extracted from the insects using a commercial kit and was sequenced by Novogene. The transcriptomes were assembled and analyzed using BLAST2GO by co-PIs Krey and Saha. BLAST2GO software identifies the putative function of gene transcripts and categorizes the genes based on gene ontology. We observed substantial differences in gene expression that were mostly consistent with the differences in biology and behavior among the three lifecycles of pear psylla. In 2020, quantitative PCR was used to confirm differential expression of a subset of genes involved in reproduction, immunity, defense, muscle function, and sensory.

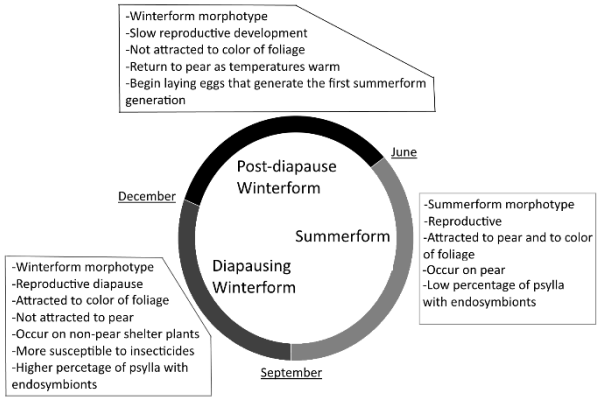

Figure 1. Annual occurrence and phenological traits associated with summerform (light grey), diapausing winterform (dark grey), and post-diapause winterform (black) pear psylla.

As expected, genes involved in reproduction were highly expressed in summerform and post-diapause winterform pear psylla. Unexpectedly, there were many genes that were categorized as reproductive genes that were upregulated in diapausing winterform pear psylla compared with summerform or post-

diapause winterforms. These reproductive genes that were upregulated in diapausing winterform psylla were distinctly different from those that were upregulated in the other seasonal morphotypes and therefore may be involved in the regulation of reproductive diapause.

Genes involved in immunity were down regulated in post-diapause winterform pear psylla compared with the other two seasonal types. We previously found that that pear psylla collected in spring are more likely than those collected in summer to harbor the plant pathogen that causes pear decline, *Phytoplasma pyri*. The decreased expression of immunity genes in post-diapause winterforms may be due to a shift in metabolic resources to reproduction, and may render psylla more susceptible to infection by *Phytoplasma* and perhaps insect pathogens. Further research is needed to examine whether pear psylla are more susceptible to entomopathogens during the orchard re-entry phase in early spring.

Previous research found that diapausing winterforms are more susceptible to certain classes of insecticides than are other seasonal types. This led us to hypothesize that genes involved in defense would be downregulated in diapausing winterforms. Although we did observe differences in the regulation of defense genes in diapausing winterforms, about half of these defense genes were upregulated in these insects. More specific analyses of differentially expressed genes are required to identify the genetic responses responsible for the seasonal changes in susceptibility to insecticides.

Genes involved in muscle function were strongly upregulated in diapausing winterform pear psylla compared with the other two seasonal types. Although diapausing winterforms disperse perhaps long distances in autumn, they are largely non-dispersing by December when specimens were collected for transcriptome analysis. We therefore did not anticipate muscle genes to be upregulated in diapausing winterforms. Some insects undergo a reorganization of muscle fibers during cold acclimation, so it is possible that the differential expression of muscle genes is related to cold tolerance in winterform pear psylla. Muscle genes were mostly down-regulated in post-diapause winterforms, while a different suite of muscle genes were upregulated in summerform pear psylla.

We identified 15 sensory receptor proteins putatively involved in sight, olfaction, or hearing that were upregulated in diapausing winterform psylla. Winterform psylla are attracted to color of foliage in autumn but are not attracted to pear specifically. Because they are attracted to the color of foliage, they often disperse from pear orchards after leaf drop and overwinter on evergreen conifers. Post-diapause winterforms are not attracted to the color of foliage and disperse from conifers to pear trees. We currently do not know what ques pear psylla use to locate pear trees, but preliminary evidence suggests that pear psylla are attracted to pear volatiles in early spring. It is currently not known whether winterform pear psylla are also attracted to volatiles released by conifers. The upregulation of sensory receptors in diapausing winterform psylla collected in December may be due to a change in which senses (olfactory versus visual) primarily drive pear psylla dispersal and behavior.

Ongoing work. Co-PI Surya Saha at the Boyce Thompson Institute has a new and improved pipeline that will more accurately assign gene functions to sequenced RNA. In year 4 with the no-cost extension, we will use this pipeline to fine-tune our sequence results and to identify genes involved in induction of cold hardiness in winterform psylla.

We were unable to finish work toward objective 2, to compare gene expression profiles between winterform that emigrate from pear versus those that remain in pear. Winterform pear psylla have been collected from various conifer shelter plants and from pear orchards. In year four with a no-cost extension, we will use qPCR to compare expression of sensory genes and genes putatively involved in cold hardiness.

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. In addition, this research will likely lead to an improved understanding of cold hardiness in winterform pear psylla, identify when pear psylla are most susceptible to entomopathogens and insecticides, and determine the factors that regulate how pear psylla locate suitable shelter plants in winter and pear trees during spring and summer. Overall, our study 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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FINAL PROJECT REPORT

Project Title: Acoustically based mating disruption of winterform psylla

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Other funding sources

Agency Name: USDA -ARS, Innovation Fund (Awarded Jan 1, 2020 – Dec 31, 2021)
Amount awarded: $21,000
Notes: To purchase equipment (speakers, minishakers, laptop computers) for field tests of disruption

Agency Name: Washington State Commission on Pesticide Registration (Awarded Dec. 2020)
Amount awarded: $24,768
Notes: Funding support for Dowen Jocson to conduct a field trial in 2021.

Total Project Funding: $155,660

Budget History:

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
WTFREC Expense				
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

[1] Salary for Dowen Jocson for the academic year
[2] Benefits for Dowen Jocson for the academic year; includes health insurance and fringe
[3] Summer wages for Dowen Jocson; summer wages for hourly employee (40 hrs/week; 12 weeks)
[4] Fringe benefits for the PhD student and time-slip employee during the non-academic year
[5] Yr 1 – acoustics equipment, cages, pear whips; Yrs 2 and 3 – acoustics equipment for playback tests and cage trials; pear whips.
[6] Yr 1 – Funds were to be used to visit the USDA facility in Gainesville, FL for training in insect acoustics, but trip was not needed. Yrs 2/3 - Vehicle lease through the state motor pool for use in conducting field research

OBJECTIVES

1. Determine whether pear psylla uses acoustic duetting in mate search activities.
2. Describe the vibrational signals used by psylla in mate location activities.
3. Show (in small cage studies with potted pear plants) 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.
5. Use data obtained in this industry-funded project to leverage funds from other sources

SIGNIFICANT FINDINGS

Years 1-2

- Recruited a Ph.D. candidate (Ms. Dowen Jocson) to complete our research objectives
- Acoustics equipment was purchased and set-up at the Pullman location, and psylla colonies were established. Acoustics assays with summerforms and winterforms were initiated.
- Acoustic signals from male summerform psylla were detected, quantified, and described. This is the first evidence that this species communicates acoustically.

Year 3

- Playback tests were conducted using both live males and the synthesized male signal to confirm that the male signal induces female acoustic response (duetting).
- Males were more likely to sing when they had recently been exposed to female presence.
- Male song syllables increase in pitch (frequency in Hz) as temperatures get warmer.
- ***Leveraged funding***: Applied for Innovation Fund grant (USDA-ARS) that was funded ($21,000)

Year 4

- Conducted a four-week, replicated cage trial with winterforms as a first effort to examine whether psylla mating can be disrupted acoustically
- Both male song playback and white noise (random acoustics) playback reduced total number of offspring per plant at four weeks by ~60% compared to control (no acoustic disruption) plants.
- ***Leveraged funding***: Applied for WSCPR grant to support field-based disruption trials in 2021; the proposal was funded for $24,768.
- ***Leveraged funding*****:** Applied for USDA-NIFA predoctoral fellowship (decision pending).

RESULTS AND DISCUSSION (YEARS 1-4)

Years 1-2. Ms. Dowen Jocson arrived in summer 2018 to begin a Ph.D. program in Entomology. She began her research in autumn 2018. ***Confirm that pear psylla communicates acoustically and describe the signal***. The vibrational signals of psyllids have been described for ~40 species across a range of taxa (Tisechkin 2006, Percy et al. 2006, Rohde et al. 2013, Liao and Yang 2015, Wood et al. 2016), including another pear psyllid (Eben et al. 2014), suggesting this mode of sexual communication is widespread. Our first assays confirmed that pear psylla does communicate acoustically. Assays were done in a soundproof room at the Pullman campus (Fig. 1A). We recorded signals using an accelerometer (Fig. 1B-C), which detects vibrations in the plant surface produced by signaling insects. The signal was sent to a computer (Fig. 1D) where it was translated into a readable form using free software (Raven). We confirmed pear psylla does indeed communicate acoustically. The male signal is a series of 15-25 "pulses" lasting about 10 seconds, followed by a longer phrase of more tightly packed syllables (Fig. 2: upper panel). Duration of an individual call was about 30 seconds, with consecutive calls (3 calls shown in Fig. 2: upper panel) separated by 10 to 15 seconds. The signal is superficially similar to that of a close relative of our pear psylla, the European pear psylla (*Cacopsylla pyri*; Eben et al. 2014). One noticeable difference is that the signal of our pear

psylla has substantially higher average frequency ("pitch"; 1320 Hz [Fig. 2: lower panel]) compared to the lower frequency of the *C. pyri* signal at approximately 690 Hz (Eben et al. 2014).

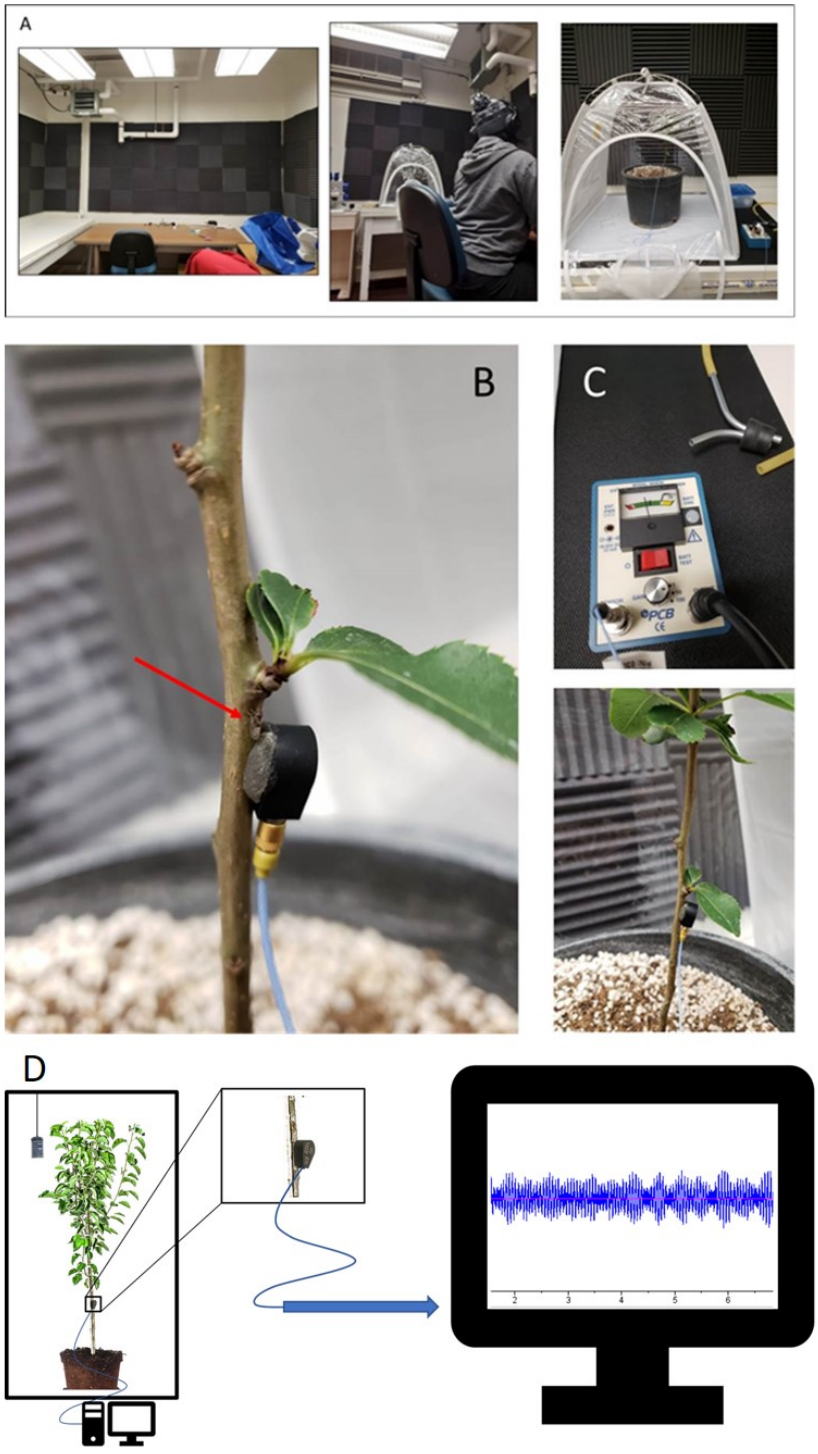
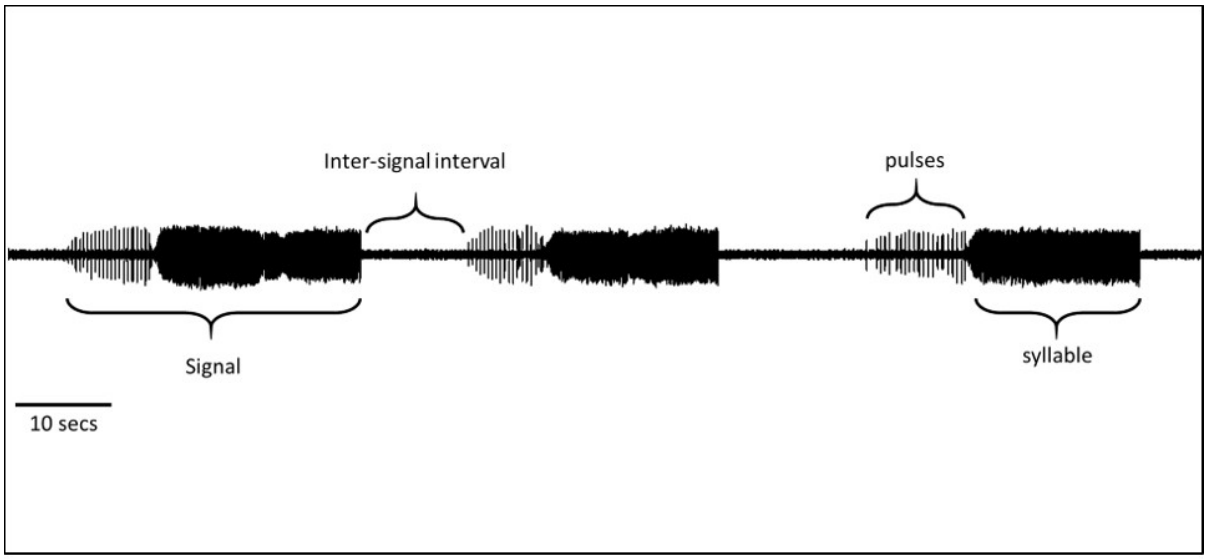


Figure 1. (A) Semi-soundproof room at Pullman location being used in our acoustics assays. (B) Head of accelerometer attached to pear whip (red arrow shows location of a psyllid). (C) Signal conditioner used to power accelerometer head and translate the vibrations. (D) Set-up.



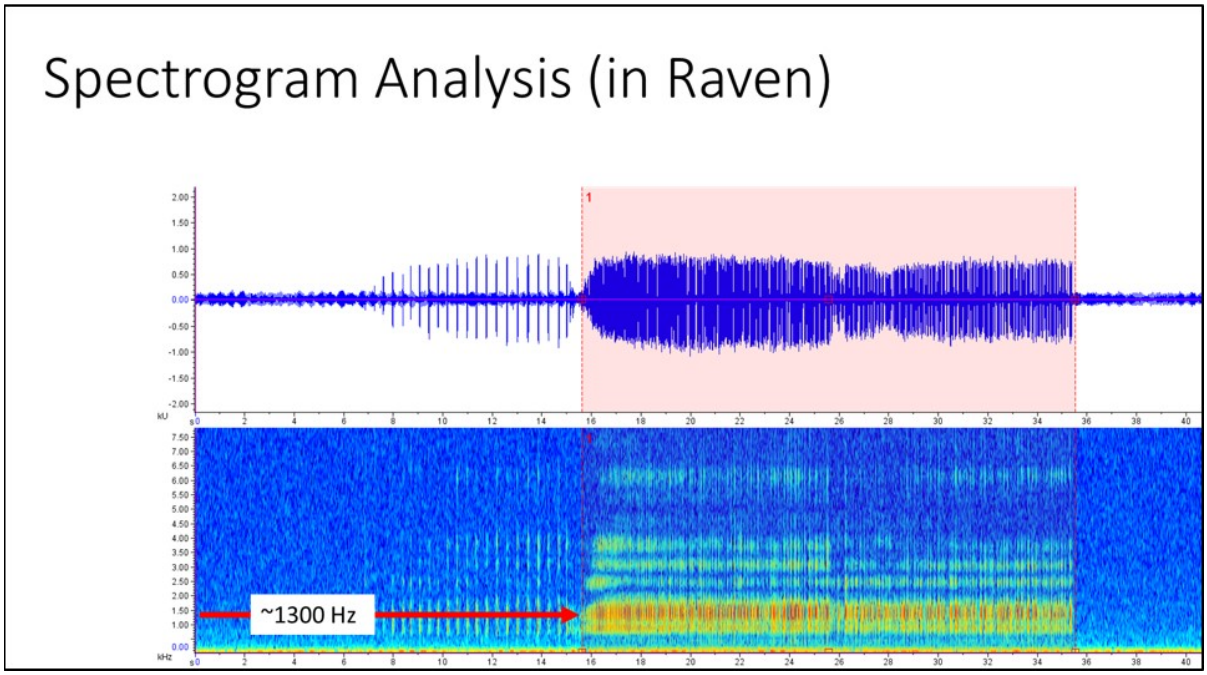


Figure 2. Upper panel shows oscillogram for signaling male summerform pear psylla; lower figure shows sonogram and spectrogram analysis depicting signal frequency concentrated at 1300 Hz.

Year 3. Research in Year 3 examined environmental and biological factors that affect signal traits and willingness to signal. ***Previous exposure of males to females*.** 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). Psyllids appear to use 3 modalities during their mate location activities (Lubanga et al. 2014): acoustic (=vibrational), visual, and olfactory. The exact relationship among these modalities in bringing the sexes together has yet to be fully defined. One prevailing belief is that vibrational duets bring a male and female psyllid to the same general neighborhood on the plant (e.g., perhaps on the same shoot), and that visual and chemical cues are then used to prompt courtship, physical contact, and mating (Lubanga et al. 2014). A second idea, yet

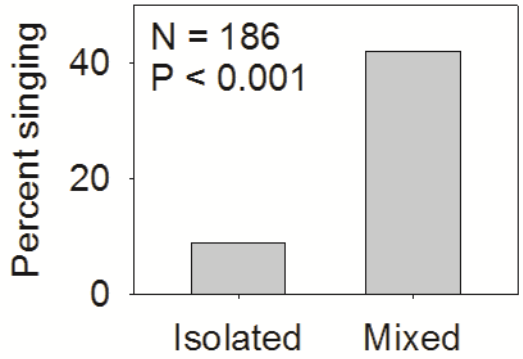


Figure 3. Probability a male summerform will sing as a function of pre-exposure to females. "Isolated": males from single-sex culture; "Mixed": males from mixed-sex culture.

to be tested, is that the sex pheromone of pear psylla (the hydrocarbon 13-methylheptacosane) discovered by scientists at the ARS laboratory in Wapato, when detected by males, is a "trigger" that prompts males to initiate singing activities.

Temperature. Temperature is likely to affect how rapidly the stridulatory structures of singing psylla vibrate and therefore affect properties of the vibratory signal (see Jocson et al. 2019). Male assays were conducted across a range of temperatures (74 to 90 °F) to test whether this environmental factor affected signal characteristics. Pitch of the male signal increased with increasing temperature between 74 and 90 °F (Fig. 4). This result suggests that characteristics of the acoustic signal will shift seasonally under orchard conditions.

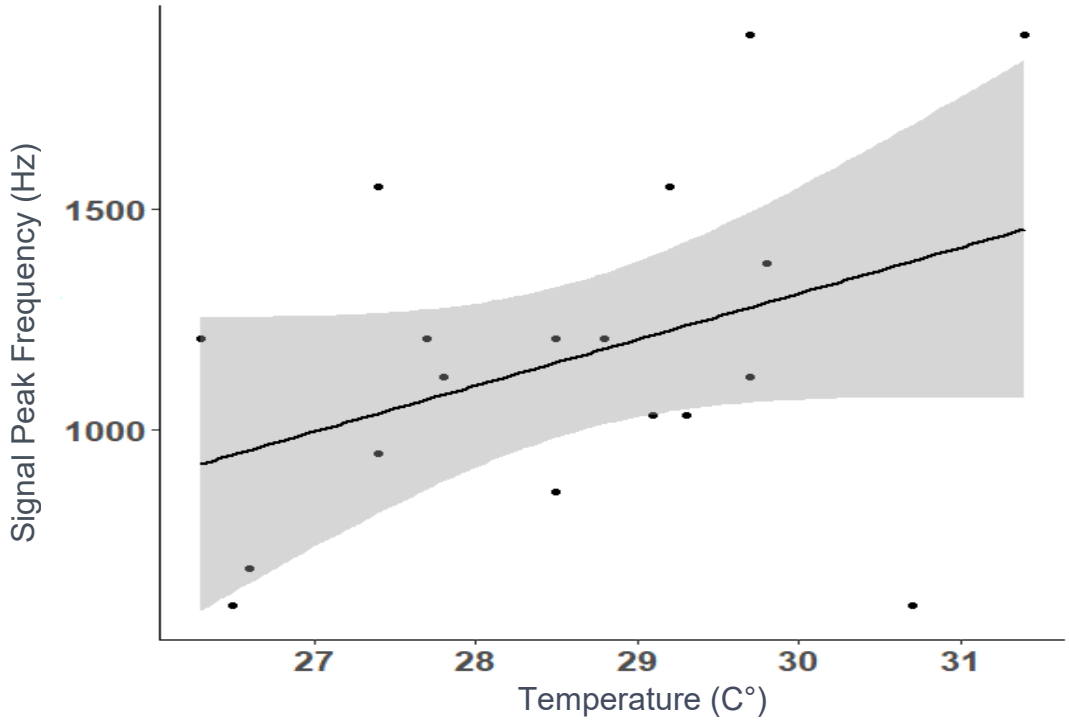


Figure 4: Linear regression that shows that there is a trend of increasing pitch (frequency in Hz) of the male signal as temperature increases (from 74 to 90 °F). The gray bar shows the 95% CI around the regression line. Assay was conducted with summerforms.

Playback tests of signal and duetting. Our practical aim for this project is to show that a mimic of either the female signal or the male signal, transmitted through pear trees under field conditions, disrupts the mate-seeking behavior of males. While we have detected and described the male signal, we now 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. We assayed recordings from live summerform males as well as the synthetic mimics of the male signal. Signals 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 a plant hosting one or more female psylla. Our assays showed that female summerforms responded to both the live male signal and to male recordings by sending out vibrational 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).
Innovation Fund. We applied for and obtained a USDA-Innovation Fund grant ($21,000). Funds are being used to purchase equipment (speakers, minishakers, laptop computer) for cage- and field-based trials of mating disruption through application of acoustic signals.

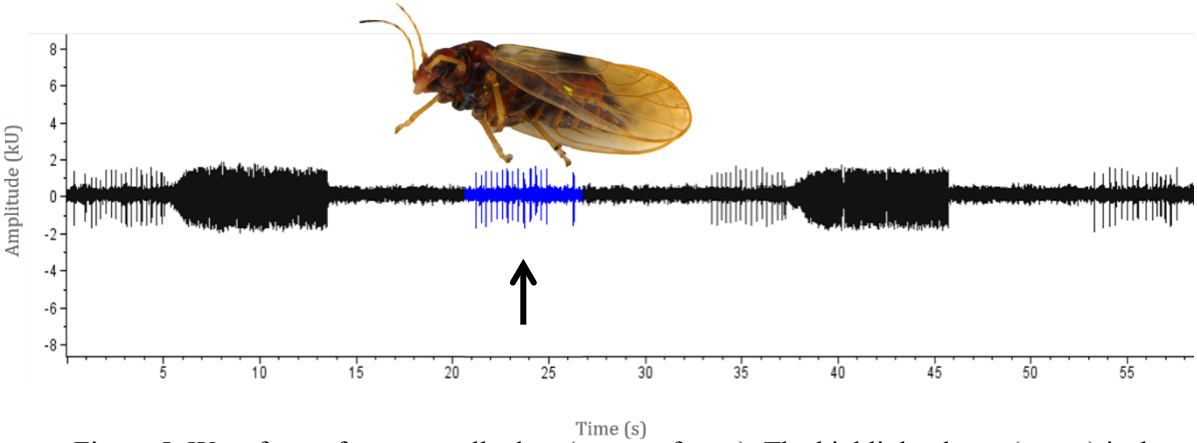


Figure 5: Waveform of a pear psylla duet (summerforms). The highlighted part (arrow) is the female response to the male signal that preceded it.

Year 4. ***Cage trial to examine prospects for disruption.*** Use of acoustic disruption of mating to manage plant-feeding pests has yet to be shown for any insect except as demonstration plots in vineyards, for leafhopper pests (Polajnar et al. 2016). Efforts to disrupt psyllid mating are limited to a cage study with citrus psyllid (Lujo et al. 2016). Our first look at the potential viability of this strategy for psylla control was done in a greenhouse using bug dorms, each containing a potted pear whip (Fig.6C). Twelve playback devices (LRAs; linear resonance actuators) were constructed for the cage study; raspberry pi (small computers) were programmed to do playbacks 24/7 in the greenhouse (Fig. 6A and 6B). Equipment was purchased using Innovation Fund dollars. We had 4 cages per treatment and 3 treatments: male signal playback, white noise playback, and no playback (control). Twenty female winterforms were added to each cage and allowed to settle on the whips. After 48 hours, 10 male winterforms were added to each cage, and the playback devices were activated. After 4 weeks, all adults were removed. Eggs, nymphs, and any new adults were counted from five randomly selected and fully-leaved branchlets per plant, providing estimates of reproduction. Results showed that plants exposed to either the male song playback or the white noise playback had fewer first generation psylla per 5 branchlets than control (no playback) plants (Fig. 7). The male signal and white noise playbacks did not differ significantly, suggesting that even a constant but random background acoustics signal interfered with production of next generation offspring. The results suggest that the playbacks led to delays in mating and thus delays in onset of egg-laying.

Where to next? Funding to support Dowen through 2021 was obtained in December 2020 through a grant submitted to the Washington State Commission on Pesticide Registration (funded for $24,768). Dowen also submitted a pre-doctoral fellowship proposal to the USDA-NIFA (decision pending). We hope to use those dollars to conduct a field test of the disruption concept under an orchard

situation. Ideally, 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 male acoustic signal and a white noise signal to trees. A laptop computer will control the minishakers and signal production (all equipment to be purchased using dollars from the Innovation Fund grant). 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.

Figure 6. (A) raspberry pi computer for playing back signals. (B) audio interface with a playback device connected to the back. The LRA tip (blue tape) is attacked to the stem of the plant to mimic vibrational communication. (C) Bugdorms set up with playback devices attached to the plants.

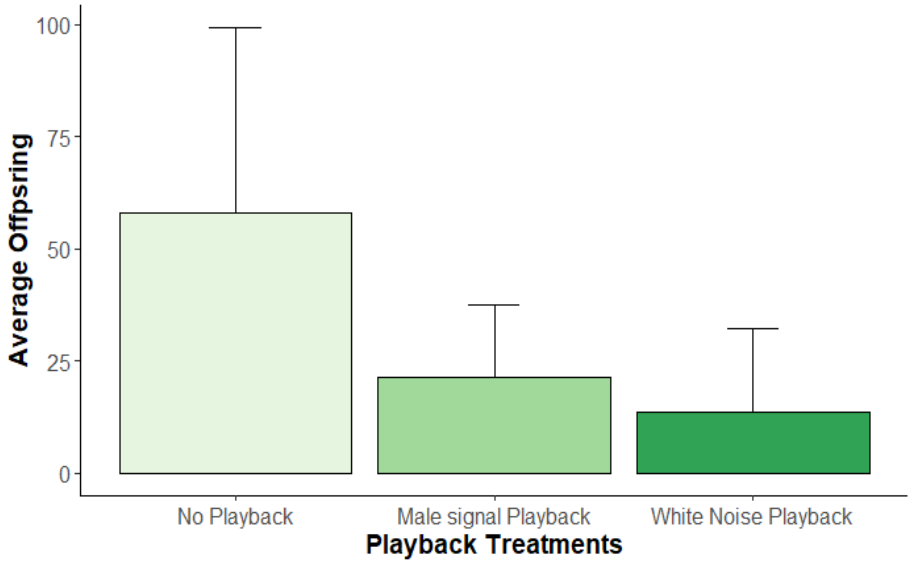


Figure 7: Average number of winterform-produced offspring per 5 branchlets from plants that received no playback, male signal playback, and white noise playback. This shows that the playback treatments were effective in reducing the number of psylla offspring (egg, nymphs, and new adults) compared to plants that did not receive any vibrational playback.

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EXECUTIVE SUMMARY
Project Title: Acoustically based mating disruption of winterform psylla

Key words: pear psylla, pear IPM, mating disruption, psyllid biology

Abstract:
Mate-searching behavior by psyllids includes use of vibratory cues sent through the plant surface as a male-female duet. These signals have been described for over 40 species of psyllids including one species of pear psyllid (*Cacopsylla pyri*), a close relative to the highly damaging species found in North America (*Cacopsylla pyricola*). We confirmed through a series of assays that our pear psylla also engages in a vibrational duet. The male signal is a series of 15-25 "pulses" lasting about 10 seconds, followed by a longer phrase of more tightly packed syllables. Duration of an individual call was about 30 seconds, with consecutive calls separated by 10 to 15 seconds. The signal is superficially similar to that shown by *C. pyri*. Pitch increases with temperature. Males are more likely to initiate signaling if they are pre-exposed to females. Females respond only following detection of the male signal. The female signal is less complex than that of the male, consisting only of a series of pulses.

Practical goals of this work include developing methods to saturate pear orchards with the male or female signal as a way to delay mating of winterform psylla in late winter as they emerge from wintering in an unmated condition. A replicated cage study with virgin winterforms was conducted showing that production of first generation offspring was about 60% lower on potted trees exposed to synthetic versions of the male signal or to random vibration ("white noise") compared to that on control (no signals applied) trees. The results, while highly preliminary, are consistent with the idea that either source of vibration delayed or interfered with successful mating of post-diapause females.

Future objectives include a field trial in a high-density, trellised orchard, in which signals are to be dispersed through trellis wires. These trials are to be supported by funding obtained elsewhere. We used data obtained in this industry-supported project to leverage funding from the USDA Innovation Fund program and from the Washington State Commission on Pesticide Registration.

Presentations (all by Dowen Jocson):
- Field Day August 2019 in Wenatchee, WA (Invited Speaker)
- Pear Day January 2020 in Wenatchee, WA (Invited Speaker)
- Entomological Society of America 2019 10-minute paper presentation, St. Louis, MO
- 3-minute Thesis presentation competition for the College of Agricultural, Human and Natural Resource Sciences (1st place)
- 3-minute Thesis presentation competition for Washington State University (4th place)

Interview with Dowen Jocson:
- Good Fruit Grower: March 1, 2020 issue
  - https://www.goodfruit.com/portfolio-items/march-01-2020/?portfolioCats=983
- ARCS Fellowship Speaker for 2020 Luncheon
  - https://www.youtube.com/watch?v=zCHwh5g-LSU&feature=youtu.be

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

Project Title: Fire blight product testing for effective recommendations

PI: Tianna DuPont
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Email: tianna.dupont@wsu.edu
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Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee WA 98801

Cooperators: advisory committee

Total Project Request: **Year 1:** $14,255 **Year 2: $14,686** **Year 3:** $15,132

Other funding sources: **Awarded**
Amount: $15,000
Agency Name: USDA NIFA IR4
Notes: 2019-34383-29950

Other funding sources: **Awarded**
Amount: $22,500
Agency Name: Gift Grants from Product Companies

Other funding sources: **Awarded**
Agency Name: USDA Specialty Crop Research Initiative
Amount: $416,000
Notes: 9/1/20 to 8/31/24

Budget 1
Organization Name: WSU **Contract Administrator:** Shelli Thompkins/Kate Roberts
Telephone: 509.293.8800/509.335.2885 **Email address:** shelli.tompkins@wsu.edu /arcgrants@wsu.edu

Item	2020	2021	2022
Salaries[1]	$7,800	$8,112	$8,436
Benefits[2]	$2,955	$3,074	$3,196
Wages			
Benefits			
Equipment			
Supplies	$500	$500	$500
Travel			
Miscellaneous			
Plot Fees	$3,000	$3,000	$3,000
Total	$14,255	**$14,686**	$15,132

Footnotes: [1]Salaries for a scientific assistant 2 month/ yr.
[2]Benefits at 38% for scientific assistant.

OBJECTIVES

1. Test new fire blight prevention products.
2. Provide research-based information to growers and consultants.

SIGNIFICANT FINDINGS

- Two applications of hydrogen peroxide (27%), peracetic acid (5%) products at 128 fl oz/ 100 gal resulted in blossom blight infections no different than water controls. Additional applications may be necessary to suppress infections. However, 2019 data showing extensive fruit marking from multiple post petal fall applications suggest that caution should be used with late applications of these products.
- Of plant essential oil/ extract products trial ET91, an unlabeled experimental product provided control comparable to organic standard (Blossom Protect/Buffer + Previsto). Other plant essential oil/ extract products performed no better than the water-treated check.
- Of mineral products, the copper sulfate product TDA-NC-1 provided control comparable to organic standard (Blossom Protect/Buffer + Previsto). The long-term average for Alum is 80% control, however control was no different than the water-treated check in 2020.
- The biological control phage product provided control no different than the water-treated check in 2020. In 2019 while strikes per 100 clusters were 50% of that from the water-treated check they were highly variable and not statistically different than the water-treated check.

METHODS

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

Plots: Five blocks of 24 trees were designated for bloom trials. 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: Frozen-preserved cultures 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: Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml. A 3-gallon backpack sprayer (solo) was used to lightly wet 100+ clusters per plot.

Treatments: Products were applied by tree to the area of the tree to be inoculated according to manufacturer recommendations using a Stihl SR420 blow mister backpack sprayer with a wetting agent (Biolink, organic; Regulaid, conventional). Bloom trial products were applied to wet, previously calibrated to equal 100 gal/A. 2020 application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall).

Included in this trial as a comparison and 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., and critical for comparisons as long-term standards). An untreated and inoculated check treatment and an untreated non-inoculated check treatment were included. An organic standard (Blossom Protect/ Buffer Protect followed by soluble copper) was also included for comparison.

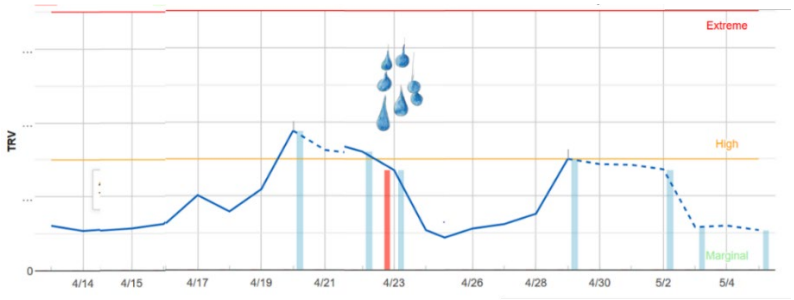
Environmental Conditions: Conditions during product application were as follows. The day of inoculation was overcast during the morning a very small amount of precipitation.

Table 3. Environmental Conditions

DATE	MIN AIR TEMP F	MAX AIR TEMP F	HUMIDITY	LEAF WETNESS	WIND SPEED
14-Apr	47.9	74	25.5	0	4.9
15-Apr	47.1	65.4	38.6	0	5.9
16-Apr	37	65.4	39.5	0	4.4
17-Apr	36	75.4	36.1	0	4.5
18-Apr	51.2	69.1	47.4	0	4.3
19-Apr	46.3	72.8	45.5	0	4.1
20-Apr	42.9	77.3	42	0	4
21-Apr	49.7	70	34.9	0	6.3
22-Apr	49.2	56.3	65.4	0.17	3.1
23-Apr	46.9	67.7	49	0.08	5.4
24-Apr	43.2	69.3	46.4	0	3.4
25-Apr	48.3	68.7	50.7	0	5.2
26-Apr	44	67.6	33.1	0	4.4
27-Apr	49.5	68.5	39.6	0	5.8
28-Apr	42.1	71.9	44.5	0	3.8
29-Apr	50.8	75.1	39	0	3.2
30-Apr	50.7	65.4	36.8	0	4.8
1-May	41.5	70.5	37.6	0	4.5
2-May	48.6	67.5	47.9	0.02	5.8
3-May	42.9	64.3	40.7	0	4.5
4-May	37.8	69.4	40.9	0	3.5
5-May	49.7	77.6	38.4	0	3.5
6-May	49.4	62.7	39.1	0.01	7.4
7-May	40.7	74.7	38.2	0	3.2
8-May	44.5	76.4	41.4	0	4.5

Fire Blight Risk and Pressure: During full bloom fire blight risk was moderate with warming temperatures right after full bloom. Petal fall sprays went on the evening before a significant rainfall event. See output from the Cougar Blight DAS model for full bloom and petal fall (Figure 1).

Figure 1. Cougar Blight Fire Blight Risk.



Evaluation: Trees were visually evaluated for flower cluster infection every week following treatment. Symptoms became visible 13 days after inoculation. Strikes were counted for 4 weeks. Blighted clusters were removed immediately after counting. Cluster infection counts were summed across all dates. Fruit were evaluated for russet fruit skin marking on Jun 26, 2020. 25 fruit per tree were rated. Russet ratings

were on a 1 to 15 scale with individual values lower than 3 consider insignificant for commercial packing.

Erwinia enumeration: Additionally, *Erwinia Amylovora* was enumerated from flowers collected at full bloom, petal fall and one week after petal fall. Five flower clusters per tree were sampled at each time point. A bulk sample was immersed in sterile water and sonicated for three minutes. After sonication a 10- µl sample of the wash and two 1:100 dilutions will be spread on CCT medium amended with nalidixic acid (50 µg/ml) to selectively enumerate Ea153N.

Analysis: Statistical analysis was performed using an analysis of variance ANOVA and multiple means comparison t tests (LSD) (SAS).

RESULTS & DISCUSSION

Table 1. Effect of hydrogen peroxide, peracetic acid treatments applied to Red delicious apple trees on infection from *Erwinia Amylovora* in apple blossoms.[‡]

	Rate per 100 gal	Timing	strikes per 100 clusters**			
Streptomycin standard (Firewall 17)[z,y]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	±	1.2	a
Oxytetracycline standard (Fireline 17) [z,y]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2	b
Organic standard (Blossom Protect/ Buffer Protect + Previsto)	1.24 lb 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5	±	1.3	b
hydrogen peroxide (26.5%), peracetic acid (4.9%) (Jet Ag)	128 fl oz	Day after inoc and 3 days after inoc*	28	±	3.9	c
hydrogen peroxide (27%), peracetic acid (5%) (Oxidate 5.0)	128 fl oz	Day after inoc and 3 days after inoc	24	±	3.8	c
hydrogen peroxide (27%), peracetic acid (5%) (Oxidate 5.0)	50 fl oz	Day after inoc and 3 days after inoc	28	±	4.1	c
Untreated check	----	100% bloom, +1 day, petal fall	31	±	7.1	c

** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.

[Z] Buffered to 5.6 pH.

* Note inoculation is done at dusk. Day after spray is done early morning next day. 3 days after inoculation coincides with petal fall sprays.

[‡]Application dates were: April 15, pink, April 19 (20% bloom), April 21 (50% bloom), April 23 (full bloom), April 24 (full bloom plus 1 day), April 28 (petal fall). Inoculation was conducted on the evening of April 23, 2020 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10^6 CFU per ml.

Figure 1. Effect of treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers at Full Bloom (FB), Petal Fall (PF) and Petal Fall + 1 week (PF+1).

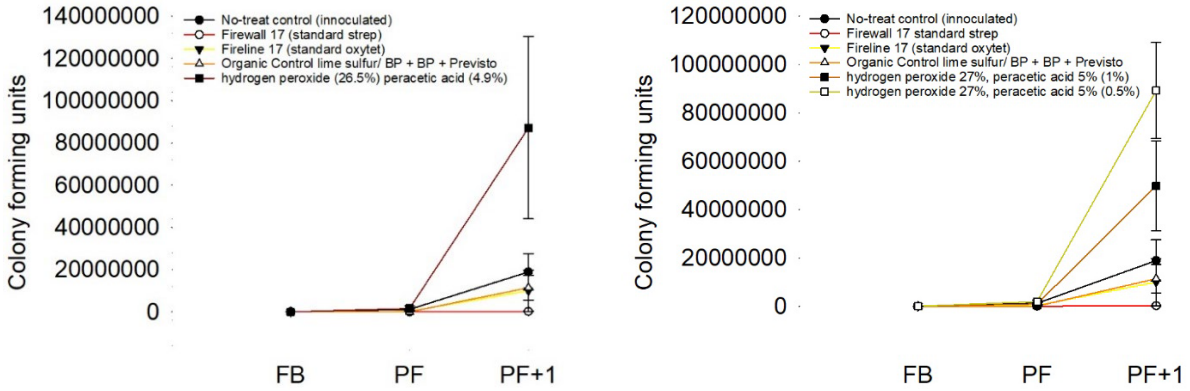


Table 2. Effect of Essential Oil/ Plant Extract Treatments on infection of *Erwinia Amylovora* in apple blossoms. ‡

Treatment	Rate per 100 gal	Timing	strikes per 100 clusters	
Streptomycin standard (Firewall 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8 ± 1.2	a
Oxytetracycline standard[y] (Fireline 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2 ± 2	b
Organic Standard (Blossom Protect/Buffer) + Soluble Copper (Previsto)	1.24 lb 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5 ± 1.3	bc
Thyme oil (23%) (Thyme Gard 0.5%)	2 qrt	80% bloom, 100% bloom +1 day, petal fall	17 ± 2.3	cd
Thymol (23%) (Thymox 0.5%)	2 qrt	80% bloom, 100% bloom, petal fall	22 3.5	d
Cinnamon oil (60%) (Cinnerate)	1 qt	50% bloom, morning after inoc, petal fall	19 ± 3.5	d
TS28	21.9 ml	100% bloom, +1 day, petal fall	23 ± 5.5	cd
TS108	25 ml	100% bloom, +1 day, petal fall	31 ± 5.8	d
ET91	38.4 oz	100% bloom, +1 day, petal fall	10 ± 6.6	b
Lupine[u] (Problad)	40 oz	50% bloom, morning after inoc, petal fall	22. 6 ± 4.1	cd
Water-treated check	NA	100% bloom, +1 day, petal fall	31 ± 7.1	d

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.
[z]Buffered to 5.6 pH.
‡Application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.
[u]Banda de Lupinus albus doce (20%).

Figure 2. Effect of essential oil/ plant extract treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers at full bloom (FB), petal fall (PF) and petal fall + 1 week (PF+1).

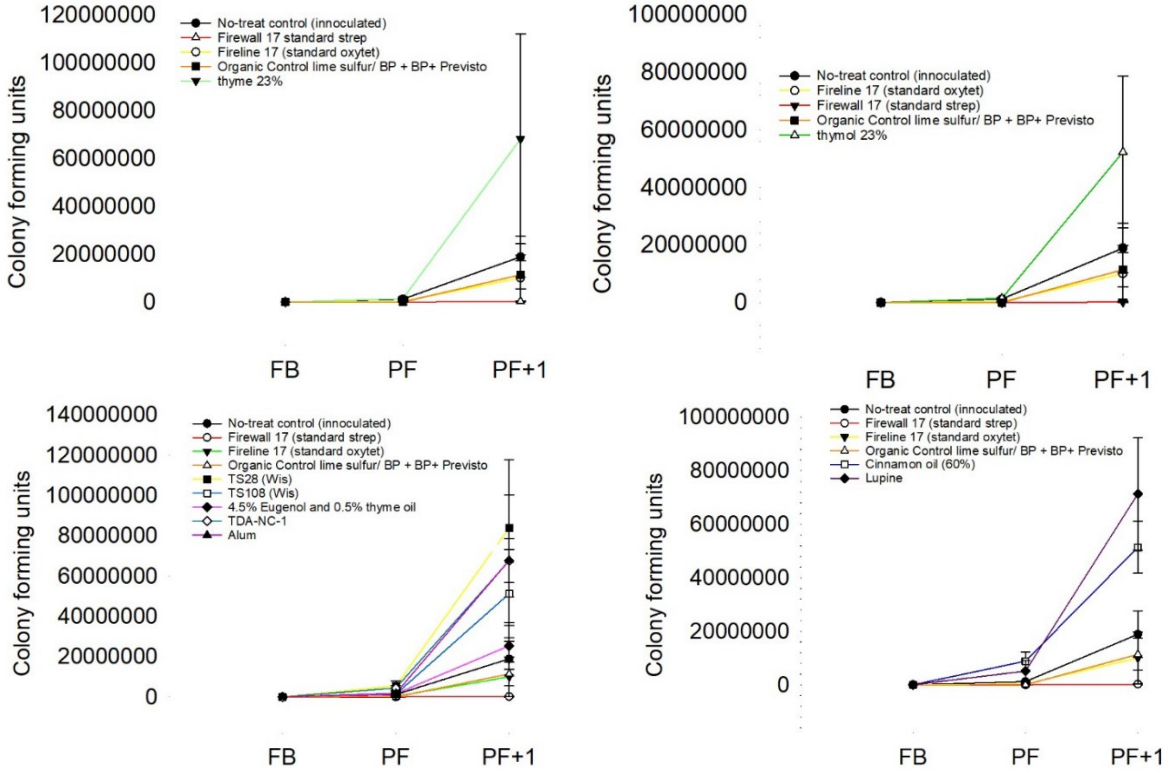


Table 3. Effect of Mineral Product Treatments on *Erwinia Amylovora* infection of apple blossoms.‡

Treatment	Rate per 100 gal	Timing	strikes per 100 clusters	
Streptomycin standard (Firewall 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8 ± 1.2	a
Oxytetracycline standard[y] (Fireline 17) [yz]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2 ± 2	b
Organic Standard Blossom Protect/Buffer + Soluble Copper (Previsto)	1.24 lb 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5 ± 1.3	bc
Alum[y]	8 lb	100% bloom, petal fall	22 ± 4.2	d
TDA-NC-1[x]	17.1 g	Tight cluster, 50% bloom, 100% bloom + 1 day, petal fall	13 ± 2.3	bc
Water-treated check	NA	100% bloom, +1 day, petal fall	31 ± 7.1	d

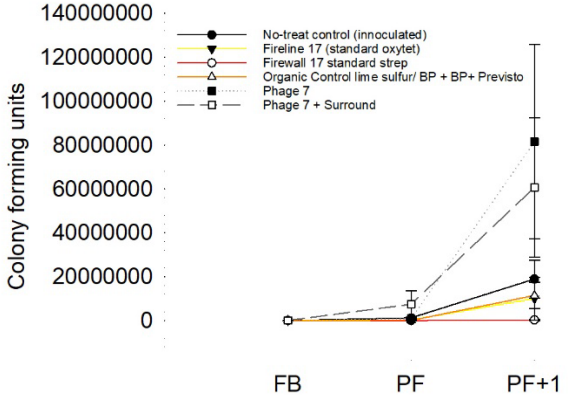
[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.
[Z]Buffered to 5.6 pH.
[x] Amended with Silwet oil at 0.0125%. Copper sulfate product.
‡Application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.

Table 4. Effect of Biological Control Product Treatments on *Erwinia Amylovora* infection of apple blossoms.‡

Treatment	Rate per 100 gallons water	Timing	Strikes per 100 clusters**			
Untreated, Inoculated Check	water	100% bloom, +1 day, petal fall	31	±	7.1	c
Streptomycin standard (Firewall 17)[zy]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	±	1.2	a
Oxytetracycline standard (Fireline 17) [zy]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2.0	b
Organic standard (Blossom Protect/Buffer Protect +Previsto)	1.24 lb 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5	±	1.3	b
Phage7 (Agriphage)	2 qt	100% bloom 12hr before ap, +1 day, +3 days	24	±	4.8	c
Phage7 (Agriphage +Surround)	2 qt + 0.1 lb	100% bloom 12hr before ap, +1 day, +3 days	31	±	3.7	c

** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.
[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.
[Z] Buffered to 5.6 pH.
‡Application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.

Figure 3. Effect of Biological Control treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers.



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

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

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Budget: **Year 1**: $24,202 **Year 2**: $24,686 **Year 3:** no cost extension

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' (FireLine) or '-calcium complex' (Mycoshield)) influences the pH-buffering enhancement of oxytetracycline.

SIGNIFICANT FINDINGS:

- In both apple and pear, a lowered pH improved the efficacy of oxytetracycline for fire blight suppression
- Pathogen populations on flowers were suppressed to a greater degree with acidified oxytetracycline compared non-acidified oxytetracycline.
- pH-adjustments of oxytetracycline caused negligible to slight effects to fruit finish quality (russeting).
- A prolonged inhibitory residual is the likely reason that fire blight control is improved by acidification of oxytetracycline.
- In more limited trialing, acidifying an alternative antibiotic, kasugamycin, also improved fire blight control.

RESULTS:

Summary of orchard trials. Fire blight suppression trials (8 total) were arranged in randomized complete block designs of 4 to 12 treatments applied to single-tree plots replicated four times. The number of flower clusters on individual trees were counted prebloom and this count as well as tree location were considered in the assignment of trees to blocks in the plot design. Experimental protocols were similar among trials with the fire blight pathogen inoculated onto the trees near full bloom followed by sprayed treatments at full bloom and prior to petal fall. Trial-specific dates of inoculation and sprayed treatments are summarized in Table 1.

Table 1. Mean number of flower clusters per tree and dates of experimental actions related to evaluation of acidified oxytetracycline treatments for fire blight control in experimental pear and apple orchards located near Corvallis, OR.

				Date of experimental action			
Year	Orchard	Cultivar	Mean flower clusters per tree [x]	Pathogen [y] inoculation	Full bloom treatment	Treatment prior to petal fall	Post-petal fall sample [z]
2017	Pear	Bartlett	171	18 April	20 April	22 April	1 May
	Apple	Golden Delicious	399	28 April	29 April	3 May	10 May
2018	Pear	Bartlett	1012	12 April	14 April	20 April	27 April
	Apple	Gala	375	24 April	25 April	1 May	8 May
2019	Pear	Bartlett	156	18 April	20 April	26 April	2 May
	Apple	Gala	218	24 April	26 April	2 May	9 May
2020	Pear	Bartlett	455	8 April	10 April	14 April	21 April
	Apple	Gala	431	15 April	17 April	21 April	28 April

[x] Mean number of flower clusters per replicate tree. Each treatment was replicated four times. [y] The pathogen, *E. amylovora* stain Ea153N, was misted onto the trees (1 x 10^6 CFU/ml) 12 to 36 h prior to the full bloom treatment. [z] Flower clusters were sampled to measure epiphytic *E. amylovora* populations on the day after the 'full bloom' and 'prior to petal fall' treatments, and at 6 to 9 days after petal fall.

Objective 1:

Infection suppression. As a result of pathogen inoculation, fire blight developed in the water-treated control trees of each trial with infection incidences ranging from 6% (pear 2018) to 73% (apple 2018) of total flower clusters; among the eight trials, mean incidence of infection for the water control was 26% (Table 2). The acidifier controls, Buffer Protect or citric acid only, reduced fire blight incidence significantly ($P \leq 0.05$) in five of eight trials, but averaged over trials, this reduction in infection incidence averaged only 15% (Fig. 1).

In all eight orchard trials, relative to the water-treated control, treatment of pear or apple with a non-acidified antibiotic resulted in a significant reduction ($P \leq 0.05$) of fire blight incidence (Table 2). Moreover, also relative to the water-treated control, acidified antibiotics always suppressed fire blight significantly ($P \leq 0.05$). Within-trials, the differences in fire blight suppression by acidified oxytetracycline (OTC) compared to OTC by itself was frequently smaller than least significant difference for the trial, and therefore, many of the direct comparisons of acidified to non-acidified materials did not differ significantly ($P > 0.05$). Nonetheless, for 26 of 30 within-trial comparisons, acidified OTC showed better suppression than OTC only.

Averaged across orchard trials, relative infection suppression from Mycoshield (OTC-calcium complex) was improved from a mean of 57% (± (standard error) 13.5) without acidification to 80% (± 4.9%) when amended with citric acid or Buffer Protect (Fig. 1). Acidified FireLine (OTC-hydrochloride formulation), in contrast, was improved to a lesser degree with a relative infection suppression of 88.8% ± 1.7 compared to 79.7% ± 3.7 for the antibiotic only. In more limited trialing, acidifying kasugamycin (Kasumin 2L) increased relative infection suppression to 94% (± 2.7%) compared to 83% (± 5.7%) for the non-acidified kasugamycin.

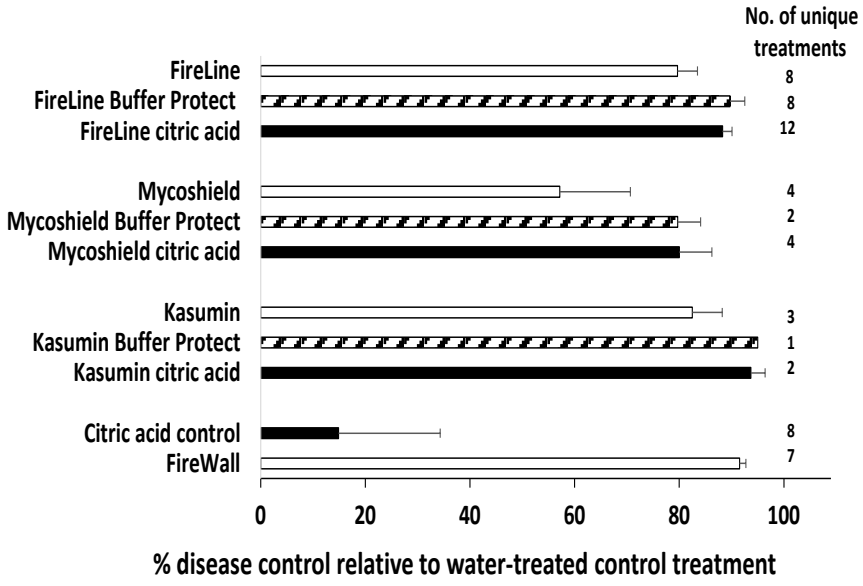

Fig. 1. Effect of acidified oxytetrcycline materials for fire blight control in Bartlett pear and Golden Delicious or Gala apple expressed as 'percent infection suppression relative to the water-treated control'. Data are from eight orchard trials conducted from 2017 to 2020 and depict the mean and standard 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 16 to 32 oz./100 gal and amount of Na_2HPO_4 raged between 0 and 16 oz./100 gal (see Table 2). The citric acid control was Buffer Protect in 2017, and citric acid with Na_2HPO_4 (2018), K_2HPO_4 (2019), or no buffer amendment (2020). Rates of antibiotic materials were held constant among trials and are shown in Table 2.

Table 2. Incidence of fire blight on pear and apple flower clusters as affected by oxytetracycline and various materials used to acidify the sprays applied the trees in eight orchard trials [t] conducted near Corvallis, Oregon from 2017 to 2020.

Treatment	Rate [u] oz./100 gal	Incidence (%) [v] 2017 Pear		2017 Apple		2018 Pear		2018 Apple		2019 Pear		2019 Apple		2020 Pear		2020 Apple	
Water control		**6.0** (13)	**a** [w]	**13.6** (62)	**a**	**44.0** (440)	**a**	**73.4** (223)	**a**	**8.5** (15)	**ab**	**15.0** (30)	**a**	**14.5** (67)	**a**	**30.7** (126)	**a**
Buffer Protect	75	4.9	b	15.8	a	---		---		---		---		---		---	
Citric acid buffer [x]	16 8	--- [y]		---		23.9	b	15.1	b	16.5	a	8.0	b	16.4	a	14.6	b
FireWall	8	0.7	c	---		5.5	d	3.3	c	0.8	e	1.4	c	1.0	e	1.5	cd
FireWall Citric acid	8 16	---		---		---		---		---		---		1.0	e	1.1	d
FireLine	16	1.6	c	1.4	b	10.9	c	11.1	b	3.6	cd	1.5	c	2.9	de	4.1	c
FireLine plus Buffer Protect	16 75	0.3	c	0.8	b	6.4	d	2.2	c	1.8	de	1.8	c	---		---	
FireLine Citric acid	16 8	---		---		---		---		---		---		2.3	e	4.2	c
FireLine Citric acid	16 16	---		---		---		---		1.5	de	1.6	c	1.6	e	1.3	cd
FireLine Citric acid $Na2HpO_4$	16 32 16	---		---		5.3	d	2.3	c	---		---		---		---	
FireLine Citric acid $Na2HpO_4$	16 16 8	---		---		8.9	c	3.9	c	---		---		---		---	
FireLine Citric acid $Na2HpO_4$	16 12 12	---		---		9.6	c	3.9	c	---		---		---		---	
FireLine LI70	16 64 [z]	---		---		---		---		2.5	cde	1.2	c	2.1	e	2.1	cd
FireLine TRIFOL	16 64 [z]	---		---		---		---		2.6	cde	1.6	c	---		---	
Mycoshield	16	---		---		18.4	b	---		6.3	bc	---		6.8	bcd	2.6	cd
Mycoshield Buffer Protect	16 75	---		---		7.0	cd	---		2.1	de	---		---			
Mycoshield Citric acid	16 16	---		---		---		---		3.0	cde	---		3.3	de	1.7	cd
Mycoshield Citric acid $Na2HpO_4$	16 16 8	---		---		7.2	cd	---		---	cde	---		---			
Kasumin	64 [z]	0.2	c	---		---		---		---		---		2.7	de	2.2	cd
Kasumin Buffer Protect	64 [z] 75	0.3	c	---		---		---		---		---		---			
Kasumin Citric acid	64 [z] 16	---		---		---		---		---		---		1.3	e	1.1	d

[t] Single-tree plots were arranged in a complete randomized block design with four replications per treatment. All treatments applied twice except FireWall, which was applied once at full bloom. Dates of pathogen inoculation and treatment applications are provided in Table 1.

[u] Approximated an orchard spray volume of 100 gallons per acre.

[v] Infected flower clusters divided by total number of clusters per tree. Proportional incidence data transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown.

[w] Means within a column followed by same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference.

[x] Na_2HPO_4 in 2018, K_2HPO_4 in 2019, and no buffer amendment in 2020.

[y] '---' indicates treatment was not included in that specific experiment.

[z] Fluid ounces per 100 gallons.

Pathogen populations in flowers. Inoculation of the pear and apple trees near full bloom with the fire blight pathogen resulted in measureable populations of *E. amylovora* for all sampling dates of all trials. Across trials, the full bloom sample from water-treated trees averaged 4.0 $\log_{10}$ (CFU/flower) with mean population size increasing to > 5.0 $\log_{10}$ (CFU/flower) at petal fall and exceeding 6.0 $\log_{10}$ (CFU/flower) at the post-petal fall sample (Fig. 2A).

Over all trials, relative to the water control, FireLine reduced the measured *E. amylovora* population size by 2.0 to 2.5 log units through petal fall, with this difference decreasing to one log unit by the post-petal sample (Fig. 2A). Also relative to the water treatment, acidifying FireLine increased the magnitude of the population reduction at all sampling dates with the greatest differences occurring at petal fall (3 log units). Similarly, relative to the water control, Mycoshield reduced *E. amylovora* population size by 1.5 to 2 log units from full bloom to petal fall (Fig. 2B). For the petal fall sampling date, the addition of Buffer Protect or citric acid to Mycoshield further reduced the pathogen's population size by an additional 1 to 1.5 log units compared to the suppression obtained by Mycoshield only.

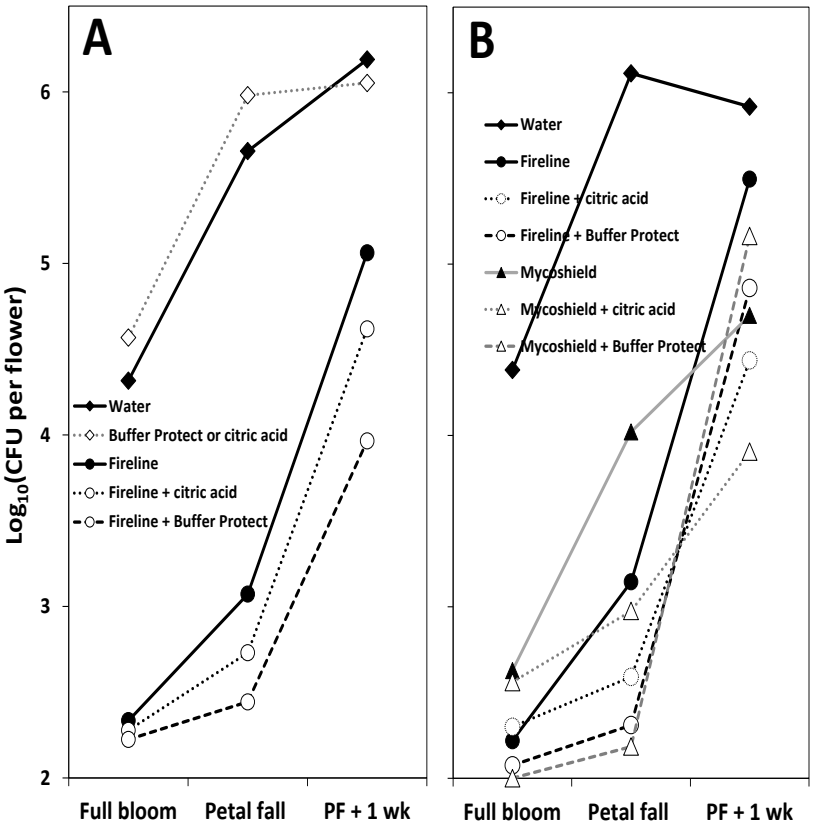

Fig. 2. Effect of acidified oxytetracycline materials on epiphytic populations of *Erwinia amylovora* on Bartlett pear and Gala apple flowers sampled from research orchards located near Corvallis, OR. A) Data depict across-trial means from up to eight pear and apple trials conducted from 2017 to 2020 (see Table 1). B) Data depict across trial means from three pear trials and one apple trial conducted from 2018 to 2020. Each treatment was applied to four replicate trees in each trial. 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. The Y-axis is on log scale for which a value of '2.0' is 100 pathogen CFU/flower (the detection limit) and a value of '6.0' is one million CFU per flower.

Pathogen population size data were further summarized with the statistic 'relative area under the population size curve' (A_{pop}), which represents the average population size ($\log_{10}$ (CFU/flower)) from full bloom to one week past petal fall weighted for length of time between each sampling date:

$$A_{pop} = \sum_{i=1}^{n-1} \{[(y_i + y_{i+1})/2] \bullet (t_{i+1} - t_i)\}/(t_n - t_1)$$

where y is $\log_{10}$ (CFU/flower) for a sample and t is days after inoculation for the ith sample date, and n is the total number of sample dates. Variation in A_{pop} was summarized utilizing individual trials as

replicates with means of common treatments across trials as the summary statistic. These means characterized the impact of treatments on the pathogen's epiphytic populations on flowers (Table 3). For example, citric acid alone had an overall A_{pop} of 5.71 $\log_{10}$(CFU/flower), which was slightly greater than the water-treated control. In contrast, streptomycin, which was applied to trees only at full bloom, had an A_{pop} of 2.67 $\log_{10}$(CFU/flower).

A_{pop}-values for OTC formulations or kasugamycin (Kasumin) by themselves were in the range of 3.13 to 3.89 $\log_{10}$(CFU/flower) (Table 3). The addition of an acidifying amendment to either OTC formulation or kasugamycin reduced A_{pop} compared to the antibiotic only. The smallest changes in A_{pop} attributable to an acidifier was observed when citric acid or LI-700 was added to FireLine (0.13 to 0.16 log units), and the largest reduction occurred when Buffer Protect was added to Mycoshield (0.70 log units).

Table 3. Values of relative area under the population size curve, A_{pop}, for epiphytic populations of *Erwinia amylovora* measured on pear and apple flowers at stages of full bloom, petal fall, and 1-wk post-petal fall as affected by antibiotic sprays and the materials used to acidify the sprays in orchard trials [t] conducted near Corvallis, Oregon from 2017 to 2020.

Treatment	Number of times treatment was represented over the eight orchard trials	Mean A_{pop} value [u]	Standard error of A_{pop}
Water	8	5.57	0.33
Citric acid [v]	6	5.62	0.18
FireWall	7	2.67	0.14
FireLine	8	3.26	0.18
" with Buffer Protect	6	2.77	0.21
" with citric acid [w]	10	3.10	0.15
" with LI 700	4	3.13	0.30
Mycoshield	4	3.89	0.53
" with acidifier [x]	4	3.09	0.30
Oxytetracycline [y]	12	3.47	0.22
" with acidifier	24	2.96	0.10
Kasumin	3	3.13	0.35
" with acidifier [z]	3	2.43	0.06

[t] In each trial, single-tree plots were arranged in a complete randomized block design with four replications per treatment. All materials applied twice except FireWall, which was applied once at full bloom. Dates of pathogen inoculation and treatment applications are provided in Table 1. Rates of materials are provided in Table 2.

[u] Mean and standard error of $A_{pop\ values}$ obtained by averaging the number of times a treatment was represented over the eight orchard trials.

[v] Includes treatments of citric acid only (2019 and 2020) and those where the antibiotic was amended with citric acid plus Na_2HSO_4 at a ratio of 2:1 (2018).

[w] Includes FireLine treatments amended with citric acid only (2019 and 2020) and those where the antibiotic was amended with citric acid plus Na_2HSO_4 at a ratio of 2:1 (2018).

[x] Includes Mycoshield treatments amended with Buffer Protect (2018 and 2019) or citric acid (2020).

[y] Includes all FireLine and Mycoshield treatments; following line includes any OTC-treatment amended with an acidifier.

[z] Includes Kasumin treatments amended with Buffer Protect (2017) or citric acid (2020).

pH of sprayed materials and floral pH as a result of treatment. Well water (pH 6.3) amended with citric acid (16 oz./100 gal) had a pH of 3.0 (Table 4). The addition of di-sodium phosphate or di-potassium phosphate (8 to 16 oz./100 gal) to citric acid (12 to 32 oz./100 gal) raised the pH to a range of 3.3 to 4.6. The commercial formulations of OTC in well water had pH-values closer to neutral (pH 6.1 to 6.6). OTC formulations with citric acid (± phosphate) had pH-values between 3.0 and 4.0. Also in well water, the pH of commercial acidifying surfactants, LI 700 (5 ml/liter) and TRI-FOL (5 ml/liter), measured 3.6 and 2.6, respectively.

Among orchard trials, the pH of the floral wash for the well water-treated control and antibiotic only treatments averaged between 5.9 and 6.0 (± (s.e.) 0.01) and declined slightly through the bloom period to 5.7 (± 0.12) (Fig. 3). In contrast, near full bloom, the pH of flower clusters treated with a citric acid or the citric acid-based Buffer Protect, or with an OTC-formulation mixed with citric acid had pH-values that averaged between 5.7 and 5.8. At petal fall, floral pH for treatments that included citric acid declined to a range of 5.2 to 5.5, but then increased to 5.6 (± 0.04) at 7 days after petal fall. Within individual orchard trials, variation in pH measurements were influenced partly by tree species with apple flowers becoming more acidic over time (e.g. water-treated apple flowers decreased to 5.5 to 5.6 at one week post-petal fall whereas water-treated pear flowers averaged 5.9). In addition, pH measurement for trials with the less rain during bloom were 0.1 to 0.3 units lower than those with more rain (data not shown).

Table 4. pH of well water, commercial oxytetracycline formulations and materials used to acidify antibiotic sprays for fire blight control.

Treatment	Acidifying amendment	Rate oz./100 gal.	pH [v]
Well water		-	6.7 ± 0.1
+	Buffer Protect	75	3.7 ± 0.0
+	citric acid	16	2.9 ± 0.1
+	citric acid plus $Na2HpO_4$	32, 16	3.4 ± 0.1[w]
+	citric acid plus $Na2HpO_4$	16, 8	3.5 ± 0.1
+	citric acid plus $Na2HpO_4$	12, 1	4.6 ± 0.0
+	citric acid plus K_2HPO_4	16, 8	4.1 ± 0.3[w]
+	LI-700		3.6 ± 0.1[w]
+	TRI-FOL		2.6 ± 0.0[w]
+ FireLine [y]		16	6.6 ± 0.1
+	Buffer Protect	75	3.6 ± 0.2
+	citric acid	15	3.2 ± 0.1
+	LI-700		3.5 [x]
+ Mycoshield [z]		16	6.1 ± 0.4
+	Buffer Protect	75	3.9 ± 0.2
+	citric acid	16	3.5 ± 0.2
+ Kasumin		64 fl. oz.	6.5 [x]
+	citric acid	16	2.8 [x]

[v] Means of measurement taken in springs of 2018 to 2020.
[w] Not measured in 2018.
[x] Measured in 2020 only
[y] '-hydrochloride' formulation
[z] '-calcium complex' formulation

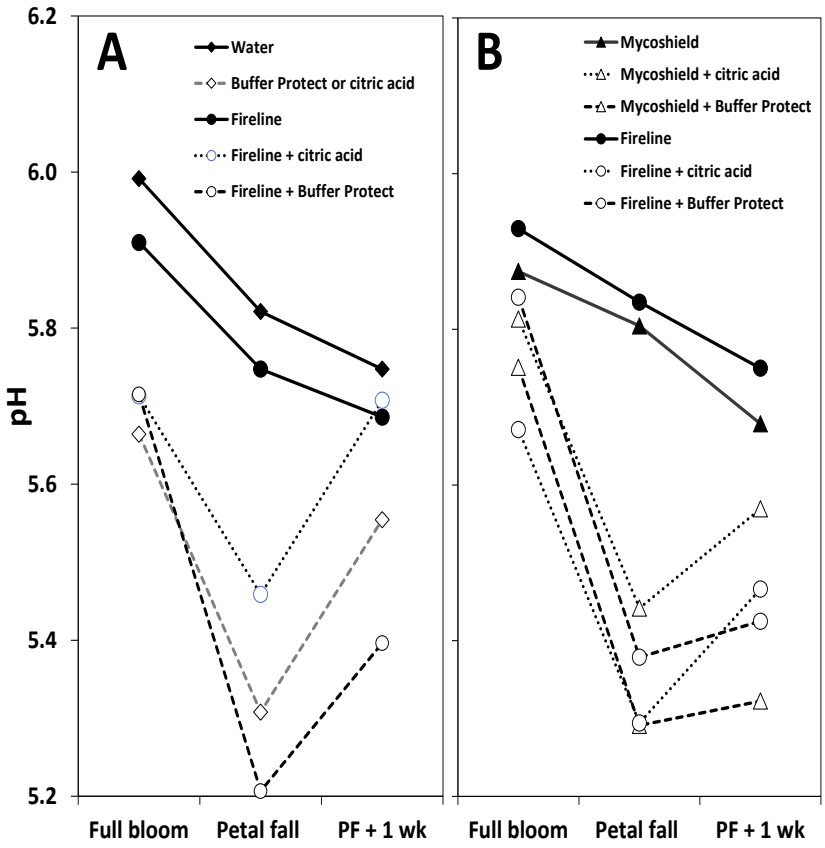

Fig. 13. Measured pH of washed Bartlett pear and Gala apple flowers after treatment with acidified oxytetrcycline in research orchards located near Corvallis, OR. Data depict across-trial means from up to six pear and apple trials conducted from 2018 to 2020 (see Table 1). At the bloom stages indicated, a pH-probe was placed in a deionized-water wash of five flower clusters (~25 flowers, bulked in 25 ml of water) from each replicate tree in each trial. A), data are from three pear trial and three apple trials conducted from 2018 to 2020. B), data are from three pear trials and one apple trial conducted from 2018 to 2020. Because panel B is mostly pear trials, the pH values for FireLine treatments are slightly higher than for comparative treatments in panel A where pear and apple trials are equally represented.

Treatment effects on fruit russeting. For both Bartlett and Comice pear, application of citric acid-based acidifying treatments at full bloom and petal fall resulted in negligible to slight effects on percent russeting of fruit surfaces (Fig. 2). For Bartlett pear, the mean difference between the most russeted treatments and the least russeted treatments was < 0.7%; for Comice pear, this difference was < 1%. Statistically, significant effects of treatment on percent fruit russeting resulted only for Bartlett pear in 2018 (Fig. 2A). Treatments with significantly ($P \leq 0.05$) greater russeting than the water-treated control included 'Buffer Protect', 'citric acid (32 oz./100 gal) plus disodium phosphate (16 oz./100 gal)', and 'citric acid (32 oz./100 gal) plus disodium phosphate (8 oz./100 gal)'. The same trends of treatment effects observed in Bartlett pear 2018 were also observed in Comice pear 2018 and Bartlett pear 2019 with the highest rates of citric acid combined with di-sodium phosphate (2018) or with di-potassium phosphate (2019) showing slightly more fruit russeting (up to 0.9%) than the water-treated control. In contrast, 'citric acid only', which had a relatively low pH among the sprayed treatments (Table 2), had the lowest percent fruit russeting in three of the four trials ((Fig. 2B-D); for the fourth trial (Bartlett pear 2018), citric acid only was the treatment most similar to the water-treated control (Fig. 2A).

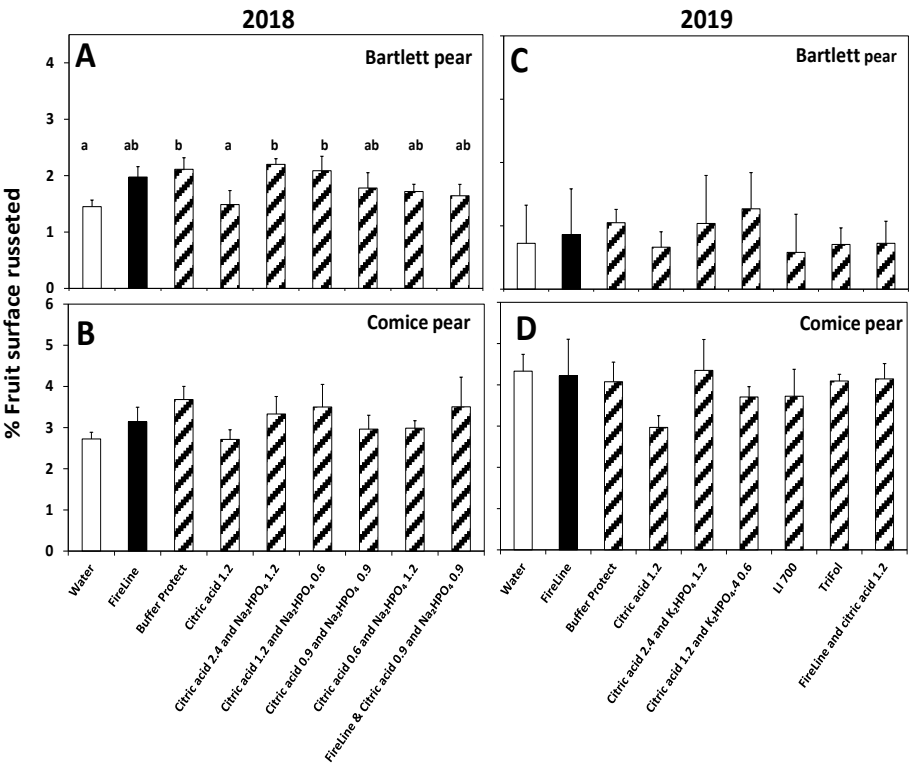

Fig. 4. Effect of citric acid-based buffers and oxytetracycline applied to A, C) Bartlett and B, D) Comice pear trees on severity of fruit russeting (%) in research orchards located near Medford, OR. Treatments were applied at full bloom and at petal fall (April 2018 and 2019). In late August, 30 fruit from each replicate tree were rated for russeting severity. Data depict mean and standard error from four replicate trees that received each treatment. X-axis: numbers in treatment labels indicate the rate of citric acid or phosphate buffer in grams per liter (Conversions: 1.2 g/l = 16 oz per 100 gal., 0.6 g/l = 8 oz per 100 gal., and 0.9 g/l = 12 oz per 100 gal.). Rates of other materials are shown in Table 2. In panel A, bars labeled with same letter are not significantly different according to Fischer's protected LSD at $P = 0.05$; in the other panels, differences among treatments did not differ significantly ($P > 0.05$).

Discussion

Antibiotics are regarded highly in conventional fire blight control because of their ability to suppress the pathogen's rate of growth on flowers and to protect flowers from infection. They also provide a longer-term benefit of reducing the amount of epiphytic inoculum available for secondary infection phases of the disease (e.g., late or secondary flowers and new vegetative shoots). In achieving these goals, acidifying oxytetracycline (and kasugamycin) enhanced infection suppression, although the observed enhancement was incremental to the degree of control achieved by the antibiotic alone. Nonetheless, we view the incremental improvement in suppression achieved with acidifiers as valuable to fire blight control as it is inexpensive and easy to implement. Moreover, because secondary phases of fire blight can be both very damaging to trees and time-consuming to clean-up, excellent infection suppression during primary bloom is considered by orchardists to be vastly superior to only good/very good infection suppression. At a minimum, the data generated by this research should result in closer monitoring of pH of antibiotics in spray tanks, and consequently, potentially improve the quality of sprays used for fire blight management.

As to why more acidic conditions enhances the efficacy of oxytetracycline on pome flowers, we hypothesized that the stability of OTC in a spray tank might be improved or the effective residual of OTC on floral surfaces could be prolonged. Although we did not measure OTC residuals directly, pathogen populations on flowers treated with acidified OTC increased more slowly than on flowers treated with OTC only (Fig. 3), which we believe reflects a prolonged half-life as a result of the more acidic conditions. In general, pear and apple flowers are not susceptible to infection after petal fall (Thomson, 2000). Therefore, on acidified OTC-treated flowers, the smaller pathogen population sizes

observed as the primary bloom period approached petal fall is likely where the benefits of acidification occur; this also was the bloom stage where we measured the lowest floral pH. The rapid increase in pathogen populations after petal fall represents inoculum that has mostly missed the window of susceptibility offered by individual flowers. Nonetheless, this inoculum may be available for later phases of infection. In this regard, we were disappointed that acidifying OTC did not extend a suppressive residual farther into the post-petal fall period.

Various researchers (McManus and Stockwell, McManus and Jones (1994), Stockwell et al. (1996a), and Stockwell et al. (2008)) have characterized oxytetracycline for fire blight suppression as being "bacteriostatic", meaning that the antibiotic slows the rate of pathogen reproduction but does not kill existing cells. To a degree, our data refutes this characterization because shortly after inoculation pathogen populations on OTC-treated flowers were so much smaller than on water-treated flowers. Our view is that OTC is best understood in terms of its effective residual (half-life), which in addition to pH sensitivity is a concept that becomes a more focused rationale for additional investigations (e.g., Christiano et al., 2010). An increased half-life as a result of a pH-adjustment appeared to be independent of OTC formulation, although, in more limited trialing, the calcium-complex formulation (Mycoshield) benefited more from a lower pH than the hydrochloride formulation (FireLine). In contrast, our hypothesis that acidification of spray tank-water (without OTC) could affect *E. amylovora* directly was not well supported by the data. This was shown by poor infection suppression with either citric acid or Buffer Protect by themselves. Because *E. amylovora* cannot grow at pH < 5, a possible explanation for ineffectiveness of citric acid by itself is that on a micro-scale, the floral surfaces on which a lower pH was achieved differs from surfaces where *E. amylovora* populations increase epiphytically (Wilson et al., 1999).

With regard to fruit russeting, moderately-sensitive Bartlett pear and highly-sensitive Comice pear provided an indication of the relative safety of a pH-lowering adjustment to the spray suspension. Surprisingly, for russeting-sensitive Comice pear, no significant effects of the treatments were observed, but in 2018 a few treatments slightly increased fruit russeting on Bartlett pear. Across all the russeting trials, compared to the low pH treatment of citric acid by itself, those with higher amounts of buffering salts in the spray suspension also showed slightly higher amounts of fruit russeting. This result was unexpected as initially we hypothesized that low pH would be a greater risk to fruit finish than the material load in the spray suspension. Consequently, the fire blight trials in 2018 utilized citric acid with Na_2HPO_4 (which is also in Buffer Protect) but then shifted to citric acid alone in 2019 and 2020. Also, in using buffering salts, a problem encountered at the time of treatment applications was that Na_2HPO_4 dissolved very slowly in the cold, well water used for spraying. Consequently, in 2019, fruit russeting treatments utilized K_2HPO_4, which buffers similar to Na_2HPO_4 but dissolves more readily in cold water; however, after the second season of fruit russeting trials, we concluded that a buffering material was unnecessary.

Compared to citric acid, the commercial acidifiers, LI 700 and TRE-FOL, also reduced pH and in limited trialing, also appeared improve fire blight control when used to acidify OTC. Some commercial pome fruit growers who have followed this research have been experimenting with phosphorus acid (and other) amendments to adjust the pH of OTC in spray tanks but we have not collected any data on potential benefits of these material(s).

Acknowledgements
Support was provided by the Northwest Fresh Pear Committee and the Washington Tree Fruit Research Commission. We thank Agrosource Inc., NuFarm Americas Inc. and UPL-NA Inc. for donating antibiotic materials used in this study.

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Executive summary

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

Keywords: fire blight control, *Erwinia amylovora*, antibiotics, oxytetracycline

Abstract

The half-life of the fire blight control material, oxytetracycline, is strongly affected by pH, increasing with increasing acidity. From 2017 to 2020, pear and apple orchard trials were conducted to evaluate if citric acid-based amendments to oxytetracycline sprays improve fire blight control. Over four seasons, acidified oxytetracycline resulted in better infection suppression than oxytetracycline by itself for 26 of 30 within-trial comparisons. Acidified oxytetracycline also suppressed epiphytic populations of *E. amylovora* on flowers to a greater degree than the antibiotic only. As sprays, commercial oxytetracycline formulations at label rate and amended with citric acid (16 oz./100 gal) in well water had pH-values near 4.0, and after spraying, the pH of flowers washed in distilled water (1 ml/flower) was reduced to a range of 5.2 to 5.5 compared to a pH near 6.0 after a treatment of oxytetracycline only. In fruit finish trials in pear orchards, sprays acidified with citric-acid based materials had negligible to slight effects on the finish quality (percent russeting) of fruit surfaces. Overall, compared to the water-treated control, infection suppression after two bloom applications of an acidified commercial oxytetracycline formulation averaged 85.9% $\pm$ 0.4 compared to 72.2% $\pm$ 1.7 without an acidifier.

FINAL PROJECT REPORT

Project Title: IPM Strategic Planning for Pears in Washington and Oregon

PI: Katie Murray*
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City/State/Zip: Central Point, OR 97502

Total Project Funding: $16,816

Other funding sources

Agency Name: United States Department of Agriculture Extension Implementation Program
Amt. requested/awarded: ~ $28,000 supports this work
Notes: The total cost for this project is estimated to be approximately $44,445, including salary/benefits support for three OSU faculty for a total of 12.5 weeks of work ($35,030), travel support for staff and participants to two events ($6,715) and event costs for two events ($2,700). Approximately 60% of the funds needed to support this work came through a USDA/EIP grant, which includes some salary support as well as funding for workshop costs and travel.

Budget History:

Item	Year 1: 2019-2020	2021	
WTFRC expenses			
Salaries	$10,710		
Benefits	$6,106		
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	16,816	0	

Goals and Outcomes:

**Due to the PI no longer with the IPPC, questions or comments regarding the report should be directed to Chris Hedstrom (chris.hedstrom@oregonstate.edu), IPM Outreach and Communications Coordinator, Oregon IPM Center (IPPC).*

Objectives:

We requested co-funding from the WA Tree Fruit Research Commission to support an IPM Strategic Plan update with the Pacific Northwest pear industry. The project evaluated progress since our 2014 Pest Management Strategic Plan for Pears, incorporating our new IPM-focused format. The overall goal was to help the Pacific Northwest pear industry describe current major pests and impacts, assess progress on critical research, education, and regulatory needs over the last 5 years, and identify continuing and emerging short- and long-term pest management challenges and goals to be shared with local research and extension faculty.

Results:

Our process engaged approximately 30 farmers, consultants, commission members, researchers, and extension agents representing four main regions across Oregon and Washington (Southern Oregon, Mid-Columbia, Wenatchee, and Yakima). This group became our formal workgroup, which was engaged via email and in person in a months-long consultation. Our in-person meeting took place in March of 2020 in Hood River, Oregon, with 21 attendees. The workgroup we brought together reached consensus about the major pest management activities, challenges, and priorities. Information gathered at this meeting was compiled into a final publication that outlines current pest management activities for the whole production season, as well as critical pest management needs for research, regulation, and education, and IPM advancement.

Discussion:

There are a number of benefits to the industry with this work, including:

- Provides a critical update to the USDA and EPA on current production and pest management issues facing the OR/WA pear industry, information critical to EPA as they review pesticides for registration or re-registration. Federal agencies rely on up-to-date information to avoid having to make assumptions and use worst-case scenarios when performing pesticide registration reviews. The update will be immediately accessible to our federal agencies through the National IPM Database.
- Brings updated information about current practices and critical pest management needs into a formal conduit for communication from growers and other pest managers to regulators, policymakers, researchers, extension professionals.
- Assists researchers in obtaining federal grant funds to work on pest management issues formally documented as important to the pear industry by providing granting organizations evidence of stakeholder input on pest management priorities.
- Provides updated stakeholder-identified priorities to the industry itself, to help focus where limited time and resources are best spent to solve the most critical pest management challenges.

The completed document is a roadmap that can be used by the industry to address critical pest management issues, and to prioritize industry time and resources. The final version will be posted on

the USDA's IPM Centers' "National IPM Database," and is also published as an OSU Extension document to increase its accessibility.

The Oregon IPM Center can provide support to the development of education events by extension agents that act upon the critical IPM needs identified from stakeholders through IPM strategic planning process. We can conduct pesticide risk assessments, and provide information about application management, pollinator and natural enemy protection and agro-ecology to support local extension IPM programing. Risk assessment data, along with the critical needs and priorities, are shared with Extension agents and researchers and used to collaboratively develop research and educational programming. Critical pest management needs identified in the document will be shared with OSU departmental researchers (crop and soil science, botany and plant pathology, horticulture, etc) and posted to our Critical Needs Database for easy searchability. We can also conduct follow-up as desired with industry members on how best to utilize the project's outputs.

At this time of this report, the finalized document was in press with Oregon State University Extension. Before that document is finalized and published, a draft of the document can be accessed here: https://beav.es/JfF

Citation:

Murray, K., Hilton, R., Jepson, P., Hedstrom, C. (2020). *Integrated Pest Management Strategic Plan for Pears in Oregon and Washington*. Oregon State University Extension Publication EM 9310 (in press). https://catalog.extension.oregonstate.edu/em9310

EXECUTIVE SUMMARY

Project title: IPM Strategic Planning for Pears in Washington and Oregon

Key words: IPM, strategic planning, integrated pest management

Abstract:

We requested co-funding from the WA Tree Fruit Research Commission to support an IPM Strategic Plan update with the Pacific Northwest pear industry (Oregon and Washington). The project evaluated progress since our 2014 Pest Management Strategic Plan for Pears, incorporating our new IPM-focused format. The overall goal was to help the Pacific Northwest pear industry describe current major pests and impacts, assess progress on critical research, education, and regulatory needs over the last 5 years, and identify continuing and emerging short- and long-term pest management challenges and goals to be shared with local research and extension faculty.

Our process engaged approximately 30 farmers, consultants, commission members, researchers, and extension agents representing four main regions across Oregon and Washington (Southern Oregon, Mid-Columbia, Wenatchee, and Yakima). This group became our formal workgroup, which was engaged via email and in person in a months-long consultation. Our in-person meeting took place in March of 2020 in Hood River, Oregon, with 21 attendees. The workgroup we brought together reached consensus about the major pest management activities, challenges, and priorities. Information gathered at this meeting was compiled into a final publication that outlines current pest management activities for the whole production season, as well as critical pest management needs for research, regulation, and education, and IPM advancement.

Citation:

Murray, K., Hilton, R., Jepson, P., Hedstrom, C. (2020). *Integrated Pest Management Strategic Plan for Pears in Oregon and Washington*. Oregon State University Extension Publication EM 9310(in process). https://catalog.extension.oregonstate.edu/em9310

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

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

PI: **Achala KC**
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Cooperators: Dr. Peever, WSU-WA; Dr. Ashley Thompson, OSU-OR; 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: $110,834**

Other funding sources
KC lab:
Agency Name: Chemical company contracts. **Amt. awarded:** $36,000
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 (1040 hrs @ $13.00)	6,240	10,400	13,520
Benefits[1]			
Post-Doctoral research associate	15,775	16,248	16,735
Undergraduate labor	749	1,248	1,623
Equipment	0	0	
Supplies[2]	1,500	1,545	1,591
Travel[3]	500	1,000	500
Hood River Plot Fees[4]		3,000	
Total	49,764	59,191	**60,492**

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 1040 hrs and 12% benefit rate. The hours request for undergraduate labor is increased for year 3 based on the requirement from 2018 and 2019 samples collection and processing time.
[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.
[4] Plot fees for trials in Hood River @ $3,000 per acre. Trials in Hood River was not possible in 2020 due to COVID-19 related restrictions. The budget request was re-distributed to cover the extra undergraduate labor expense incurred during 2020 sample collections and processing.

Budget 2: Amiri
Organization Name: WSU **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu / shelli.tompkins@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
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
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 gray 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:

- *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. Variabilities in inoculum size and dynamics throughout the season have been observed among orchards located in different districts.
- In trials conducted in Southern Oregon, *Botrytis* was detected in all orchard stages included in this study and largely present in samples at full bloom to petal fall stages in both years.
- In all locations, the size of *Botrytis* inoculum was greater in organic orchards compared to conventional orchards.
- In SO orchards, the inoculum size decreased as we moved towards commercial harvest in both organic and conventional orchards.
- In WA orchards, trials to detect *Botrytis* from bloom to late in storage were reconducted in 2020, sample analyses have been delayed by COVID-19 restrictions for lab access. Data will be available in spring 2021.
- In SO trials, fungicides showed a range of effectiveness against 20 *Botrytis* isolates indicating variability in sensitivity when exposed to preharvest fungicides with different modes of action. When tested on wound inoculated fruit assays, the efficacy of Ziram, and PhD were higher than 50% for all isolates tested in this study. Whereas 25% of the isolates showed reduced sensitivity to Manzate, and Botran. Similarly, when three postharvest fungicides (ADA 72902, BioSpectra, and Scholar) were tested for their efficacy on wound inoculated fruits, their efficacy were higher than 60% for all isolates tested in this study. However, on spray inoculated fruits, no significant differences among the treatments were observed.
- About 600 and 110 Botrytis isolates were collected from WA and OR, respectively in 2019 and 2020 and they are being processed for DNA extraction and species identification.
- Four seasonal timely field spray programs have been tested again in 2020 to improve gray mold management. Fruit are in cold storage and data will be available in spring 2021.

Methods

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

Experimental Sites: The research trials planned in this objective were conducted at three districts in the PNW. Trials in Cashmere, WA and Hood River, OR were led by Amiri including one conventional and one organic orchard (d'Anjou). Trials in Medford, OR were led by KC including one conventional and one organic orchard (Comice).

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 were collected from two orchards at each district in the spring of 2019 and 2020. Afterward, 60 fruit were collected from the same trees and orchards used for flowers sampling at fruit set, mid-summer, and at commercial maturity. Blossom and fruit samples were transported in separate clean bags to the Pathology Labs at TFREC or SOREC. Thirty samples were used for molecular quantification of *Botrytis* infections and the 30 remaining samples were used for isolation of *Botrytis* on a semi-selective medium. Flowers were freeze-dried and stored at -80°C. Fruits were peeled and the peel and the flesh of the fruit were freeze-dried separately and stored at -80°C. DNA were extracted from freeze-dried samples and the presence of *Botrytis* were detected using a quantitative polymerase chain reaction (qPCR) assay (Diguta et al. 2010). Spores of *Botrytis* were 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 were quantified and compared to weather data and fungicide 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 were 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 were collected from the Washington State University-AgWeaterNet (http://www.weather.wsu.edu/) in way to obtain data at all sampled orchards from the closest (≤ 1 mile) weather station. In Medford, the weather data were collected from Bear Creek local weather station from where the samples were 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 block at OSU-SOREC. Dr. Amiri will be conducting the trials at a commercial d' Anjou orchard in Cashmere, WA.

Trials at OSU-SOREC (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 gray 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. Trials were reconducted in 2021 using the same orchards. Samples are being processed for detection and results are expected to be available in spring 2021.

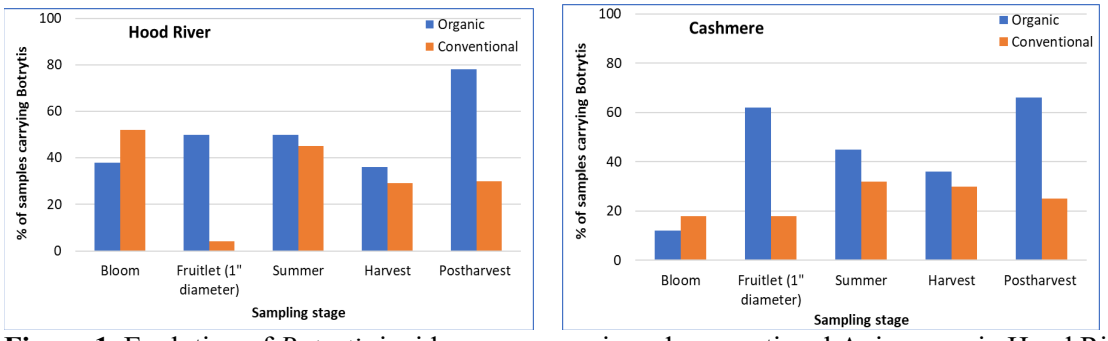

Figure 1. Evolution of *Botrytis* incidence on organic and conventional Anjou pear in Hood River and Cashmere throughout the 2019-20 preharvest growing season and after 6 months of CA storage as detected by qPCR.

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.

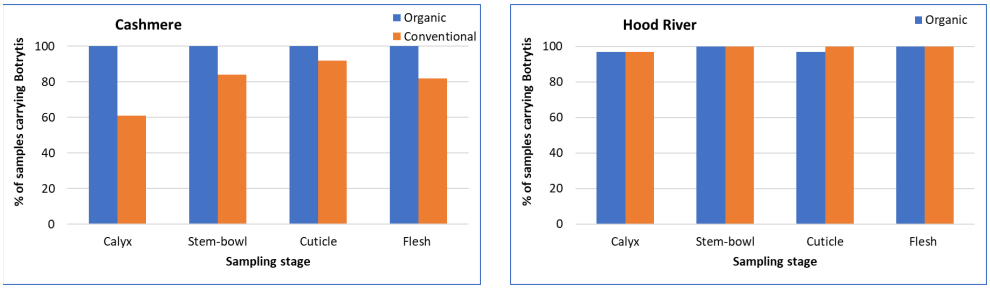

Figure 2. Incidence of *Botrytis cinerea* on different organs of the fruit at commercial maturity (harvest time) Anjou pear in organic and conventional orchards in 2019.

Trials at SO (Year 2)

Comice pears were collected in a commercial orchard in Southern Oregon starting in early April to late August of 2020 from conventional and organic blocks in 5 stages. Pears were then plated onto BSM for *Botrytis* detection and isolation. Out of the collected pears that were grown conventionally, *Botrytis* was isolated from 27, 46, 4, 21, and 2% from full bloom, petal fall/fruit set, fruitlet, mid-summer, and commercial maturity respectively. Out of the collected pears that were grown organically, *Botrytis* was isolated from 29, 46, 0, 15, and 4% from full bloom, petal fall/fruit set, fruitlet, mid-summer, and commercial maturity respectively (Figure 3).

Interestingly *Botrytis* isolation percentage was quite high at full bloom, and petal fall/fruit set stages; however, it decreased overtime in both orchards. When analyzing weather data, 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 is expected 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 *Botrytis* performing better at more moderate temperatures ranging from 65 ºF to 78 ºF. Therefore, a maximum average temperature of 91.25ºF in July and 91.65ºF in August would have been too hot and not optimal for fungal growth.

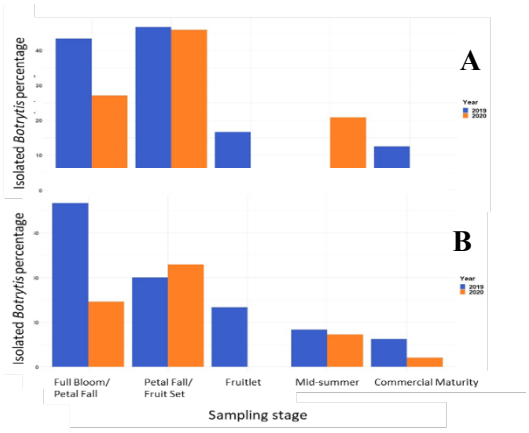

Figure 3: Percentage of *Botrytis* isolated from pears collected in Medford organic (A) and conventional (B) orchards at different stages during their development in 2019 and 2020.

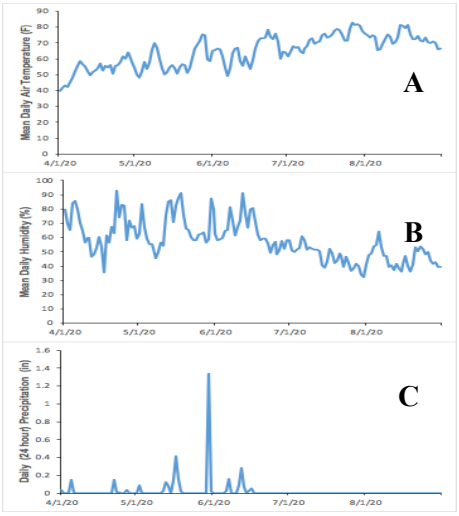

Figure 4: (A) Mean daily air temperature (ºF); (B) mean daily humidity (%); and (C) total daily precipitation (in.) from April to August of 2020 in Medford, OR

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

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

Preharvest fungicides, Manzate Pro-Stick, Ziram 76DF, Ph-D, and Botran 5F were tested for their effectiveness against 20 *Botrytis* isolates. When tested on wound inoculated fruit assays, the fungicides showed a range of effectiveness against 20 *Botrytis* isolates indicating variability in sensitivity when exposed to preharvest fungicides with different modes of action. The ranges in fungicide efficacies were 32.31% to 99.22%, 21.15% to 89.53%, 61.39% to 96.15%, and 76.35% to 100% for Manzate, Botran, Ziram, and Ph-D respectively (Figure 5). The efficacy of Ziram, and PhD were higher than 50% for all isolates tested in this study. Whereas 25% of the isolates showed reduced sensitivity to Manzate, and Botran. Based on these results, the discriminatory doses on seven fungicides (three from this study and four from previous study) have been identified. Nearly, 500 Botrytis isolates have been collected from orchards and packinghouses in southern Oregon. These Botrytis isolates will be screened for their sensitivity against these seven fungicides in 2021. Similarly, when three postharvest fungicides (ADA 72902, BioSpectra, and Scholar) were tested for their efficacy on wound inoculated fruits, their efficacy were higher than 60% for all isolates tested in this study. However, on spray inoculated fruits, no significant differences among the treatments were observed.

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

Based on these lab results and the epidemiology study (objective 1), another gray mold management program have been developed (Table 3). Both programs (Table 2 and Table 3) were applied in a research block in SOREC in 2020. Fruits from both trials were harvested on Sept. 15, 2020 and are in normal atmosphere cold storage. These fruits will be assessed for gray mold and overall rot development in Mid-March, 2021.

Table 3. Description of spray regimes tested in 2020 at SOREC, OR.

Treatment type	Spray timing within season	Number of sprays	Bloom	petal fall/fruit set	summer	7DPH	Postharvest
Control		0					
	Early	2		Ph-D			Penbotec
Conservative	Mid	2			Ph-D		Penbotec
	Late (current industry standard)	2				Ph-D	Penbotec
	Early-Early	3	Ziram	Ph-D			Penbotec
Moderate	Early-mid	3		Ziram	Ph-D		Penbotec
	Mid-Late	3			Ziram	Ph-D	Penbotec
	Early-Mid-Late-No postharvest	3		Inspire Super	Ziram	Ph-D	
Extensive	Early-Mid-Liate-Postharvest	4		Inspire Super	Ziram	Ph-D	Penbotec

Future work:

2021-2022:

Conduct the genetic analyses of *Botrytis* isolates

Obtain data from cold storage facilities on samples collected in 2020

Conduct a 2nd year of field trials in the same orchards used in Year 1 for fungicide programs and their efficacy on gray mold management

Outreach via online webinars

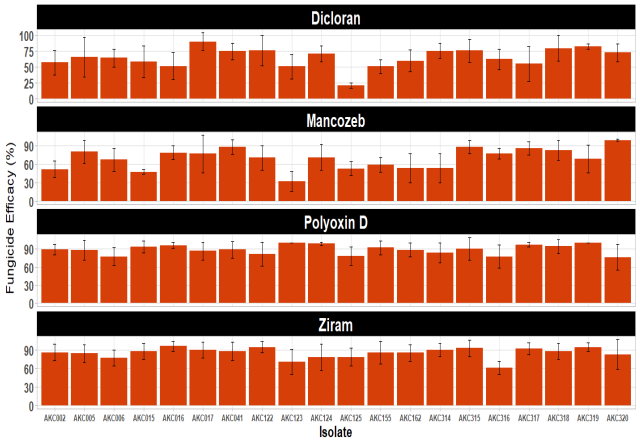

Figure 5: Fungicide efficacy values of 20 *Botrytis* isolates when inoculated on pears.

PROJECT PROPOSAL **PROPOSED DURATION:** 3 years

Project Title: New active ingredients for pear superficial scald control

PI: David Rudell
Organization: USDA-ARS, TFRL
Telephone: 509 664 2280 (ext. 245)
Email: David.Rudell@ars.usda.gov

Co-PI: Carolina Torres
Organization: WSU-TFREC (02/19)
Telephone: +56 9 6847 0541
Email: cartorres@utalca.cl

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

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).

WTFRC Budget:

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

Item	2020	2021	2022
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous*			
Miscellaneous			
Storage fees (Stemilt RCA room)		6695	6695
Total		6695	**6695**

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

Item	2020	2021	2022
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	2020	2021	2022
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	1000	1000	1000
Travel			
Miscellaneous*	11,500	4805	4805
Miscellaneous			
Plot Fees			
Total	12,500	5805	**5805**

Footnotes: One-third instrument service contract

JUSTIFICATION

Management for superficial scald control continues to be an industry challenge. Uncertainty of th continued availability of ethoxyquin, market access issues related to its use, and ripening issues with 1-MCP treated fruit all contribute to a need for alternative scald control methods. A new chemistry, developed by Dr. Carolina Torres, has been shown to control scald on 'Packham's Triumph'. The formulation contains purified squalane from vegetable oil, which is likely the active ingredient. Squalane is an oily triterpane related to many triterpene cellular membrane, wax, and, possibly, cuticular components. The mode of action is unknown although preliminary evidence indicates it does not act as an antioxidant like ethoxyquin or DPA. If so, this is a unique mode of action which, if determined, may yield new information that can be used to find additional active ingredients, risk assessment protocols, and cold chain management strategies to eliminate superficial scald. We propose to determine the efficacy and best use of the existing squalane-based formulation as well as analyze the mode of action. To accomplish this, we will treat multiple 'd'Anjou' lots from different growing regions and store them in air or CA storage evaluating appearance as well as fruit quality over commercially relevant storage durations. Multiple seasons and harvest maturities will be included in each location. We will exhaustively analyze peel chemistry at critical points in the cold chain to determine changes squalane addition may provoke focusing on cellular membrane, wax, and cutin chemistry to determine mode of action. We expect to deliver information describing best use of this formulation in relation to current CA storage and cold chain management practices, and an improved understanding of pear scald. **Knowledge gap** includes no assessment of superficial scald control efficacy of squalane on PNW pears, understanding of the mode of scald control action of squalane is lacking, and lack of understanding of impacts of squalane on appearance including defects caused by CO_2 sensitivity (See literature review). Our goal is to eliminate superficial scald from the pear supply chain.

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.

The project's specific goals (*Anticipated benefits*) is to replace ethoxyquin with a new plant-based active ingredient of known mechanism of scald control. **The goals and objectives address industry priorities** "Fruit Ripening: need for tools to control scald" and "maintain fruit visual quality on store shelf". Attainment of the goals will reduce superficial scald incidence and potentially control of postharvest physiological disorders while enhancing fruit quality throughout the cold chain.

Participant Roles and Project Locations:

Dr. David Rudell is a Research Plant Physiologist located at USDA-ARS, TFRL. He will direct the project, fruit storage study, assist in any new formulation development, and metabolic analyses to determine mechanisms by which tested products may control scald. A USDA-ARS biological sciences technician will be hired using grant funds to specifically perform project activities to meet project objectives. Dr. Rudell's program specializes in developing cold chain management tools and strategies using quality assessment and discovery of novel changes in natural chemistry indicative of cold chain and storage quality and outcome. Metabolic analysis equipment and storage facilities are located at USDA-ARS, TFRL. Drs. Rudell, Mattheis, and Torres have collaborated on regional and international projects.

Dr. Carolina Torres has been recently hired as the Endowed chair for Postharvest Systems at WSU-TFREC and will direct outreach, intellectual property related to her product, fruit treatment, and

formulation. She developed the main squalane-based product we are testing and is pursuing licensing in the US.

Dr. James Mattheis is the Research Leader at USDA-ARS, TFRL. He will be responsible for fruit acquisition, storage, and quality analysis. Dr. Mattheis's program specializes in determining storage solutions for pear industry postharvest issues.

METHODS (see timeline and deliverables in Table 1, 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

Superficial scald control using the existing formulation and other formulations containing squalane needs to be demonstrated on 'd'Anjou'. As the existing formulation is effective for controlling scald on 'Packham's Triumph', in Years 1-3 we will test the previously established rate on 'd'Anjou' pears from an orchard in each of the Hood River, Yakima, Wenatchee, and Okanogan regions. 300 pears will be harvested twice (early and late) from external canopies or randomly picked from bins immediately following harvest. Fruit will be transported to TFRL, initial fruit quality evaluated, and 100 drenched 4% squalane formulation, 100 drenched with 1000 ppm ethoxyquin, and 100 drenched with only the carriers and adjuvants but containing no squalane (control). Pears will be stored in 31°F air or CA (1% O_2, 1.5 % CO_2) for 3 and 6 months, respectively. Scald incidence and severity as well as phytotoxicity will be evaluated monthly in air stored fruit and monthly after removal following 6 months CA.

An additional experiment (Dose response) will be performed during Year 1 using fruit harvested from 3 orchards. 'd'Anjou' pears will be harvested from the external canopy at commercial harvest. Pears will be treated with 2, 3, and 4 % of the existing formulation or a control containing all inert ingredients. Pears will be stored in CA (1% O_2, 1.5% CO_2) for up to 8 months. Pear peel will be sampled from one of the orchards at 0, 3, 6, and 8 months. An additional 2 trays per treatment will be left for up to 2 weeks at 68 °F to evaluate scald incidence and severity. Fruit quality will be evaluated upon removal from CA and following the 2 week 68 °F ripening period.

Other formulations using different carriers and adjuvants will be made and tested to evaluate any changes in phytotoxicity and potentially provide other options if phytotoxicity is a problem.

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

Pear peel sampled from pears harvested from the orchard developing the most scald on control fruit during the "dose response" experiment outlined under objective 1 will be analyzed to determine the

mode(s) of action of this active ingredient. This will include samples taken from all 4 drench treatments at every sample pull out (0, 3, 6, and 8 months). We will also include tests of peel impacted by other formulations. Our method entails comprehensive analysis of natural peel chemicals that comprise the peel surface barrier and cellular natural chemicals that includes those associated with superficial scald (Serra et al., 2018). We will also include an analysis of cutin, the "plastic" surface coat of pears to indicate any changes that are related to scald and scald control.

We will determine if scald control using squalane, like ethoxyquin, is associated with control of oxidative stress or some other mechanism. Such an analysis will also provide new information about chemical changes leading specifically to pear superficial scald similar to our improved understanding of changes in peel chemistry leading up to apple superficial scald development (Gapper et al., 2017).

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

The impacts of squalane on other external and internal defects will be evaluated and cataloged on all of the pears tested in objective 1. Novel symptoms will be photographed, compiled alongside a description of storage conditions, and reported. In addition, we will perform a preliminary experiment in Year 2 evaluating the impacts of squalane compared with ethoxyquin and diphenylamine on pithy brown core and external CO_2 injury incidence under modern ultra-low O_2 CA conditions. For this test, we will purchase 800 fruit from the Hood River region and drench them with 1000 ppm ethoxyquin, 2000 ppm diphenylamine, 4% squalane formulation, or leave them untreated (200 fruit per treatment). 100 fruit from each treatment will be stored at 0.5% O_2: 5% CO_2 and 0.5% O_2: 0.2% CO_2 for 3 months. Fruit will be assessed for internal and external defects upon removal from storage. The experiment will be repeated in Year 3 if warranted.

Literature Review:

Diminishing market acceptance of ethoxyquin has made finding replacement superficial scald control tools for susceptible pear cultivars even more critical. Diphenylamine (DPA) is purportedly less effective than ethyoxyquin for superficial scald control on pears (Mellenthin et al., 1980) and is also subject to expanding market restrictions. While improved storage technologies can control scald when pears remain in storage (Chen et al, 1993), ULO (0.5–1% O_2) CA can result in black speck of peel (Chen and Varga, 1989) or blotch pit as 'd'Anjou' is especially sensitive to even low (0.5%) atmospheric CO_2 under these conditions (Mattheis et al., 2013).

Other active ingredients have shown promise for controlling apple superficial scald. While formulations containing stripped corn oil reduced scald on apples it was ineffective for controlling pear scald (Ju and Curry, 2000) and can leave an undesirable residue and are sometimes phytotoxic, causing surface lesions. Although preliminary evidence indicates squalene, may reduce scald on 'Granny Smith' (Curry, 2000), a full study supporting this claim was never reported.

However, squalane, a related compound found in olive oil, has been purified and formulated for application on apples and pears to control superficial scald (Torres, 2018). Drenches using rates of 0, 2, 3, and 4% squalane formulation indicate control of on 'Packham's Triumph' scald at all tested concentrations (Figure 1). While this formulation caused unacceptable levels of phytotoxicity on apple in some cases, less extensive injury occurred at the highest rate, similar to that provoked by DPA in the same test and appears to be related to the formulation rather than the active ingredient (Figure 2). This formulation contains ingredients acceptable for organic production in Chile but other formulations that may be less phytotoxic are possible.

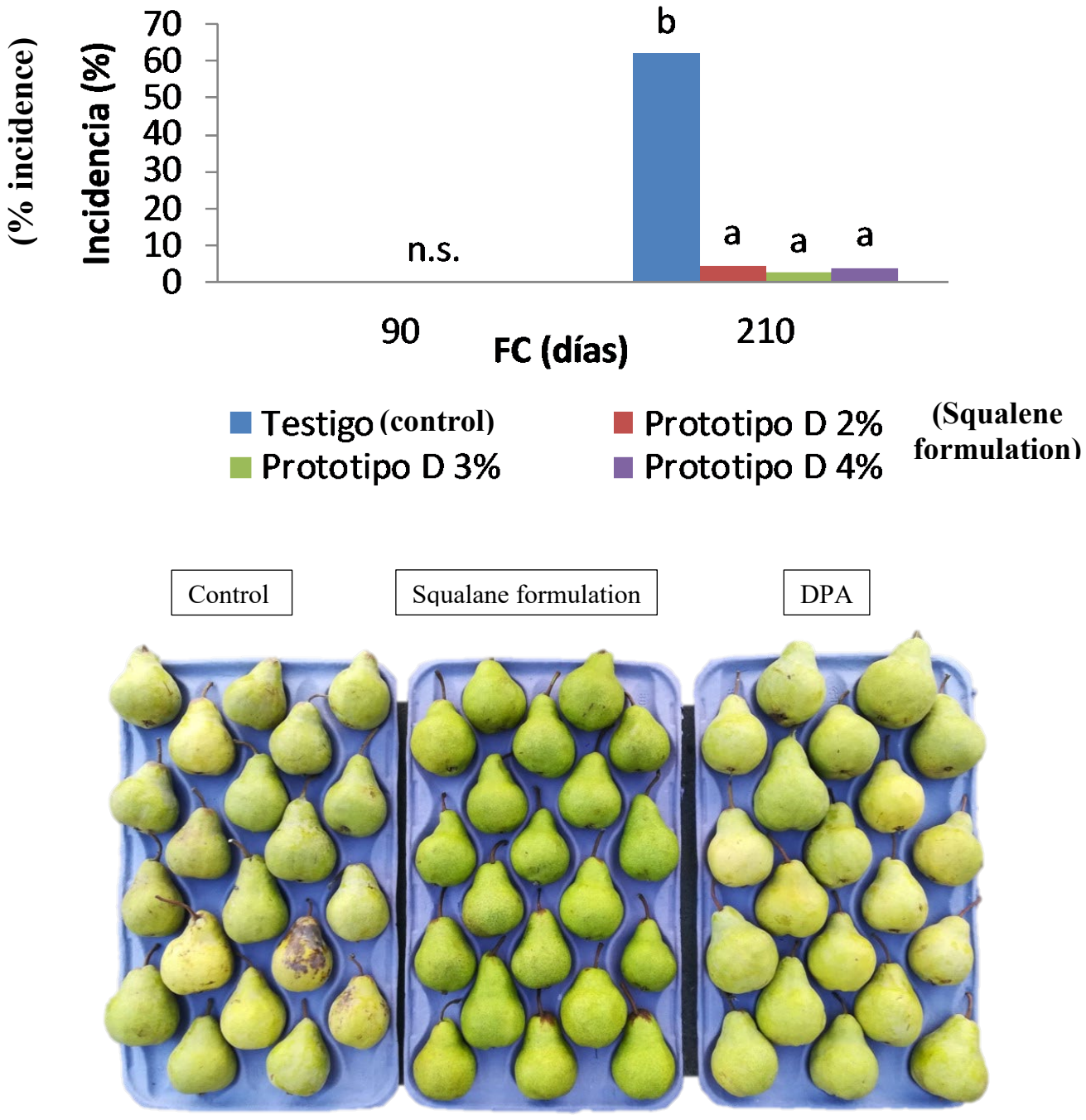

Figure 1. (top) Control of superficial scald on 'Packham's Triumph' pears following 210 d CA storage after drenching with 3 different concentrations of squalane formulation. (bottom) Control of scald after 210 d CA by squalane formulation compared with diphenylamine and no treatment.

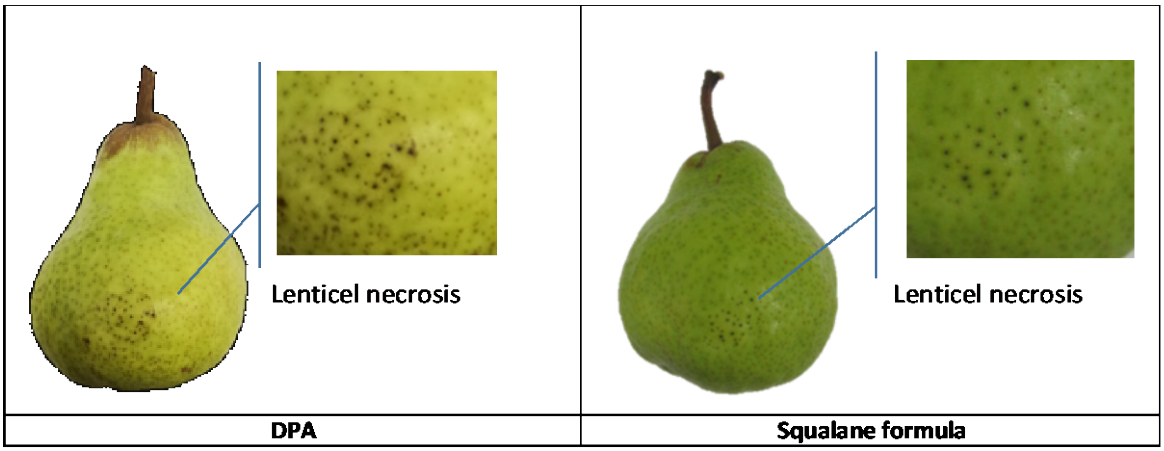

Figure 2. Slight lenticel darkening on 'Packham's Triumph' caused by at-harvest drenching with 2000 ppm DPA (right) and the highest tested level (4%) of the existing squalane formulation. Phytotoxicity caused by the squalane formulation was less than 5% incidence following 180 d CA and 7 d ripening at

The mechanism by with squalane controls scald is unknown. Antioxidants such as ethoxyquin and DPA appear to reduce oxidation of natural chemicals produced by the fruit, directly or indirectly stopping scald from developing (Lurie and Watkins, 2012). Prevention of one of these groups of natural chemicals from, conjugated trienols (CTOLs), has been the focus of the majority of scald research over the last 60 years, although recent work indicates many other oxidation of many other chemicals is also prevented by DPA (Rudell et al., 2009) and most likely ethoxyquin as well. Squalene and squalane either build or are directly related to many of these other chemicals that are "preserved" by DPA. It is unknown if oxidation of these chemicals causes scald or merely occurs at the same time as other changes provoked by the chilling temperatures during the first few months of cold storage leading to scald months later. New evidence also indicates that the metabolic machinery that keeps apples and pears alive during storage as well as the structure of the natural coating or cuticle is different in fruit that will not develop scald (Gapper et al., 2017). It is unknown if squalane reduces oxidation of some of these key metabolites or, as it is an oil soluble chemical and can "mix" with the waxy cuticle, actually can alter the fruit surface preventing scald. Understanding of this mechanism by which squalane may to lead to other products using this and other active ingredients.

Literature cited

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Serra, S., Sullivan, N., Mattheis, J.P., Musacchi, S., and Rudell, D.R. 2018. Canopy attachment position influence metabolism and peel constituency of European pear fruit. BMC Plant Biol. 18:364.

Torres del Campo, C.A. 2018. Composition for controlling superficial scald in pome fruit. International patent application WO/2018/161184.

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**: 2 of 3
WTFRC Project Number: PR-19-101

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

PI: Amit Dhingra
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Co-PI: Kate Evans
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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**

Other funding sources: **Awarded**
Amount: $73,459 (2017 – 2019)
Agency Name: Fresh & Processed Pear Committee Research
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.

Other funding sources: **Awarded**
Amount: $322,003 (2019 – 2022)
Agency Name: Fresh & Processed Pear Committee Research
Notes: "Pear Rootstock Breeding" (PI: Evans; Co-PI: Dhingra)
Synergistic project to develop and establish pear rootstock seedlings to develop dwarfing rootstocks that are suited for high-density pear production.

Budget
Organization Name: Washington State Univ **Contract Administrator: Katy Roberts**
Telephone: 509-335-4564 **Email address: arcgrants@wsu.edu**

Item	2020	2021	2022
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**

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

- Of the 65 accessions, 45 have 10 or more clones. Several of the 45 accessions have more than 10. A total of 500 clones are currently being maintained in the WSU Pullman Greenhouse.
- The broad genetic diversity represented by the accessions is evident in the rate of growth, caliper and architecture of the plants
- The clones have been potted up to 5-gallon buckets to enable further growth

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 20th, plants representing most of the accessions are being maintained 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. No new initiations were done in 2020. Due to COVID-related travel restrictions, budwood collection in April 2020 was not feasible. Previously, getting the budwood shipped to WSU Pullman did not result in successful initiation of the plant material in tissue culture. The plan is to visit USDA Corvallis in April 2021 to collect additional budwood.

A summary of status of each accession in terms of number of plants in the micropropagation process, soil or in the cold as of January 20th is presented in Table 1.

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

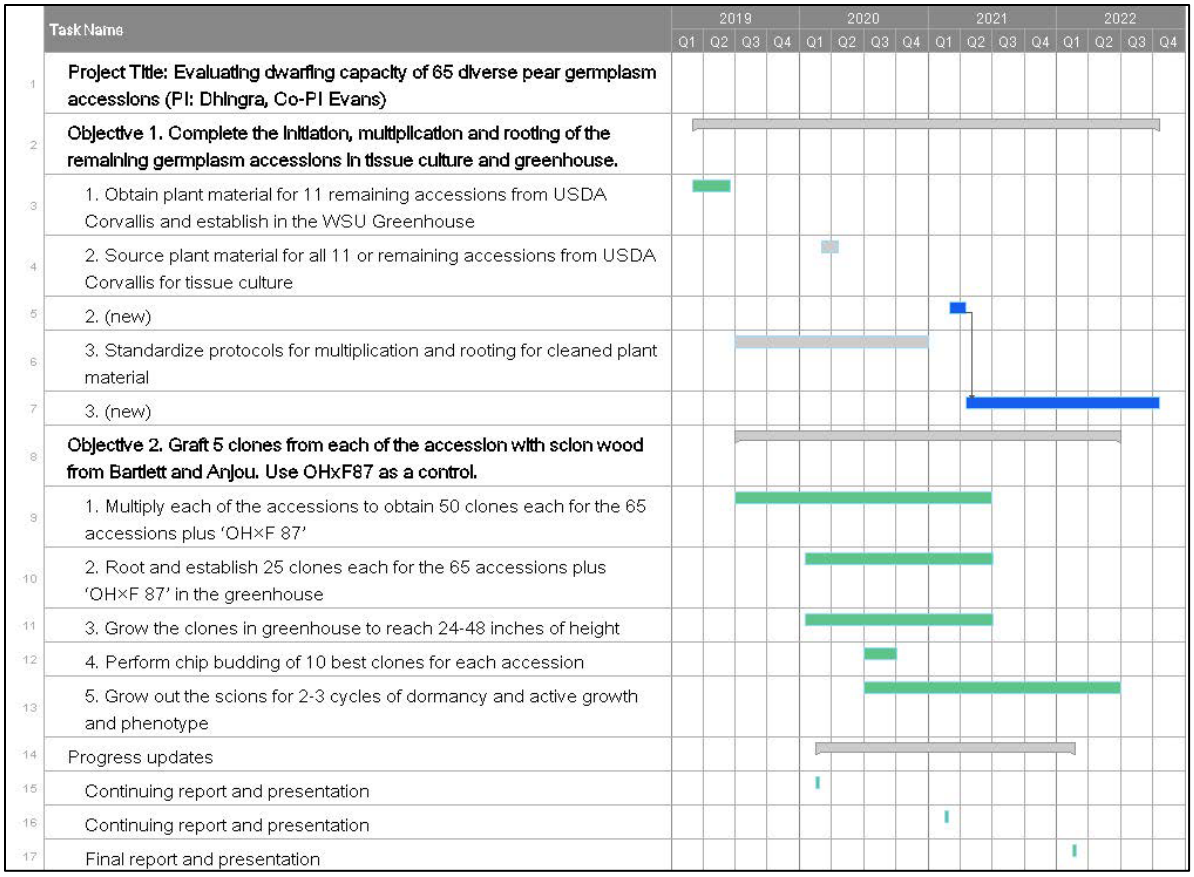
Sample#	Row	Position	# of shoots in TC at 40 deg F	# of shoots in TC at 75 deg F	Total number of shoots in TC	# of rooted saplings in greenhouse at 75 deg F	# of cuttings in greenhouse 75 deg F	# mature trees, dormant outside	# rooted young clones dormant at 42 deg F	Total number of rooted clones in soil
2	NF 23	15	5		5	20				20
3	NF 23	14	10		10	9				9
4	NF 24	11	5		5	12				12
5	NF 25	8	5	5	10	16			1	17
6	NF 28	9		5	5	15				15
7	NF 30	4	10		10					0
10	NF 33	4			0	13				13
11	NF 34	2		5	5					0
13	NF 52	1		5	5	16	7		1	17
14	1	17			0	10				10
15	1	21		5	5	30				30
16	2	3	15	5	20	7	9			7
17	2	23	10	5	15	14	4		4	18
18	2	27		5	5	13	5		2	15
19	3	15			0	8	6			8
22	4	21	10		10	10	2			10
23	4	45	10		10					0
25	5	11	5		5	19	10			19
27	6	45			0	11				11
28	8	23	10		10	4				4
29	8	25			0	8				8
30	10	13			0	12	6	2		14
31	12	25	10	5	15	1				1
33	14	3	5		5	2				2
34	14	43		5	5	2				2
35	15	19		5	5	15		1		16
36	16	29		5	5	11			1	12

37	**16**	**37**	**10**	**5**	**15**	**20**				**20**
39	**17**	**35**		**5**	**5**	**14**				**14**
41	**19**	**11**		**5**	**5**	**6**	**7**	**2**	**1**	**9**
42	**19**	**17**		**5**	**5**	**17**	**1**			**17**
43	**21**	**9**		**5**	**5**	**4**				**4**
45	**21**	**43**	**10**		**10**			**3**	**3**	**6**
46	**22**	**7**			**0**	**15**	**8**			**15**
48	**23**	**31**		**5**	**5**	**13**				**13**
49	**23**	**47**	**5**		**5**	**10**				**10**
50	**25**	**29**	**10**		**10**	**14**				**14**
51	**25**	**59**	**10**	**5**	**15**	**15**	**4**			**15**
52	**26**	**25**	**10**		**10**	**20**		**3**		**23**
53	**27**	**1**	**10**		**10**	**9**	**7**			**9**
57	**31**	**19**	**10**		**10**	**15**				**15**
58	**47**	**5**	**10**		**10**	**17**	**6**			**17**
60	**67**	**1**	**10**		**10**	**20**				**20**
63	**67**	**17**	**5**	**5**	**10**	**2**				**2**
64	**68**	**7**		**5**	**5**					**0**
65	**40**	**1**		**5**	**5**					**0**

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 from nearly 40 accessions previously established in the greenhouse were potted up into 1-gallon grow tubes to maintain vigorous growth to achieve the goals of this objective. The number of clones for each accession, their current stage and location is summarized in Table 1.

Table 2: Gantt chart representing the modified timeline (in blue) and activities underlying each objective.



As is evident from the Gantt Chart (Table 2), part of the objective 1 of the project continues to progress as proposed, while initiation of 11 accessions (depicted in blue) will be done in Spring 2021.

This project represents the diverse germplasm – (boxed and labeled as 1 in figure 1) a 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.

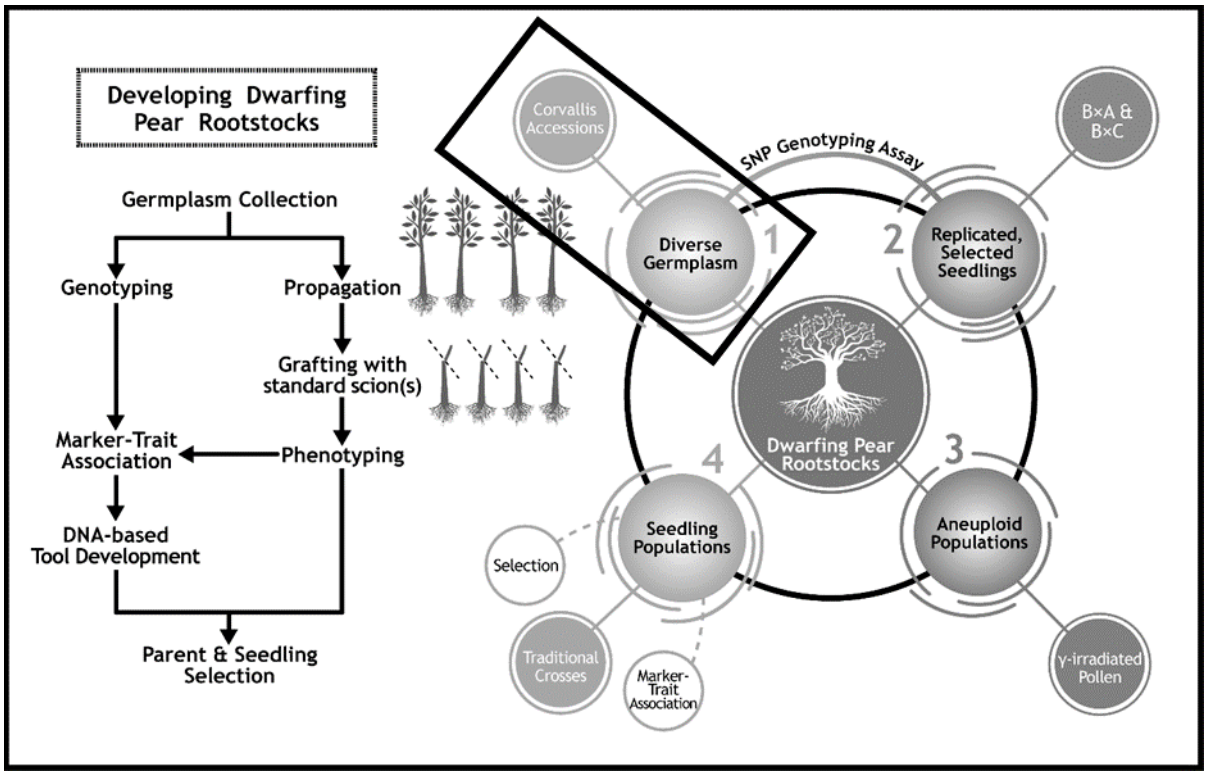


Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks.

Outreach Activities

- Amit Dhingra hosted farmers from Yakima in February 2020 and shared details about the pear rootstock breeding project
- Amit Dhingra visited Brandt nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in February 2020.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-19-108

Project Title: Pear Rootstock Breeding

PI: Kate Evans
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Cooperators: Joseph Postman (USDA-ARS Corvallis, OR), Nahla Bassil (USDA-ARS Corvallis, OR), Sara Montanari (Plant and Food Research, New Zealand), Stefano Musacchi (WSU-TFREC),

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

Other Funding Sources

Agency Name: Northwest Nursery Improvement Institute
Amount Awarded: $9,070 (2020 – 2021)
Notes: "Assessing effects of chemical and hormonal treatments on germination of hybrid *Pyrus* rootstock seeds" (PI: Teh; Co-PI: Evans)
Synergistic project to test effects of chemicals and hormones in improving germination of hybrid *Pyrus* seeds.

Agency Name: Fresh & Processed Pear Committee Research
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: Fresh & Processed Pear Committee Research
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

Organization Name: WSU-TFREC **Contact Administrator**: Katy Roberts

Telephone: 509-335-4564/509-663-8181 **Email**: arcgrants@wsu.edu

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

- ~1,100 seedlings (from 2019 crosses) are being maintained in WSU-TFREC hoop house.
- ~325 seedlings (from 2017 crosses) were planted at WSU Columbia View orchard. In August 2020, these seedlings were budded with 'd'Anjou' scions. Additional ~50 seedlings (from 2016 crosses) were rebudded with 'd'Anjou' scions.
- Pollen from diverse rootstock parents was collected at WSU Sunrise orchard.
- ~600 seedlings (from 2016 crosses) at WSU Columbia View orchard were phenotyped for various vigor-related traits.
- Two high-density genetic maps for a rootstock population (from 2016 cross) were constructed.
- ~45 replicated B × A and B × C selections (planted in 2017) at WSU Columbia View orchard were phenotyped for vigor-related traits, which were highly correlated.

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.

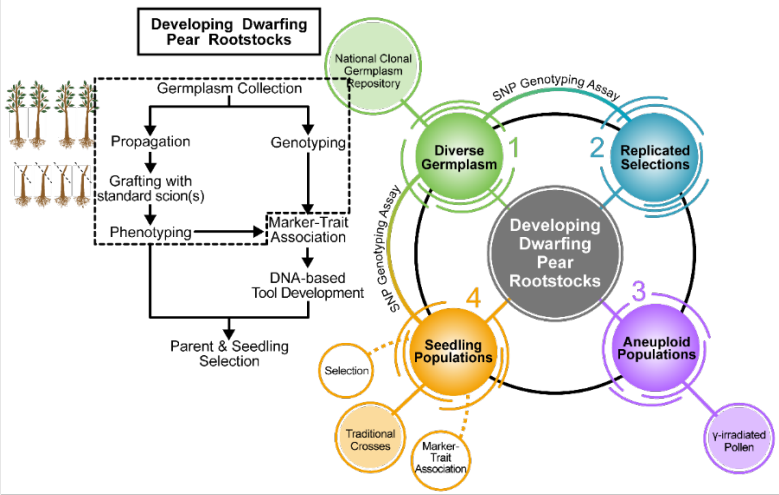


Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. Current accomplishments highlighted within the dotted box include (a) expansion of existing seedling populations, (b) propagation of rootstock seedlings with 'd'Anjou', (c) collection of vigor-related phenotypic data, (d) DNA genotyping/sequencing, and (e) construction of genetic maps for marker-trait association.

RESULTS AND DISCUSSION

Objective 1: Develop seedling populations to produce new rootstocks

(a) Seedling populations from 2016 crosses

Previously, four *Pyrus* seedling populations (n = 600) segregating for vigor, precocity and other horticultural traits were established at WSU Columbia View orchard, Orondo, WA. These seedlings were budded with a standard scion variety, 'd'Anjou' in 2018. Seedlings that failed to bud were rebudded in fall 2019 and fall 2020.

In 2019, variability in the timing of scion bud initiation resulted in rootstocks being cut back at different times. In spring 2020, standardization cuts were made prior to bud break to normalize or standardize the variability. Scions were cut back to 40 cm (i.e., 15.75 inches) from the top of bud union.

(b) Seedling populations from 2017 crosses

Seedlings (n = 325) previously maintained in WSU-TFREC greenhouse were planted at WSU

Columbia View orchard in spring 2020. In fall 2020, these seedlings were budded with a standard scion variety, 'd'Anjou'.

(c) Seedling populations from 2019 crosses

In winter 2020, ~1,200 seeds were vernalized and germinated in WSU-TFREC greenhouse. Seedlings were moved to WSU-TFREC hoop house in early summer 2020, where they were irrigated with auto-sprinklers and protected with nets (10% shade factor). Hoop house space, irrigation set-up and protective nets were kindly provided by Dr. Lee Kalcsits.

These seedlings are currently covered with straw mulch, as they overwinter in the hoop house. In spring/summer 2021, they will be planted at WSU Columbia View orchard.

All seedlings will be maintained in the field for the remainder of the project, where annual shoot length, trunk cross-sectional diameter, internode length, scion branch angles and various horticultural traits (and precocity, if relevant) are being evaluated as a measure of vigor.

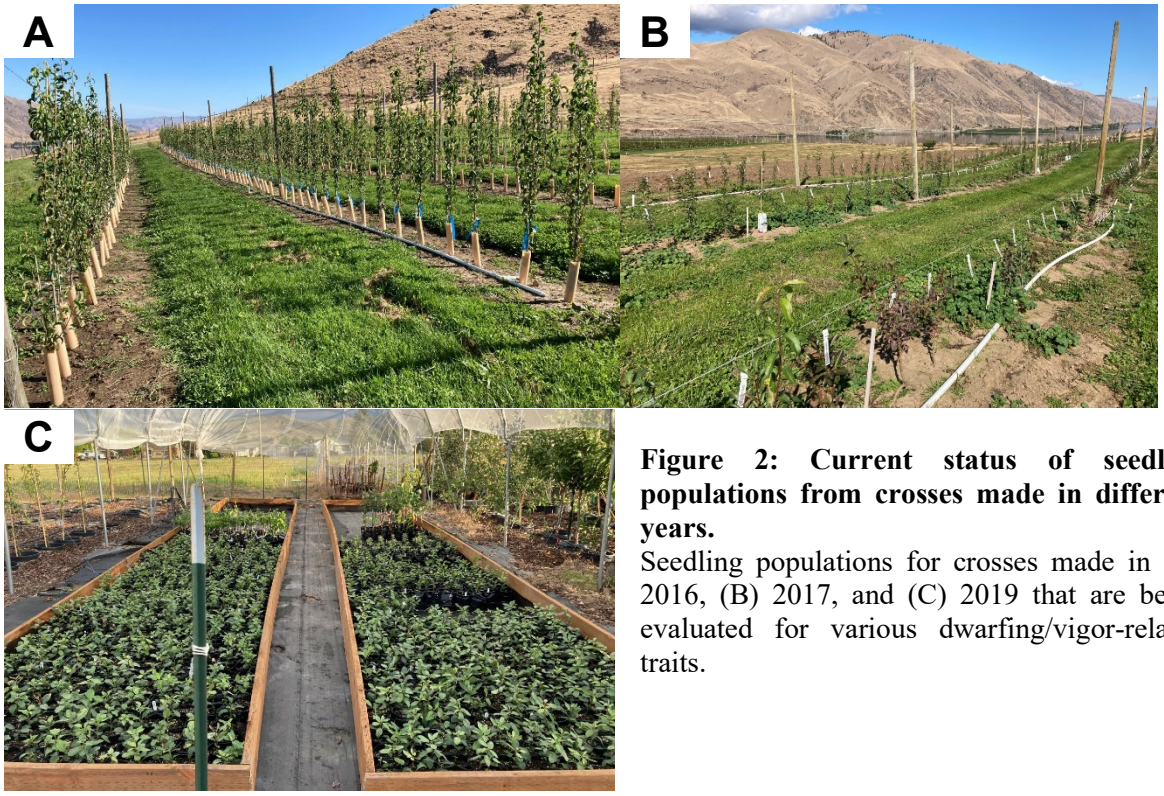


Figure 2: Current status of seedling populations from crosses made in different years.
Seedling populations for crosses made in (A) 2016, (B) 2017, and (C) 2019 that are being evaluated for various dwarfing/vigor-related traits.

Objective 2: Validate published markers for parent and seedling selection

This experiment will be performed in spring 2021.

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

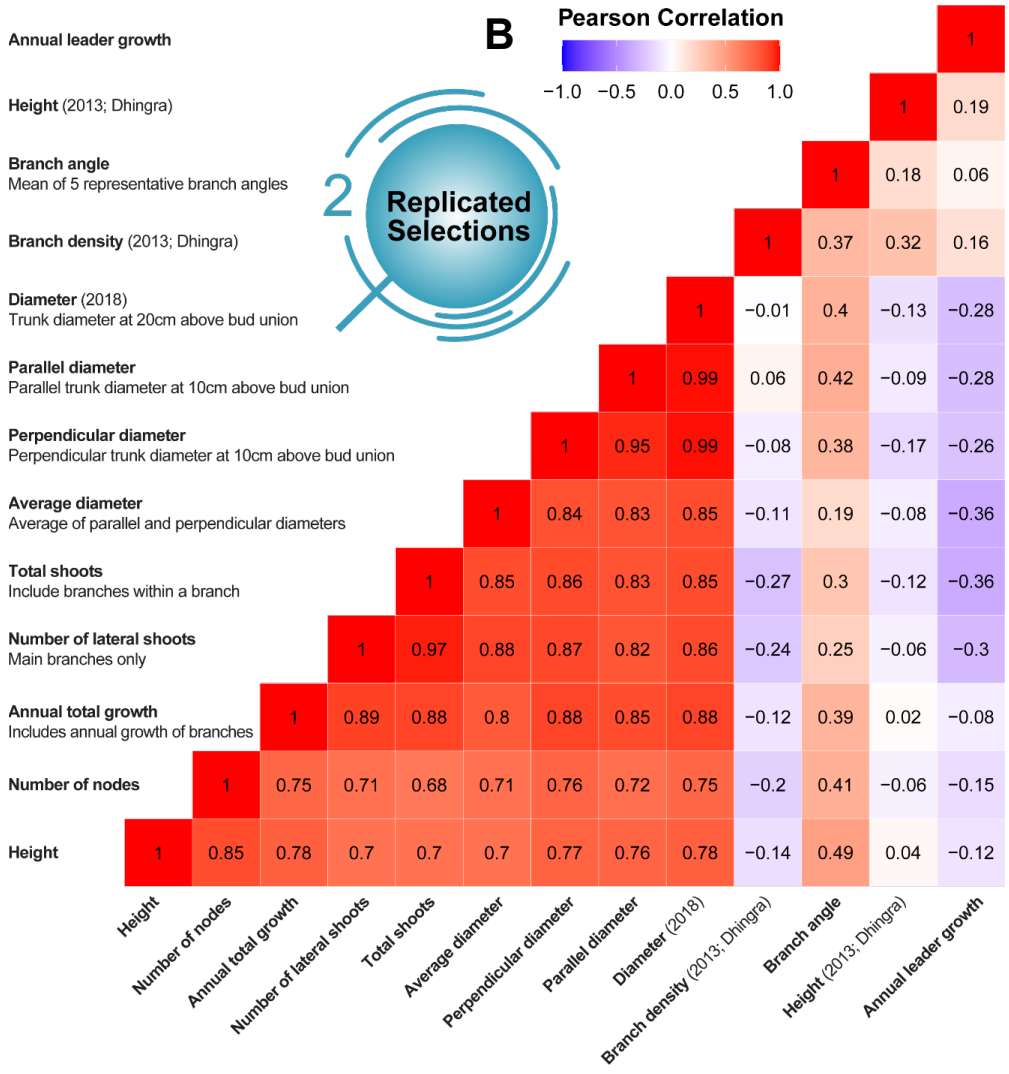
High-resolution pear genotyping/sequencing array data were used to construct two high-resolution genetic maps. In combination with phenotypic data collected in *Objective* 1, these maps will be used to identify genetic determinants associated with dwarfing and/or vigor-related traits (i.e., marker-trait association).

Additionally, we anticipate the construction of two additional high-resolution genetic maps that will also be used for marker-trait association.

Objective 4: Expand the pear rootstock parent germplasm

No additional rootstock parents were added this year. Overall, the existing pear rootstock parents at WSU Sunrise were well maintained.

Figure 3: Current status and correlation analysis of replicated B × A and B × C selections.
(A) Trees were pruned and trained to induce fruit production. (B) Several vigor-related traits (e.g., tree height, annual total shoot growth, number of nodes, and trunk cross-sectional diameter) are high correlated.


B
Pearson Correlation
−1.0 −0.5 0.0 0.5 1.0
2 Replicated Selections
Annual leader growth
Height (2013; Dhingra)
Branch angle
Mean of 5 representative branch angles
Branch density (2013; Dhingra)
Diameter (2018)
Trunk diameter at 20cm above bud union
Parallel diameter
Parallel trunk diameter at 10cm above bud union
Perpendicular diameter
Perpendicular trunk diameter at 10cm above bud union
Average diameter
Average of parallel and perpendicular diameters
Total shoots
Include branches within a branch
Number of lateral shoots
Main branches only
Annual total growth
Includes annual growth of branches
Number of nodes
Height
Height
Number of nodes
Annual total growth
Number of lateral shoots
Total shoots
Average diameter
Perpendicular diameter
Parallel diameter
Diameter (2018)
Branch density (2013; Dhingra)
Branch angle
Height (2013; Dhingra)
Annual leader growth

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

The 14 unique selections grown in triplicate (total of ~45) are being maintained at WSU Columbia View orchard. Overall tree growth was good this year. The trees were pruned (except central leaders) and trained to induce fruit production in future years. We thank Dr. Stefano Musacchi for his advice on training these trees.

In winter 2020, the trees were phenotyped for various vigor-related traits. High correlation coefficients were reported among tree height, annual total shoot growth, number of nodes, and trunk cross-sectional diameter. Consistent segregations for vigor-related traits were observed among triplicated selections; however, it is still too early to draw meaningful conclusions.

OUTREACH

- Soon Li Teh presented "Initiating pear rootstock breeding at Washington State University" at the 10th Rosaceae Genomics Conference (virtual/online) on December 9 – 11, 16 – 18, 2020.
- Graduate student, Zara York presented "Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock populations" at WSU Department of Horticulture – Research Proposal Expo via Zoom on April 21, 2020.
- Zara York, Soon Li Teh and Kate Evans presented "Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock populations" at the 2020 Annual Meeting for National Association of Plant Breeders via Zoom on August 18, 2020.

FINAL PROJECT REPORT **YEAR**: 3 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 a 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, 5 of 31 combinations had mortality rates greater than 30% 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. Higher mortality in OR was partly due to the site having more direct grafted Bartlett trees and possibly due to gopher damage in the establishment year.
- Sources of mortality will require additional years to determine. Tree mortality was not always associated with a given scion (i.e., incompatibility).
- 8 of 24 combinations had a 20% or more trees struggling to grow in 2020 at WA. A similar scenario occurred in OR. A high proportion of trees with poor growth likely indicates incompatibility. Twelve combinations had no struggling trees.
- There is a wide range of trunk size variation 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 trunk size range 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 and tree height exceeded 8' in 80% of the combinations, with the tallest combinations reaching ~10 ft. The OR site was not pruned after the 2019 season due to COVID and will be pruned in early March 2021. The WA site was pruned in 2020. Pruning weights varied across rootstocks and generally agreed with trunk size data.
- Nearly all quince rootstocks conferred precocity. Bartlett generally had more flower clusters in 2020 than ‘d’Anjou’. The fact that the OR had two to three-fold more flower clusters than WA was because trees were not pruned in 2020 and thus had larger canopies with greater bearing potential. OR trees were hand thinned based on trunk size to balance the fruit to canopy ratio. Final fruit numbers per tree were not too dissimilar between sites; ‘d’Anjou’ trees generally had fewer than 10 fruit per tree and ‘Bartlett’ had 20 to 40 fruit per tree.
- ‘d’Anjou’ tree yield in 2020 was minor (1-4 kg/tree [2-9 lbs]). Bartlett yields were roughly double (averaging around 5kg [11 lbs] per tree with maximum yields of 7 kg [15 lbs per tree or 18 bins per acre based on the planting density]. Fruit size was good (typically between 200 and 250 g [80 to 90 fruit per box]).
- To date, three rootstocks appear very promising for dwarfing, precocity and productivity.

Results and Discussion:

Tables 1 and 2 report the percentages of failure trees (tree mortality) for each combination for WA and OR sites, respectively. At both sites, among the combinations with interstems, Anjou/68.002 had the highest mortality rate (Tables 1 and 2). Interestingly, Anjou/99.002 (direct graft) had the highest tree failure incidence in WA but not OR. For Bartlett/Comice, 68.002, 99.002 and 118.001 had the highest mortality in both WA and OR (Tables 1 and 2). However, Bartlett/99.002 direct grafts had 0% mortality at both sites. The highest proportion of trees struggling at the end of the 2020 season were Anjou/Comice/99.002 and Anjou/Comice/118.001 (60% and 36%, respectively) and Bartlett/Comice/118.001 and Bartlett/Comice/68.002 (37% and 31%, respectively). In summary, combinations of both varieties with Comice on CYD 68.002 resulted in the highest proportion of dead trees after more than 3 years from planting. Observed failure for many of these combinations occurred even in the presence of an interstem, indicating that, at least for some CYD accessions, Comice may be incompatible.

Table 1: Mortality data expressed as % failure at 17, 29 and 41 months from planting and percentage of trees struggling in November 2020 for both Anjou and Bartlett grafted on 9 different quince accessions in Entiat (WA) (table sorted by cv and CYD acc. #).

Cv	Quince rootstock	Interstem	Count of tree planted (06/06/2017)	Count of tree alive+struggling (11/12/19)	Count of alive +struggling (11/10/20)	Count of dead trees (11/10/20)	Count of struggling trees (11/10/20)	% failure in 17M	% failure in 29 M	% failure in 41 M (2020)	% healthy and alive in 41 M (Nov 2020)	% struggling trees in (Nov 2020)
Anjou	22.001	Comice	22	22	22			0	0	0	100	0
Anjou		*None*	20	20	20		4	0	0	0	80	20
Anjou	23.001	Comice	12	12	12			0	0	0	100	0
Anjou	57.001	Comice	17	17	17			0	0	0	100	0
Anjou		*None*	11	10	10	1		9	9	9	91	0
Anjou	65.001	Comice	17	17	17			0	0	0	100	0
Anjou	67.001	Comice	12	12	12			0	0	0	100	0
Anjou	68.002	Comice	14	9	8	6	3	0	36	43	36	21
Anjou	70.001	Comice	39	33	33	6	5	13	15	15	72	13
Anjou		*None*	13	12	12	1	1	8	8	8	85	8
Anjou	99.002	Comice	42	42	42		25	0	0	0	40	60
Anjou		*None*	12	2	2	10		67	83	83	17	0
Anjou	118.001	Comice	11	10	10	1	4	9	9	9	55	36
Anjou		*None*	10	10	10			0	0	0	100	0
Bartlett	22.001	Comice	24	24	24		1	0	0	0	96	4
Bartlett	23.001	Comice	12	12	12			0	0	0	100	0
Bartlett	57.001	Comice	15	15	15			0	0	0	100	0
Bartlett	65.001	Comice	17	17	17			0	0	0	100	0
Bartlett	67.001	Comice	13	13	13		2	0	0	0	85	15
Bartlett	68.002	Comice	16	12	8	8	5	25	25	50	19	31
Bartlett	70.001	Comice	29	29	28	1	7	0	0	3	72	24
Bartlett	99.002	Comice	54	49	42	12	10	9	9	22	59	19
Bartlett		*None*	10	10	10			0	0	0	100	0
Bartlett	118.001	Comice	35	35	29	6	13	0	0	17	46	37
		total	*477*	*444*	*425*	*52*	*80*	*5*	*7*	*11*	*72*	*17*

Table 2: Mortality data from Parkdale (OR) through January 2021 for both Anjou and Bartlett grafted on 9 different quince accessions (table sorted by cv and CYD acc. #).

Cv	quince rootstock (CYD accession)	type of graft	Count of trees planted June 2017	Count of alive trees Jan 2019	Count of alive trees Jan 2020	Count of alive trees Jan 2021	%Mortality 2017-2021
ANJOU	22.001	Comice	22	22	22	22	0
		Direct graft	22	22	22	22	0
	23.001	Comice	12	11	11	11	8
		Direct graft	10	10	10	10	0
	57.001	Comice	17	15	14	14	18
		Direct graft	10	10	10	10	0
	65.001	Comice	20	20	20	20	0
		Direct graft	13	12	12	12	8
	67.001	Comice	12	12	12	12	0
	68.002	Comice	15	14	10	8	47
	70.001	Comice	42	42	42	42	0
		Direct graft	10	9	9	9	10
	99.002	Comice	56	55	54	53	5
		Direct graft	12	11	11	11	8
	118.001	Comice	11	10	10	10	9
		Direct graft	10	7	7	5	50
BARTLETT	22.001	Comice	24	24	24	24	0
		Direct graft	15	15	15	15	0
	23.001	Comice	12	12	12	12	0
	57.001	Comice	16	16	16	16	0
		Direct graft	14	13	13	13	7
	65.001	Comice	19	19	19	19	0
		Direct graft	11	9	9	9	18
	67.001	Comice	14	13	13	13	7
	68.002	Comice	16	15	15	11	31
	70.001	Comice	43	41	41	41	5
		Direct graft	16	15	14	14	12
	99.002	Comice	57	37	34	30	47
		Direct graft	12	12	12	12	0
	118.001	Comice	48	41	39	29	40
		Direct graft	12	11	11	11	8

Table 3: 2019-2020 winter pruning (kg/tree) and cumulative pruning weights (2018-2020) in Entiat (WA) for Anjou and Bartlett with Comice interstems grafted on 9 different quince accessions (table sorted by cv and CYD acc. #).

Cultivar	Rootstock	Interstem	Pruned Weight (kg/tree) 2019		Count of reps 2020	Pruned Weight (kg/tree) 2020		Pruned weight in 3 years 2018-2020 (kg/tree)	
d'Anjou	22.001	Comice	0.34	ab	3	0.49		0.91	ab
	23.001	Comice	0.44	a	3	0.50		1.04	a
	57.001	Comice	0.39	a	4	0.50		1.02	a
	65.001	Comice	0.42	a	3	0.62		1.15	a
	67.001	Comice	0.27	ab	3	0.51		0.80	ab
	68.002	Comice	0.11	b	3	0.23		0.36	b
	70.001	Comice	0.21	ab	7	0.31		0.54	ab
	99.002	Comice	0.28	ab	4	0.33		0.66	ab
	118.001	Comice	0.19	ab	3	0.32		0.52	ab
Significance			**			NS (0.0597)		**	
Bartlett	22.001	Comice	0.58	ab	8	0.67	ab	1.37	ab
	23.001	Comice	0.57	ab	3	0.70	ab	1.38	ab
	57.001	Comice	0.58	ab	3	0.70	ab	1.44	ab
	65.001	Comice	0.68	a	3	0.84	a	1.67	a
	67.001	Comice	0.32	bc	4	0.47	abc	0.83	bc
	68.002	Comice	0.19	c	3	0.16	c	0.37	c
	70.001	Comice	0.37	bc	4	0.52	abc	0.91	abc
	99.002	Comice	0.42	abc	10	0.52	abc	1.00	abc
	118.001	Comice	0.18	c	6	0.27	bc	0.46	c
Significance			***			**		***	

Combinations on direct graft (interstem= none) have been excluded from statistical analysis. Significance: *, p<0.05, **, p<0.01, ***, p<0.001, NS= not signifcant. Letters separate means in combination with interstem by SNK for (alpha=0.05).

Pruning weights were calculated as the total weight of wood removed per plot, then divided by the number of trees pruned in the plot to account for differences in tree numbers. Data are presented as wood removed in kg/tree. Pruning data for 2020 are only provided for WA; OR trees were not pruned due to travel restrictions that prohibited the scheduled pruning in late March. Previous pruning data from OR can was reported in earlier 2018 and 2019 reports. Relatively little wood was removed in 2020 on a tree basis in WA. However, significance was detected for Bartlett combinations with interstems (Table 3). The most vigorous combinations were Bartlett/Comice/65.001, and the least vigorous combination was Bartlett/Comice/68.002. There were no differences in 2020 pruning weights for 'd'Anjou' combinations. Three-year-cumulative weight data, however, did reveal differences in vigor across the 9 rootstock-interstem combinations (Table 3). Overall, the most vigorous combinations were Anjou/Comice/23.001, Anjou/Comice/57.001, Anjou/Comice/65.001. Anjou/Comice/68.002 was the least vigorous. Because some combinations had a high portion of their total trees categorized as failing on direct graft, these were not included in the analysis.

Trunk cross sectional areas (TCSA) of scions measured in November 2020 at 10 cm above the graft union (always on the scion) are reported in Figures 1 and 2.

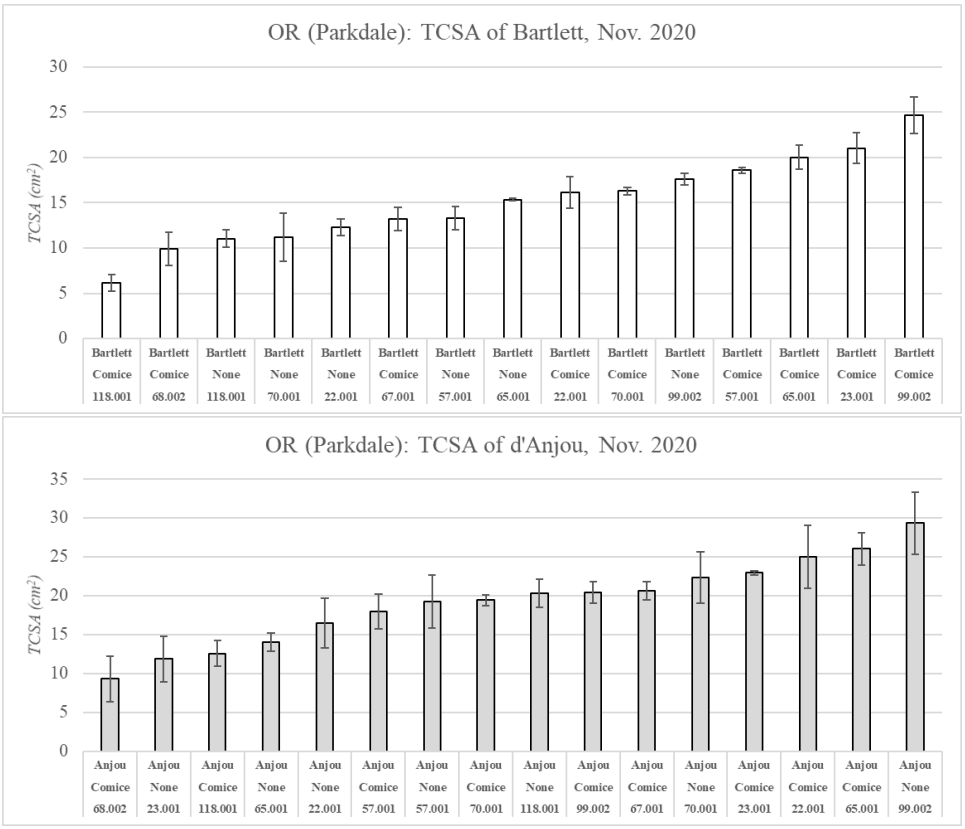

Figure 1: Trunk cross sectional area (TCSA) measured November 2020 with for Bartlett (A) and d'Anjou (B) grafted with Comice interstem on 9 different quince accessions in Parkdale (OR). Data are sorted by ascending TCSA for each variety. Bars are ±1SE.

There was roughly a three-fold difference in tree sizes across the 9 rootstock combinations at each site (Figs. 1 and 2). For a given genotype, trees in OR were generally much larger than WA and this was especially true for vigorous combinations (Figs. 1 and 2). We attribute this difference to climate and soil differences between sites (more precipitation and higher organic matter content in Parkdale than Entiat). The more vigorous and less vigorous combinations were similar between sites with some exceptions. For OR, 99.002 produced the largest trees and 65.001 and 23.001 were similarly vigorous between cultivars. 118.001 and 68.002 were the weakest trees. In WA, the largest trees were on Comice/65.001 and Comice/57.001, and the smallest trees were on Comice/68.002 and Comice/118.001, irrespective of the scion cultivar (Figure 2). The low vigor of Anjou/Comice/68.002 was similarly shown by pruning weight data (Table 3). In November 2020, a vigor evaluation was conducted in WA, assigning a score from 1 to 5 to trees still alive (a score of 1 equates to very low vigor/struggling and 5 equals high vigor). This evaluation confirmed results from other vegetative parameter data: Anjou/Comice/65.001 and Anjou/Comice/57.001 were the strongest trees and Anjou/Comice/99.002, 118.001, and 68.002 were the weakest (data not shown). A very similar trend was reported for Bartlett with Comice interstem (data not shown).

Suckering was observed in nearly all combinations with Comice interstem in WA, although ~ 78% of combinations (with interstems) had only 3 or fewer suckers/tree. Comice/67.001 had the highest number of suckers (approximately 10/tree), significantly higher than all the other combinations and Comice/23.001, 70.001, and 118.001 had the fewest. Similar results were documented in OR (data not shown here but was included in earlier reports).

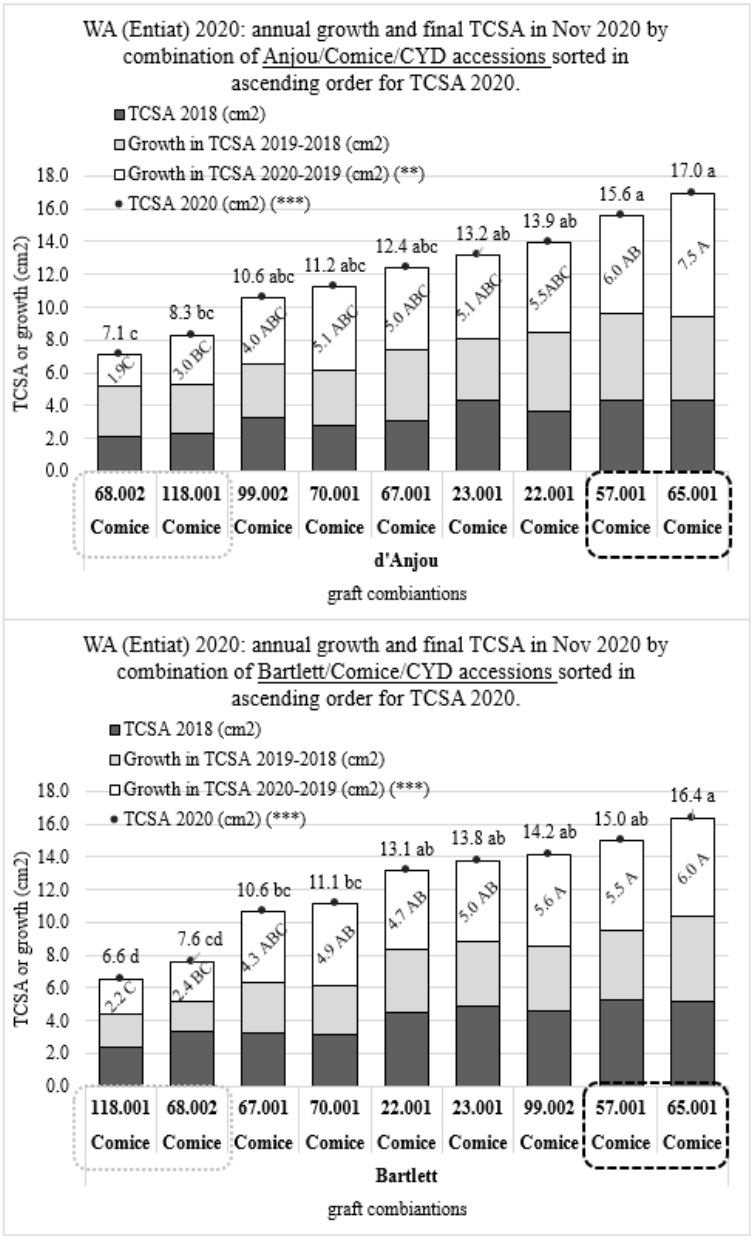

Figure 2: Annual growth and trunk cross sectional area (TCSA) in 2020 with historical data for 2018 and 2019 for Anjou (A) and Bartlett (B) grafted with Comice interstems on 9 different quince accessions in Entiat (WA). The chart is sorted by ascending TCSA 2020 for each variety. Accessions with the highest (57.001 and 65.001) and lowest (118.001 and 68.002) TCA were the same for each cultivar .

In spring of 2020, we counted flower clusters to estimate the precocity of the 9 rootstocks. Flower cluster counts were performed at both sites. No statistically significant differences emerged from the assessment across the 9 CYD accessions given high variability between young trees, however, cultivar differences were clear. The average number of clusters was between 23 and 82 for Anjou and 48 to 280 for Bartlett (Tables 4 and 5). The markedly higher number of flower clusters for Bartlett was observed at both sites, but >100 clusters per tree were only observed in OR since those trees had not been pruned and thus had significantly greater fruiting wood (Table 5). The amount of wood did not necessarily correlate to the number of clusters (Table 5). Hand thinning was performed in OR to balance

the crop load thereby resulting in a similar number of fruit per tree as WA (Tables 4 and 5). On 17-April 2020, the percentage of clusters in full bloom was higher in Anjou than in Bartlett (81% and 15% respectively on average) but there was no difference found among combinations in bloom timing for a given cultivar. In general, Bartlett fruit set was more than 4-fold higher than Anjou (6.9% and 1.6%, respectively). No tendency of a specific rootstock having an effect on fruit set was observed. Secondary (late) bloom was also assessed in WA given its relationship with fire blight infection. Despite the lack of statistically significant differences, some combinations tended to show a higher secondary bloom at the end of May (for example, Bartlett/Comice/23.001, 65.001, 118.001). Another period of secondary bloom was observed in August 2020, with the combination Bartlett/Comice/118.001 having the highest average number of secondary blooms per tree (Table 4).

Table 4: Bloom, fruit set, bloom timing and secondary bloom characteristics in spring 2020 in Entiat (WA) for Anjou and Bartlett with Comice interstem grafted on 9 different quince accessions (table sorted by cv and CYD acc. #).

Cultivar	Interstem	Rootstock	Rep N (for cluster count)	Num flower clusters/tree (4/17/20)	% Clusters in full bloom/tree (04/17/20)	Num fruit/tree (05/28/20)	% Fruit set on total flowers (05/28/20)	Num secondary bloom clusters/tree (5/28/2020)	Num secondary bloom clusters/tree (8/19/2020)
d'Anjou	Comice	22.001	3	73	90.7	6	1.7	0.3	1.3
	Comice	23.001	3	36	69.4	5	1.5	0.3	0.0
	Comice	57.001	3	26	61.8	7	3.6	0.7	0.0
	Comice	65.001	3	26	73.2	3	1.9	0.0	0.0
	Comice	67.001	3	62	91.2	6	1.1	0.7	0.0
	Comice	68.002	3	56	84.0	5	1.6	0.7	0.3
	Comice	70.001	3	25	88.2	1	0.8	0.0	2.0
	Comice	99.002	3	34	89.3	2	1.0	0.0	0.0
	Comice	118.001	3	60	78.6	5	1.4	1.0	0.0
Significance				NS	NS	NS	NS	NS	NS
Bartlett	Comice	22.001	3	59	15.2	22	6.3	1.3	0.3
	Comice	23.001	3	96	15.7	21	5.4	2.0	0.0
	Comice	57.001	3	74	16.6	26	6.5	1.3	1.0
	Comice	65.001	3	66	26.1	23	6.7	2.0	0.0
	Comice	67.001	3	74	25.3	21	5.3	1.7	0.3
	Comice	68.002	3	61	6.0	19	7.7	0.7	0.0
	Comice	70.001	3	50	8.7	22	6.0	1.0	1.0
	Comice	99.002	3	48	13.6	18	9.2	0.7	0.0
	Comice	118.001	3	48	10.1	25	8.8	2.0	2.3
Significance				NS	NS	NS	NS	NS	NS

Fruit set did not differ among the rootstocks and fruit numbers per tree ranged between 1-17 for Anjou and 16-44 for Bartlett when averaging across sites (Tables 4 and 5). Further observations and assessments on return bloom, rootstock induced precocity and fruit set will require further investigation in the following years.

The 2020 harvest represented the first crop for both cultivars since blocks were established in 2017. Although this is the 4th leaf, trees were restarted in their second leaf due to poor uniformity and small tree size; thus, trees were easily one year behind. Bartlett were harvested 20-Aug and 3-Sep in WA and OR, respectively and Anjou were harvested 3-Sep and 6-Oct in WA and OR, respectively. Harvests were performed at commercial timings for the farms they were located. Tree yield was markedly lower for Anjou than Bartlett at both sites (Figs. 3 and 4), as would have been predicted from bloom and fruit set data as well as the inherent differences in precocity between the two cultivars. Trees have largely filled the orchard space and are expected to achieve high yield potential in 2021. Fruit size was very good for both cultivars and sites, particularly for first-crop fruit (~200-250 g) and supports the well-known benefit of quince rootstocks for imparting large fruit size to pear. Statistical differences

among rootstocks was not detected within cultivar in WA, likely due to the variability associated with young trees. Similar variability was also observed in OR (see error bars, Figure 4). In WA, the combinations that produced greater than 1.5 kg/tree of Anjou were Comice/118.001 (despite producing the smallest trees) and Comice/57.001. Tree yields were ~double in OR but these two rootstocks also produced the highest yield (~4kg per tree; Fig 4). In WA, Comice/70.001 resulted in the least productive of all 9 combinations, with below 1 kg/tree on average and was similarly low in OR, though not the lowest (Figs. 3 and 4).

Table 5: Vegetative growth, bloom, fruit set, number of fruit thinned and fruit per tree in spring 2020 in Parkdale (OR) for Anjou and Bartlett with Comice interstem grafted on 9 different quince accessions (table sorted by Total length of 1 yr shoots in meters). Trees were not pruned after 2019 harvest due to covid restrictions that limited the PI from traveling to OR, hence the larger canopies and greater bloom compared with WA.

Scion	Rootstock	Interstem	No. of 1yr old Shoots 3/15/2020	Total length 1yr shoots (m) 3/15/2020	No. clusters 5/13/2020	No. Fruit Thinned 6/17/2020	% Fruit Set 6/17/2020	No.Fruit per tree 6/17/2020
d'Anjou	23.001	None	26.7	3.4	72.0	1.0	10.9	5.2
	68.002	Comice	29.0	7.4	39.7	2.3	23.8	12.3
	65.001	None	30.6	7.8	54.0	1.0	4.5	2.0
	118.001	Comice	27.3	9.0	34.3	1.0	24.8	11.8
	22.001	None	42.6	10.8	46.5	2.0	10.2	6.1
	118.001	None	41.3	11.8	69.0	19.0	21.9	14.8
	57.001	Comice	50.0	16.2	59.0	3.0	8.5	5.7
	57.001	None	67.3	17.6	73.8	2.3	19.2	12.8
	67.001	Comice	55.0	17.9	82.3	3.8	16.5	17.8
	23.001	Comice	63.2	18.7	33.6	0.0	6.8	3.0
	70.001	Comice	54.5	19.5	38.2	1.9	11.9	4.7
	65.001	Comice	67.3	20.3	46.7	0.0	4.7	1.8
	70.001	None	64.7	20.9	23.2	0.0	11.0	6.5
	22.001	Comice	67.6	22.2	59.0	1.0	9.0	3.7
	99.002	Comice	62.4	24.7	46.1	2.0	13.0	4.5
	99.002	None	78.5	27.0	69.8	2.3	11.5	8.8

Scion	Rootstock	Interstem	No. of 1yr old Shoots 3/15/2020	Total length 1yr shoots (m) 3/15/2020	No. clusters 5/13/2020	No. Fruit Thinned 6/17/2020	% Fruit Set 6/17/2020	No.Fruit per tree 6/17/2020
Bartlett	118.001	Comice	11.8	4.1	66.3	7.8	28.9	16.3
	68.002	Comice	30.9	9.0	137.1	7.4	15.3	17.4
	22.001	None	42.8	12.5	212.2	19.4	16.0	22.9
	118.001	None	40.8	12.5	211.8	25.0	24.9	28.2
	67.001	Comice	38.0	13.7	126.4	7.9	22.8	19.3
	70.001	None	51.4	14.6	169.9	25.6	36.4	33.9
	99.002	None	54.4	15.9	272.9	17.8	20.5	32.2
	22.001	Comice	44.3	16.5	220.7	12.7	19.7	27.8
	70.001	Comice	52.9	19.9	128.7	11.7	35.2	30.6
	57.001	Comice	50.4	20.4	204.7	10.9	22.1	31.1
	23.001	Comice	50.0	20.7	199.4	11.3	27.1	38.1
	57.001	None	63.2	23.4	281.2	31.4	23.7	32.4
	65.001	Comice	57.7	24.0	225.6	16.6	23.7	35.3
	65.001	None	61.8	24.1	285.5	21.5	22.3	25.2
	99.002	Comice	61.0	28.2	209.8	17.5	27.2	44.0

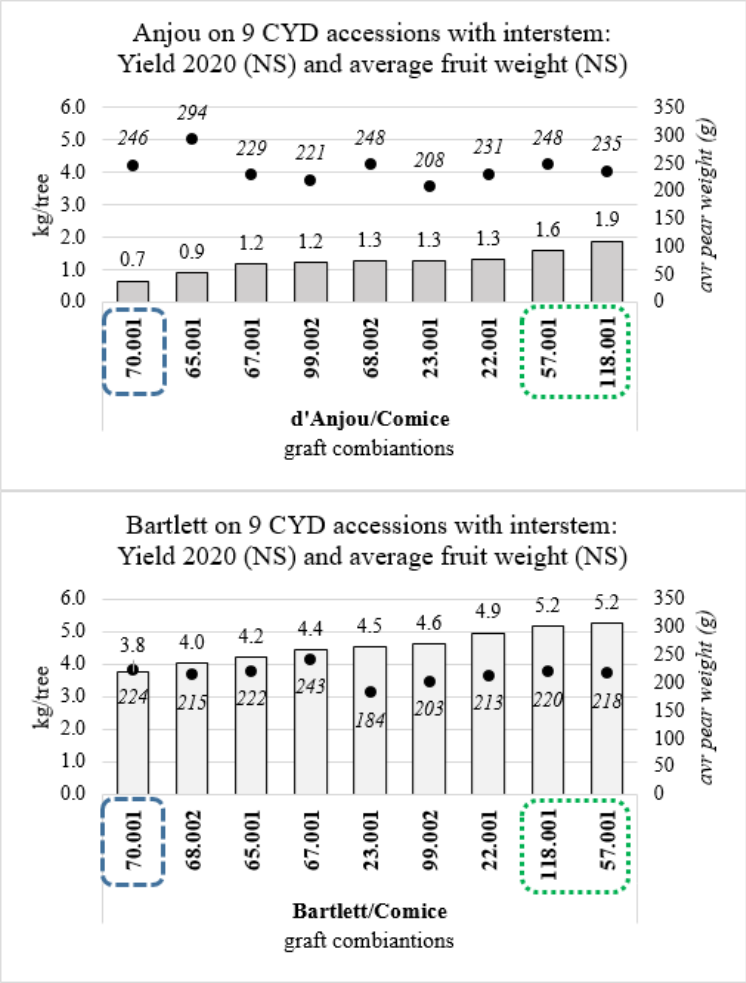

Figure 3: First crop yield data 2020: kg fruit/tree and average pear weight (g) for Anjou (A) and Bartlett (B) grafted on 9 different quince accessions in Entiat (WA). The chart is sorted by ascending yield/tree for each variety. Within a cultivar differences in either measure were not significant (NS). Dashed boxes on the x-axes show consistency in rootstock performance between cultivars.

As previously stated, Bartlett yields were markedly higher than Anjou. In WA, similar rootstock-yield trends were evident for Bartlett as observed with Anjou (Comice/57.001 and Comice/118.001 produced the highest yields) but this was not observed in OR. Again variability in young trees, relatively few trees and replicates, and the fact that this was the first crop made it difficult to determine treatment (rootstock) effects. Despite the relatively low first-crop yields, a yield of 30 Bartlett pears per tree (average across rootstocks in OR) produced ~15 lbs per tree, or 18 bins per acre at the tree density of the plantings (Fig. 4). The large fruit sizes reported were not affected by crop loads across the nine rootstocks, however a few rootstocks did produce small fruit size (<200 g), and this may be an indicator of future issues. In OR, 118.001 had both the lowers yield and fruit weight (for Bartlett). In WA, combinations with Comice/23.001 had the lowest numerical average fruit weight (Fig. 3).

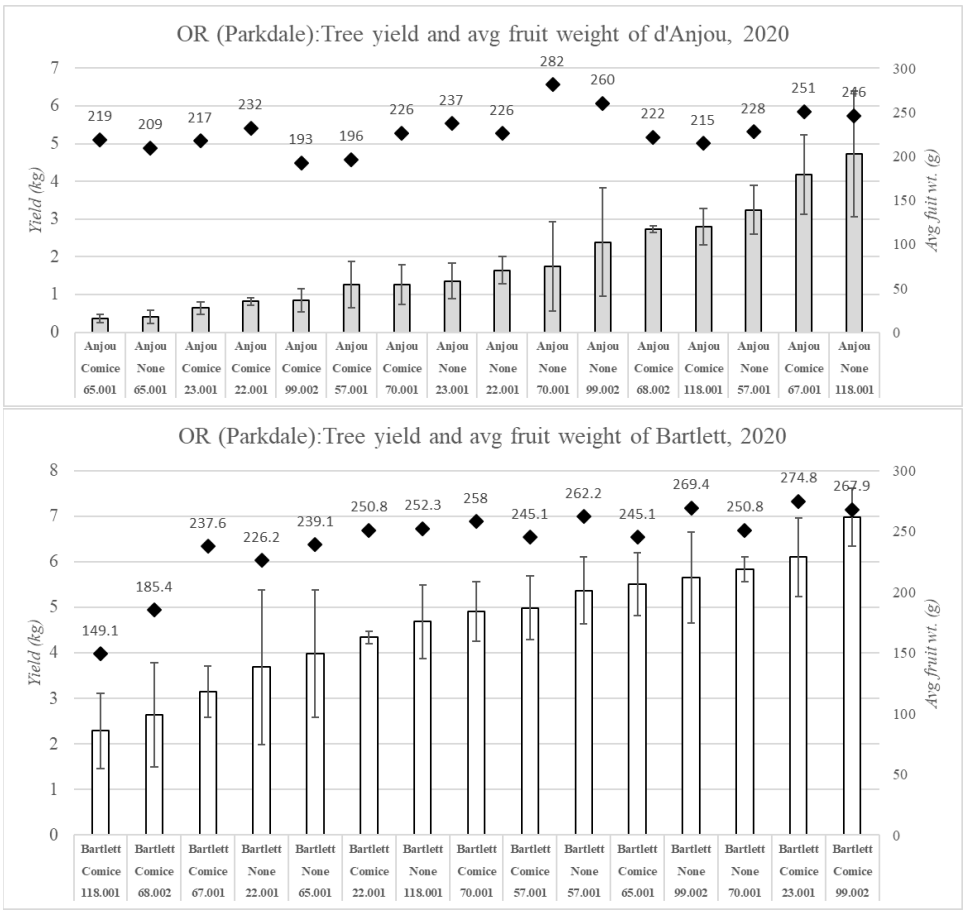

Figure 4: First crop yield data 2020: kg fruit/tree and average pear weight (g) for Anjou (A) and Bartlett (B) grafted on 9 different quince accessions in Parkdale (OR). The chart is sorted by ascending yield/tree for each variety. Bars on tree yield data are ±1SE.

In WA, fruit quality was analyzed for Bartlett combinations following 2.5 months of regular air (RA) cold storage and a 7-day (room temperature) ripening treatment; Anjou were not evaluated due to an insufficient number of fruit. In OR, Bartlett fruit quality was evaluated at harvest and after 1 month RA postharvest cold storage and a 7-day ripening period and Anjou fruit analysis only occurred following 3 months RA cold storage and a 7-day ripening period, again due to lower Anjou yield. A minimum of 14 days and 60 days cold storage is necessary to induce ripening capacity of Bartlett and Anjou, respectively, following exposure to room temperatures. Bartlett fruit quality data from WA are summarized in Table 6, for the most interesting parameters: average pear weight at harvest, pear weight drop during cold storage, pear weight drop during the 7 days of ripening at room temperature, I_{AD} (by DA meter) at harvest, I_{AD} drop during cold storage, I_{AD} drop during the 7 days of ripening at room temperature, firmness, soluble solids content (SSC), titratable acidity (TA), and pH. Despite the narrower range of pear size selected for instrumental quality analysis (in order to eliminate the confounding effects of fruit size on fruit quality attributes), significant differences in terms of weight and weight drop emerged. In particular, Bartlett/Comice/70.001 pears had the highest average fruit weight, and the greater losses in weight in both intervals (up to 5% of their initial weight; Table 6). The other combinations did not differ with respect to fruit weight loss. Differences in fruit maturity were observed across the rootstocks with Bartlett/Comice/68.002 and 70.001 appearing riper (1.90 and 1.94 I_{AD} respectively) than others (which averaged 2.03 to 2.13) based on the non-destructive DA meter

assessment (Table 6). After 7 days of ripening, Bartlett/Comice/68.002 and 70.001 pears showed the lowest drop in $I_{AD,}$ probably reflecting their more advanced maturity stage at the time of harvest with respect to Bartlett/Comice/118.001, which had the highest I_{AD} drop in 7 days, and one of the highest value at harvest (Table 6). Bartlett/Comice/118.001 also had the lowest SSC among the 9 combinations (11.7 °Brix) and Bartlett/Comice/65.001 had the highest SSC (15.3 °Brix) which was similar to Bartlett/Comice/68.002 (14.9 °Brix). The wide range of SSC needs to be confirmed in the future to determine if specific CYD accessions have an impact on fruit quality. Titratable acidity (TA) and pH between were statistically similar among the 9 combinations evaluated in WA (Table 6).

Table 6: Bartlett fruit quality carried out October 2020 (2.5 months after harvest and RA storage) for all 9 combinations with Comice interstem. Fruit (N=15) were selected in size range 70-75 mm for Entiat WA.

CV/interstem	CYD	N=	Avr. pear weight (g) at harvest		Weight drop (g) harvest-T0 day1		Weight drop (g) in 7 days RT		I_{AD} at harvest		I_{AD} drop harvest-T0 day1		I_{AD} drop in 7 days RT		Firmness (kg) (day7)		SSC (Brix) (day7)		TA (% ac. malic) (day7)	pH (day7)
Bartlett/Comice	22.001	15	232	ABC	3.6	B	10.6	AB	2.13	A	0.52	AB	1.43	ABC	0.89	A	13.7	B	0.59	3.70
Bartlett/Comice	23.001	15	213	C	3.5	B	10.3	B	2.11	A	0.55	AB	1.43	ABC	0.92	A	13.7	B	0.48	3.84
Bartlett/Comice	57.001	15	238	AB	3.6	B	9.9	B	2.03	AB	0.57	AB	1.33	BCD	0.95	A	13.4	B	0.52	3.73
Bartlett/Comice	65.001	15	242	AB	4.1	B	11.7	AB	2.05	A	0.59	A	1.36	ABCD	0.89	A	15.3	A	0.46	3.79
Bartlett/Comice	67.001	15	237	ABC	3.6	B	10.1	B	2.07	A	0.58	A	1.39	ABCD	0.81	A	13.2	B	0.47	3.79
Bartlett/Comice	68.002	15	236	ABC	4.0	B	10.1	B	1.90	C	0.57	AB	1.27	CD	0.91	A	14.9	A	0.47	3.75
Bartlett/Comice	70.001	15	250	A	5.1	A	12.4	A	1.94	BC	0.62	A	1.24	D	0.85	A	13.9	B	0.50	3.71
Bartlett/Comice	99.002	15	222	BC	3.8	B	11.2	AB	2.11	A	0.47	B	1.48	AB	0.89	A	14.1	B	0.51	3.77
Bartlett/Comice	118.001	15	236	ABC	4.3	B	11.4	AB	2.11	A	0.46	B	1.51	A	0.64	B	11.7	C	0.47	3.77
Significance			**		***		**		***		***		***		***		***		*NS*	*NS*

Bartlett harvest: 08/20/20. Harvest-T0 day1=2.5 month in storage at 33F RA. Destructive analyses were done after ripening for 7days at room temperature (RT). Firmness as avr. of 2 cheeks in the more external pear cortex layer. SSC= solible solids content in Brix degree. TA and pH means are averages of 3 juices/combination (N=3, not single fruit).
*Significance: **= $p<0.01$, ***=$p<0.001$, NS=not significant. Post doc letters separation by SNK for alpha=0.05. Same letters identify similar means for each parameter and column.*

In OR, there were only minor differences among rootstocks for fruit quality attributes of Anjou (data not shown to conserve space). These were deemed unimportant given the relatively low yields. For Bartlett, fruit were harvested at the appropriate commercial maturity range (i.e., ~19 lbs FF). Maturity did not appear to be affected by rootstock and all fruit ripened to acceptable eating quality (<4 lbs FF), though direct grafted Bartlett to 22.001 was numerically more firm than other treatments (Table 7). The range in SSC was 11.7 to 14.2 with most fruit having a SSC of ~12.5. TA levels were not affected by treatment and remained relatively high following cold storage and ripening, though this would be expected from the short PH storage period.

Table 7: 2020 Anjou fruit quality attributes measured after a postharvest (PH) cold storage period of 3 months and a 7-day ripening period and Bartlett fruit quality carried out at harvest and again following 1 month of cold storage and a 7-day ripening period, Parkdale (OR).

Scion	Rootstock	Interstem	2020 Harvest FF (lb)	2020 Harvest SS (%)	2020 Harvest TA (%)	2020 PH FF (lb)	2020 PH SS (%)	2020 PH TA (%)
Bartlett	22.001	Comice	19.3	12.1	0.41	2.3	12.7	0.37
	22.001	None	20.3	13.8	0.41	4.4	15.2	0.37
	23.001	Comice	19.1	11.7	0.37	2.2	12.8	0.39
	57.001	Comice	19.3	11.9	0.44	2.1	13.1	0.37
	57.001	None	19.9	13.2	0.39	2.2	14.2	0.40
	65.001	Comice	19.2	12.4	0.42	2.3	13.1	0.44
	65.001	None	21.4	13.3	0.38	2.4	14.2	0.39
	67.001	Comice	20.6	12.4	0.40	2.4	13.6	0.38
	68.002	Comice	18.3	14.2	0.47	2.3	15.2	0.44
	70.001	Comice	20.0	12.0	0.37	2.1	12.8	0.36
	70.001	None	20.0	13.5	0.32	2.5	14.3	0.39
	99.002	Comice	19.6	12.7	0.39	2.3	13.8	0.38
	99.002	None	20.2	12.6	0.40	2.2	13.2	0.41
	118.001	Comice	20.2	12.5	0.40	2.2	13.0	0.40
	118.001	None	20.1	12.7	0.37	2.3	13.4	0.38

Executive Summary

Project title: Field Evaluation of Pear Cultivars on Cold Hardy Quince Rootstocks

Keywords: Pear rootstocks, dwarfing, cold hardy, quince, efficiency

Abstract: Quince is the preferred rootstock for intensive pear production, facilitating high-density, efficient orchards that are amenable to mechanization. Quince rootstocks are highly dwarfing, precocious, and confer high productivity and large fruit size to pear; however, they often have insufficient cold hardiness for cold regions like the PNW. With previous funding from the NW Pear Committee, we identified 20 quince accessions from the germplasm repository at the USDA-NCGR, in Corvallis, Oregon that were as hardy or hardier than the commercial pear rootstocks, Old Home X Farmingdale (OH × F) 87 and 97. Nine of the 20 accessions were micropropagated and budded to the scions 'd'Anjou and 'Bartlett' (with and without a 'Comice' interstem) and planted in two high-density (3 ft × 12 ft., 1210 trees per acre) field performance trials in 2017 with industry collaboration (Entiat, WA and Parkdale, OR). Trees were trained to a spindle architecture and vegetative and fruiting data were recorded over a three-year period. At the completion of this project (2020, 4th leaf), tree survival varied markedly by rootstock; in OR, 13 (approx. 1/3rd) of the combinations had 0% mortality and 5 of 31 combinations had mortality rates greater than 30%. In WA, survivability was higher; only 3 of 24 combinations tested had mortality rates exceeding 20% and two-thirds of the combinations had 0% mortality. Annual vegetative growth data indicated that 8 of 24 combinations had 20% or more trees struggling at the completion of 2020. A rootstock with a high proportion of struggling trees suggests incompatibility between the quince rootstock and either the interstem (Comic) or the scions. Twelve combinations had no struggling trees. Quince rootstocks conferred significant dwarfing to d'Anjou and Bartlett. Similar genotype and/or combination effects are beginning to show after the fourth-leaf at both sites with the most vigorous or weakest trees belonging to the same rootstock. Eighty percent of the combinations filled the orchard space (in height and spread) by the fourth leaf. Annual pruning weight data across the rootstocks generally agreed with trunk size data. Nearly all quince rootstocks conferred precocity to the scions with significantly more flower clusters for Bartlett compared to 'd'Anjou in 2020. Final fruit numbers per tree were not too dissimilar between sites; 'd'Anjou' trees generally had fewer than 10 fruit per tree and 'Bartlett' had 20 to 40 fruit per tree. Differences among rootstocks were relatively minor in the first cropping year. 'd'Anjou' tree yield was insignificant (2-9 lbs per tree) but Bartlett yields averaged around 11 lbs and reached as high as 15 lbs per tree or 18 bins per acre [based on the planting density]. Fruit size was very good for rootstocks (typically between 200 and 250 g [80 to 90 fruit per box]), a common benefit of quince rootstocks. To date, three to four rootstocks appear very promising for their overall effects on dwarfing, precocity and productivity for either Bartlett and/or 'd'Anjou. Future work has been proposed to continue evaluated cropping in the 5th leaf and beyond with the aim of advancing one or more dwarfing and yield efficient quince rootstocks for PNW pear producers.

FINAL PROJECT REPORT

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

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

Total Project Request: **Year 1:** $118,792 **Year 2:** ~~$84,137~~ 64,137 **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 supported Victor's salary and benefits He was able to participate in the research objectives of this project and expand on the physiology research being conducted.

Budget 1
Organization Name: Washington State University Contract Administrator: Shelli Tompkins
Telephone: 509-663-8181 Email address: shelli.tompkins@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	~~84,137~~64,137	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 have a) showed what impact irrigation decisions have on fruit size, cork spot, and other fruit quality metrics b) documented the return on investment of different case studies; c) document the changes in the water efficiency of each of these strategies. We have combined research and Extension-based approaches to collect and deliver industry-relevant information on pear irrigation practices in Washington State.

SIGNIFICANT FINDINGS

Cork spot was the highest in the research orchard when trees were watered fully or when water was withheld later in the season. When water was withheld early in the season or a stem water potential based decision process was used, cork spot % was lower.

Late season water deficits also had lower fruit firmness suggesting that it affects fruit maturity going into storage.

Irrigation strategy had no major impact on fruit nutrient composition.

For a commercial orchard (Caudle-Dryden Case Study) located on a large hill, irrigation distribution was improved using microsprinklers versus impact sprinklers. Fruit weight in the upper part of the orchard was equal to the bottom section when microsprinklers were used but there were large differences between the upper and lower sections in the section with the old irrigation system. These improvements were enough to pay for the irrigation system in just one year of larger fruit.

Five different irrigation case studies were used to demonstrate problem solving and value to the industry for changing irrigation practices in pear orchards. These pear orchards were located in the Wenatchee Valley in 2018-2020. Two of the case studies had challenges with distribution on sloped terrain either from poor pressure or from excessive runoff and uneven distribution within the orchard. Two other orchards had the desire to use soil moisture monitoring to make more precise irrigation decisions to control vigor and cork spot. The last orchard had issues with dynamic pressure from intake filter plugging in the canal and was fixed with a simple change to the filter that saved man hours and ensured consistent delivery and water pressure to their orchards.

Stem water potential based irrigation appeared to be a better approach to managing irrigation decisions but it still remains labor-intensive. We will be pursuing plant-based irrigation sensors as part of a two-year technology proposal beginning in 2021 on both apple and pear.

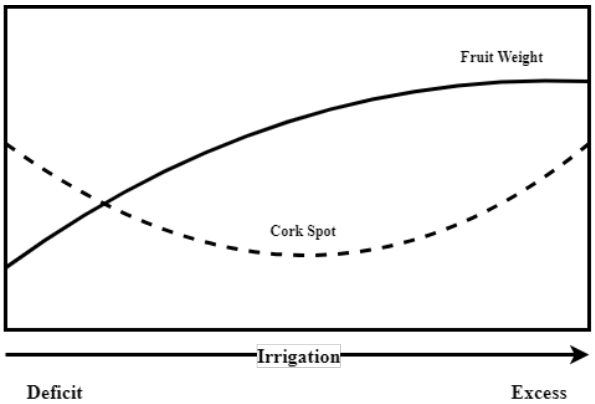

Figure 1. Conceptual figure showing the relationship between irrigation strategy and fruit weight and cork spot incidence in pear

Results and Discussion

Research Study

For this objective, 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 August 30, 2018, September 2, 2019, and August 28, 2020 from sample trees. Fruit was stored in regular atmosphere at 33 °F for 12 weeks. After storage, fruit was placed on racks to ripen for 7 days at 68 °F. 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 K, Ca, and Mg to look for changes in the ratios among these competing nutrients that may correspond to differences in cork spot or fruit size.

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} was assessed using a 3005 Series Plant Water Status Console (Soilmoisture Equipment Corp, Goleta, CA, USA). Leaves used for measurement of Ψ_{md} were 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.

Soil moisture was substantially lower during the early season period in late June and early July and for late July and August for the late summer deficit period (Figure 2). Where trees were watered based on stem water potential, soil moisture followed similar patterns as the control. During hot periods, water was turned on so that delivery was equal to the control during this period because stem water potential was below the threshold for much of this time (Figure 3). Stem water potential appeared to be a good approach to ensuring that over irrigation did not occur. By sampling twice per week, we were able to gauge tree stress in each block and associate it with plant demand at that time. Later in the season, regardless of irrigation frequency, stem water potential at this site was above the -1.0 MPa threshold because of hot, dry conditions and sandy soil. A richer soil with a greater water holding capacity may not have produced the same results.

Cork spot was the highest for the excessively irrigated control and when deficit irrigation was applied during late summer before harvest. Cork spot was lowest for when stem water potential was used all season and when early season water deficits were applied (Figure 4; P=0.09). Fruit quality was relatively unaffected by irrigation treatments (Table 1) although physiological metrics indicate that the tree was affected (data not presented here and will be presenting in publications or Extension material). Fruit weight, height: width ratios, and soluble solids content were unaffected by treatments in both 2019 and 2020. Fruit firmness had a tendency to be lower for fruit from the treatment where late summer deficits were applied indicating that there may be an effect on fruit maturity. Abiotic stress in fruit has been shown to accelerate ripening in apple and other tree fruit and may also be related to elevated cork spot incidence observed in this treatment.

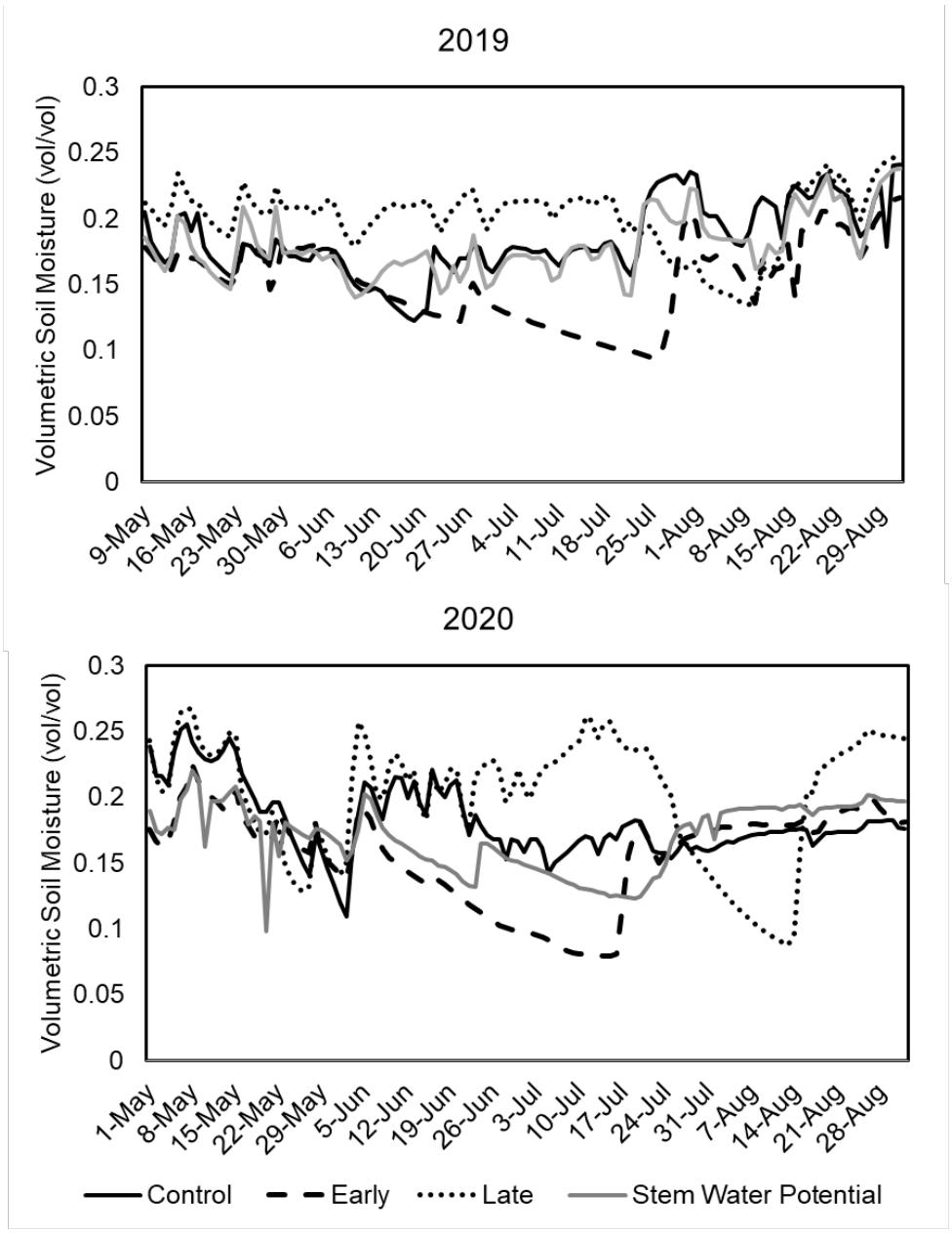

Figure 2. Soil moisture at 12" depth From May 1 to September 1 for 2019 and 2020 for each of early summer deficit (Early), late summer deficit (Late), stem water potential based irrigation (Stem Water Potential) treatments compared to an fully irrigated control (N=3) measured with a Meter Group EC-5 volumetric soil moisture sensor

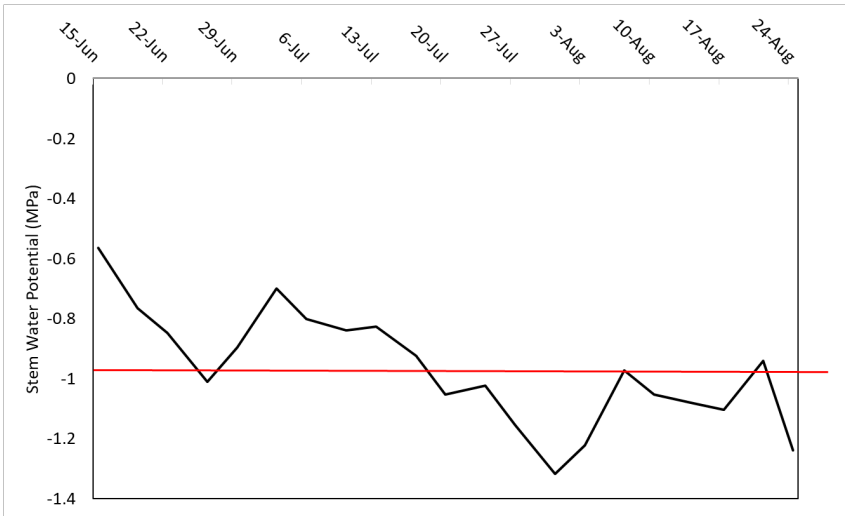

Figure 3. Stem water potential measured every Monday and Friday from June 15 to August 24, 2020. Red line represents the threshold for irrigation to occur.

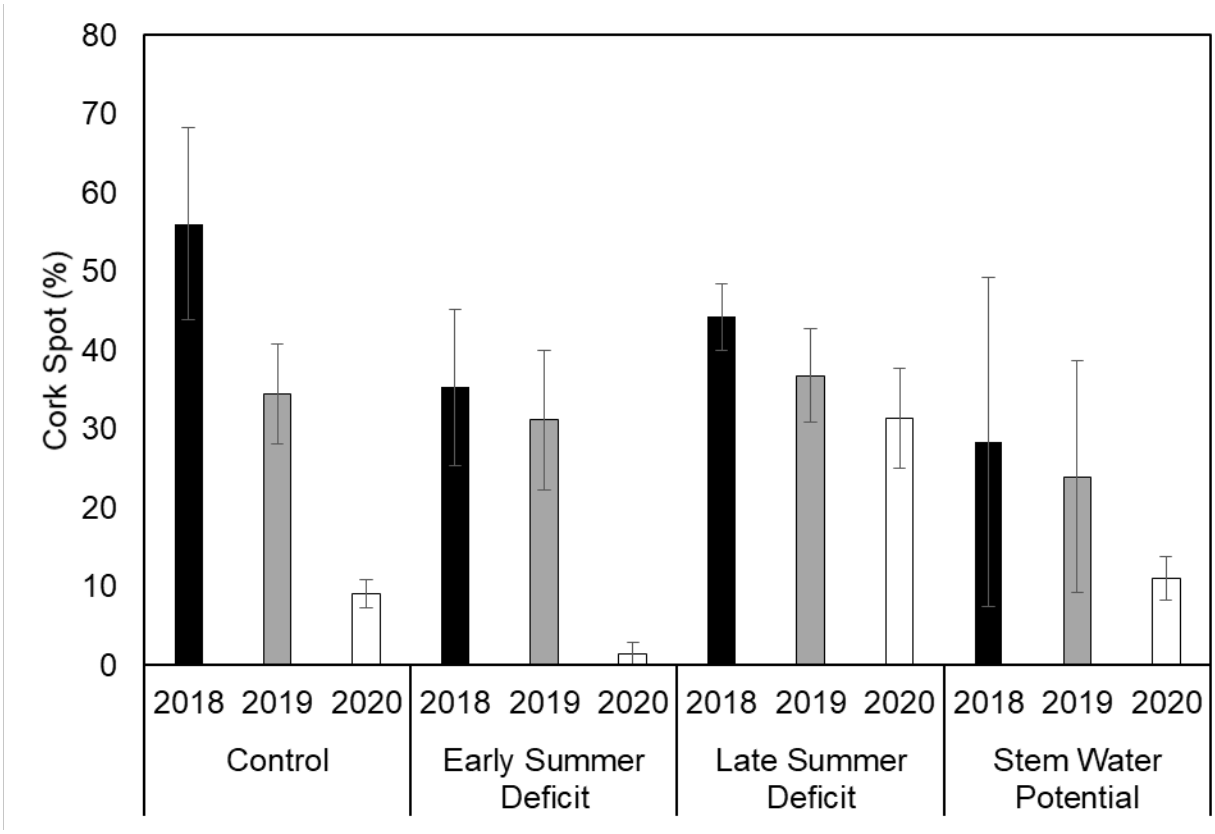

Figure 4. Cork spot incidence (%) for Anjou pear for each of early summer deficit (Early), late summer deficit (Late), stem water potential based irrigation (Stem Water Potential) treatments compared to an fully irrigated control (N=3).

Table 1. Mean fruit weight, shape, soluble solids content (°Brix), and fruit firmness (lb) of D'Anjou pears harvested in 2019 and 2020 after two months of storage at 33°F and then ripened for 7 days at 68°F

	Weight (oz)	Height: Diameter	Soluble Solids Content (°Brix)	Fruit Firmness (lb)
2019				
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
2020				
Control	6.99 a	1.13 a	14.58 a	7.8 b
SWP	6.95 a	1.18 a	14.5 a	6.5 ab
Early	6.35 a	1.17 a	15.18 a	7.1 ab
Late	7.48 a	1.15 a	15.05 a	5.7 a

Dryden Case Study

Challenge: Excessive runoff and small fruit size, particularly at the top of the hill.

Solution: This change was completed by the grower in 2017-2018. The cost for this conversion, not including labor, was $1,489 per acre. The changes resulted in an additional ~$2,400 per acre for three years with improvements to fruit quality. 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) installed at a 18' x 18' spacing (134 heads/ A). At 50% efficiency the standard system delivers 0.15 in/hr, at 70% efficiency the upgrade delivers 0.09 in/hr and 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.

Summary: 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).

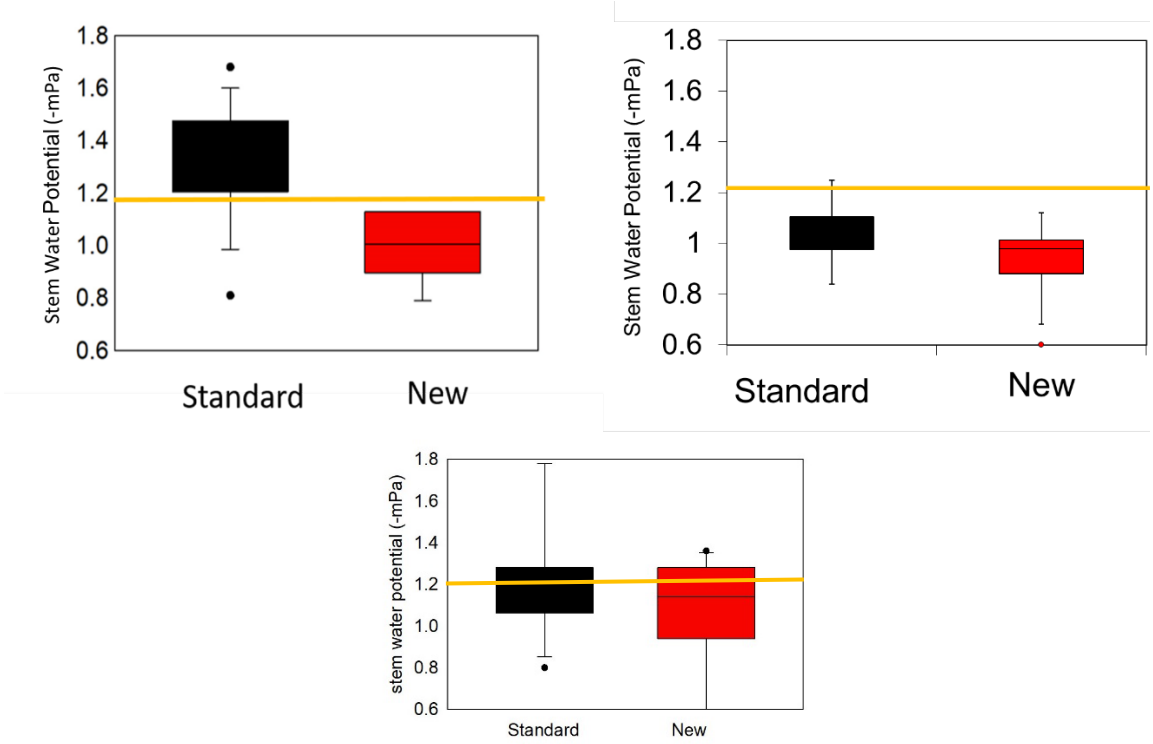

Figure 5. Box plot distribution of stem water potential for trees irrigated using the standard system compared to the modified (new) irrigation system.

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).

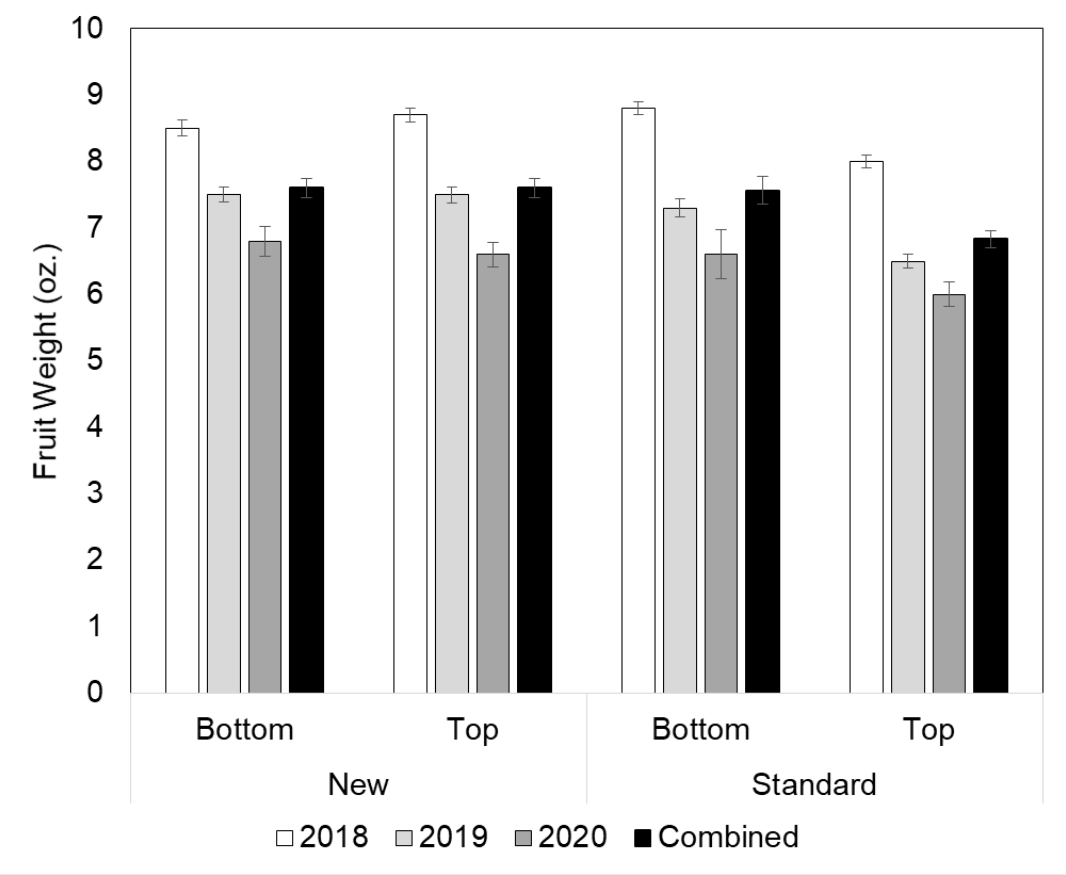

Figure 6. Fruit weight for D'Anjou pears with either the new system or old system in 2018 (solid bars) or 2019 (patterned bars).

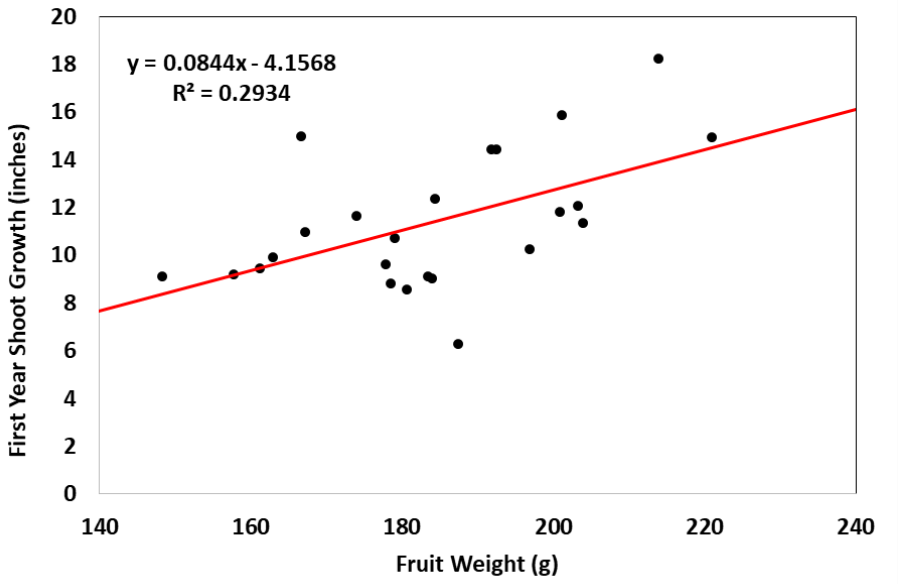

Figure 7. Fruit weight plotted against first-year shoot growth for commercially grown Anjou in Dryden.

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 , 2018 (Washington Tree Fruit Association Weekly Grower's Bulletin) dollar values were assigned to each size class for US #1 fruit. In 2019 the upgrade packed 470 boxes of large fruit (90+) US#1 compared to 441 in the standard, a total of 521 vs 519. In 2019 assuming prices per box of 60s to 90s:$29.7; 100: $27.2; 110:$25.5; 120+:$23 revenue per acre was $23,482 in the upgraded block compared to $21,248 in the standard.In 2020, the packout for the upgraded block was 91.9% compared to the standard of 91.07%. 8% of the culls in the standard block were due to small fruit and 4% to cork whereas the upgraded block had 0% small fruit and 0% cork resulting in culls. The majority of culls in 2020 were due to stem punctures 50-54%. In 2020 assuming prices per box of 90s+:$26.13; 100:$23.23; 110:$21.63; 120-:$24.38 revenue per acre was $21,542 in the upgraded block compared to $21,833 in the standard but with reductions in culls from small fruit and cork in the upgraded block.

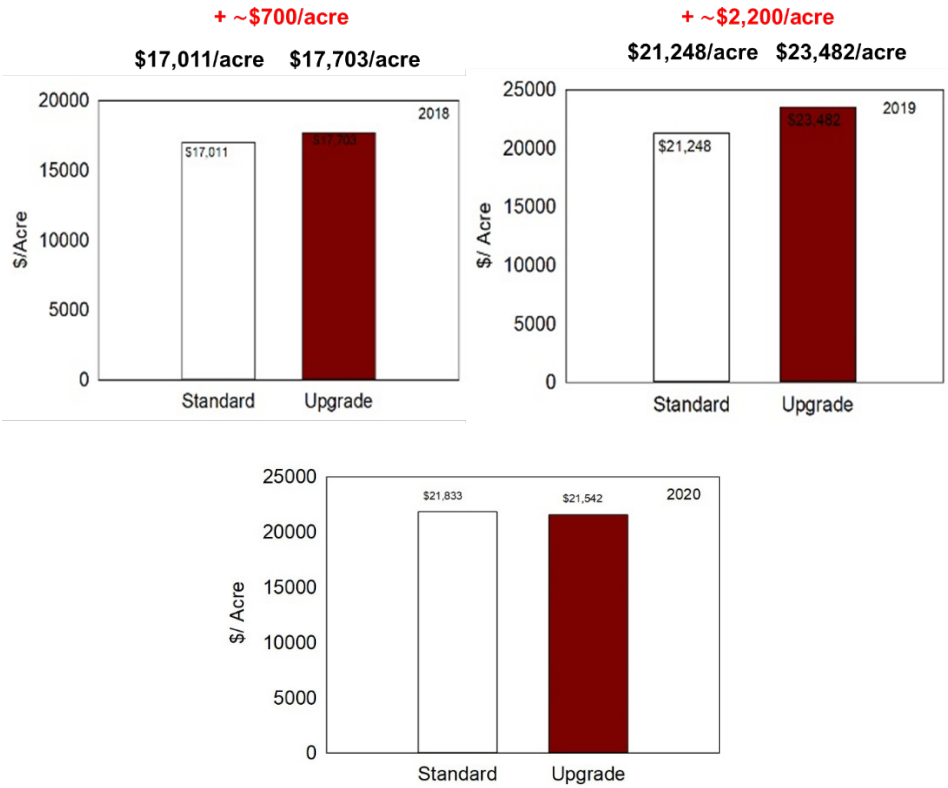

Figure 8. Estimated returns per acre in 2018-2020 for the Dryden upgraded orchard section compared to the standard irrigated control. Significant improvements were observed in 2019, largely due to increased yields and more consistent fruit size reported above. In 2020, there were no culls from small fruit and cork (compared to 8 and 4% of culls from small fruit and cork for the standard block, respectively) in the upgraded block. The packout for the upgraded block was 91.9% compared to the standard of 91.1% and was mostly due to higher stem punctures in the upgraded block.

Cashmere A Soil Water Content Monitoring Case Study

Challenge: **S**evere cork. The block was not picked in 2017 due to 80% cork. In general, there was poor control over water delivery and a concern of over-irrigation

Solution: Soil moisture sensors were installed in 2019 to inform watering decisions with the goal to meet an irrigation window. The costs for this monitoring was $304/acre annually for approximately 10 acres.

Summary: Water monitoring was installed in 2019 to help the grower make decisions on when to water and provided an irrigation target window to try to keep soil moisture within. The part of the block with irrigation decisions made using the window had substantially lower cork culls than the block that was irrigated using the normal, traditional schedule. In 2020, the block lease was not renewed but the single year data indicated substantially improved packouts and reduced culls from not excessively watering in the block with soil moisture monitoring in place.

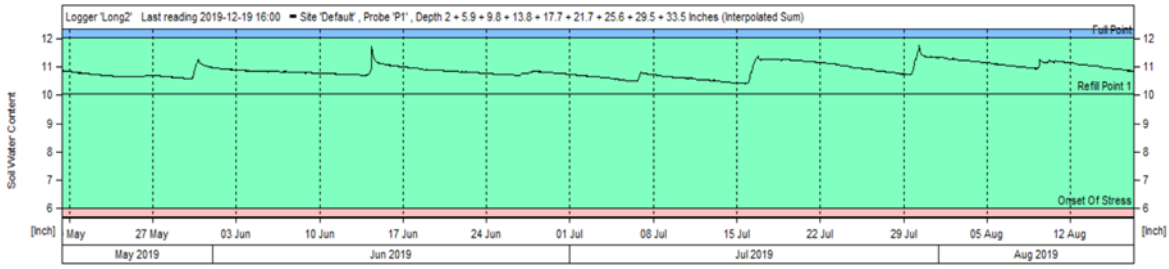

Figure 9. Output from soil water content monitoring indicating full point, refill point, and onset of stress with the goal of maintaining soil moisture between the refill and full point.

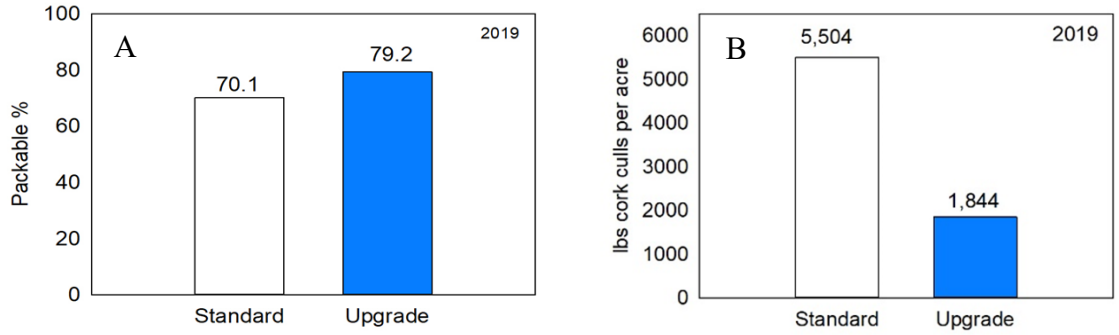

Figure 10. A. A comparison of packout percentage for the normally irrigated standard compared to the soil water content informed scheduling section. B. A comparison of the total weight of cork culls per acre in the normally irrigated standard compared to the soil water content informed scheduling section.

Cashmere B Water Distribution Improvement Case Study

Challenge: Poor water pressure at the top of the orchard and non-uniform irrigation sprinkler heads.

Solution: A pressure elevating pump at the canal at the top of the orchard was installed to improve distribution new sprinkler heads installed for uniform distribution throughout the orchard. The cost of making these changes was approximately $475/acre for 4.2 acres. These changes were made in early season of 2020. Stem water potential measurements were made during a hot period at the end of August and then fruit was sampled from six trees per plot in upper and lower sections that were either changed or were equal to the old system.

Summary: In 2020, there were no differences in returns per acre. This may be a result of this change being applied too late in the season to have a major effect. We will continue monitoring this site in 2021 if the grower is receptive to this to gather more information on this change. We did, however, observe more stable and higher stem water potential in the upgraded orchard section indicating more uniform water distribution among the trees.

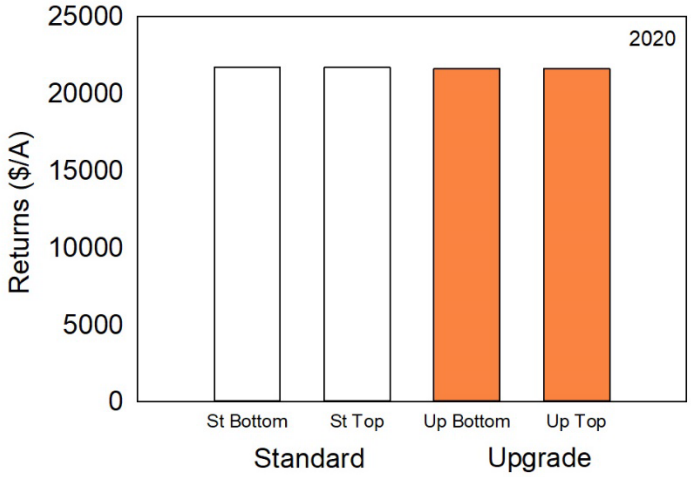

Figure 11. Return estimates per acre for the old system (Standard) compared to the upgraded system (Upgrade) for the bottom and top of the orchard. There were no differences between the sections in total returns after three months of the change in place.

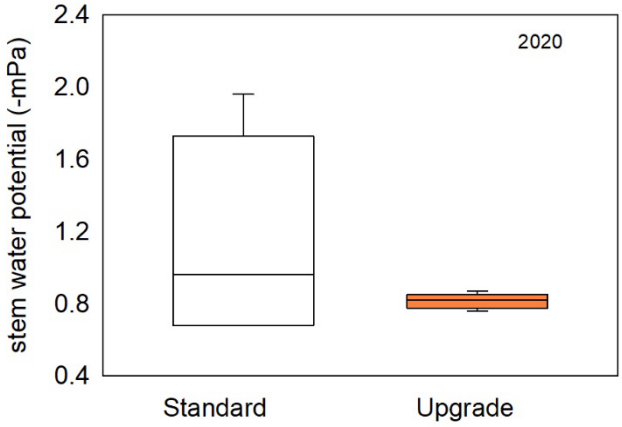

Figure 12. Stem water potential for the standard block compared to the upgraded block at a sloped site where water distribution is a problem.

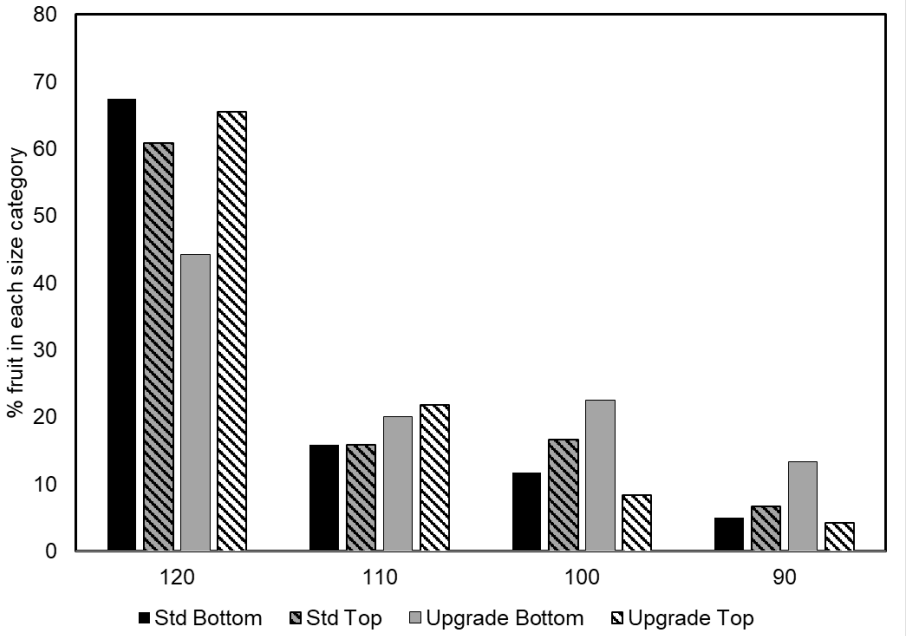

Figure 13. The % of fruit belonging to each size category (90 box size and higher, 100 box size, 110 box size, and 120 box size and smaller) for fruit harvested from six random trees within each section in 2020.

For additional details on case studies visit **http://treefruit.wsu.edu/orchard-management/irrigation-management/improving-irrigation-efficiency/**

Extension Outputs

May 27, 2020. Pear Irrigation Virtual Field Day. – 60 participants

January 28, 2020. Irrigating for Fruit Quality. Pear Day. Wenatchee, WA. Kalcsits, L., DuPont, S.T.

Improving Irrigation Efficiencies in Pears Case Studies. DuPont, S.T., Kalcsits, L. 2020. http://treefruit.wsu.edu/orchard-management/irrigation-management/improving-irrigation-efficiency/

Using Irrigation Sensors Video with Troy Peters. Improving Irrigation Efficiency in Pears Virtual Field Day. *DuPont, S.T., Peters, T., Kalcsits, L. May 2020.* http://treefruit.wsu.edu/videos/using-irrigation-sensors-troy-peters/

Irrigation Sensors with Jac LeRoux. Improving Irrigation Efficiency in Pears Virtual Field Day. *DuPont, S.T., Peters, T., Kalcsits, L. May 2020.* http://treefruit.wsu.edu/videos/irrigation-sensors-with-jac-leroux-improving-irrigation-efficiency-in-pears-virtual-field-day/

Long Case Study. Improving Irrigation Efficiency in Pears Virtual Field Day. *DuPont, S.T., Peters, T., Kalcsits, L. May 2020.* http://treefruit.wsu.edu/videos/long-case-study-improving-irrigation-efficiency-in-pears/

Caudle Case Study. Improving Irrigation Efficiency in Pears Virtual Field Day. *DuPont, S.T., Peters, T., Kalcsits, L. May 2020.* http://treefruit.wsu.edu/videos/improving-irrigation-efficiency-in-pears-caudle-case-study-summary/

EXECUTIVE SUMMARY

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

Key words: Volumetric soil moisture content, stem water potential, Anjou, cork spot,

Abstract: Irrigation is essential for the production of high quality pears in the Pacific Northwest (PNW). PNW pear orchards are distributed among varying soil types, topographies, and environments and older, low density pear orchards have root systems that extend deep into the soil profile combined with older irrigation systems with uneven distribution can create problems of over watering or under watering occurring within, or between blocks. With this project, we sought to identify water management factors contributing to poor sizing or losses due to cork spot in PNW orchards. We conducted a three year research experiment looking at the timing and frequency of irrigation in an Anjou orchard. We found that irrigation within a given soil moisture window did not substantially alter fruit quality metrics in a uniform orchard block. However, late summer water deficits promoted higher cork spot and a trend towards reduced firmness after two months of storage and 7 days of ripening at room temperature. This project also conducted five case studies to improve water delivery and solve common problems experienced in pear orchards in the PNW. These included two orchards with poor distribution on a hilly site, two orchards with the heavy soils and issues with over watering and one orchard with a filter that clogged causing losses in pressure between cleaning. In two of the most documented cases studies, we observed significant improvements in packouts and returns per acre that were economically feasible for the adoption in other orchards experiencing these problems. The other case studies demonstrated the value of soil moisture monitoring, even water distributions, and filter cleaning. Incremental changes to irrigation systems to ensure that neither excess nor deficits are being experienced in an orchard as both of these scenarios can cause increased losses from cork spot in the packing house and can affect overall fruit sizing. In this project we show the value in meeting irrigation needs through enhanced monitoring, more uniform distribution, or less variable supply in pear orchards.

California Pear Update – Bob McClain
No report submitted