2023 NW Pear Research Review

Bloom counts and trunk measurements for a bartlett chemical thinning trial.

Photo Source: Gera Garcia

February 16, 2023

Hybrid Format
Wenatchee, WA

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

Project # PR-20-100

Report Type: Final Project Report

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Project Duration: 3 Year

Total Project Request for Year 1 Funding: $89,984
Total Project Request for Year 2 Funding: $33,794
Total Project Request for Year 3 Funding: $101,308

Other funding sources: Awarded
Funding Duration: 2020-2023
Amount: $249,926
Agency Name: WSDA – Specialty Crop Block Grant
Notes:

WTFRC Collaborative Costs: none

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Item	2020	2021	2022
Salaries[1, 2]	$53,592	$1,900	$57,965
Benefits	$18,641	$569	$20,162
Wages[3]	$9,600$	$9,984	$10,383
Benefits	$901	$937	$974
Equipment[4,5]	$6,000	$8,280	
Supplies[6]	$1,250	$11,400	$11,100
Travel		$724	$724
Miscellaneous			
Plot Fees			
Total	$89,984	$33,794	$101,308

Footnotes:
[1]Research Assistant Professor (Nottingham) = 2% FTE, $7,612.50/month for 12 months x 1.04/year + 29.9% benefits [2]Postdoctoral Research Associate = 100% FTE, $4,313.75/month for 12 months x 1.04/year + 35% benefits [3]Summer Time Slip = $15.00/hr x 40 hr/week x 16 weeks x 1.04/year + 9.4% benefits [4]Toward vehicle purchase [5]Meter Group weather sensors and data loggers for field plots [6]Sampling supplies, pesticides and labor for commercial plot experiments (spraying, pruning, washing) [7]Gas for travel to orchard sites = $3.25/gallon at 20 mpg for 2,000 miles/year + $100 maintenance (years 2 and 3)

OBJECTIVES:

Obj. 1. **Build a pesticide effects database**. Compile information on psylla life-stage susceptibility and non-target effects data from previous studies and perform new experiments to fill knowledge gaps. Use this database in conjunction with the pear psylla phenology model to design the phenology-based management program in Obj. 3.

Obj. 2. **Enhancing the management program with cultural techniques**. Perform field trials to determine optimal timings for kaolin applications, tree washing, and summer pruning at strategic timings.

Obj. 3. **Design and validate the pear psylla phenology-based management tool**. Use the current phenology model and findings from Obj. 1 and 2 to design an optimal spray program for pear psylla. Test this program against standard conventional programs on 2-4 acre plots in commercial orchards and compare costs, pests, natural enemies, and pest injury.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS:

(In order of importance)

- **Phenology-based IPM Program Development**: An phenology-based IPM program for pear psylla was developed and made publicly available on the WSU Tree Fruit Extension website (http://treefruit.wsu.edu/crop-protection/psylla-phenology-model/) and DAS (https://decisionaid.systems/). The program includes a degree day model and appropriate timings for insecticide sprays, kaolin sprays, honeydew washing, and summer pruning.
- **Testing the Program**. The phenology IPM program was tested in large commercial plots in 2021 and 2022. The phenology program provided equal control of honeydew fruit injury as conventional orchards. The IPM phenology program resulted in 95% reductions in psylla overwintering adult populations in October compared with conventional orchards. 2022 Seasonal results are publicly available at the WSU Tree Fruit Extension site: http://treefruit.wsu.edu/crop-protection/pear-ipm/2022-pear-pest-scouting/
- **Economics:** The IPM phenology program developed in this project cost $280/acre less than conventional programs, on average. If implemented throughout the 20,000 acres of pears WA, it would save the WA industry $5.6 million per year.
- **Providing Extension:** All information from the project is available online, including the model, recommendations, and real time scouting data. Additionally, we broadcasted summaries of results and reminders of our online resources via 3 Fruit Matters Newsletter articles in 2021 and 4 in 2022. We also hosted two major Extension events including a pear IPM field day at one of our IPM orchards in Peshastin (Sept 2022) and a day-long pear IPM Fruit School in Wenatchee (Dec 2022, organized by T. DuPont).
- **Insecticide Efficacy:** Insecticides shown to be effective on pear psylla and pose low risk to natural enemies include Surround (kaolin), Celite (diatomaceous earth), Esteem (pyriproxyfen), Ultor (spirotetramat), Centaur (buprofezin), Cinnerate (cinnamon oil), Aza-Direct (azadirachtin), and 440 IAP oil. Additional products that are effective on pear psylla, but should be limited due to high risk to natural enemies include Bexar (tolfenpyrad), Assail (acetamiprid), and Actara (thiamethoxam). Malathion, while effective in the lab, has shown low efficacy in the field.
- **Surround timings:** Delayed dormant was the most effective Surround timing. A second spray significantly improved suppression of eggs and nymphs, particularly if applied at budburst. Late fall (early November) Surround sprays helped orchards that cannot be sprayed in the early spring due to wet terrain, but should not replace the early spring spray as they are less effective.

METHODS AND RESULTS:

Obj. 1. Build a pesticide effects database.

Methods. A literature review was conducted to determine all known results from pesticide tests on pear psylla and spider mites in pears. New bioassays were conducted in the summers of 2020, 2021, and 2022 to determine the psylla life stages most susceptible to various selective insecticides. Sprays targeting adults, eggs, and early nymphs were compared for each product. All bioassays followed similar methods with some minor alterations between experiments. Bioassays were conducted using potted d'Anjou pear trees grafted on OHFD rootstocks, 3-5 years old. Adult pear psylla were collected from an untreated pear psylla orchard at the TFREC, gently anesthetized with CO_2, and separated into groups of 6 females and 4 males. Adults were place in 23 x 17cm mesh bags and secured over first-year shoots with at least 4 leaves. Each bag of adults was assigned an insecticide treatment (product and rate) and timing (adult, egg, or nymph). Sprays were made through mesh bags using a 0.5 L aluminum misting bottle. Applications applied to adults were made the same day adults were collected and bagged on shoots. Four to seven days after bagging, all bags were removed, adults were brushed off plants, eggs were counted, and bags were replaced over shoots. The group selected for egg treatments were sprayed in the same manner, then re-bagged. After 7 to 10 days, nymphs were counted and nymphs sprays were made. Further counts occurred every 5 to 7 days until all late instars had become adults, which were counted.

Results: Results from literature review and past years insecticide bioassays have been incorporated into the Crop Protection Guide for Tree Fruit https://cpg.treefruit.wsu.edu/. This includes efficacy rating for effective and non-effective products. In collaboration with Tianna DuPont, we incorporated recommendation information for most effective materials in to an updated Pear IPM fact sheet that has been peer-reviewed by the Extension-review board and published on the WSU Tree Fruit Extension website: http://treefruit.wsu.edu/crop-protection/opm/pear-psylla/. Table 1 shows generalized results from efficacy tests conducted in the past three years. These products were selected based on efficacy demonstrated in past work. Not all products tested are displayed; those displayed had repeated efficacy when sprayed on a given life stage (i.e., eggs) in at least two trials. Life stage sprayed does not necessarily mean life stage killed. Selective materials often prevent development, so mortality occurs at future life-stages. However, it is more important for growers to know when to spray instead of what stage is affects, hence our designation "life stage sprayed."

Table 1. Insecticide demonstrating efficacy for selected products relevant to the phenology model. A **+** indicates that the product caused significant mortality, relative to the check, in at least two trials.

	Life stage sprayed*			
Product	**Adult**	**Egg**	**Instars 1-3 (young nymphs)**	**Instars 4-5 (hardshells)**
Surround (kaolin)	+	+	+	
Celite (diatomaceous earth)	+	+	+	
Oil 440	+	+	+	
Esteem (pyriproxyfen)	+	+		
Ultor (spirotetramat)	+	+		
Cinnerate (Cinnamon oil) 60 fl oz/100 gal [1]	+	+		
Aza-Direct (azadirachtin)				
Bexar (tolfenpyrad)[2]	+	+	+	+
Assail (acetamiprid) [2]	+	+	+	+
Actara (thiamethoxam)	+		+	

*Not necessarily the life stage killed.

[1] Lower rates of 30 and 40 fl oz/100 gal were not effective.

[2] Should not be used more than once per season due to high disruption of natural enemies

Obj. 2 Enhancing the management program with cultural techniques.

2a. Surround Timings

Methods. To determine optimal timings for kaolin applications, Surround WP (kaolin) was applied at 50 lb/acre (200 gpa for large trees, 100 gpa for small trees) to small, replicated plots at various timings in the fall of 2020 and spring of 2021, and again the following year. Each timing was considered a treatment, and received 5 replicate 4-tree plots at both the Wenatchee (TFREC, large trees) and Rock Island (Sunrise, small trees) orchard (10 replicates, 40 trees per treatment timing, total). In year 1, each set of trees was treated at one of the following phenological timings: fall (10 Nov), delayed dormant (4 Mar), budburst (30 Mar), 60% petal fall (21 Apr). Due to the clear advantage observed from the delayed dormant timing in year 1, and considering that this is the most common spray performed commercially, in year 2, we examined which spray timing was optimal in addition to the delayed dormant spray. Therefore, in year 2, all trees (including checks) were sprayed at delayed dormant (3 Mar) in additional to another treatment at either: fall (3 Nov), bud burst (25 Mar), bloom (26 Apr), or petal fall (11 May).

Results. In year 1 (2020-2021), the delayed dormant spray resulted in the greatest decrease in psylla compared to check plots for adults, eggs and nymphs in both large and small trees (Fig. 1, data only displayed for eggs and nymphs in large tree plots). The fall and budburst sprays also significantly suppressed eggs and nymphs compared with the checks, but to a lesser degree than delayed dormant. The 60% petal fall spray did not provide suppression of eggs or nymphs compared with the check.

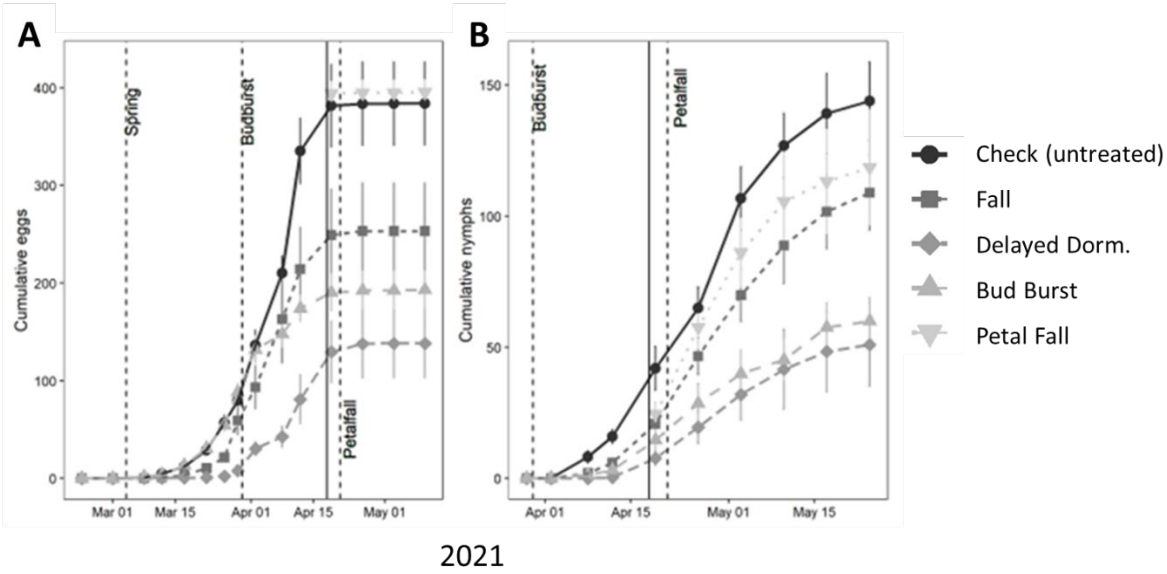

Fig 1. 2021 cumulative psylla densities (new count averages added to previous date) for eggs (A) and nymphs (B) resulting from Surround sprayed at various application timings.

In year 2 (2021-2022), we tested to see which spray timing would be optimal in addition to a ubiquitous delayed dormant spray (Fig. 2). Both budburst and fall sprays provided significant and similar egg suppression to the check, while budburst and petal fall provided significant and similar nymph suppression to the check. Interestingly, the petal fall spray had the least egg suppression compared with the check. The fall spray provided intermediate suppression of eggs, but no additional control of nymphs.

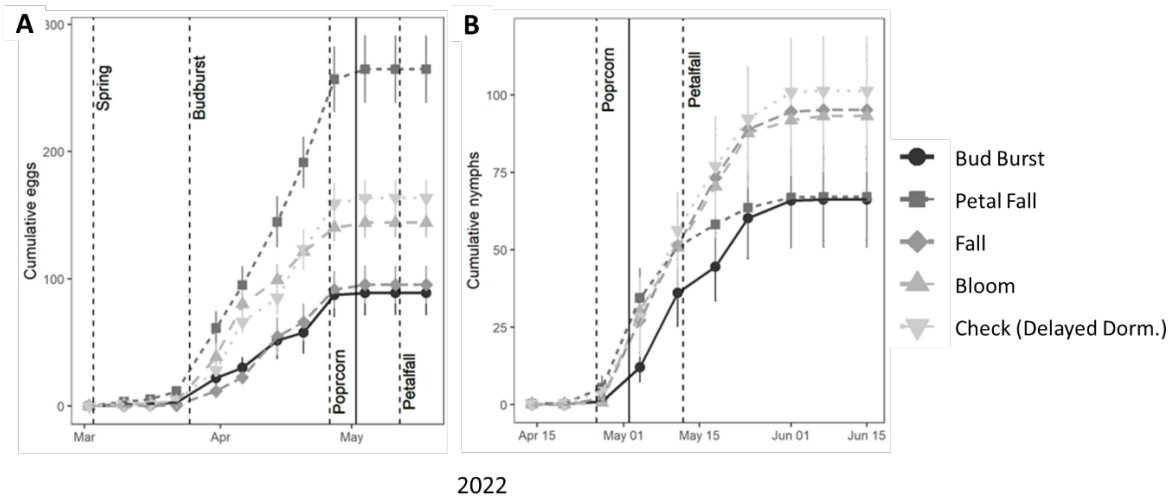

Fig 2. 2022 cumulative psylla densities (new count averages added to previous date) for eggs (A) and nymphs (B) resulting from Surround sprayed at various application timings. All trees were treated once at delayed dormant.

Conclusions: If Surround is only applied once, delayed dormant is the optimal timing to suppress psylla; nevertheless, other prebloom spray timings will also improve suppression. A single spray at petal fall does not appear to improve suppression

In addition to the optimal delayed dormant spray, a second Surround spray will likely improve suppression further, particularly the at the budburst timing. Adding a petal fall spray may worked well to suppress nymphs, but lack of egg suppression is concerning. Adding a fall spray suppressed eggs, but not nymphs, suggesting that this may not be a good addition to a delayed dormant spray. Fall Surround sprays are probably best for situations when a delayed dormant spray cannot be made.

2b. Honeydew Washing Timing:

Methods: An experiment was conducted to establish honeydew washing thresholds based on visual leaf inspections for honeydew droplets. The number of leaves with honeydew droplets was counted on trees each week in 10 commercial orchards (3 conventional, 3 organic, and 4 IPM). Ten trees in each orchard were used, on which 10 leaves and 20 fruit were sampled for presence or absence of honeydew. The number of leaves with honeydew per 100 leaves and number of fruit with honeydew per 200 fruit were determined in each orchard every week. Five percent of fruit affected by honeydew was considered the tolerance threshold.

A second experiment was conducted to determine how many leaves need to be sampled per orchard to accurately estimate the percentage of honeydew affected leaves. One shoot with at least 10 leaves was collected for each of 100 trees at 6 orchards (100 shoots per orchard). The percentage of honeydew affected leaves was calculated for each shoot, and averages for increments of 5 shoots leading up to 100. The monitoring level was established as the number of shoots at which the average honeydew level did not differ from the full 100 shoot sample (i.e., sampling 7 or more shoots provided the same percentage honeydew affected leaves and error as sampling 100 shoots).

Results. The IPM and organic orchards stayed below 5% of honeydew affected fruit throughout the summer. Percentage of honeydew affected fruit increased in conventional orchards in week 8, hitting 20% followed by over 30% in week 9 (Fig. 3). For affected leaves, IPM orchards and conventional orchards both hit 20% in week 6, but only conventional orchards continued to rise. Prior

to week 8, honeydew on leaves hit 35%, suggesting that the visual threshold is between 25 and 35%. Therefore, our honeydew washing threshold is 30% of leaves with visible honeydew droplets.

Between 5 and 10 shoots per orchard area provided the same results as sampling 100 shoots, therefore, 7 was established as the minimum number of shoots to be sampled per orchard area to measure leaf honeydew levels for threshold monitoring. In orchards with known differences in pressure, the 7 shoot rule should be used per "pressure zone".

Conclusions: About 7 shoots with 10 leaves each (70 leaves total) should be monitored for honeydew in each orchard zone. If 30% of the total (21 out of 70 leaves) have visible honeydew droplets, washing should be performed.

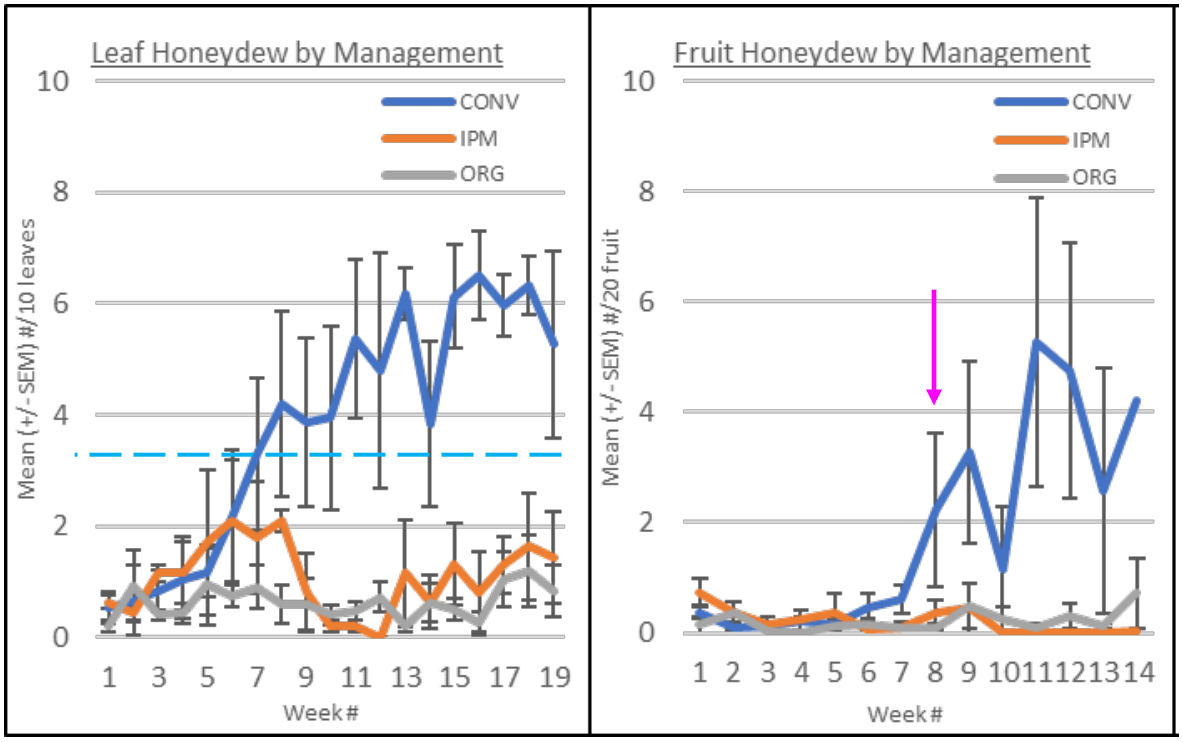

Fig. 3. Left: Mean (+/- SEM) no. of leaves with visible honeydew bubbles per 10 leaves from 10 trees per orchard per week. Right: Mean (+/- SEM) no. fruit with visible honeydew per 20 fruit from 10 trees per orchard perweek. Pink arrows show where fruit injury signficantly increased (week 8). Blue dashed line shows the level of honeydew on leaves (measured in no. of leaves with visible honeydew droplets) preceding fruit injury where signficant differences in honeydew are estimated to occur, indicating leaf honeydew thresholds preceding fruit injury.

Obj. 3 .Design and validate the pear psylla phenology-based management tool

3a. Model Recommendations Development:

Methods: An optimized spray program was developed using Surround (kaolin), Esteem (pyriproxyfen), Ultor (spirotetramat), Aza-Direct (azadirachtin), Cinnerate (cinnamon oil) and horticultural oil at strategic timings. Selective materials and timings for mites, mealybugs, and codling moth were also included. The program was developed using a holistic approach that not only aligned materials with their best psylla life stage target, but also considered elements like cost savings, potential non-target effects, vulnerable tree stages, convenience (i.e., grouping materials into single sprays when possible), logical constraints (i.e., avoiding bloom, particle film residues on fruit, etc.) and label restrictions (spray and pre-harvest interval minimums). Degree day timings for tree washing and pruning were incorporated based on pear psylla phenology (presence of nymphs) and

practical orchard management considerations (i.e., avoiding washing near bloom to avoid fire blight and pruning after shoots are fully developed).

Results: The pear psylla degree day model and corresponding recommendations timings have been made publicly available on in the Decision Aid System (https://www.decisionaid.systems/) and within the WSU Tree Fruit Extension Pear IPM website (http://treefruit.wsu.edu/crop-protection/psylla-phenology-model/). A shortened, two page handout has also been created for printing, and is available at http://s3.us-west-2.amazonaws.com/treefruit.wsu.edu/wp-content/uploads/2022/02/24171655/PDD-2022-Recs-and-Timings.pdf. The two-page handout is copied below in Fig 4 and Table 2.

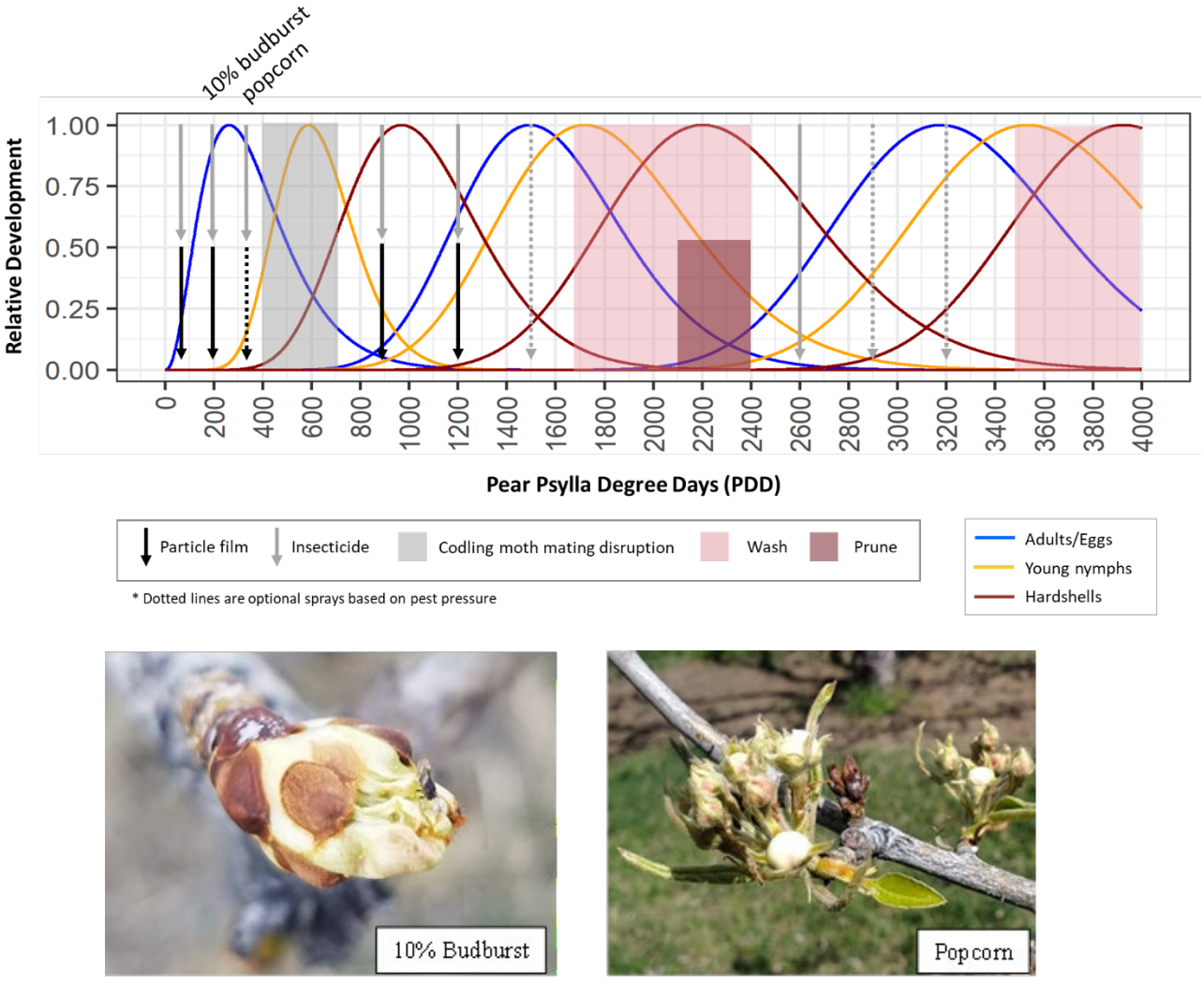

Fig. 4. Pear psylla degree day (PDD) model with overlayed management recommendations. Two timings are based on bud phenology instead of PDD (10% budburst and popcorn, pictures displayed under graph). Solid line arrows indicate "mandatory" sprays (recommended timings regardless of psylla pressure), dotted lines are for high pressure areas and/or years, and blocks are timeframes for cultural techniques. *Growers must follow labels above all else. While these suggestions fall in line with label recommendations, misinterpretations could lead to label breaches. For example, Esteem has three possible timings, but only two applications are allowed per season; therefore, only two of the possible timing can be used for Esteem.

Table 2. Recommendations and timings (either PDD or bud development) for management of pear psylla and other pests.

PDD or bud stage timing	Conditions	Conventional recommendations	Organic recommendations
75 PDD	winterform adults colonizing orchards	**Pear Psylla**: Surround CF or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Mites**: Lime Sulfur	**Pear Psylla**: Surround CF or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Mites**: Lime Sulfur
10% Budburst	10 % of buds opening from the tip.	**Pear Psylla:** Surround CF or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Pear Psylla/Scale**: Esteem	**Pear Psylla:** Surround CF or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Pear Psylla/Mealybug/Scale**: Cinnerate and/or Azadirachtin.
Popcorn	Before bloom. All buds have closed white petals.	**Pear Psylla/Scale**: Esteem **Mealybug/Psylla:** Centaur **Pear Psylla**: Surround or Celite @ 50lb/a if only one previous was made. A third Surround or Celite spray at 25 or 50 lb/ac can be made if psylla pressure is high (3 or more adult per tray)	**Pear Psylla/Mealybug/Scale**: Cinnerate and/or azadirachtin. **Pear Psylla**: Surround or Celite @ 50lb/a if only one previous was made. A third Surround or Celite spray at 25 or 50 lb/ac can be made if psylla pressure is high (3 or more adult per tray)
50% Bloom	egg lay and hatching nymphs	**Codling Moth:** Mating Disruption	**Codling Moth:** Mating Disruption
900 PDD	1-5% summerform adults/eggs	**Pear Psylla:** Surround WP or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Pear Psylla:** Ultor/Movento + Non-ionic surfactant **Codling Moth**: 1% Oil (375 CM DD)	**Pear Psylla:** Surround WP or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Pear Psylla:** azadirachtin and/or Cinnerate **Codling Moth**: 1% Oil (375DD)
1200 PDD	25% summerform adults/eggs	**Pear Psylla:** Surround WP or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Pear Psylla:** Ultor/Movento + Non-ionic surfactant **Codling Moth**: 1% oil + Altacor (525 CM DD)	**Pear Psylla:** Surround WP or Celite @ 50lb/ac. Add Spreader sticker for added residual efficacy, but mix carefully. **Pear Psylla:** azadirachtin and/or Cinnerate **Codling Moth**: 1% oil + Virus (525DD)
1500 PDD	50% summerform adults/eggs	**Pear Psylla**: Oil if low to moderate pressure (1-2 adults per tray. If high pressure (3 or more), use oil + Dimilin or Esteem **Codling moth:** 1% oil + Esteem or Dimilin based on moth capture	**Pear Psylla**: 1% oil if low to moderate pressure (1-2 adults per tray. If high pressure (3 or more), use oil + Dimilin or Esteem **Codling moth:** 1% oil + Virus based on moth capture
1700 - 2400 PDD	hardshells increasing	**Pear Psylla**: Honeydew washing if 30% of leaves have visible honeydew bubbles. If using overhead sprinklers, wash for no more than 12 hours at a time. If using an airblast sprayer, use volume of 800 gpa or greater.	**Pear Psylla**: Honeydew washing if 30% of leaves have visible honeydew bubbles. If using overhead sprinklers, wash for no more than 12 hours at a time. If using an airblast sprayer, use volume of 800 gpa or greater.
2200 PDD		Particle films are should not be used for the rest of the season because they can disrupt natural enemies and flare mites.	Particle films are should not be used for the rest of the season because they can disrupt natural enemies and flare mites.
2100 – 2500	hardshell peak, adults low	**Pear Psylla:** Summer prune to remove hardshell nymphs. Target shoots with visible honeydew for removal.	**Pear Psylla:** Summer prune to remove hardshell nymphs. Target shoots with visible honeydew for removal.
2600 PDD	15% summerform adults (2nd gen)	**Pear Psylla/Codling moth:** Dimilin or Esteem	**Pear Psylla:** 1% Oil, azadirachtin and/or Cinnerate. Be care with sensitive varieties. Do not use azadirachtin products on Comice.
2900 PDD	35% summerform adults (2nd gen)	**Pear Psylla/Codling moth:** oil 1%. **Pear Psylla:** If 2 or more psylla adults per tray, include Dimilin or Esteem.	**Pear Psylla:** 1% Oil. **Pear Psylla:** If 2 or more adults per tray, include azadirachtin and/or Cinnerate. Be care with sensitive varieties. Do not use azadirachtin products on Comice.
3200 PDD	50% summerform adults (2nd gen)	**Pear Psylla/Codling moth:** oil 1%. **Pear Psylla:** If 2 or more psylla adults per tray, include Dimilin, Esteem, or an organic material such as azadirachtin or Cinnerate. Be care with sensitive varieties. Do not use azadirachtin products on Comice.	**Pear Psylla/Codling moth:** oil 1%. **Pear Psylla:** If 2 or more psylla adults per tray, include azadirachtin or Cinnerate. Be care with sensitive varieties. Do not use azadirachtin products on Comice.
3500 PDD – Harvest	hardshells increasing to peak	**Pear Psylla**: Honeydew washing if 30% of leaves have visible honeydew bubbles. If using overhead sprinklers, wash for no more than 12 hours at a time. If using an airblast sprayer, use volume of 800 gpa or greater.	**Pear Psylla**: Honeydew washing if 30% of leaves have visible honeydew bubbles. If using overhead sprinklers, wash for no more than 12 hours at a time. If using an airblast sprayer, use volume of 800 gpa or greater.

3b. Testing the Pear Psylla IPM Phenology Model:

Methods: A pilot study to test outcomes of the phenology-based IPM program was conducted in 2021 in commercial orchard blocks being used for another pear-IPM focused project led by Nottingham and DuPont (USDA-NIFA grant award #2019-70006-30443). Plots for this project were either managed as conventional, bIPM (biological-IPM), or organic. In previous years, bIPM plots simply avoided use of broad-spectrum materials (primarily using kaolin, Aza-Direct, Cinnerate, Esteem, Ultor; full list found in DuPont et al. 2021). However, in 2021, bIPM plots followed the phenology program established in this project (Obj. 3a). For each treatment (conventional, bIPM, and organic) there were 4 orchards plots at least 4 acres in size (16 plots total). Plots were sampled weekly throughout the season for all pear psylla life stages, mites, and natural enemies using standard methods of beat trays, bud inspections, leaf brushing, and sticky cards.

In 2022, the same treatments were examined ("bIPM" now called "phenology") in 19 orchards (8 conventional, 8 phenology, and 3 organic). The only change was that 4 phenology orchards were allowed one Bexar (tolfenpyrad) spray at delayed dormant, when risk of harming natural enemies is lowest. Each phenology plot had a corresponding conventional plot within approximately 200 m. All phenology plots used for 2022 were not previously used in 2021, and had not previously been IPM or organic. All orchard groups, except one in Rock Island, were in high pear psylla pressure areas of the Wenatchee Valley (Fig. 5) and involved large old trees. Two of the organic plots had been organic for many years, and one was in its first year of transition. The same sampling methods were used in 2022 as 2021.

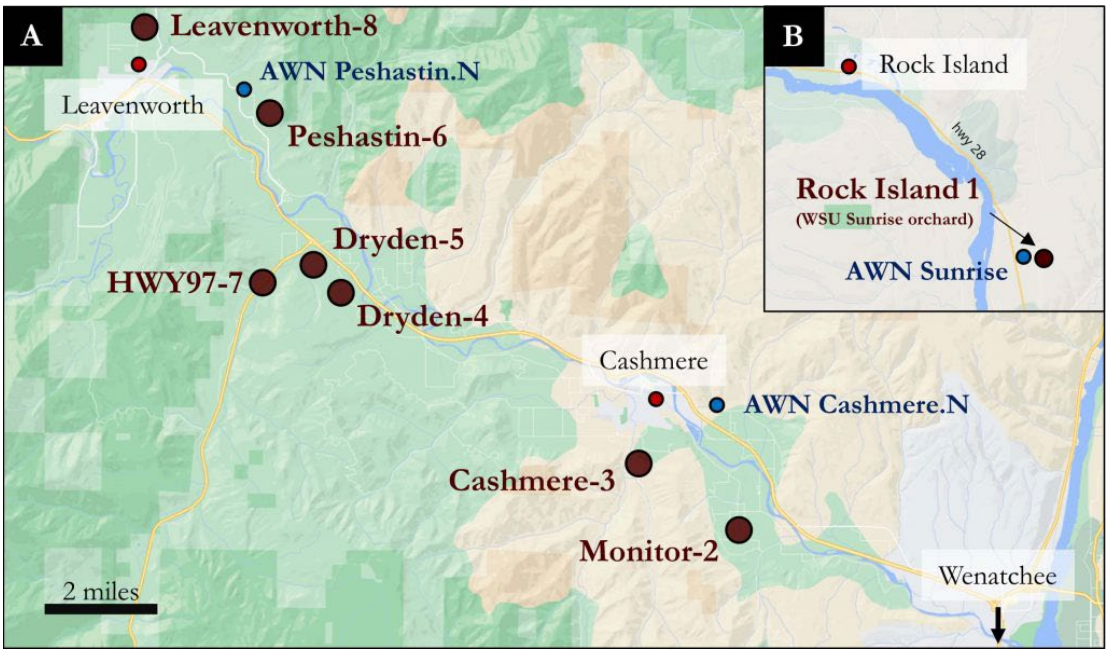

Fig. 5. 2022 sites for insect monitoring in paired commercial pear orchards (phenology and conventional combined as 1 dot, organic not shown). AgWeatherNet (AWN) temperature sensor locations are indicated with blue points.

Results: In 2021, the phenology model program (bIPM) resulted in consistent control of pear psylla nymphs, keeping populations below the treatment threshold of 0.3 nymphs/leaf throughout the season (Fig. 6). Natural enemies in the phenology model program were conserved similar to organic plots, and were significantly greater than conventional plots throughout the season. Data from the 2021 individual plots can be accessed online at http://treefruit.wsu.edu/crop-protection/pear-ipm/2021-pear-ipm-scouting/.

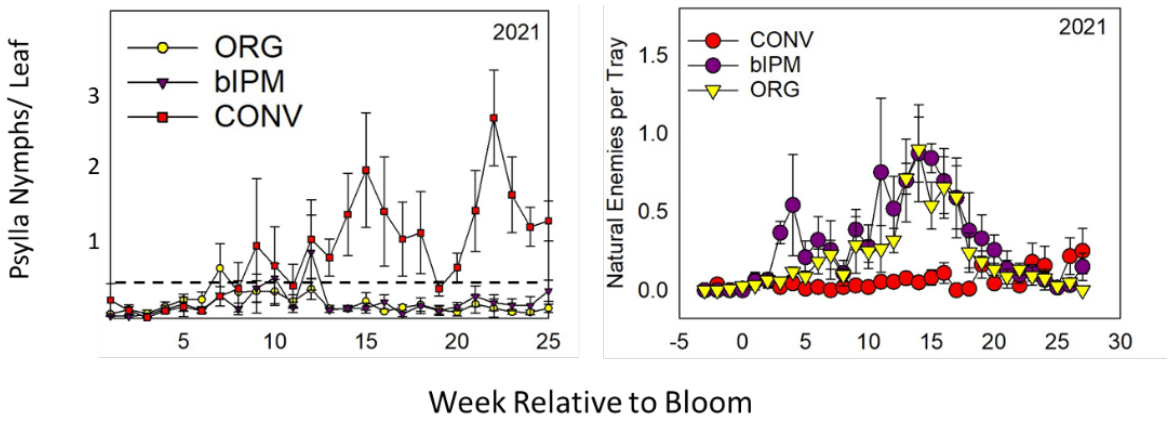

Fig. 6. Pear psylla and combined natural enemy densities in commercial orchard blocks following either the phenology model-based program ("bIPM"), conventional, or organic management, 2021. The dotted line is the treatment threshold of 0.3 psylla nymphs per leaf.

In 2022, phenology (IPM) and organic programs had fewer first generation pear psylla eggs and nymphs than conventional programs; adults were not different (Fig. 7 [left]). For the first summer generation of pear psylla, organic orchards had the most nymphs, phenology was intermediate, and conventional had the fewest (Fig. 7 [right]). For the second summer generation, psylla life-stages were similar among treatments at first (early-Aug); but as harvest approached, psylla nymphs increased in conventional plots relative to phenology and organic (Fig. 7 [right]). The final generation of psylla adults, which would go into overwintering (September and October), were around 10-fold greater in convention plots relative to phenology and organic (Fig. 7 [right]). Natural enemies increased in phenology and organic orchards in early August and remained through the fall, but never established in conventional plots (Fig .7 [right]). While not displayed in this report, we saw no difference in any other pest densities among treatments including codling moth, spider mites, and mealybug.

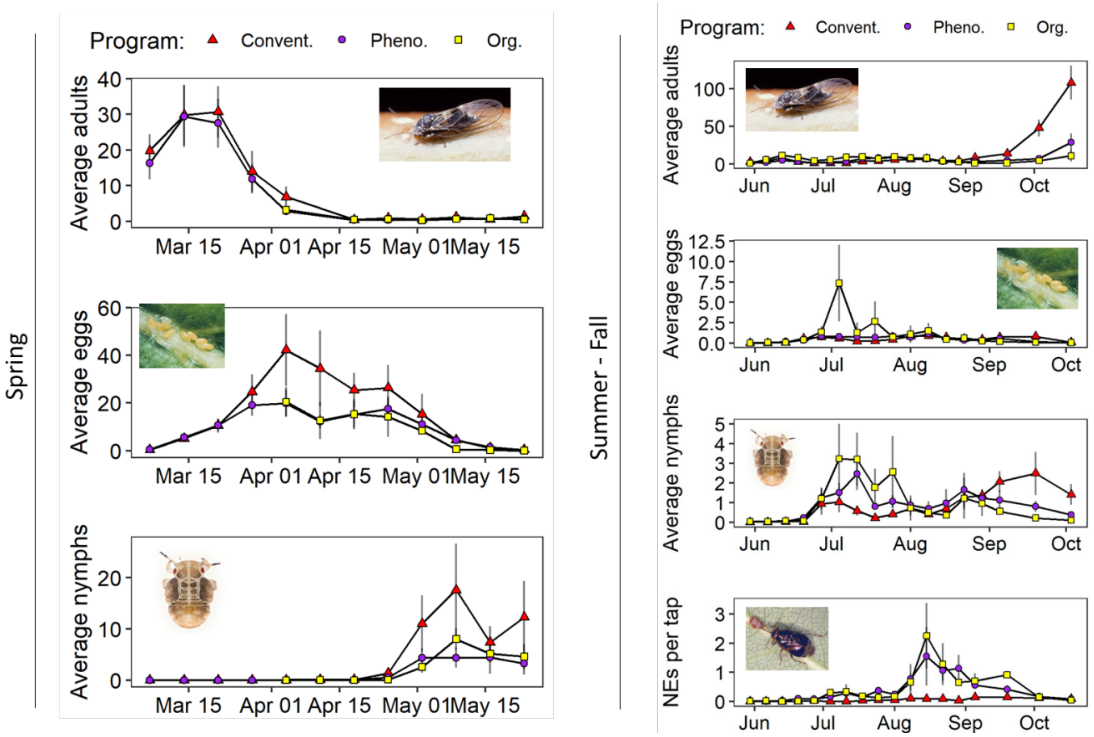

Fig. 7. 2022 weekly averages of psylla adults, eggs, and nymphs in conventional, phenology, and organic orchard treatments.

Season-long phenology spray programs cost $280/acre less than conventional programs, on average (Fig. 8 [left]). The average percentage of fruit rated as US-1 (highest quality, less than 1% honeydew injury) was not different among phenology and conventional treatments for Bartlett (not shown) or d'Anjou (Fig. 8 [right]). It is important to note that some phenology plots experienced greater injury than growers considered "desirable", however, so did conventional.

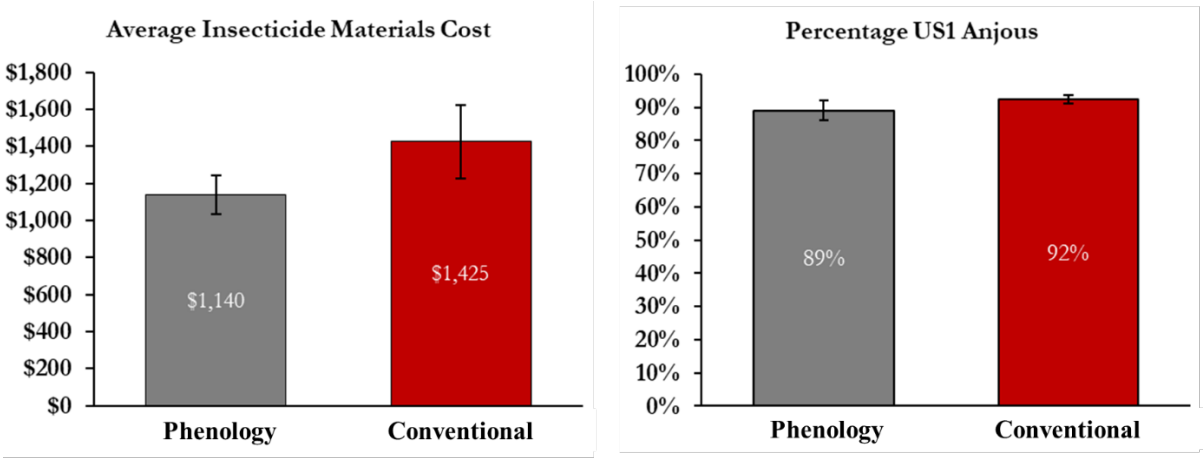

Fig 8. (Left) Average full season cost for all insecticide and miticide spray materials per acre for phenology ($1,140) and conventional ($1,425) programs. (Right) Average percentage of d'Anjou pears (100 sampled per plot) rated as US-1 quality (less than 1% injury) for phenology and conventional programs.

Conclusions: In 2021, the phenology based IPM program provided clearly superior suppression of pear psylla; however, we do not yet have economic data for these plots because they were associated with a different project and it was not originally planned to conduct field trials in this season.

In 2022, the phenology program provided similar control of pear psylla to the conventional program, as demonstrated by the equal percentage of pears rated US-1 across treatments. While some phenology plots experienced more injury than desirable, so did some conventional plots. This shows that the phenology program is not perfect at controlling psylla, as some plots faired better than others; but again, this was also true for conventional plots. The phenology program consistently was less expensive thank conventional, by $280 per acre on average, and used either no broad-spectrum materials or only one (four phenology orchards used one Bexar spray at delayed dormant) per season. This demonstrates that the phenology-based IPM program can effectively manage pear psylla with selective materials and at a lower cost, which was the primary goal of this project. If implemented throughout the 20,000 acres of pears WA, it would save the WA industry $5.6 million per year.

Psylla densities among treatments were more dynamic in 2022 than 2021. The phenology program provided improved psylla suppression to conventional programs early in the season, demonstrating that two Surround sprays early (without added broad-spectrum tank mix sprays) is as or more effective than one Surround spray coupled with multiple tank mixes of broad-spectrum materials like Malathion, Rimon, Assail, and Bexar (also demonstrated in Nottingham et al. 2022).

The first summer generation presented a issue that will be a challenge to gaining adoption of this phenology program. Nymphs were higher in the phenology program than conventional programs for about three weeks in July, which caused significant stress to growers—surprisingly, no one dropped out of our program. Many of cooperators expected that the high psylla pressure in phenology blocks would continue to increase and result in greater injury than conventional. To the contrary, psylla pressure neutralized among treatments around August, and then increased in conventional plots near harvest. Phenology plots ended with similar injury to conventional plots. Similar injury

outcomes were likely the result of the late season psylla serge in conventional and/or the effective use of honeydew washing via overhead sprinklers or airblast sprayers (used in both conventional and phenology).

Just prior to the last generation of psylla, natural enemies (mainly Trechnites, Campylomma, and Deraeocoris) increased in phenology and organic plots, but never developed in conventional. The differences in natural enemies almost certainly explains the steep increase in psylla nymphs and concomitant winterform adult in conventional plots at the end of the season. This trend suggests that areawide adoption of programs that conserve natural enemies (IPM or conventional) will lead to regional reductions in pear psylla for future years, due to massive decreases in adults going into overwintering and increased establishment of natural enemies. It is critical that growers and crop advisors understand these trends, as it will make management easier, less expensive, and more sustainable in future years.

In this project, we have not only developed an IPM program that is effective, strategic, and economical, we have debunked the idea that adopting IPM is "risky", particularly in the first year. Again, our phenology orchards experienced no differences in injury from psylla or any other pest injury. Meanwhile, they cost $280/acre less and produced 10-fold fewer winterform adults, so if anything, there is more risk in remaining conventional. As an industry there is certainly greater risk in not using IPM. It should also be noted that there was nothing special about the orchards in which we tested phenology programs. They were all in their first year of IPM, they were located in high pressure areas of the Wenatchee River Valley (not isolated), and they had large, old d'Anjou and Bartlett trees. The phenology program will remain publicly available within the Tree Fruit Extension website (http://treefruit.wsu.edu/crop-protection/psylla-phenology-model/) and via subscription in the Decision Aid System. We hope growers and crop advisors will not only use it appropriately, but share their results so adoption spreads.

3c. Extension and Outreach: All information from the project is online, including the model, recommendations, and real time scouting data. Additionally, we broadcasted summaries of results and reminders of our online resources via 3 Fruit Matters Newsletter articles in 2021 and 4 in 2022. We hosted two major Extension events including a pear IPM field day at one of our IPM orchards in Peshastin (Sept 2022) and a day-long pear IPM Fruit School in Wenatchee (Dec 2022, organized by T. DuPont).

REFERENCES CITED:

Beers, E. H., and B. M. Greenfield. 2015. Pear psylla insecticide test, 2013. Arthropod Manage. Tests 39.

Burts, E. C. 1983. Effectiveness of a soft-pesticide program on pear pests. J. Econ. Entomol. 76: 936-941.

Burts, E. C. 1992. Pear, soft pesticide test, 1991. Insecticide and Acaricide Tests 17.

DuPont, S. T., C. Strohm, L. Nottingham, and D. Rendon. 2021. Evaluation of an integrated pest management program for central washington pear orchards. Biol. Control 152: 104390.

Higbee, B. S., D. R. Horton, and J. L. Krysan. 1995. Reduction of egg hatch in pear psylla (homoptera: Psyllidae) after contact by adults with insect growth regulators. J. Econ. Entomol. 88: 1420-1424.

Nottingham, L. B., R. J. Orpet, and E. H. Beers. 2022. Integrated pest management programs for pear psylla, cacopsylla pyricola (förster) (hemiptera: Psyllidae), using kaolin clay and reflective plastic mulch. J. Econ. Entomol.

Executive Summary

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

Keywords: Pear Psylla, *Cacopsylla pyricola*, IPM, Phenology, Degree Days

Abstract: Pear psylla has been the most costly pest of pear orchards in Washington since it arrived in the 1940's, particularly in the Wenatchee River Valley, the state's largest pear production region. Coventional pear growers here make 10-15 sprays per season to control psylla, costing about $1,500 per acre on average. Most sprays involve tank mixes of multiple broad-spectrum insecticides that dessimate natural enemy populations. This is not only expensive, but it has led to extremely high areawide populations of pear psylla in Wenatchee due to lack of biological control from natural enemies. Growers in other pear-growing regions, like Hood River, OR, use around three selective sprays for pear psylla per season, then allow natural enemies to do the rest.

The purpose of this project was to develop an effective and economical IPM program for pear psylla, by strategically timing selective techniques (such as IGRs, kaolin, and honeydew washing) with pear psylla degree days and tree phenology. We performed a literature review followed by experiments to determine optimal timings of selective techniques, then incorperated them into a pear psylla phenology model. The final phenology-based IPM program is availble in the WSU Tree Fruit Extension website (http://treefruit.wsu.edu/crop-protection/psylla-phenology-model/) and in the Decision Aid System (https://decisionaid.systems/). The phenology program was tested against standard conventional programs in replicated 2-4 acre commercial orchards throughout the Wenatchee River Valley (four reps in 2021, eight in 2022). In both years, the phenology program controlled psylla densities similar to or better than standard conventional orchards, and led to major increases in natural enemies. The phenology orchards also produced 10-fold fewer psylla adults going into overwintering than conventional orchards. Fruit downgraded by honeydew (only measured in 2022) was not different between phenology and convnetional programs (Fig 1. Right). No differences among programs were seen for other pests including codling moth, mites, and mealybug. The phenology programs cost $280 per acre less than the conventional programs, on average (Fig 1. Left). Across the 20,000 acres of pears WA, this program could save the WA industry $5.6 million per year.

Our results demonstrate that this phenology-based IPM program is effective, economical, and extremely low-risk, even in the first year of adoption. Moreover, areawide adoption results in a regional suppression of overwintering pear psylla, due to conservation of later season natural enemies. This will greatly reduce the areawide populations of pear psylla, making management in future years easier and cheaper for all growers.

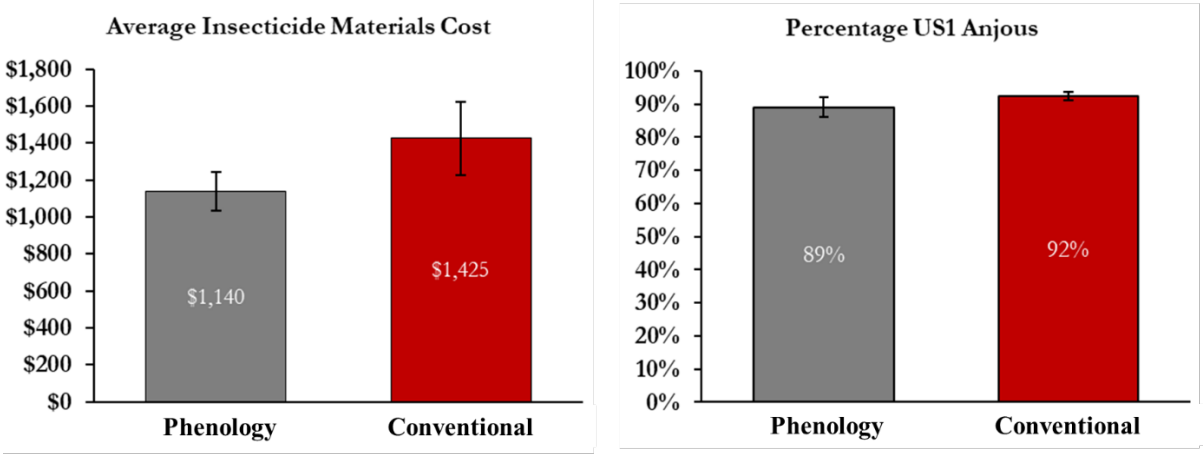

Fig 1. Left graph: 2022 average full season cost for all insecticide and miticide spray materials, per acre, for phenology ($1,140) and conventional ($1,425) programs. Right graph: 2022 average percentage of d'Anjou pears (100 sampled per plot) rated as US-1 quality (less than 1% injury) for phenology (89%) and conventional (92%) programs.

CONTINUING PROJECT REPORT **YEAR**:2 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:** $6,000

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	2020	2021	2022
Salaries	$8650	$8866	
Benefits	$2768	$2837	
Wages			
Benefits			
Equipment			
Supplies	$17582	$16,797	$5000
Travel		$500	
Miscellaneous			
Plot Fees	$1000	$1000	$1000
Total	$30,000	$30,000	$6000

Footnotes:

OBJECTIVES: Goals, Years 2-3 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 designed a method to allow us to perform simultaneous collections from multiple trees, which incorporated powerful air and vacuum pumps and manifolds. These materials were purchased and used to build the collection system for implementation in year 2. The volatile collectors that were used in the collections were purchased as a prefabricated item (http://www.volatilecollectiontrap.com/) and were found to be contaminated. Therefore, we had to create our own volatile collectors that have been determined to be free of contaminants. We will use these new collectors for volatile collections in year 3.

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 year 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 Year 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 Year 3. Attempts to collect and identify volatiles prior to Year 1, were conducted by a former WSU graduate student in winter 2019. Differences in volatiles were found when comparisons 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 analyses will be conducted using the brand-new instrument, which is more reliable and sensitive than the older instrument. In addition, the new instrument is equipped with an autosampler, which allows us to process samples faster and more accurately. The lab was equipped with a GC-EAD instrument that was nonfunctional. However, in the fall of 2021, necessary repairs and replacements were made to the instrument which will allow us to use the GC-EAD for assays in year 3.

Expected results. Using GC-MS and GC-EAD 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

- In preliminary studies, there was a difference found 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 and methods used for volatile collections were only suitable to collect from one tree at a time.
- Method for collecting volatiles was modified to allow for simultaneous collection of volatiles from multiple trees and a control.
- Prefabricated volatile collectors were found to be contaminated with several chemicals, which prevented volatiles emitted by pear trees to be properly analyzed. New, cleaner, and cheaper collectors have been made for volatile collections
- New GC-MS was purchased, installed, and used for analyses of volatile collections. GC-EAD instrument was repaired and will be used for future analyses.
- 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.

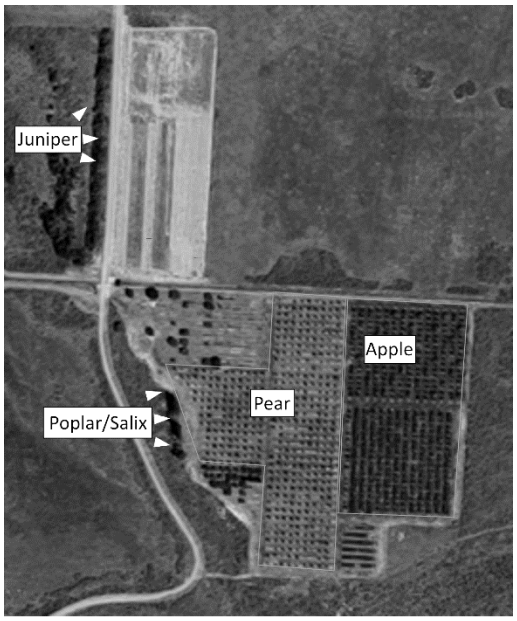

Figure 1. Layout of pear orchard at the USDA experimental farm in Moxee, where winterform psylla will be collected and where volatile collections will take place.

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 5 trees in orchards in Moxee, WA (Figure 1). Methods similar to Giacomuzzi et al. (2017) will be used to collect volatiles from pear trees (Figure 2a). 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 be attached to the air pump (before the manifold) to introduce clean air into the inlet of the bag (Figure 2b). A volatile collector will be connected to the outlet and to the manifold of the vacuum line (Figure 2b). The tubing that is connect to the inlet and outlets of each bag are fitted with a flow meter to ensure contant flow over the trees (Figure 2c). 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.

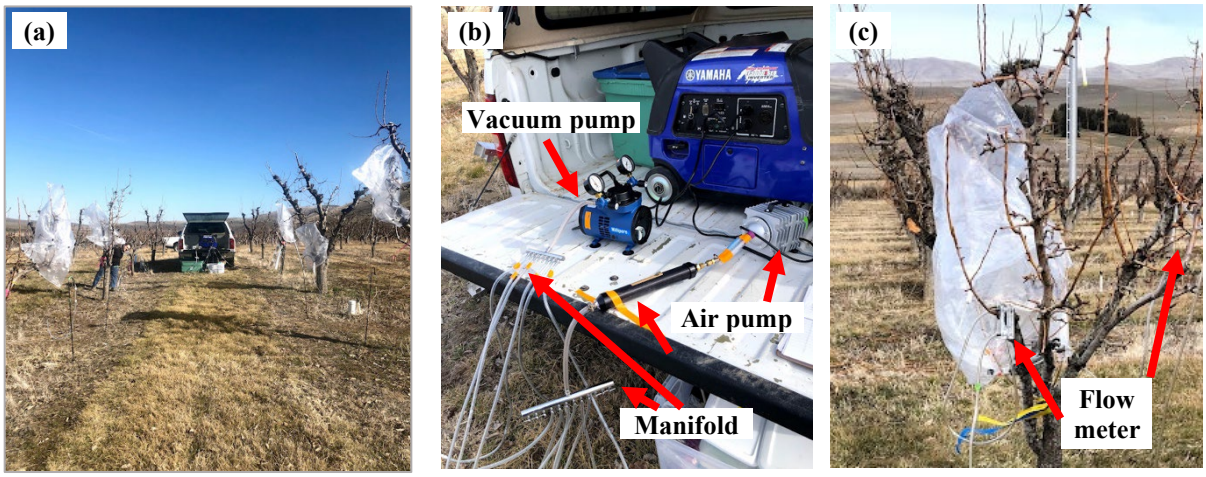

Figure 2. Example of volatile collection set up: (a) Volatiles being collected from 5 Bartlett pear trees at the USDA experimental farm in Moxee; (b) air pump, vacuum pump, and tubing set up; (c) up close image of volatile collection set up on pear tree.

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 from one tree on a semi-weekly basis 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 (data not shown). During the analyses, there appeared to be issues with old GC-MS instrument used for analyses.

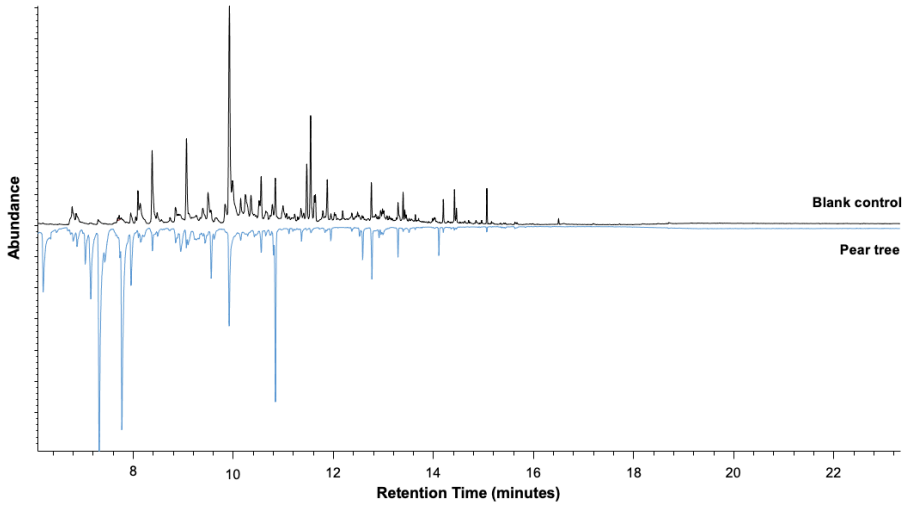

Figure 3. Representative GC analysis of volatiles sampled from an empty sampling bag (control), and a sampling bag that contained portions of a pear tree. Top trace: GC chromatogram of blank control. Inverted trace: GC chromatogram of sampled pear tree.

In 2021, a new GC-MS was purchased and installed in the lab and all extracts of volatiles from 2021 were analyzed on the new instrument. It appeared that each of the analyzed extracts contained many peaks/compounds. However, compound identifications revealed that the extracts contained several contaminants, including some related to plastics (e.g. diethyl benzenes; Figure 4a). To determine the source of the contaminants, GC-MS analyses were conducted during a simulated extraction. New collectors (that had not been used for volatile collections) were extracted with solvent ($MeCl_2$) and analyzed on the GC-MS. The analyses revealed most of the same contaminants as the collectors used for the pear trees (Figure 4b), and some were at a higher abundance. The source of solvent ($MeCl_2$; Optima Grade from Fisher Scientific) was also analyzed on the GC-MS, however only one contaminant was found, but at significantly lower levels than the extracted ("clean") collector (Figure 4c). These results indicated that the solvent was not contaminated, and that the collectors were indeed the source of contamination. There were two peaks that only appears in the extracts of volatiles (fist two peaks with asterisks in Figure 4a), however these peaks were present in pear extracts and the controls, which indicates that these compounds are not unique to the trees.

Due to the fact that the source of contamination were the volatile collectors, a newer collector needed to be developed and used. The collectors that will be used from now on, are similarly made to the previous used collectors in that glass tubing was used to house the adsorbent. However, the adsorbent was changed from Porapak Q to thermally desorbed charcoal and there were no plastic components (Figure 5). Solvent ($MeCl_2$) was used to extract the new charcoal collectors for GC-MS analyses, which revealed fewer contaminants, both quantitatively and qualitatively (Figure 5).

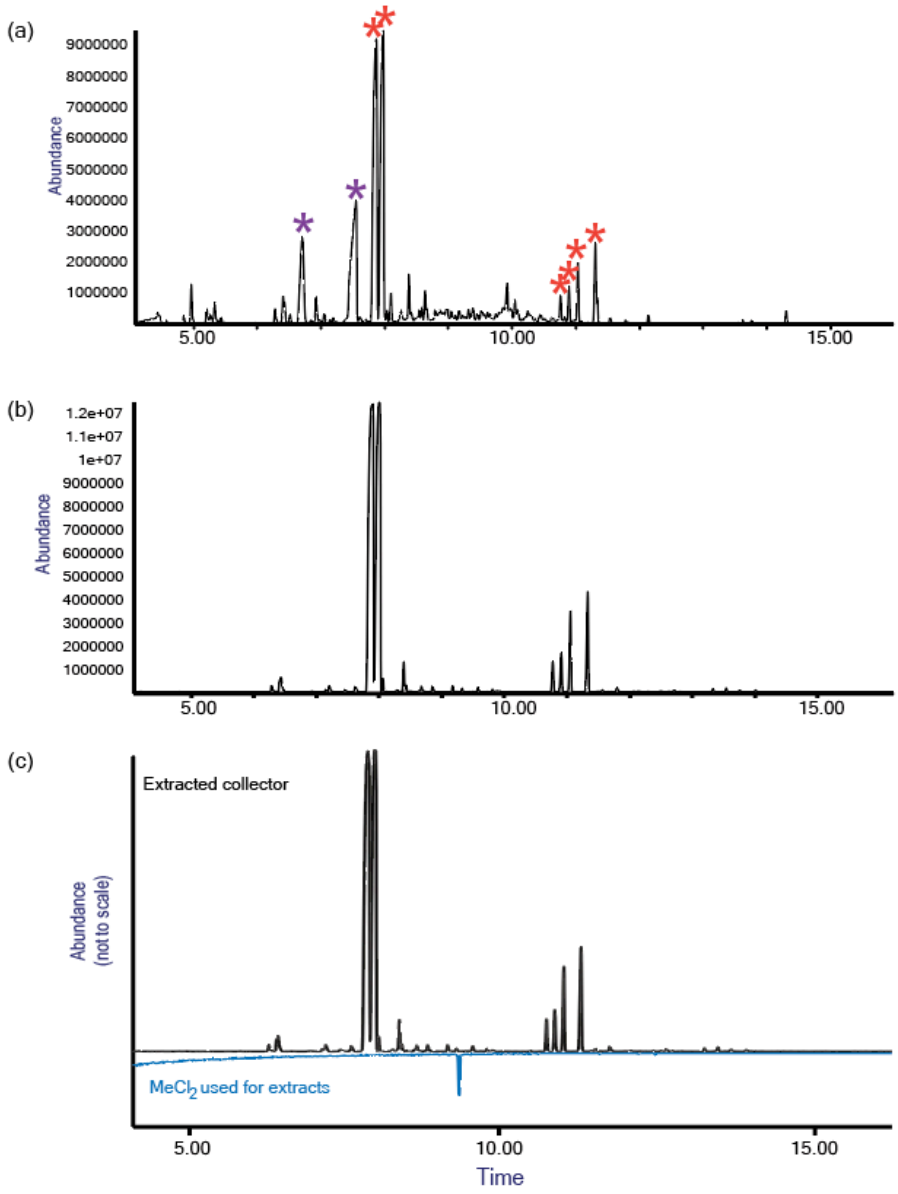

Figure 4. Representative GC-MS analyses of: (a) extracts of volatiles from a Bartlett pear tree collected in early April (the first two asterisks represent compounds identified in all extracts of volatiles, including the control and the remaining asterisks represent compounds that were identified as contaminants); (b) extract of an unused volatile collector; and (c) a comparison of an extract from an unused collector (top) and the solvent ($MeCl_2$) used for all extracts (inverted trace, not to scale).

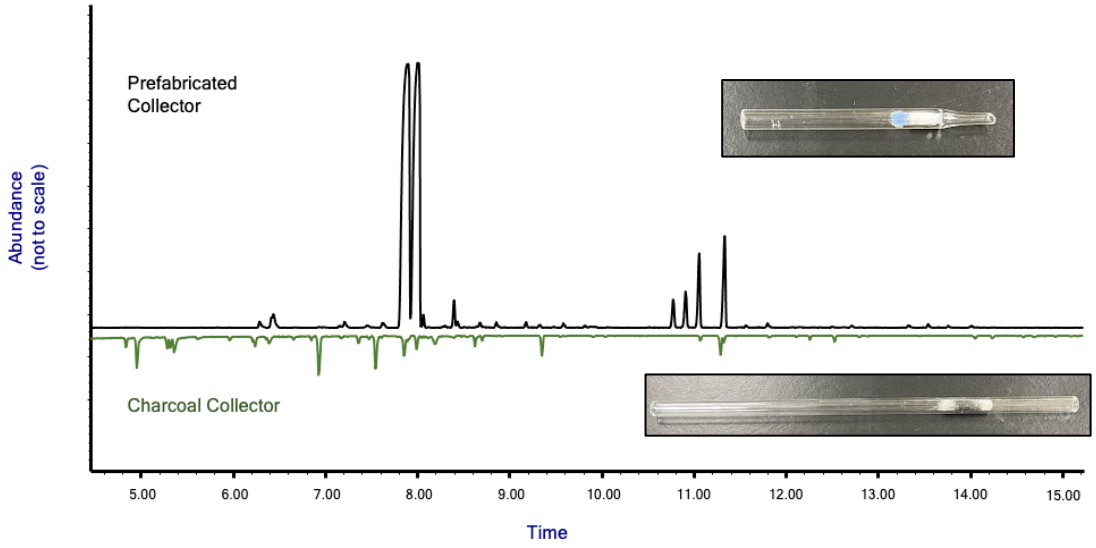

Figure 5. Representative chromatograms of an extract from an unused collector (top trace) and the extract from the new charcoal collectors (inverted trace). The trace representing the extract of the charcoal was scaled up for demonstration purposes.

Preliminary bioassays

Results from caged bioassays were promising and suggest that pear tree volatiles may be attractive to winterform psylla (Figure 6). 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.

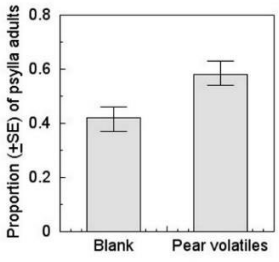

Figure 6. Mean (±SE) number of pear psylla caught in traps baited with a nontreated piece of filter paper ("Blank") and pear volatiles.

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.

Project/Proposal Title: Tactics to improve natural enemy releases in tree fruit

Report Type: Continuing Project Report (NCE)

Primary PI: Rebecca Schmidt-Jeffris
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Address: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

Cooperators: Steve Arthurs (BioBee), Chuck Weaver (Parabug), Rudy Prey [note: apple grower cooperators are specified in apple report]

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ $102,558*
Total Project Request for Year 2 Funding: $106,033*
*50% by WTFRC Apple Crop Protection, 50% by FPC/PPC Pear

Other related/associated funding sources: awarded

Funding Duration:	2020-2023
Amount:	$36,614
Agency Name:	BioBee
Notes:	In-kind match of commercial insectary insects, Artemac (brine shrimp cysts on tape), and shipping costs for beneficials to be used in this project. Itemized estimate provided by BioBee.

Funding Duration:	2020-2023
Amount:	$720
Agency Name:	Parabug, Chuck Weaver private contractor
Notes:	In-kind match of drone pilot labor for releasing insects as part of Obj. 2. ~$18/acre × 10 drone-treated acres per trial × 2 trials (apple & pear) × 2 years.

Funding Duration:	2021-2022
Amount:	$29,968
Agency Name:	Western IPM Center, project initiation grant
Notes:	This project expands the efforts in this grant by providing support to conduct grower input sessions and a needs assessment survey. The WIPMC grant will also be used to start a grant team and stakeholder advisory group that will submit a federal grant application to expand

this work (likely to USDA OREI). The data collected in this grant will be used as preliminary data in the OREI submission. The results in this report are due to this grant award.

Funding Duration: 2020-2023
Amount: $348,733
Agency Name: Western SARE
Notes: This is a complementary (non-overlapping) project, specifically focusing on earwig releases in apple and pear, on the ground and by drone.

WTFRC Collaborative Costs: none

Budget 1*
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: 510-559-5769 **Email address:** Chuck.Myers@usda.gov
Station Manager/Supervisor: Rodney Cooper **Email Address:** rodney.cooper@usda.gov

Item	2021	2022
Salaries[1]	$17,458	$17,894
Benefits[1]	$5,587	$5,726
Wages	$0	$0
Benefits	$0	$0
Equipment	$0	$0
Supplies[2]	$6,500	$6,500
Travel[3]	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$29,545**	**$30,120**

Footnotes:
[1]GS-5 technician for 6 months per year, 100% FTE at 32% benefits, Year 2 includes 2.5% COLA increase. Technician would assist WSU postdoc (see below) with sampling in all locations. This technician will also assist the postdoc with surface sterilization and PCR for gut content analysis.
[2]Funds to purchase PCR reagents and other PCR supplies for gut content analysis, trapping supplies, and some commercial nutritional supplement products (others provided as in-kind match).
[3]Fuel to field sites will be provided by USDA base funds and is not requested.
*50% by WTFRC Apple Crop Protection, 50% by FPC/PPC Pear

Budget 2*
Organization Name: WSU
Contract Administrator: Stacy Mondy
Contract administrator email address: anastasia.mondy@wsu.edu
Station Manager/Supervisor: Chad Kruger **Email Address:** cekruger@wsu.edu

Item	2021	2022
Salaries[1]	$52,827	$54,940
Benefits[2]	$18,373	$19,108
Wages[3]	$1,200	$1,248
Benefits[3]	$113	$117
Equipment	$0	$0
Supplies	$500	$500
Travel	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$73,013**	**$75,913**

Footnotes:
[1]Nottingham salary ($7,612.50/mo × 12 mo × 2% FTE = $1,827 Year 1, Year 2 reflects 4% COLA increase) + Postdoc salary ($4,250/mo × 12 mo × 100% FTE = $51,000 Year 1, Year 2 reflects 4% COLA increase). Nottingham to supervise data collection efforts in pear in the Wenatchee area and advise on project methods and data summary. WSU Postdoc will be based at the USDA-ARS facility in Wapato, WA and supervised by Schmidt-Jeffris. The postdoc will be responsible for leading data collection and summarizing project results. Due to difficulties in finding a qualified postdoc candidate, we have expanded our search to also include an associate in research, which would have a similar salary, but be hired at the M.S. level.
[2] Benefits rate for Nottingham is 29.9% ($547 Yr 1, $569 Yr 2). Benefits rate for postdoc is 35% ($17,826 Yr1, $18,539 Yr2).
[3]Summer technician at $15/hr×8 hr/wk ×10 wks, 9.4% benefits rate, salary includes 4% COLA increase in Year 2
*50% by WTFRC Apple Crop Protection, 50% by FPC/PPC Pear

OBJECTIVES

1. Improve retention of released natural enemies. A primary complaint from growers is that natural enemies disperse from the orchard immediately after release. Nutritional supplements such as pollen (Nutrimite, Biobest) and brine shrimp cysts (Artemac, BioBee) are commercially available and have been shown to improve retention and survival of natural enemies in greenhouses, but this has not been tested in tree fruit orchards. Using methyl salicylate lures, which attract natural enemies, in combination with nutritional supplements may further improve natural enemy retention with little additional effort on the part of the grower. We will test supplements and lures in combination and individually in plots where commercially available predators, lacewings and minute pirate bugs, have been released. We will collect data on pest control levels, retention of released natural enemies, and recruitment of resident natural enemies. This objective was modified to test *Ephestia* eggs instead of pollen, due to greater ease of application.

2. Determine cost-effectiveness and efficacy of natural enemy release by drone. One method for reducing natural enemy release labor costs is to conduct releases by drone. However, the ability of natural enemies to survive release by drone into orchards and whether this method significantly decreases natural enemy abundance relative to hand-releases is unknown. We will compare released predator abundance, pest control levels, and labor costs for releases by hand and by drone of lacewings and mealybug destroyers in apples. In apple, this objective was modified to include comparison of additional treatments, including mealybug destroyer larvae, lacewing cards, multiple species of lacewings, and releasing lacewings as larvae versus eggs.

SIGNIFICANT FINDINGS

- Thanks to a no-cost extension, we were able to delay the main parts of this the project to begin in 2022. The main delay was due to our inability to find a qualified postdoc. Instead, we readvertised the position as an associate in research, open to individuals with M.S. degrees. Daniel Hausler was hired to manage the project in early 2022. Some data was still collected in 2021 because of funding from other sources.

- **Grower survey and discussion, 2021-2022**. In collaboration with Tianna DuPont and Ashley Thompson, we collected survey data on apple and pear grower perspectives of releasing natural enemies in tree fruit. 132 growers and consultants responded, representing 43,868 apple and pear acres. 37 respondents (28%) are using biocontrol releases occasionally or annually on 7,842 acres costing them $153 per acre on average. The main natural enemies they are releasing are lacewings (29%), lady beetles (28%), and predatory mites (25%). The main barrier to adoption of releases was lack of knowledge/recommendations on how to release successfully (52%). Five stakeholder input sessions were conducted in 2021-2022 in Omak, Wenatchee, Yakima, Hood River, and Medford with a total of 60 participants. The input sessions identified the following as critical research areas: (1) information to make natural enemy releases more effective/useful, (2) evidence of efficacy, (3) what species to release, (4) where to purchase, (5) release timings, (6) release rates, (7) a list of common release mistakes and how to avoid them, (8) on farm success stories, (9) consistent supply, (10) proper placement in the tree/orchard, and (11) pesticide toxicity to natural enemies. Feedback from the survey will be used to determine future research directions and to obtain federal funding to expand the work in this project.

<u>Pear</u>

- **Improving retention, 2022.** In an organic commercial pear orchard, releases of *O. insidiosus* and *C. carnea* did not decrease pear psylla populations and the use of Predalure and food supplements

also had no effect on psylla. Similarly, none of the resident natural enemy groups increased in response to the lure or food treatments. Two individual *O. insidiosus* were recovered a week after release, but were not found later in the experiment. Two *C. carnea* larvae were also found, but molecular analysis will be needed to determine if they were from the release or are resident to the orchard. All natural enemies from tap counts were kept and stored in alcohol for PCR gut content analysis. This will allow us to determine which natural enemies are the most important predators of pear psylla. The most abundant natural enemies in tap counts were *Campylomma*, whirligig mites, and spiders. Ongoing work at USDA-ARS Wapato indicates that whirligig mites are voracious predators of potato psyllid and it is likely that they are important control agents of pear psylla also.

- **Efficacy of hand releases versus drone, 2022.** In the same orchard, we also tested releases of *O. insidiosus* and *C. carnea* by hand and by drone and compared results to a no-release control. There were no differences in psylla abundance between any of the treatments. We did not find any *O. insidiosus* or *C. carnea* following release.

Apple

- **Mealybug destroyers, 2020-2022.** In 2020, mealybug destroyers released by hand either early (mid-May) or late (mid-June) at either 2,000 or 5,000 per acre caused ~3× decrease in mealybug populations, but this effect was highly variable between plots. The drone release did not cause a decrease. In 2021, we examined mealybug destroyer releases in one-acre plots, comparing drone versus ground releases of 1,000 mealybug destroyers per acre to a no-release control. We found very few mealybug destroyers 1 day after release and no mealybug destroyers 8 days after release; they likely dispersed due to low pest density in this orchard. In 2022, mealybug destroyers were not recovered after release, despite the presence of mealybugs in the plots, and no differences were observed between treatments. It is possible that a fire blight spray affected this release. In general, mealybug destroyers do not appear to be a reliable control method for mealybugs in apples and cannot currently be recommended due to their high cost.

- **Lacewings, 2021.** We tested releases of two species of lacewings as eggs or larvae: *Chrysoperla rufilabris* and *Chrysoperla carnea*. We found that the *C. carnea* larvae (which came from a different insectary than the eggs) were actually *C. externa*. While lacewings in the *C. carnea* species group are suited to our arid climate, *C. externa* is not. This quality control issue was reported to the insectary. A release of *C. carnea* as eggs (100,000/acre) was the most successful treatment at suppressing woolly apple aphid and green apple aphid in this study. A release of *C. rufilabris* larvae was also effective (20,000/acre). Seasonal counts of aphid colonies were reduced by 57% and 43% in these treatments, respectively. Low numbers of larvae of the released lacewing species were found throughout the trial (1-5 per treatment, across 8 weeks of sampling). Therefore, when determining efficacy of beneficial releases, scouts should focus on pest numbers, not necessarily natural enemy recovery.

- **Lacewings, 2022.** We compared releases of (1) *C. carnea* eggs by hand, *C. rufilabris* eggs by (2) hand, (3) card, and (4) drone, (5) *C. rufilabris* larvae, and (6) a no-release control. None of the treatments caused a reduction in aphids. Lacewing larvae were recovered from ground-based release treatments (5-14 total per treatment, across 8 weeks), but were not recovered from the control or the drone treatments.

- **Improving retention, 2022.** In a commercial apple orchard, releases of *O. insidiosus* and *C. carnea* decreased green apple aphid populations. However, the food supplements and Predalure caused an increase in aphids compared to the treatments where they were not used. It is likely that complex interactions between released and resident natural enemies are occurring. Possible

interactions will be explored via molecular gut content analysis, which is currently in progress. In the commercial and research orchard trials, Predalure showed potential for recruiting resident natural enemies for pest mite control and decreased brown mite abundance.

METHODS

The methods below are for the apple portion of the project only. They have been updated to reflect how the work was conducted in 2022.

Fig. 1. Ladybeetle feeding on Artemia tape

1. Improve retention of released natural enemies.
This two-year (2022-2023) study will be conducted in an organic commercial pear orchard in Peshastin, WA. The release day will target when early season pear nymph populations begin to rise, approximately bloom. There will be a total of five treatments made of combinations of lure use (Predalure, methyl salicylate), food supplements (Artemac, brine shrimp cysts + *Artemia* eggs), and releases (100,000 lacewing eggs + 2,000 *Orius insidiosus* per acre): (1) Predalure (methyl salicylate) + Foods + Release, (2) Predalure + Release, (3) Food + Release, (4) Release only, and (5) No-release control. Each combination will be replicated in the orchard 5 times in 0.25 acre plots. One week prior to release, we will conduct precounts of pear psylla (and mites, if present) by collecting a random 30-leaf sample for brush counts in the lab. At this point, one methyl salicylate lure will be added to one tree in the center of each plot to allow the volatiles sufficient time to dissipate prior to releasing the natural enemies. One week after this, we will apply *Ephestia* and Artemac throughout each plot at the insectary recommended rate. Artemac (Fig. 1) will be applied by tying tape with attached cysts to trees and *Ephestia* eggs will be applied by hanging cards. Then, we will release by hand two natural enemy species across the entire trial at insectary recommended release rates: 100,000 *Chrysoperla carnea* eggs per acre (green lacewing, BioBee) and 2,000 *Orius insidiosus* per acre (minute pirate bug, Beneficial Insectary). Post-release sampling will occur at weekly intervals following release for 4 weeks. Pear psylla and mites will be sampled as previously described. Beat tray samples will be collected from the 9 center trees of each plot. All natural enemies from the tap counts will be collected and stored in ethanol. Lacewings and *Orius* collected will be identified to species in the laboratory to determine if they are from the insectary. These specimens will be used for gut content analysis to determine: 1) if released beneficials are consuming pests at high rates and 2) if either released beneficials or resident natural enemies are consuming the nutritional supplements. We will also place two sticky cards on trees within the center of each plot to count all natural enemies to species. DNA analysis will be conducted on any captured *C. carnea* to distinguish resident from released individuals.

Determine cost-effectiveness and efficacy of natural enemy release by drone.

This two-year (2022-2023) study will be conducted in a commercial pear orchard in Peshastin, WA. We will test the two most common natural enemies released by growers for pear psylla control: green lacewings and minute pirate bugs. However, we will use a lacewing species that has not yet been tested for efficacy when released in pear, *Chrysoperla carnea*.

The treatments will be 1) minute pirate bug (*O. insidiosus*) drone release, 2) minute pirate bug ground release, 3) lacewing (*C. carnea*) drone release, 4) lacewing ground release, and 5) no-release control. There will be four 0.25-acre replicates per treatment (20 plots total). One week prior

to release, pear psylla counts will occur (as described in Obj. 1) and treatments will be randomized based on pest levels. We will use the release rates of 100,000 lacewing eggs/acre and 2,000 *Orius*/acre, as recommended by the insectary. Ground releases will be conducted by ATV and the amount of time spent conducting the release in each replicate will be recorded. The released natural enemies (*O. insidiosus* and *C. carnea*) will be counted by sticky card and beat trays and pear psylla will be counted by leaf samples and beat trays, as in Obj. 1. All sample types will be collected once weekly for four weeks following releases.

RESULTS AND DISCUSSION

Results and discussion from pear trials only. For apple results, see the ACP report.

Retention Trial. None of the treatments in our study differed from each other in pear psylla abundance (Fig. 2); releases of *C. carnea* and *O. insidiosus* did not reduce pear psylla counts and

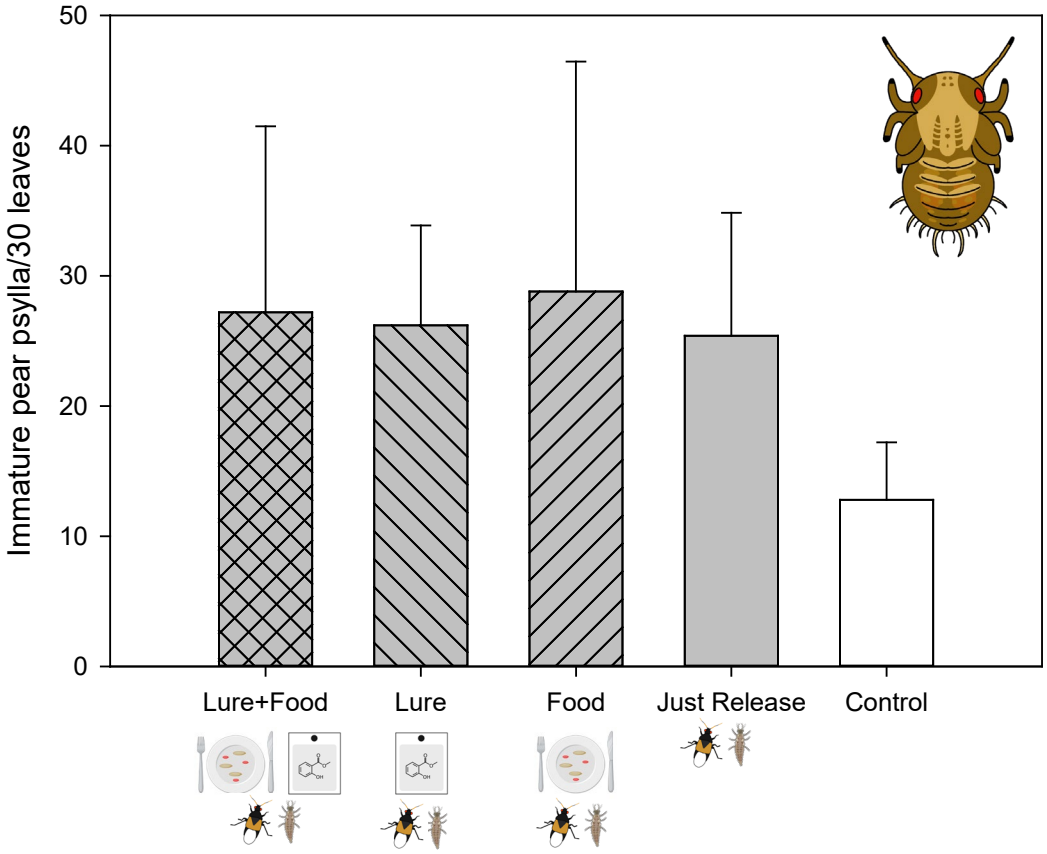

Fig. 2. Seasonal sums of immature pear psylla per plot for 3 weeks post-release in the 2022 retention trial in pears.

lures and food supplements did not alter treatment efficacy. We were able to recover our released predators: 2 *O. insidiosus* were found one week post-release and two *C. carnea* larvae were found three weeks post-release. The populations of resident natural enemies were not affected by our treatments. Across our samples, the most prevalent natural enemies were *Campylomma*, whirligig mites, and spiders. *Deraeocoris* and lacewings were also present, but far less abundant. In 2023, these samples will be used to conduct PCR-based gut content analysis to determine (1) which predators are most commonly found to have consumed pear psylla, (2) if any predators consumed the food

supplements, and (3) if any pear psylla predators commonly eat each other (intraguild predation). This will provide growers with better recommendations on which natural enemies to focus on as part of conservation efforts. We are particularly excited to find whirligig mites in abundance; this is an important natural enemy of potato psyllids in weedy hosts near potato fields (Fig. 3). It is likely to also be an important pear psylla predator. Currently, whirligig mites are available for purchase in Canada, but not the United States. Seeking permitting in the U.S. partially depends on consumer demand.

Fig. 3. Whirligig mite eating a potato psyllid.

Drone Efficacy Trial. None of the treatments resulted in a decrease in pear psylla abundance (Fig. 4). We were also unable to recover any of our released *O. insidiosus* and *C. carnea*. A limited number of resident green lacewings (all *Chrysopa* species) were found. However, due to time limitations, we were unable to release the natural enemies until a week after arrival (they were kept at 50 °F). It is possible that the quality of the natural enemies declined during storage, although we did confirm that they were alive prior to release. However, even in the retention trial, when natural enemies were immediately released, no effect was observed on pear psylla. It may be that natural enemies that are currently commercially available are not appropriate for pear psylla management. Whirligig mites and *Anthocoris* species are more likely to be suitable for pear psylla control, but are currently not available for purchase in the U.S. Finally, both pear trials were conducted in the same commercial orchard; there is the potential that this site is not hospitable to these natural enemies. We will select another organic pear orchard in 2023 to repeat the study.

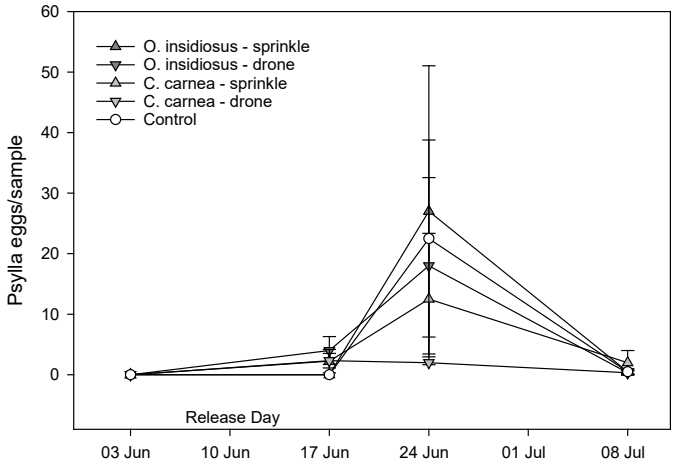

Fig. 4. Brush counts of pear psylla eggs from 30-leaf samples in natural enemy release trial.

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

Report Type: Final Project Report

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Cooperators: Steve Castagnoli/Christopher Adams (OSU-MCAREC)

Project Duration: 3 Year

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

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2023
Amount: $245,974
Agency Name: WSDA SCBG
Notes: This grant was submitted using Year 1 data from this project as preliminary data.

Funding Duration: 2022
Amount: $20,596
Agency Name: WSCPR
Notes: This grant was submitted using Year 3 data from this project as preliminary data.

Funding Duration: 2023
Amount: $14,980
Agency Name: WSCPR
Notes: This grant was submitted using Year 3 data from this project as preliminary data.

Funding Duration: 2023-2024
Amount: $224,688
Agency Name: USDA NIFA Postdoctoral Fellowship
Notes: This grant was submitted using data generated from samples collected as part of this project.

Budget 1

Organization Name: USDA-ARS **Contract Administrator:** Mara Guttman

Telephone: 510-559-5619 **Email address:** mara.guttman@usda.gov

Item	2019	2020	2021
Salaries[1]	$17,404[2,3,4]	$17,839[2,3,4]	$18,286[2,3,4]
Benefits	$4,529[2,3,4]	$4,642[2,3,4]	$4,759[2,3,4]
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	2019	2020	2020
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	2019	2020	2021
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.
In Year 3, we completed assays to compare methods for monitoring *Trechnites* and for estimating parasitism rates. Percent parasitism was estimated using only PCR of pear psylla nymphs, which we have determined to be the most efficient method. A USDA-ARS Post-doc was hired for model development and further testing and will continue for the next ~2 years.

Expected Results. Preliminary results from trap catch, dissections/emergence, and PCR have been summarized. Full model and building of the grower tool will continue in spring & summer 2023. The most efficient method for trapping *Trechnites* and which trap best reflects percent parasitism was completed at conclusion of Year 3 and a peer reviewed manuscript is currently in progress.

2. Define the relationship between counts of adult *Trechnites* and parasitism of psylla nymphs
We will continue processing data to define this relationship. We need to define the relation within time as well to account for rising and possible falling parasitism rates that fluctuate with the life cycle of both *Trechnites* and pear psylla.

Expected results. Using machine learning we have developed a model that can accurately predict parasitism rates within a low margin of error. 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.
Experiments to test non-target effects of pesticides on *Trechnites* require a reliable source of *Trechnites* adults and psylla mummies (immature stages of *Trechnites* still in psylla nymphs). Rearing *Trechnites* has proven to be challenging in part because of inconsistencies in the availability of colony-reared early instar pear psylla. An alternative to rearing is collection of mummies directly from the field. We found adequate numbers of mummies could be collected in cardboard bands wrapped around pear tree branches. The cardboard bands are placed in trees in autumn when the parasitized psylla nymphs search for overwintering shelters, and a retrieved in mid-winter. Cardboard bands were placed in trees in winter of 2021 & and we completed pesticide bioassays of *Trechnites spp*. adults in the spring of 2022. Assays on mummies will be conducted in Spring 2023.

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.

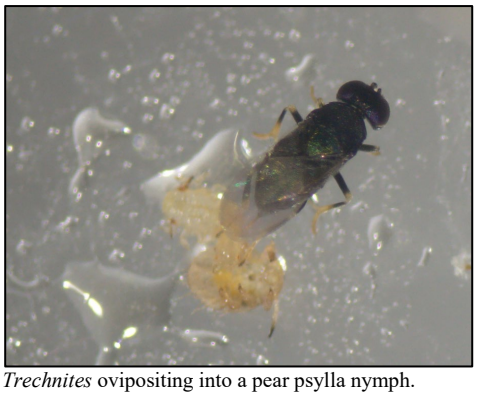
4. Examine native psyllids from multiple locations for *Trechnites*.

We concluded examining native psyllid species for parasitism by *Trechnites* through the final year of this project. We have found *Trechnites insidiosus* attacking native, non-pest *Cacopsylla* spp occurring on willows. 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, but all emerging wasps were *T. insidiosus*. Fresh and Processed Pear Committee funds were used to leverage additional funds from WSDA to expand this work to include a larger geographical area.

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

- 3D-printed tube traps and screened sticky cards continue to be successful at capturing adult *T. insidiosus*
- PCR was determined to be the most effective way of assessing parasitism levels
- Overwintering bands can be effective at obtaining large numbers of *T. insidiosus* for bioassay work and at assessing hyperparasitism levels. We learned in 2022 timing of band placement greatly affects the number of psylla mummies obtained.



Trechnites ovipositing into a pear psylla nymph.

- We produced a model that accurately predicts parasitism rates to within 7.5% for 95% of the observations in the field. The model was trained on a portion of the data collected for Objectives 1 and 2 and tested against the remaining data. Surprisingly, the location of the data was often of least importance to producing accurate results. We believe this model will be generalizable to much of the pear growing region in the PNW.
- 48 *Trechnites sadkai* were found from June to October in beat tray samples from bitterbrush (*Purshia*) located near Tieton, WA. This parasitoid was potentially attacking psyllids that occur on this plant. Tube traps placed near stands of bitterbrush captured both *T. sadkai* and *T. insidiosus*. Several other parasitoid species were collected, including *Tamarixia* spp. from psyllids occurring on bitterbush.

METHODS

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

Adult Trechnites. At each of the four locations, five plots were laid out in an orchard. Collection of all data occurred from April-late September at all locations. We discontinued this sampling in the two Oregon research orchards, as *Trechnites* populations remained low. We expanded the use of traps in Oregon but removed the random leaf/targeted nymph samples described below.

Within each plot, we placed 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 replaced the unscreened sticky cards at all locations. Beat tray samples, which were conducted in Year 1, were discontinued, as they did not adequately reflect *Trechnites* abundance. Leaf samples consisted of up to 20 leaves that are found to contain psylla nymphs, when sufficient quantities were present. An additional sample of 25 leaves was randomly collected from each plot to determine the age distribution of psylla nymphs. We also used 3D-printed cylinder traps to sample for *Trechnites*.

Percent parasitism. PCR was used to detect percent parasitism every year. In Year 1, we also dissected psylla nymphs to assess parasitism. In Year 2, we discontinued dissection and 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.

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

The percent parasitism data allowed us to model how counts of the adult parasitoid in orchards via the three different methods (sticky cards, tray counts, traps) related 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 were compared to percent parasitism to determine if the relationship was consistent between locations and which trap type most closely predicted parasitism levels.

Model development: The postdoctoral researcher has produced a preliminary model that can accurately predict parasitism rates in WA locations. We are currently collecting weather data from our OR cooperators to finish modelling across all PNW locations. At present the model incorporates both sticky card and cylinder trap data to predict parasitism. Optimization of the model will continue in 2023 to reduce potential scouting labor for use in the grower tool.

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

We tested 12 products (Actara, Altacor, Assail, Bexar, Centaur, Delegate, Entrust, Fujimite, Lime-Sulfur, Malathion, Neemix and Rimon) in 2022. For each pesticide tested, we examined effects on sprayed adults (% mortality) compared to a water sprayed control. Mummies have been collected to test as well and will be assayed in 2023.

We were unable to test sprayed adults for sublethal effects as removing the adults from the container led to high mortality of *Trechnites*. *Trechnites* adults can only be collected once per year from psylla mummies and 2022 mummy collection was particularly low. We will be able to test the pesticide mortality on the mummies collected in 2022 and may be able to repeat adult exposure bioassays depending on survival numbers.

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

Each year, we located *Salix scouleriana*, *Salix prolixa*, and *Ribes* patches in early spring and *Salix exigua*, and *Purshia tridentata* in spring and summer. 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 were used to determine if adult psyllids were present. From these samples, psyllid mummies were isolated and the emerging parasites and psyllid host were identified. Collection occured 2-3 times per season, with the timing focused on life cycles of known psyllid species that feed on these plants.

RESULTS AND DISCUSSION

Obj. 1. We completed sampling orchards at four locations. Full analysis and tool building is in process, we discuss preliminary results under Obj 2. Fig. 1 shows comparison of trap types and psylla counts and Fig. 2 shows the comparison between trap types and *Trechnites* catch. Both 3D-printed tube traps and sticky cards collected high numbers of *T. insidiosus*. Cylinder traps and sticky

cards both monitor *Trechnites* and psylla much better than beat trays. Screened sticky cards would be effective if the numbers of psylla and *Trechnites* were the only species of interest. For studies also examining larger insects (e.g. lacewings), unscreened sticky card would need to be used. Cylinder traps are better if preservation of the insect for additional research is needed. Parasitism increases with rising adult pear psylla numbers. At peak parasitism we see a decline in adult pear psylla (approx. 2 weeks post adult psylla peak). This led to a population peak of *T. insidiosus* adults captured and continued suppression of pear psylla. We can observe a linked phenology of *Trechnites* and psylla in Fig 3 and 4.

Obj. 2. In 2021, we successfully obtained funding from the WSDA to expand this work and hired a postdoc (Zilnik) with expertise in modelling. Zilnik has prepared a preliminary model to predict parasitism based on trap capture and PCR results from all three years. The model was constructed using machine learning tools. Model training was conducted with 2/3 of the data collected between 2019-2021. The remaining 1/3 of the data were used to test the accuracy of the model. Currently, the model can accurately predict parasitism rate 95% of the time within 10% of the observed value (Fig. 5). The most important variables in the model that contribute to the high accuracy were psylla degree days, psylla nymph counts, and cylinder trap psylla adult counts (Fig 6). *Trechnites* counts from sticky cards and cylinder traps also contributed substantially to model accuracy. At present, the model does not appear to gain more accuracy from location information and it therefore could be generalized to most locations in WA. We will obtain psylla degree day data from Medford, OR and complete the model. During the spring of 2023, additional testing and optimization of the model will be performed. Additional validation with grower orchard data will be conducted in 2023 in Yakima Valley, Wenatchee Valley, and Hood River as part of other ongoing projects.

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. 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. We repeated this process in 2020 but returned too few psylla mummies to complete this objective. In 2021, we adjusted our banding procedure and obtained 474 psylla mummies from the 1,200 bands placed (37.8% of bands contained at least 1 mummy). We were able to conduct the pesticide bioassays on 12 compounds in 2022. The results are summarized in Fig 7. As expected, broad spectrum compounds resulted in high mortality rates of *Trechnites* adults. Altacor and Rimon had the highest 24-hour survival rates. Only Rimon showed no difference in survivorship from the control at 48 hours. Compounds recommended to include in IPM programs such as Neemix, Centaur, Lime-Sulfur, and Spinosad had very low survivorship of *Trechnites*. *Trechnites* are very susceptible to many commonly used insecticides and care should be taken to avoid spraying these compounds when *Trechnites* adults are present in the orchard. *Trechnites* is likely more protected in the mummy stage, which will be tested in Spring 2023.

Obj. 4. In 2019, we found *Trechnites* emergence from *Cacopsylla americana* and *C. alba* collected from *Salix rigida/prolixa* and *S. exigua*. *Cacopsylla alba* occurs on catkins of the host or in galls produced by a small midge, and more occasionally on foliage; parasitized psyllids were collected from all structures, but especially from catkins and galls. These are the first records world-wide that *Trechnites* 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.

In 2020, we also found *T. sadkai* in beat samples and tube traps in bitterbrush in Tieton, WA, but *T. sadkai* did not emerge from psyllid mummies collected from bitterbush. Old samples from the Tieton area (2002-2003) from both bitterbrush and a neighboring soft pear orchard were consulted. While the bitterbrush samples contained *Trechnites spp.*, the pear orchard samples were only *T. insidiosus*.

In 2021, we found no *Trechnites spp.* in surveys of *Salix rigida/prolixa*. The parasitoid *Prionomitus* was collected frequently in West Yakima and Union Gap. Closer examination of the reproductive morphology of the *T. sadkai* samples revealed that previous findings were likely incorrect and instead we are observing *T. alni*. It remains unclear if *T. alni* would specialize on psyllids and thus be good biological control agent for pear. We were able to collect many psyllid mummies containing *Prionomitus spp*. however further testing is needed to determine if they will attack pear psylla.

In 2022, we collected 20+ parasites from *Cacopsylla alba* mummies spring through late summer, from locations along the Yakima River and Ahtanum Creek, where *Salix exigua* (host of *Cacopsylla alba*) is common. Sex ratio of the *Trechnites* from these mummies was slightly male-biased. This is a multivoltine psyllid, quite different from the typical univoltine life cycle of *Salix Cacopsylla*. Collections of mummies from other *Cacopsylla* (from *Ribes*), univoltine *Cacopsylla* from other *Salix*, and *Cacopsylla* relatives on *Purshia* produced only *Prionomitus*, apparently a poor natural enemy of pear psylla in North America although a better parasite for pear psylla in Europe.

Our multi-year survey indicates that *Salix exigua* is a potential reservoir of *Trechnites*. Studies are planned to confirm that *Trechnites* specimens reared from willow psyllid will attack pear psylla.

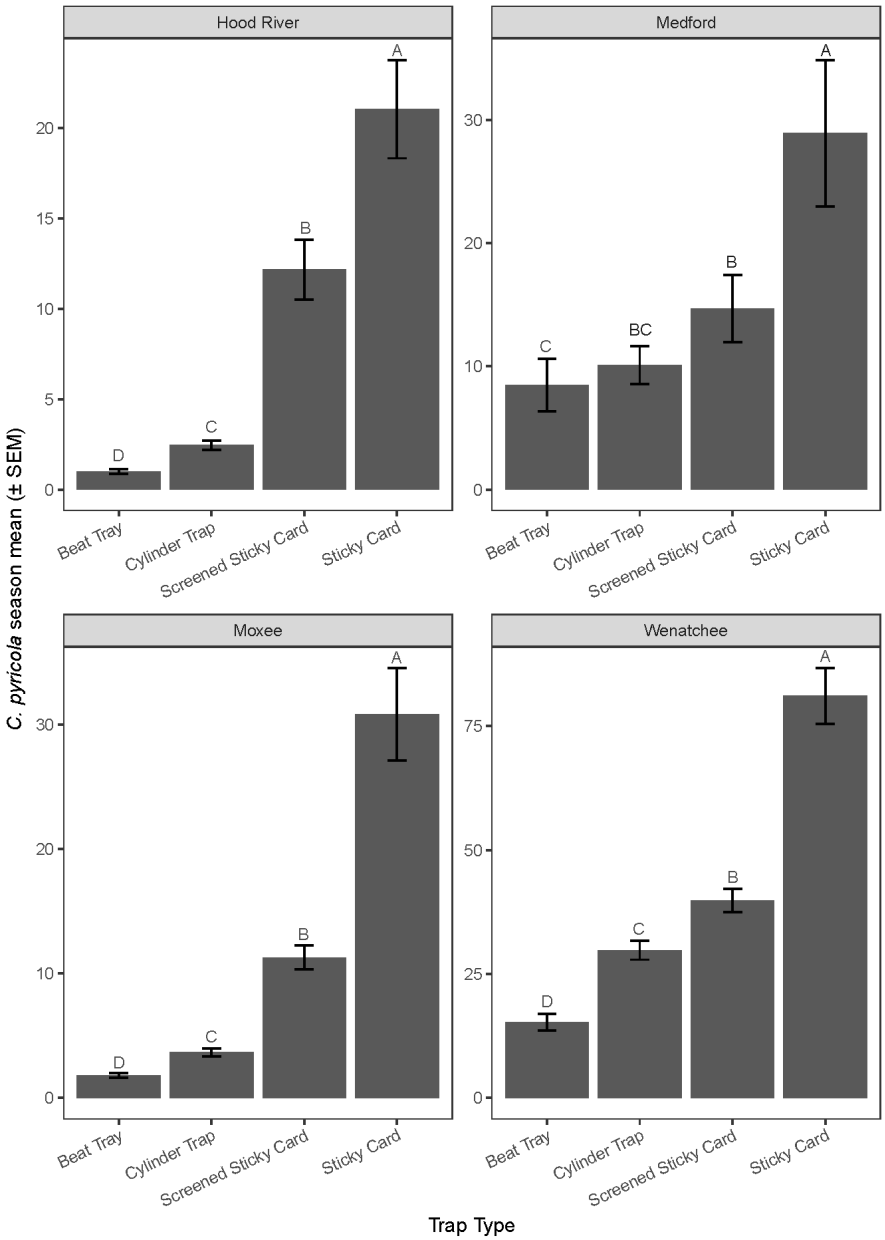


Fig 1. Seasonal mean number of *C. pyricola* by trap type in 2019 (± SEM). Letters indicate means separation between trap type. Note that y-axis varies between locations. Sticky cards (including screened sticky cards) returned higher numbers of *C. pyricola* than all other traps except in Medford, OR. All traps returned higher numbers of *C. pyricola* than the beat tray sampling method.

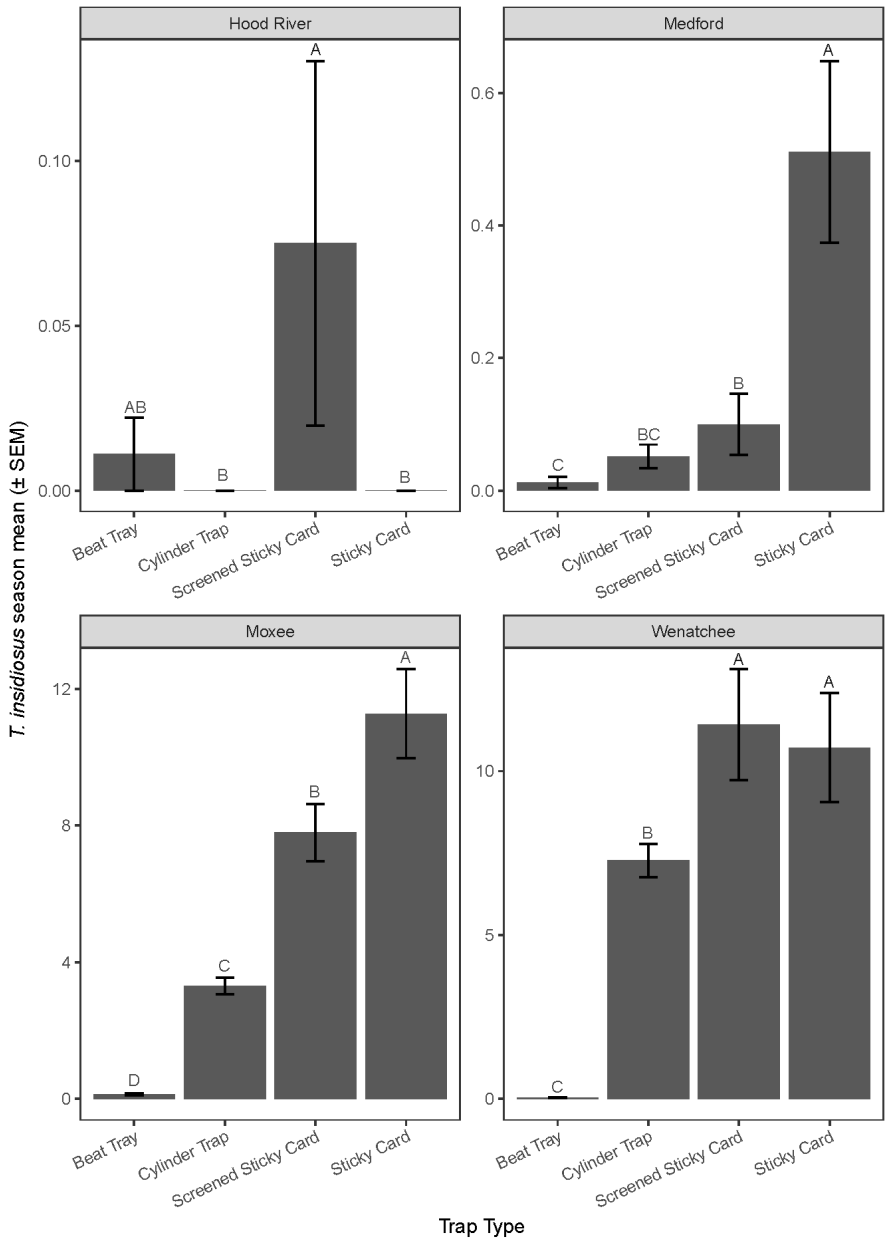


Fig 2. Seasonal mean number of *T. insidiosus* by trap type in 2019 (± SEM). Letters indicate means separation between trap type. Note that y-axis varies between locations. Moxee and Wenatchee, WA returned an order of magnitude larger number of *T. insidiosus* than the Hood River and Medford sites. Hood River, OR was the only location where beat tray sampling recorded more *T. insidiosus* than any trap type except screened sticky cards, likely due to very small numbers of wasps.

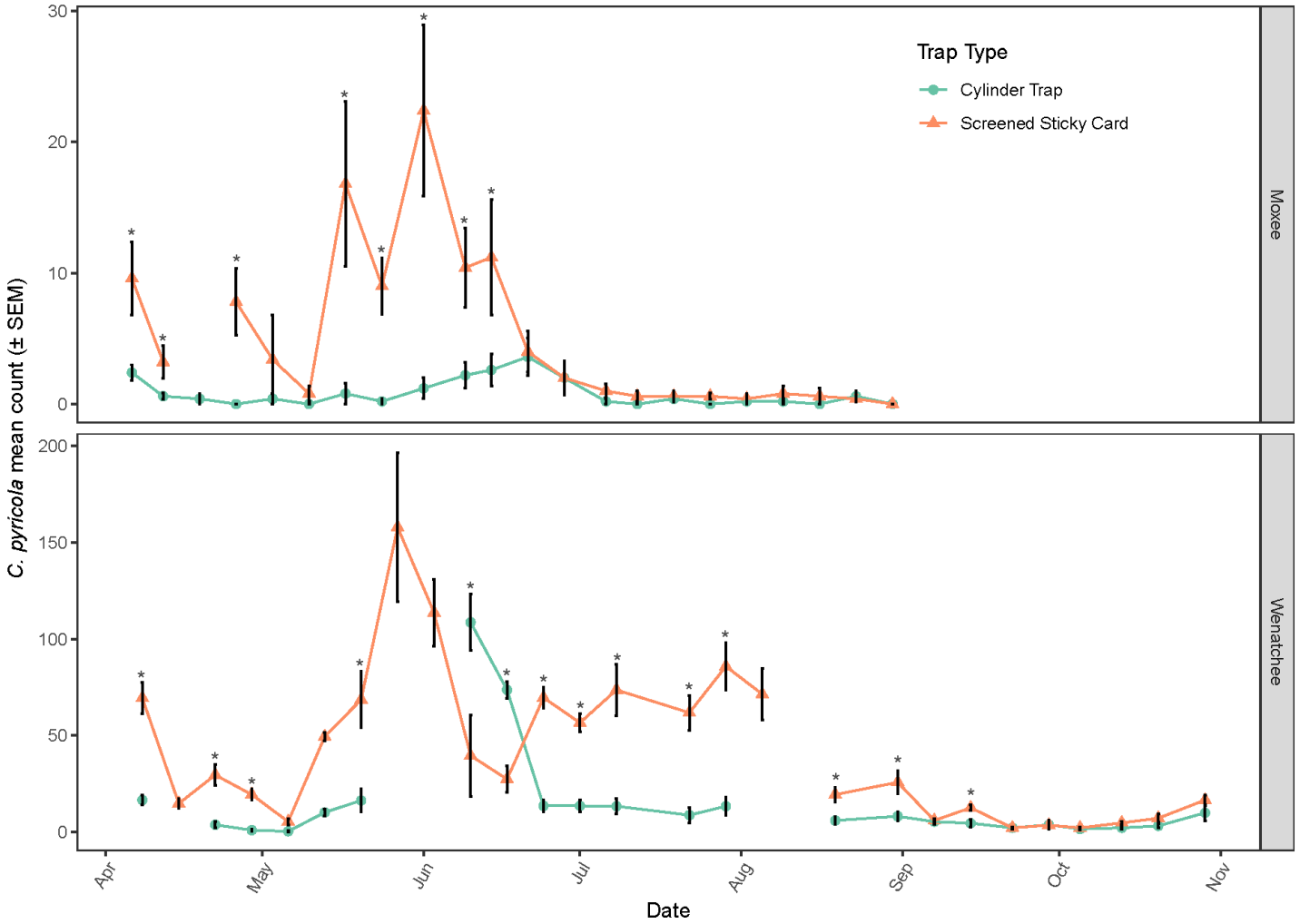


Figure 3. Mean (± SEM) weekly counts of adult *C. pyricola* in 2021. The cylinder trap nearly missed the population peaks in Moxee, though overall trap catch did qualitatively track population growth. Cylinder traps appear to track *C. pyricola* population peaks more accurately at higher population densities

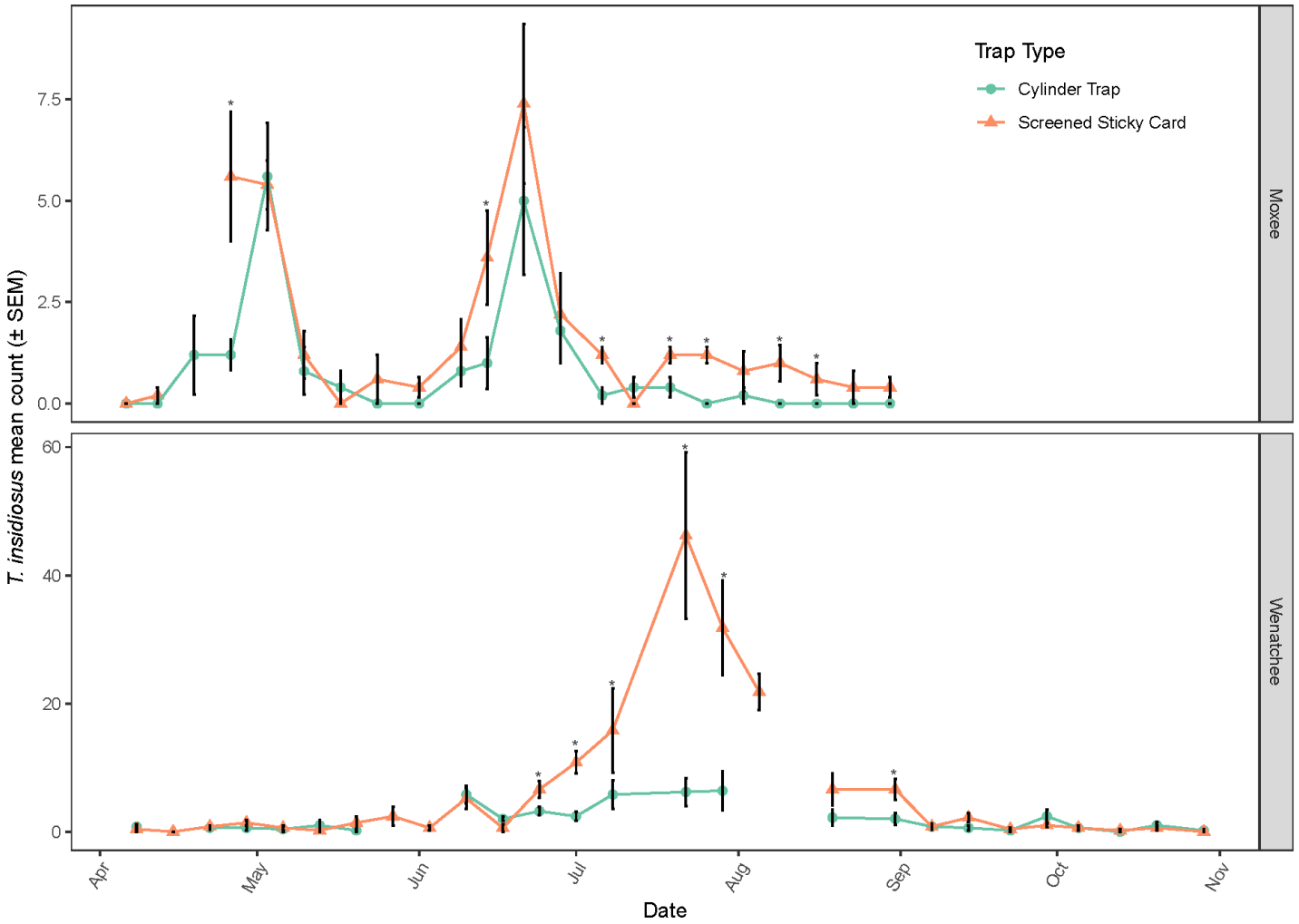


Figure 4. Mean (± SEM) weekly counts of adult *T. insidiosus* in 2021. Wenatchee had a single population peak with more total *T. insidiosus* and Moxee had two population peaks. Notice that cylinder trap and screened sticky card values almost always overlap at lower population densities. Screened sticky cards appear to track high population densities much better than cylinder traps.

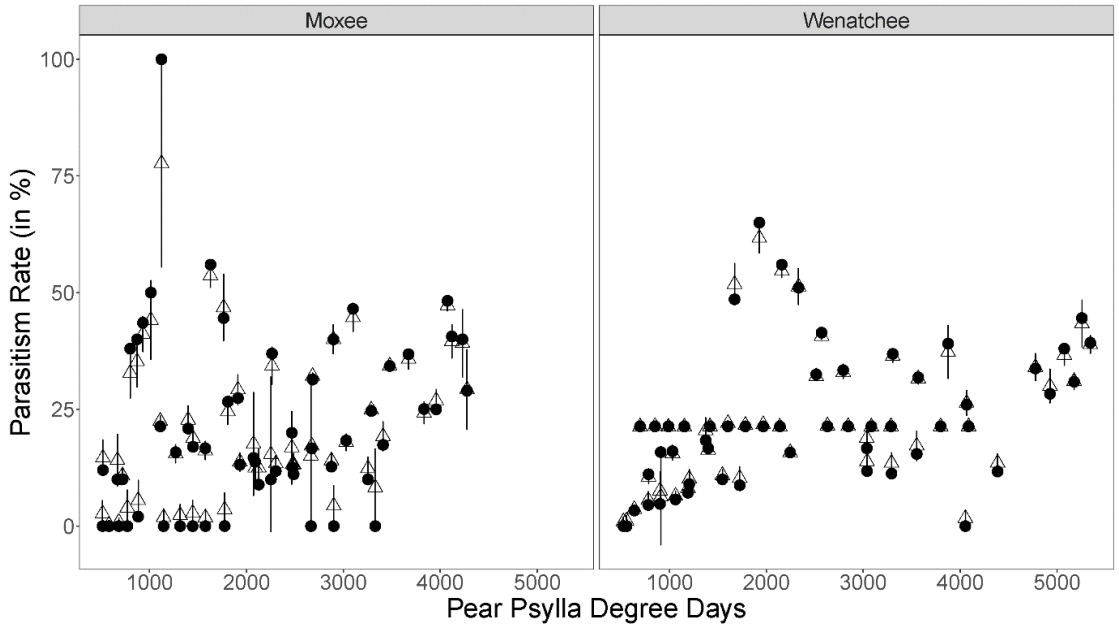


Fig 5. Observed mean parasitism rate (solid circles) vs mean predicted parasitism rate (open triangles) with prediction error. Prediction error generally increased at extreme ends of the parasitism rate (100% and 0%). The model generally performs well (low error) at predicting parasitism rates between 10% and 80%.

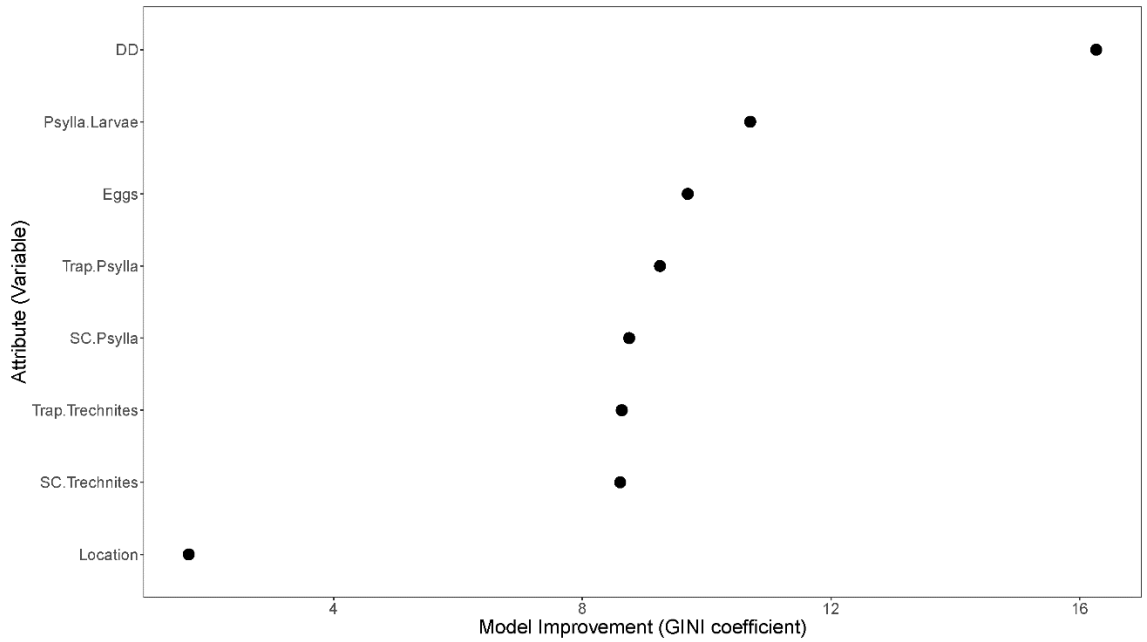


Fig 6. Plot of variable performance in improving predictive power of the parasitism rate model. Psylla degree days (DD) contributed to the most to improving model performance. Further testing will reveal which methods growers should use to monitor psylla and *Trechnites* to get an estimate of their biological control services.

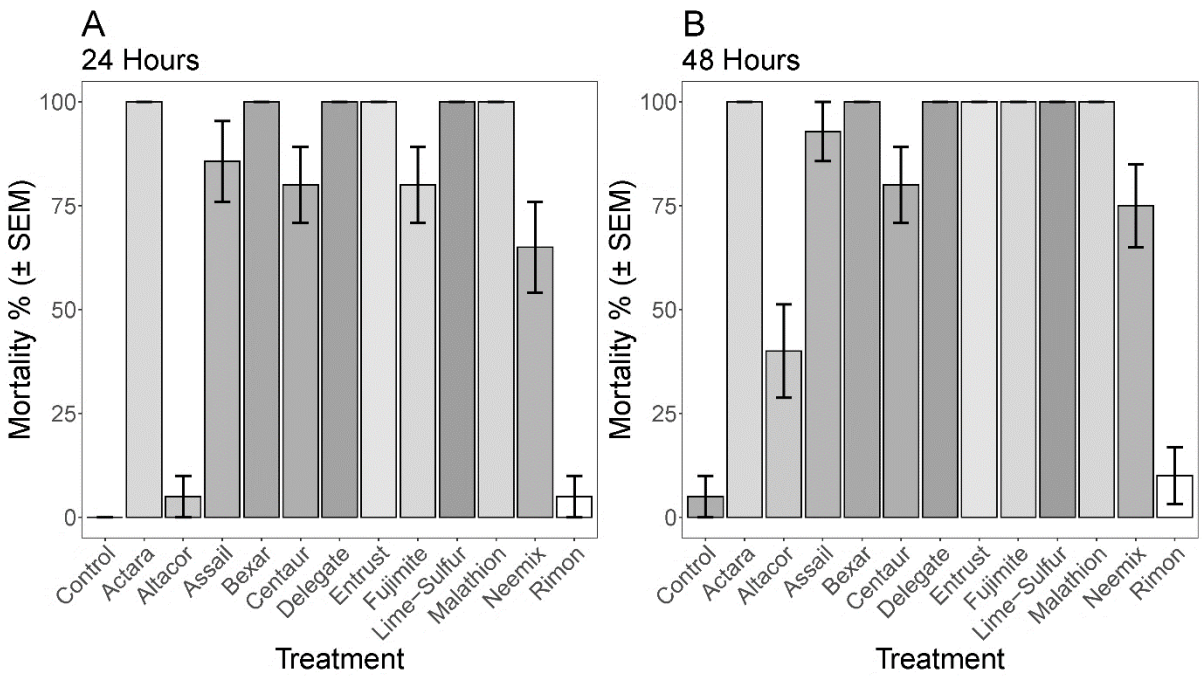


Fig 7. Percent mortality of adult *Trechnites* (± SEM) for the compounds tested at 24 hours (A) and 48 hours (B). All compounds except Altacor and Rimon had increased mortality above the control at 24 hours and only Rimon did not differ from the control mortality at 48 hours. *Trechnites* appears to be extremely susceptible to the majority of compounds used in pear for pest management.

CONTINUING PROJECT REPORT **PROPOSED DURATION**: 3 Years

Project Title: **Calibrating current NE action thresholds with lure-baited trap catch**

PI: Christopher Adams

Organization:Agricultural Research Foundation
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Co-PI (3):
Organization:
Telephone:
Email:
Address:
City/State/Zip:

Cooperators: GS Long, Wilbur-Ellis, W. Ag. Improvement, Chamberlin

Total Project Request: Year 1: $45,000 **Year 2:** $45,000 **Year 3:** $45,000

Other funding sources

Agency Name: WSARE

Amt. requested: $339,668

Notes: To continue and expand this research a WSARE proposal was submitted. If funded, this grant would provide $339,668 for another 3 years of research.

Budget 1

Organization Name: OSU - ARF **Contract Administrator:** Elizabeth Etherington/Cody Hess

Telephone: 541-740-0002/ 541-737-1275

Email address: Elizabeth.etherington@oregonstate.edu / cody.hess@oregonstate.edu

Item	2021	2022	2023
Salaries[1]	13,000	13,000	13,000
Benefits[2]			
Wages			
Benefits			
Equipment			
Supplies[3]	$6,000	6,000	6,000
Travel[4]			
Miscellaneous			
Plot Fees			
Total			

Footnotes:

[1] new student position
[2] 11.3%
[3] Research consumables
[4] In state travel

Budget 2

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

Telephone: 509-454-4463 **Email address:** Chuck.Myers@ars.usda.gov

Item	2020	2021	2022
Salaries	13,000	13,000	13,000
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total			

Footnotes:

Budget 3

Organization Name: WSU **Contract Administrator:** Shelli Tompkins/Katy Roberts

Telephone: 509-665-8271, ext 2/ 509-335-2885

Email address: shelli.tompkins@wsu.edu / arcgrants@wsu.edu

Item	2021	2022	2023
Salaries	13,000	13,000	13,000
Benefits			
Wages			
Benefits			
Equipment			
Supplies[1]			
Travel			
Plot Fees			
Miscellaneous			
Total			

Footnotes:

Recap of Original Objectives

Biological control services provided by natural enemies (NE) are a key part of pear integrated pest management in the Mid-Columbia region. Considerable work has been done studying the role of natural enemies in pear IPM in the PNW (DuPont and Strohm 2019, DuPont et al. 2021) and the economic value of these biological controls (Gallardo et al. 2016). Through careful management of these natural enemies, pear psylla populations can be substantially reduced below economic action thresholds (Amarasekare and Shearer, 2017, Westigard and Moffitt, 1984). While it is universally understood that these natural enemies can play an important role in pear orchard IPM, relative abundance of these beneficial arthropods has not been converted into action thresholds that can be used by crop consultants. Establishing natural enemy thresholds has been identified as an important priority for maintaining current IPM programmes (DuPont et al. 2021). This research is designed to establish meaningful action thresholds by partnering with experienced crop consultants to calibrate trapping numbers using a lure-baited yellow sticky card, with experience based decisions about pest management.

1. **Use plant volatile baited monitoring traps to describe NE communities in orchard ecosystems through the season.**
   Following the specifications of Jones et al. (2015) lures where manufactured in the lab and deployed with yellow sticky cards as NE traps. Traps were placed at 20 pear orchards throughout Hood River County and were checked weekly for natural enemies (Fig. 1). These traps were maintained from April – October.

2. **Compare capture of several key species of NEs in lure-baited traps with numbers measured from standard scouting techniques.**
   Weekly natural enemy data was collected via lure-baited traps. Pear psylla numbers were measured each week by randomly collecting 10 pear shoots from target blocks and counting the number of eggs, young nymphs, and old nymphs from five leaves on each shoot. This method is regularly used by crop consultants to help guide management decisions.

3. **Establish action (or in-action) thresholds for key NEs.**
   In collaboration with crop consultants, we have begun to create target thresholds for key natural enemy species that we hope indicate populations are building at a rate sufficient to control pear psylla. These numbers will need to be verified and refined over the next year.

Significant Findings

- 2021 Graphs are from Oregon Data only as Washington data was not collected in 2021.

- **Other:** Crop consultants already feel that this data is highly valuable, and have requested that the data be sent out to all stakeholders in the Hood River area every week. This data is now part of our weekly updates to the stakeholders (Fig. 2). Consultants currently use the area wide average of NE catch numbers to decide if a specific block is above or below average, which helps them decide how best to manage each block.
- **Other:** To continue to expand this research a Western SARE proposal was submitted. If funded this grant would provide $339,668 for an additional 3 years of research providing time to fine tune natural enemy thresholds and management decisions.

- **Objective 1 (33% complete):** In 2021 at total of 5,037 natural enemies were collected, with green lacewings (1,680) and Dereaocoris (1,836) being the most abundant NE found in Hood River Co. In

2022 at total of 5,315 natural enemies were collected, with green lacewings (1,091), Dereaocoris (1,303), and yellow jackets (1,040) being the most abundant NE found in Hood River Co. These data suggest that lure baited monitoring traps can be used to gauge NE populations.

- **Objective 1 (66% complete):** A weekly natural enemy report containing the average number of NE found in each region of Hood River Co. was sent out to pear growers. Averages were shown week by week, allowing growers to see if NE populations were increasing or decreasing.
- **Objective 2 (50% complete):** Lure baited trap data accurately describe seasonal phenology of many key natural enemy populations throughout the growing season. These data allow us to predict relative populations of these key natural enemies.
- **Objective 3 (25% complete):** We have begun to produce target thresholds and timing for key natural enemy species that can provide suppression of pear psylla, in consultation with crop advisors. These will be tested and adjusted as needed in the next field season.

Methods

1. Use plant volatile baited monitoring traps to describe NE communities in orchard ecosystems.

NE lures containing acetic acid, methyl salicylate, phenylacetaldehyde, and 2-phenylethanol, a combination that has been shown to attract key indicator groups of NE, were made at the OSU MCAREC lab. These lures were hung on yellow sticky traps and placed at 20 pear orchards that were recommended by collaborative crop consultants. Traps were checked and replaced weekly from April to September. Captured insects were identified to family level, species complex (e.g. Lacewings), or to species when possible.

Expected outcomes: At the end of this project, we hope to be able to correlate numbers of natural enemies with relative levels of pear psylla control, and supply crop consultants with reliable action thresholds. While this project will likely require years of refinement, I believe that this first step is critically important to setting the expectation that action threshold for natural enemies can be quantified. Additionally, we hope to direct private industry to manufacture specific lures according to our specifications that will target key natural enemies and be available for commercial use.

2. Compare capture of several key species of NEs in lure-baited traps with numbers measured from standard scouting techniques.

To evaluate the usefulness of NE traps we will need to show that trapping can be as good or better at measuring the building NE populations, as scouting. Scouting for NE gives only provides snap shot in time pest and predator populations, and may be negatively influenced by weather or sampling technique, which makes it difficult to know if you have an accurate picture of the insect community. Traps have the advantage of collecting data continually over the time period between trap checking. Lure baited traps provide a more consistent measure of the local arthropod community and does not vary with the person checking the trap. Catch data was shared with consultants in real time during the study and reviewed retrospectively to see how recommendations and predictions of pest and NE populations matched with catch data. Cooperating crop consultants have been asked to keep detailed notes of psylla and NE counts made as part of their normal scouting routine, as well as recommendations they made for each week. At the end of the season, we will compare crop consultant's management decisions and scouting counts with trap capture for that same period of time.
For the 2022 field season, the addition of scouting for psylla each week was added to the trapping protocol. Weekly psylla counts were sampled by randomly collecting 10 pear shoots from each site and counting the number of eggs, young nymphs, and old nymphs from 5 leaves from each shoot.

This method is regularly used by crop consultants to help guide management decisions. The addition of this data will give a clearer image of how psylla populations grew or decreased each week at each site.

Expected outcomes: This research aims to provide data for the establishment of a standard lure for the attraction of natural enemies, for the purpose of monitoring populations. At the conclusion of this research, we hope to encourage/collaborate with the private industry (e.g. AlphaScents) to develop a commercial lure that can be used by crop consultants.

3. Establish action (or in-action) thresholds for key NEs.

In year three we will, in collaboration with our crop consultant partners, establish target thresholds for key natural enemy species that indicate that populations are building at a rate sufficient to control psylla numbers. We will attempt to make management decisions based on these target numbers.

Expected outcomes: This research aspires to establish action threshold for natural enemies that would allow crop consultants to confidently recommend withholding pesticide sprays based on catch data. This project plans to arrive at these action threshold in collaboration with the crop consultants that will one day use them.

Results and Discussion

Objective 1. Use plant volatile baited monitoring traps to describe NE communities in orchard ecosystems.
We are currently still collecting and processing spray recommendations and confirming actual spray application timings.

The traps placed at 20 pear orchards in Hood River Co (Fig 1.A.) yielded a total of 5,037 natural enemies in 2021. Of these the most common insects found were green lacewings (1,680), Dereaocoris (1,836), Yellow Jacket's (809), and earwigs (232) in 2021. In 2022 traps placed in the same 20 orchards yielded a total of 5,037 natural enemies. Of these the most common insects found were green lacewings (1,091), Dereaocoris (1,303), Yellow Jackets (1,040), Syrphidae (615), Trechnites (696), and earwigs (274) (Fig. 3 A and B).

In Chelan County, WA 9 traps placed along US route 2 near Cahsmere (Fig 1.B.) that yielded a total of 3,773 natural enemies. Of these the most common insects found were green lacewings (1,112), Trechnites (1,743), and Dereaocoris (462), in 2022 (Fig. 3 C).

In Yakima County, WA 10 traps placed in pear orchards (Fig 1.C.) yielded a total of 2,668 natural enemies. Of these the most common insects found were green lacewings (994), Dereaocoris (409), Coccinellidae (322), and Yellow Jackets (320) (Fig. 3 D) in 2022.

Total number of natural enemies was similar in all three regions (although Hood River is double Yakima) suggesting that pear psylla control by natural enemies could be achieved in all three regions. Relative abundance graphs (Figure 4 A-D) illustrate timing of these natural enemies and will help us predict the arrival of these key insects. The irregular (or spotty) relative abundance of natural enemies seen in Chelan Co (Figure 4 C) is likely the result of insecticide sprays. An interruption in relative abundance may indicate that these insects were recruited from outside the orchard after their numbers were reduced or eliminated. Natural enemies in Yakima county (Figure 4 D) show an unbroken building

of the population numbers. While total numbers where only half of what we found in Hoor River, the consistent presence of these insects is encouraging.

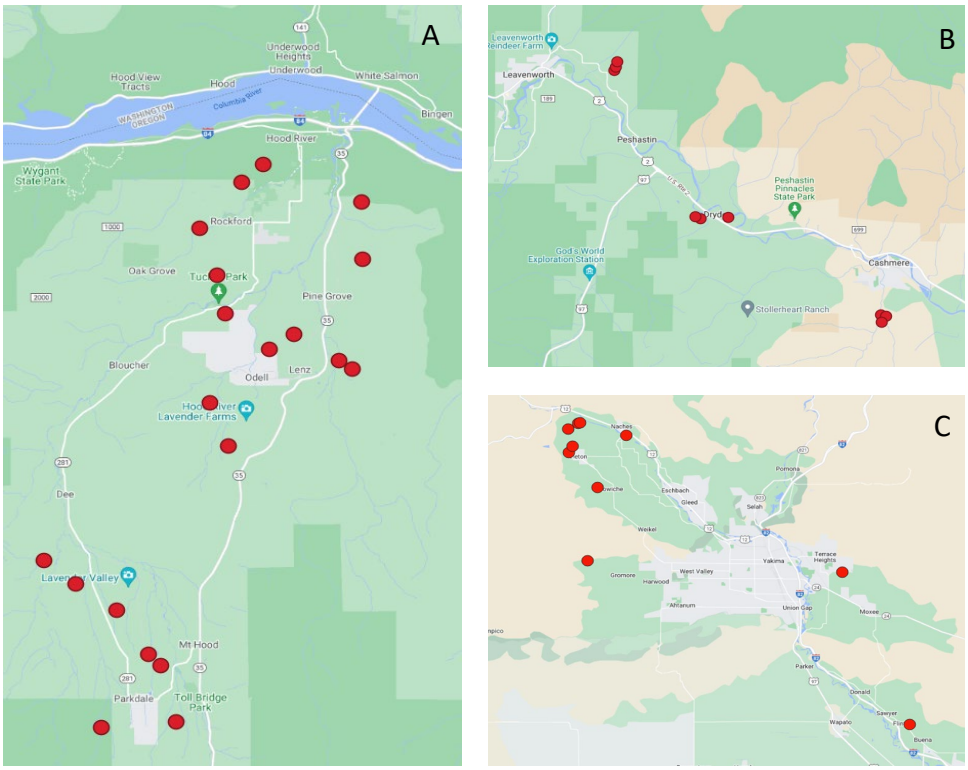
Objective 2. Compare capture of several key species of NEs in lure-baited traps with numbers measured from standard scouting techniques.

Lure baited yellow sticky cards effectively collected 12 key natural enemies season long and represent significant time savings over scouting the orchard with beat trays. In Addition, lure baited yellow sticky cards collected insects not typically collected in beat trays such as yellow jackets, bald faced hornets, and adult syrphid flies. Lure baited yellow sticky card provide the additional benefit of collecting data all day long over an entire week (or more). This benefit addresses some of the limitations of beat trays which are impacted by the time of day the traps are checked or from the high wind conditions. Beat tray data can also be impacted by variation between people conducting the sample, or the limb of tree selected.

Objective 3. Establish action (or in-action) thresholds for key NEs.

Earlier researchers have suggested that natural enemies need to be present in large numbers early in the season to be effective at rendering biological control against pear psylla. In Orchards identified by crop consultants as “easy” to control with natural enemies, we find large populations of natural enemies early in the season and at ratios of up to 100:1 (NE:PP). Where populations of NE are not present early in the season or when ratios of NE to PP is not sufficient, we see lack of control. Tracking natural enemies with lure baited sticky cards also indicates where psylla sprays are impacting natural enemies and, in some cases, we can see where insecticide sprays were applied when no psylla were present. This tool will allow for improved management decisions and better-timed sprays.

We will begin to establish target thresholds for key natural enemy species that indicate that populations are building at a rate sufficient to control psylla numbers after collecting NE data in 2022 and compiling it with the 2021 data. There is much work to be done before we can confidently make recommendations from these trapping data. However, we are encouraged by the high level of enthusiasm from our crop consultant collaborators, who feel that this data is informative to them.



Figures 1 (A-C). Maps showing the sites where traps were placed in A. Hood River County, OR in 2021 and 2022, B. Chelan Co. in 2022, and C. Yakima Co. in 2022.

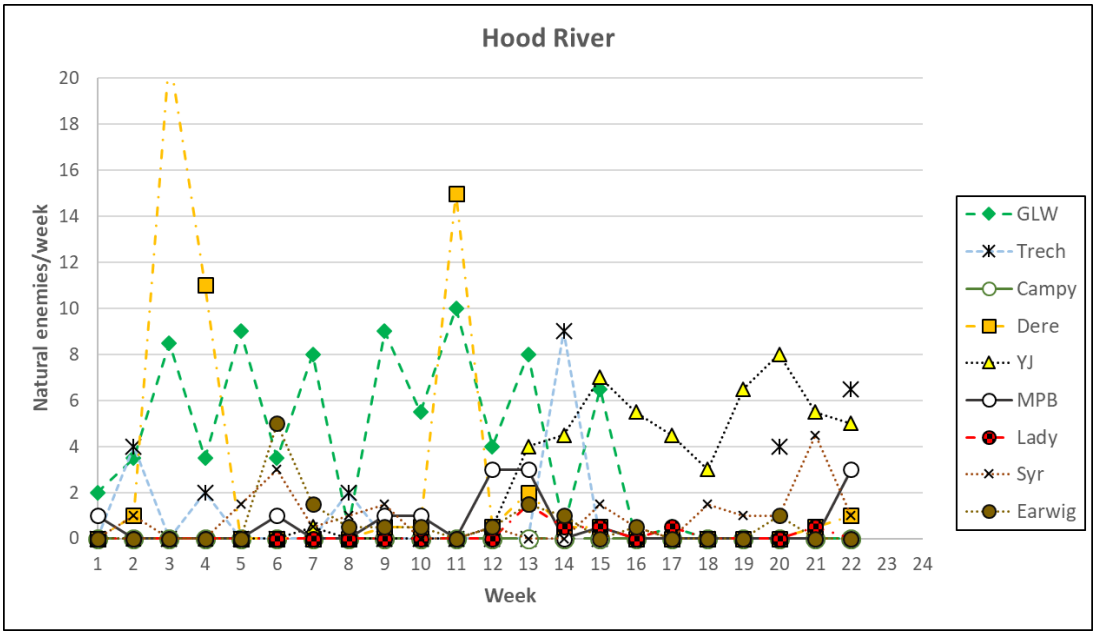


Figure 2. An example of the average natural enemy counts found in Hood River region, sent out weekly to growers and crop consultants in 2021 and 2022.

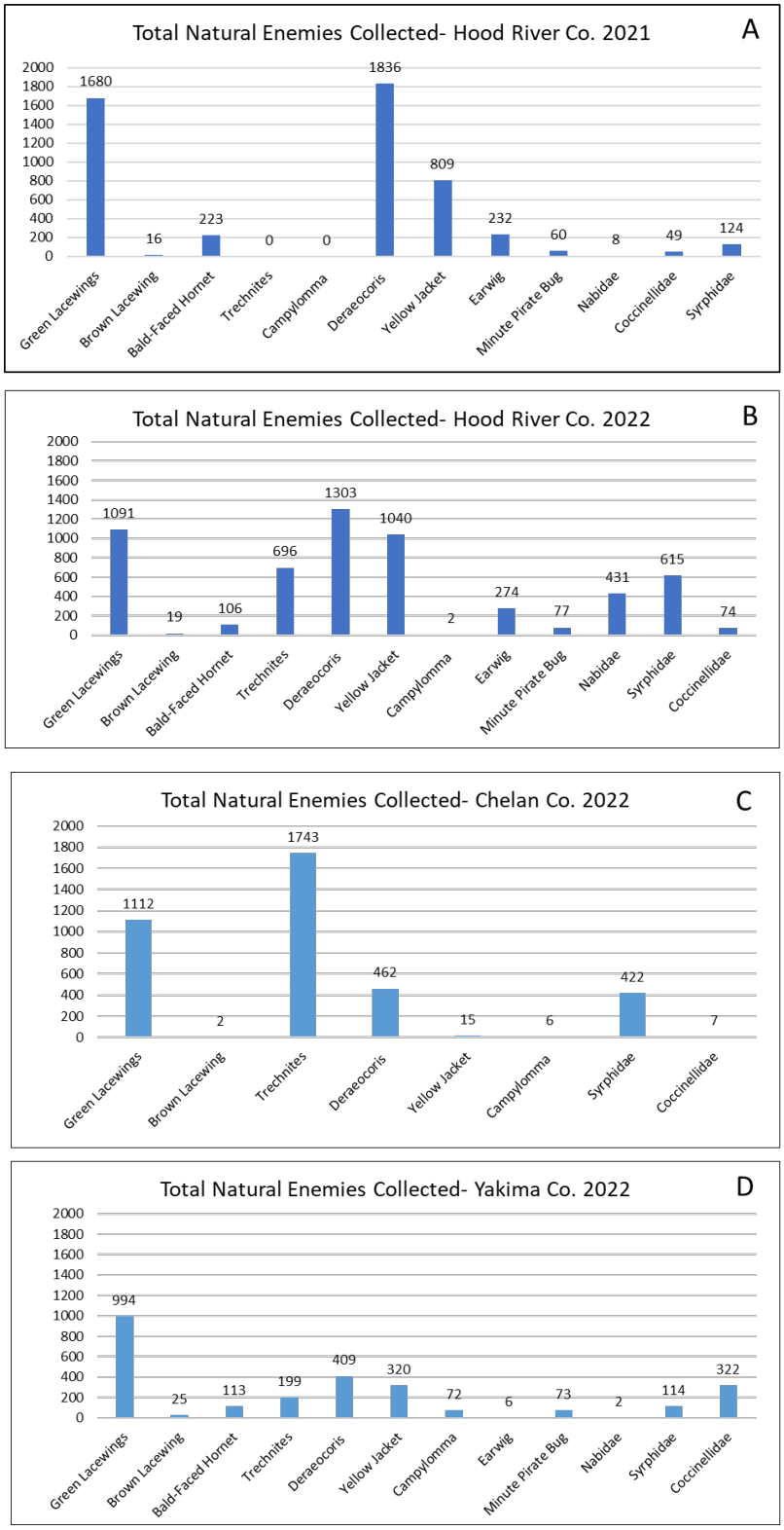


Figure 3. Total number of natural enemies collected from Hood River Co. in 2021 (A), 2022 (B), Chelan Co. in 2022 (C) and, Yakima Co. in 2022 (D). Showing variation in abundance and species diversity by region.

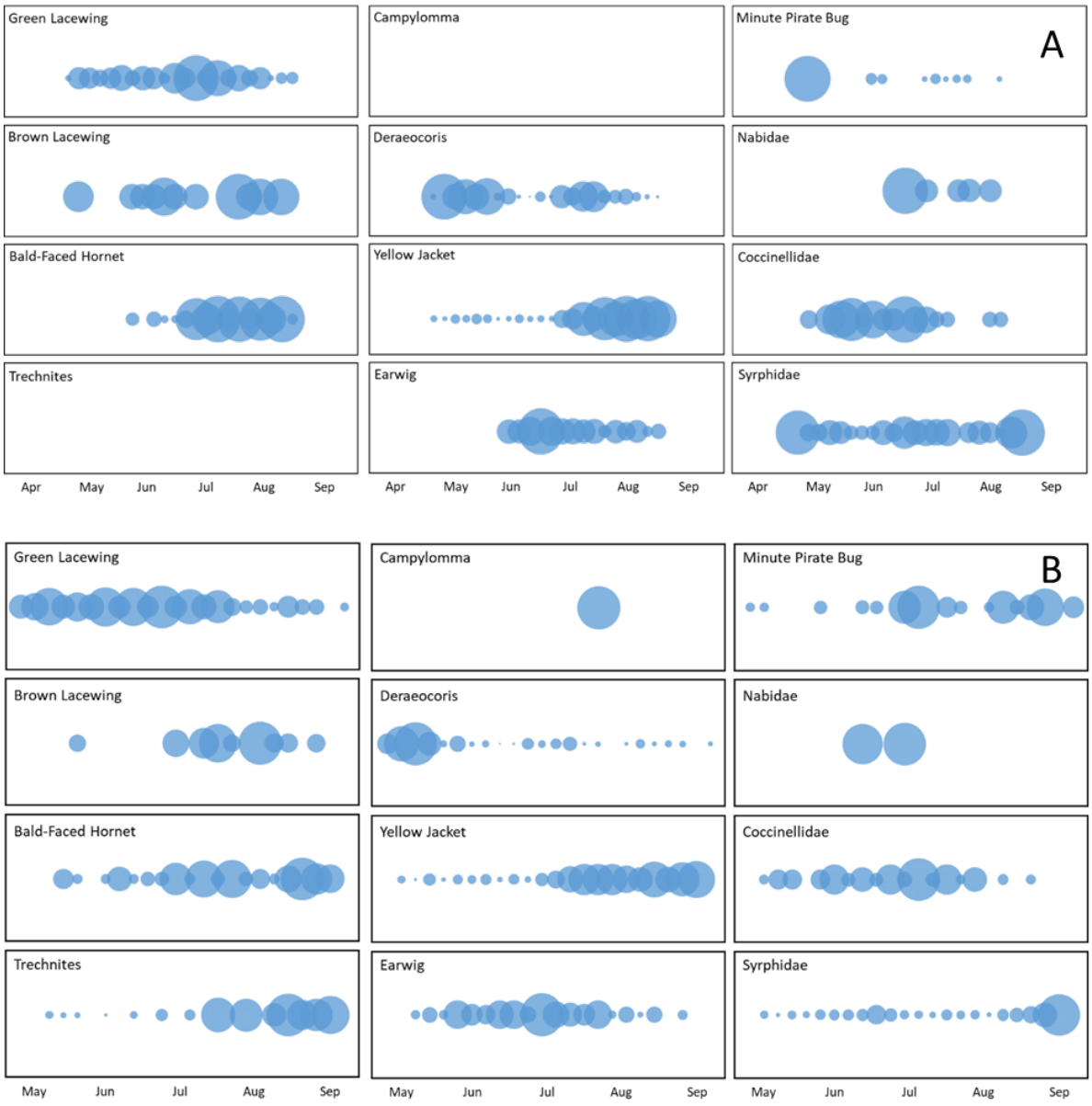


Figure 4, A and B. The relative abundance of selected natural enemies throughout the season in Hood River Co. in 2021 (A) and 2022 (B)

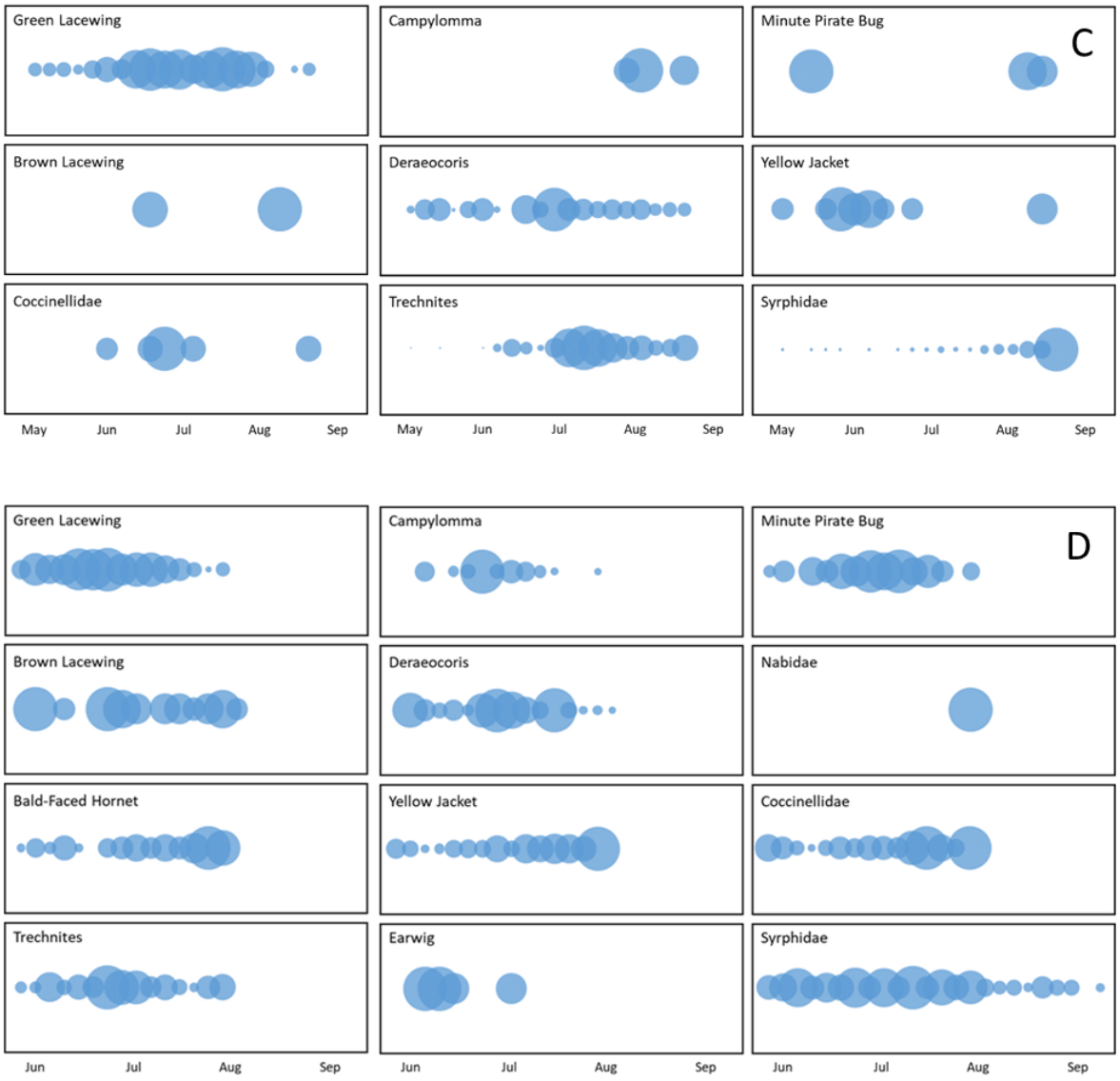


Figure 4, C and D. The relative abundance of selected natural enemies throughout the season in Chelan Co. in (C), and Yakima Co. (D) in 2022.

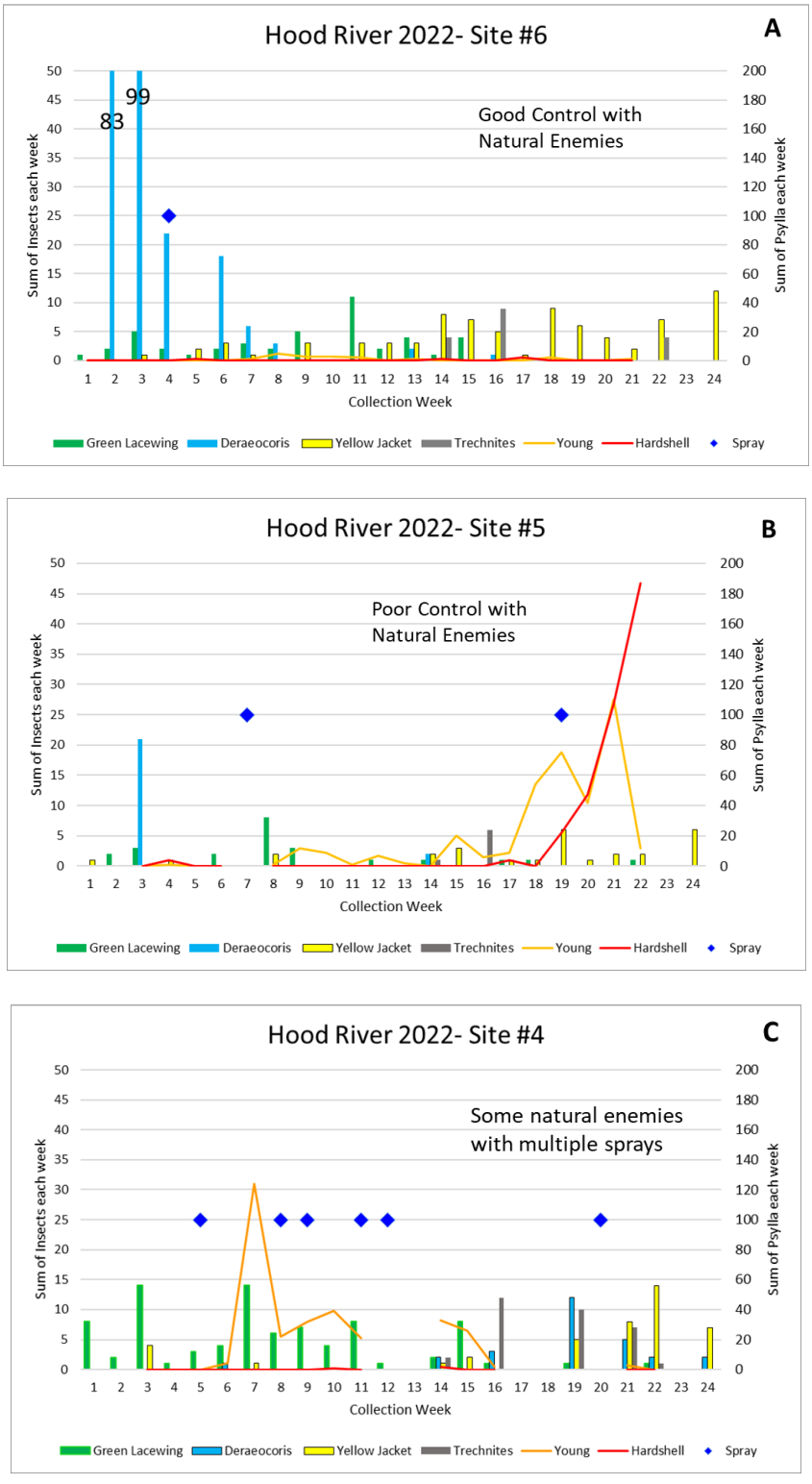


Figure 5. Counts of Natural enemies (NE), young pear psylla nymphs (young), and mature psylla nymphs (hard-shell) at select sites in Hood River Co. Figure A shows ideal NE control. Figure B shows lack of NE control. And Figure C shows insufficient NE control with sprays.

Literature Reviewed.

Amarasekare K. G., and P. W. Shearer. 2017. Stability of Cacopsylla pyricola (Hemiptera: Psyllidae) Populations in Pacific Northwest Pear Orchards Managed with Long-Term Mating Disruption for Cydia pomonella (Lepidoptera: Tortricidae). Insects, (8) 105, 1-12.

DuPont S. T., C. J. Strohm. 2020. Integrated pest management programmes increase natural enemies of pear psylla in Central Washington pear orchards. J. Appl. Ent. 144:109-122.

DuPont S. T., C. Strohm, L. Nottingham, D. Rendon. 2021. Evaluation of an integrated pest management program for central Washington pear orchards. Bio. Control. 152 (2021) 104390.

Gallardo R. K., J. F. Brunner, S. Castagnoli. 2016. Capturing the economic value of biological control in western tree fruit. Bio. Control. 102 (2016) 93-100.

Gut L.J., P. H. Westiguard, C. Jochums, W. J. Wiss. 1981. Variation in Pear Psylla (*Psylla pyricoloa* Foerster) Densities in Southern Oregon Orchards and its Implications.

Jones V. P., D. R. Horton, N. J. Mills, T. R. Unruh, C. C. Baker, T.D. Melton, E. Milickzy, S. A. Steffan, P. W. Shearer, K. G. Amarasekare. 2015. Evaluating plant volatiles for monitoring natural enemies in apple, pear and walnut orchards. Bio. Control, 102: 53-65.

Jones V. P., S. A. Steffan, N.G. Wiman, D. R. Horton, E. Miliczky, Q. Zhang, C. C. Baker. 2016. Evaluation of herbivore-induced plant volatiles for monitoring green lacewings in Washington apple orchards. Bio. Contorl, 56: 98-105.

Mills N., J., V. P. Jones, C.C. Baker, T. D. Melton, S.A. Steffan, T.R. Unruh, D.R. Horton, P.W. Shearer, K.G. Amarasekare, E. Milickzy. 2016. Using plant volatile traps to estimate the diversity of natural enemy communities in orchard ecosystems. Biological Control, 102: 66-76.

Westiguard P.H., H. R. Moffitt. 1984 Natural Control of the Pear Psylla (Homoptera: Psyllidae): Impact of Mating Disruption with the Sex Pheromone for Control of the Codling Moth (Lepidoptera: Tortricidae). J. Eco. Ent. 77(6), 1520-1523.

CONTINUING PROGJECT REPORT **PROPOSED DURATION**: 3 Years

Project Title: **Biological control of BMSB using Trissolcus japonicus**

PI: Christopher Adams
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Co-PI (3):
Organization:
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Email:
Address:
City/State/Zip:

Co-PI (4):
Organization:
Telephone:
Email:
Address:
City/State/Zip:

Cooperators:

Total Project Request: **Year 1:** $30,550 **Year 2:** $31,347 **Year 3:** $32,167

Other funding sources

Agency Name: Columbia Gorge Fruit Growers

Amt. requested/awarded: $30,324

Notes:

Budget 1

Organization Name: Agricultural Research Foundation **Contract Administrator:** Charlene Wilkinson

Telephone: 541-737-3228

Email address: Charlene.wilkinson@oregonstate.edu

Item	2021	2022	2023
Salaries[1]	$ 7,975	$ 8,215	$ 8,461
Benefits	$ 5,575	$ 5,742	$ 5,914
Wages			
Benefits			
Equipment			
Supplies[2]	$ 2,000	$ 2,000	$ 2,000
Travel[4]	$ 1,000	$ 1,000	$ 1,000
Miscellaneous			
Plot Fees			
Total	$ 16,550	$ 16,957	$ 17,375

Footnotes:

[1]Faculty Research Assistant at 0.15 FTE, with 3% increase in years 2 and 3; OPE 70%

[2]Research consumables

[3]Travel to field plots

Budget 2

Organization Name: Agricultural Research Foundation **Contract Administrator:** Charlene Wilkinson

Telephone: 541-737-3228

Email address: Charlene.wilkinson@oregonstate.edu

Item	2021	2022	2023
Salaries[1]	$ 9,100	$ 9,373	$ 9,654
Benefits[2]	$ 3,900	$ 4,017	$ 4,138
Wages			
Benefits			
Equipment			
Supplies			
Travel[3]	$ 1,000	$ 1,000	$ 1,000
Plot Fees			
Miscellaneous			
Total	$ 14,000	$ 14,390	$ 14,792

Footnotes:

[1]PhD student in Wiman lab at 0.15 FTE with 3% increase in years 2 and 3; OPE 30%
[3]Travel to field plots

Recap of Original Objectives

This project addresses management of the invasive brown marmorated stink bug (BMSB) using a biological control agent, *Trissolcus japonicus* (Tj), a small egg-parasitoid wasp of BMSB. This project aims to raise and then release large numbers of this wasp, in and around managed pear orchards in Hood River, and then measure establishment and impact in subsequent years.

1. **Raise and release Tj for release in key locations. (every year)**
   A colony of *T. japonicus* was established in 2021, and new wasps were reared from BMSB eggs collected from the MCAREC lab colony. Releases of the wasps occurred weekly from June 1st- October 3rd at 12 sites.
2. **Measure establishment using sentinel egg masses and yellow sticky traps (years 2 & 3)**
   Sentinel egg masses were placed at the 2021 release sites and left for 24 hours on 6-Jul and 20-Jul, 2022. Three yellow sticky cards were placed at each site and left for two weeks on 6-Jul, 20-Jul, and 1-Aug.
3. **Describe the habitats where wasp establishment is most successful (years 2 & 3)**
   The sites that appear to have successful establishment from the 2021 releases were bordered by mixed oak and conifer forest. This habitat provides the brown marmorated stink bug additional host plant resources, as well as refugia for both the stink bug and Tj from pesticide sprays applied in the pear orchards.
4. **Measure the effectiveness of Tj biocontrol for preventing fruit damage (years 2 & 3)**
   BMSB populations will be measured with lure baited (congregation pheromone) traps to measure BMSB populations in year zero (before releases of wasps) and then during each subsequent year, to measure change in populations. Growers hosting release sites will be asked to share cull reports from the packing houses.

Significant findings / outcomes

- **Other:** As part of these efforts, we have been sending out weekly reports of BMSB captured across the network of traps. This report allows stakeholders to see if BMSB numbers are building across the region.
- **Objective 1 (66% complete):** A total of 8,434 Tj were reared at the MCAREC insectary, and released at 15 pear orchards (14 pear and 1 peach) located throughout Hood River County in 2021. A total of 44,200 Tj were reared at the MCAREC insectary, and released at 12 pear orchards (11 pear and 1 peach) located throughout Hood River County in 2022.
- **Objective 1 (66% complete):** The Oregon Department of Agriculture donated 1,400 Tj from their colony for release in Hood River in 2021, and an additional 1,700 Tj in 2022.
- **Objective 2 (50% complete):** Tj was recovered on yellow sticky traps at 4 out of 14 of the 2021 release sites**.** The inability to collect Tj on sticky cards from each release site does not mean Tj did not successfully establish at the site. The traps rely only on the wasps' attraction to the color yellow, which only measures wasps in close proximity to the sticky traps.
- **Objective 3 (50% complete):** The 2021 sites where Tj was successfully recaptured were surrounded by mixed oak and conifer forest bordering the pear orchard. Additional analysis will be done to look for correlation between wasp recapture and habitat.
- **Objective 4 (33% complete):** There was no correlation between wasp release site and reduced BMSB capture.

Methods

1. Raise and release Tj for release in key locations.

We currently have a dozen cages of stink bugs housing about 30 insects each that regularly produce several hundred eggs per week (Figure 5). Stink bugs require daily fresh food and water, colony maintenance, and egg collection, requiring serval hours per day 7 days per week. Stink bug eggs are collected daily and newly emerged wasps are placed in small cup containers with fresh stink bug eggs (Figure 5). Releases occurred every week from August through October at 15 sites in 2021, and from June- October at 12 sites in 2022 (Figure 1). Weekly release numbers varied in 2021, depending on the amount of wasps available each week. In 2022, 200-300 wasps were released at each site each week.

To maintain colony heath, wild caught Tj wasps and wasps from other regional rearing programs will be occasionally be added to our colony to prevent genetic drift within the colony.

Expected outcomes: We expect to release 200-300 wasps weekly at 12 new sites during the final 2023 field season.

2. Measure establishment using sentinel egg masses and yellow sticky traps (years 2 & 3)

We began to measure Tj establishment in 2022 using yellow sticky cards and sentinel egg masses at each of this year's release sites. Cards and sentinel eggs were be placed at sites where Tj was previously released and checked after 24 hours (eggs) or 2 weeks (traps) to see if any wasps were recovered. Sentinel eggs were brought back to the lab and held in cages until wasps emerged. Parasitism by Tj in subsequent years will be considered evidence of establishment. Yellow sticky cards were examined under microscope for presence of Tj wasps (Figure 6). Capture of adults in subsequent years will be considered evidence of establishment.

Expected outcome: Early results from research done by Dr. Wiman's PhD student show recapture (establishment) at 25% of the sites wasps where she released in 2018 and 2019 (13 sites in Hood River County). Considering the minute size of these wasps, the size of the landscape they are occupying, and the small number of traps used (3 sticky cards per site), the 25% recapture rate is very encouraging. We expect similar recapture rates from our releases.

3. Describe the habitats where wasp establishment is most successful (years 2 & 3).

Orchard border habitat will be recorded capturing species richness (diversity), size of habitat, and distance from managed orchard. Establishment data will be analyzed against habitat parameters to determine if successful establishment is strongly correlated with surrounding habitats.

The sites that appear to have successful establishment from the 2021 releases were bordered by mixed oak and conifer forest. This habitat provides the brown marmorated stink bug additional host plant resources, as well as refugia for both the stink bug and Tj from pesticide sprays applied in the pear orchards.

Expected outcome: Results of this research could lead to planting recommendations to increasing the probability of wasp establishment in future efforts.

4. Measure the effectiveness of Tj biocontrol for preventing fruit damage (years 2 & 3)

Year zero stink bug populations were measured using pyramid traps containing the Trécé BMSB dual pheromone lure to measure the abundance of BMSB within each orchard. Pheromone baited traps will be maintained at each release sites and traps checked weekly. Abundance of stink bugs will be

used as one measure of effectiveness of biocontrol. Packing house cull report with be gathered from each grower to see how fruit damage changes from year to year.

Expected outcome: We expect to increase the population and expand the range of the egg parasitoid (Tj) throughout the Hood River pear growing region. While it may take several years to measure the impact, we expect that BMSB fruit damage will decrease near these 36 sites and that this established population will continue to spread to other orchards.

Results and Discussion

In the first two years, we successfully established and maintained a colony of BMSB large enough to produce a steady supply of eggs. These BMSB eggs were used to establish and maintain a colony of Tj wasps, and to date we have released 56,942 wasps at 27 locations across the Hood River growing region from this colony. A portion of these released wasp are from a collaboration with ODA to assist with the distribution of Tj from their state-wide program. This collaboration added 1,400 wasps in 2021 and 1,700 wasps in 2022. In addition, we are assisting Dr. Nik Wiman's PhD student with her Tj wasp release in the Hood River area. Her project added another 1,200 wasps to the Total released.

In 2022 we began trapping efforts to look for establishment of the wasp in these locations. Sentinel egg masses were placed at the 2021 release sites and left for 24 hours on 6-Jul and 20-Jul, 2022. None of the recovered egg masses were parasitized. Three yellow sticky cards were placed at each site and left for two weeks on 6-Jul, 20-Jul, and 1-Aug. While confirmation is needed from an expert taxonomist, it appears that Tj was recovered at 4 sites. A total of 100 probable Tj wasps were collected at these four sites (n= 1, 3, 25, and 71). A total of 41 wasps that appear to be another *Trissolcus* species were recovered from 7 sites. Wasp releases will continue in 12 new locations in the final 2023 field season, and trapping will continue at the first two locations. Establishing this wasp near fruit growing regions will help control BMSB populations in and around orchards.

Challenges: In 2021 the population of BMSB was extremely low (Figure 3) statewide, likely due to the warm winter, dry spring, and summer heat dome. These low catch numbers slowed the establishment of the stink bug colony and delayed the timing of our first wasp releases. Low wild numbers of BMSB will also make it more difficult for released wasps to find stink bug eggs to parasitize. With the current established BMSB colony we were able to ramp up production, start the 2022 field season earlier, and were able to release more wasps each week.

The 2022 season had much higher BMSB abundance everywhere. This makes it difficult to measure the impact from our released wasps. However, high wild BMSB populations should increase the success rate of released wasps, and may benefit the Tj program in the long term.

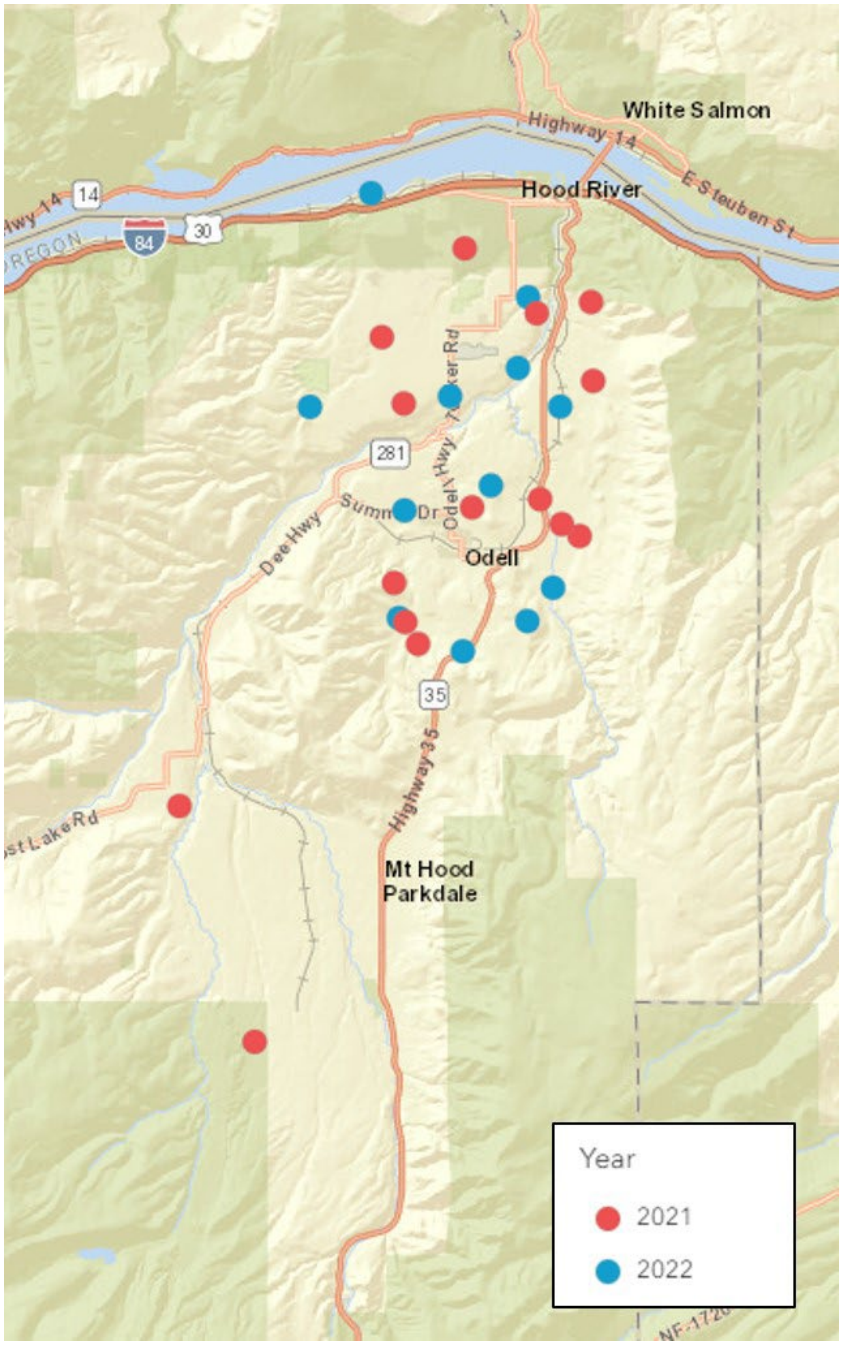

Figure 1: Sites where *Trissolcus japonicus* were released in 2021 and 2022.

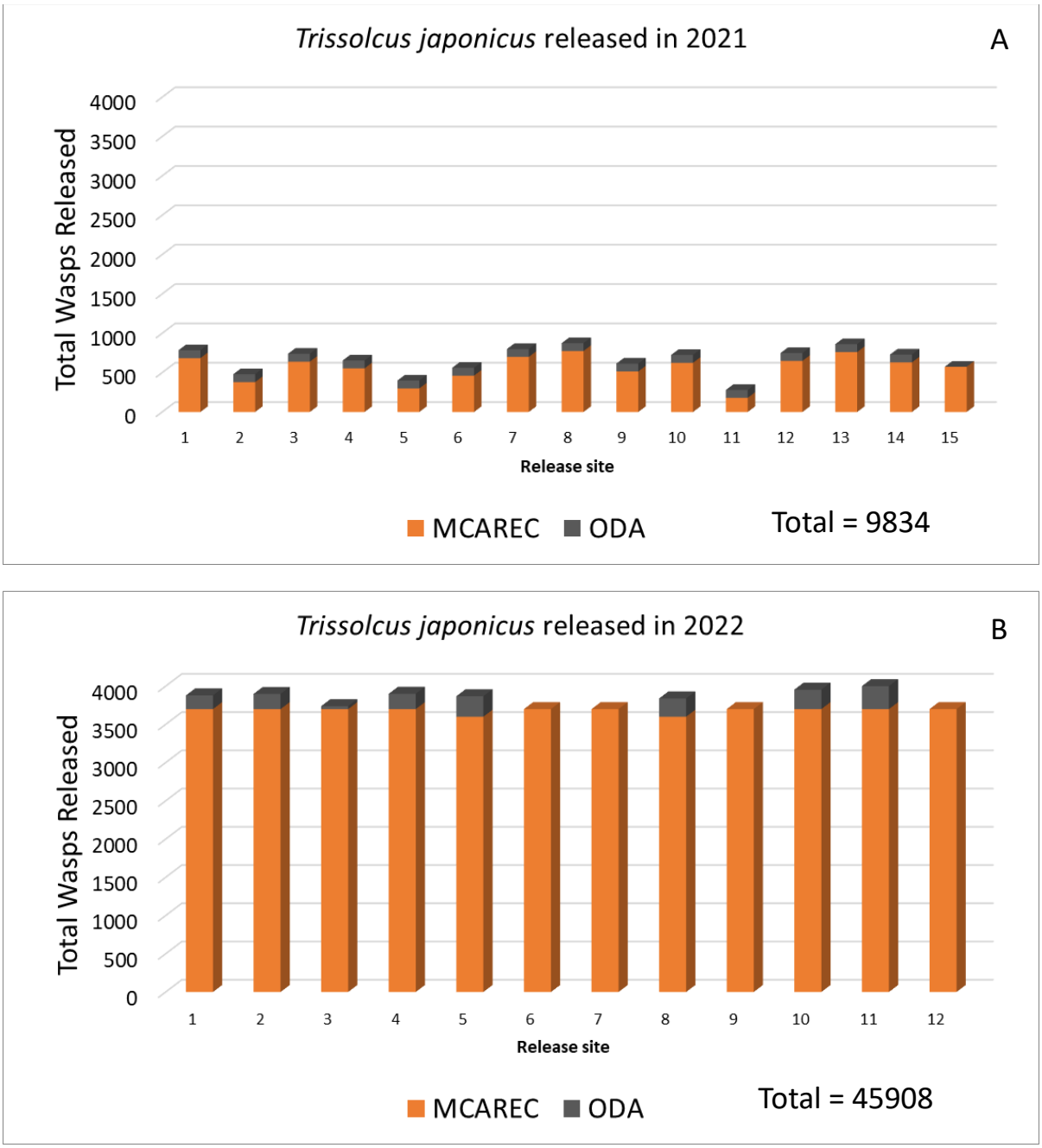

Figure 2. Number of *T. japonicus* released at each site reared by MCAREC and ODA in 2021 (A) and 2022 (B).

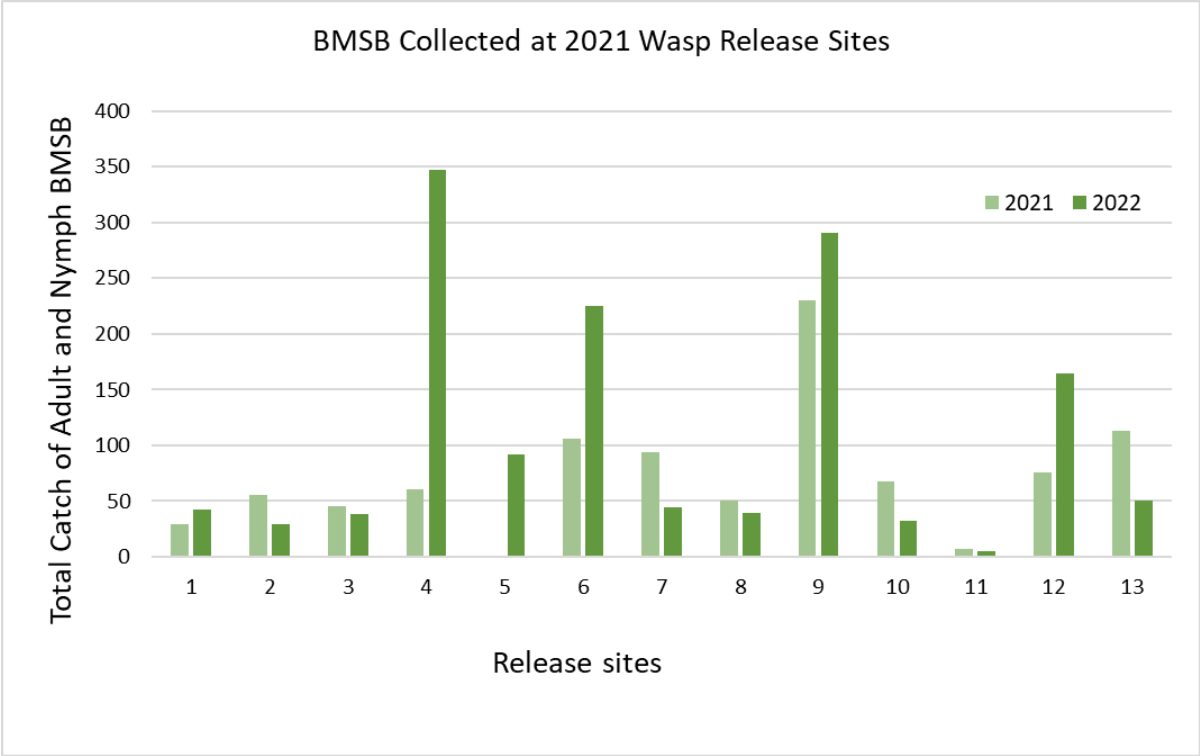

Figure 3. Weekly catch totals of BMSB adults and nymphs at 2021 wasp release sites.

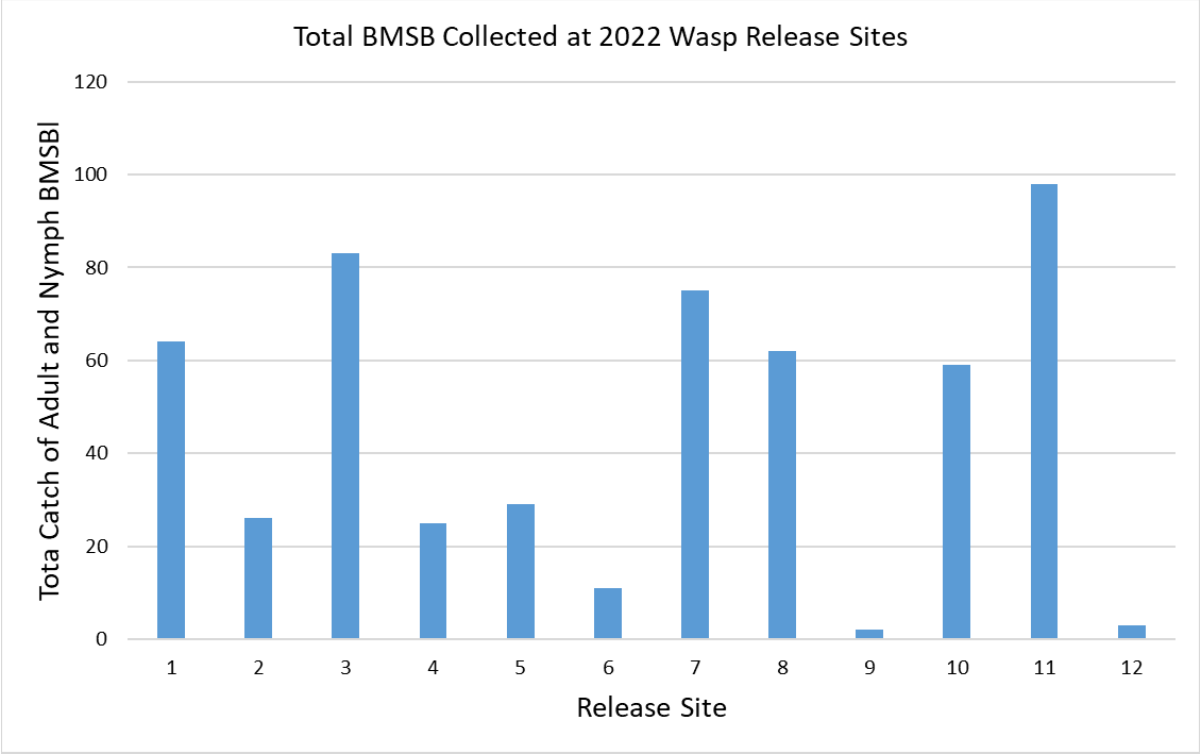

Figure 4. Seasonal total of BMSB adults and nymphs collected at each 2022 wasp release site.

Figure 5. BMSB colony cages, *Trissolcus japonicus* colony, and Release of wasps in field.

Figure 6. Sticky card placed at a 2021 release site to measure catch (considered establishment) of *Trissolcus japonicus*. Red lines indicate a suspected *Trissolcus japonicus*.

FINAL REPORT **PROPOSED DURATION**: 3 Years

Project Title: Pear Psylla Pheromone Lures for Monitoring and Mating Disruption

PI: Christopher Adams
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Address:3005 Experiment Station Drive
City/State/Zip: Hood River, OR 97031

Cooperators: Pete McGhee, Pacific Biocontrol. Andy Rust, Chamberlin distributing.

Total Project Request: **Year 1:** $20,000 **Year 2:** $20,000 **Year 3:** $20,000

Budget 1
Primary PI: Christopher Adams
Organization Name: Oregon State University
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228
Contract administrator email address: charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Stuart Reitz
Station manager/supervisor email address: stuart.reitz@oregonstate.edu

Item	2022	2023	2024
Salaries	$12,475	$12,475	$12,475
Benefits	$6,825	$6,825	$6,825
Wages			
Benefits			
Equipment			
Supplies	$700	$700	$700
Travel			
Miscellaneous			
Plot Fees			
Total	Total year 1 $20,000	Total year 2 $20,000	Total year 3 $20,000

- **I would like to return the 2022 funds and place this project on hold.**

Objectives

1. Compare pheromone baited monitoring traps to beat tray sampling for measuring early season phenology and action thresholds (year 1&2).

2. Conduct dose response experiment to determine dispensers per acre needed to reduce catch in monitoring traps (year 2&3).

After this funding was approved, it was brought to my attention that several other projects were funded to look at pear psylla pheromone for attraction and retention to potted trees and traps. These trials were never published but the reports of the work were in the WTFRC archives. They did not have great success, so I scaled back my plans and tried to look at basic attraction of psylla to its pheromone, on a smaller scale. Moving forward I will look through the WTFRC archives as part of my literature review.

Significant Findings

- Laboratory flight cage choice tests studies to pear limbs coated with pear psylla cuticular sex pheromone, 13-methylheptacosane, was not significantly different from control limbs.
- Field tests of wood dowels coated with pear psylla cuticular sex pheromone, 13-methylheptacosane, was not statistically different form controls.

Results and Discussion

1. Cage studies were performed in a lab setting at 72 F (22 C) and 40% RH. For each of the 6 replicate, 100 mixed sex winter form pear psylla were caged with two sets of pear shoots placed at the far end of a cage. Shoots were either left untreated or coated with 13-methylheptacosane. Insect were allowed to respond to pear shoots over a 24 hour period. Data was collected by visually inspecting location of psylla on shoots without disturbing psylla. Sex of insects was not assessed because psylla were inclined to jump off shoots when disturbed.

Results

No significant difference was fond between the two treatments. Only about one quarter of the psylla in the cage made it up onto one of the two treatments. Most remained on the cage floor or landed on the mess cage.

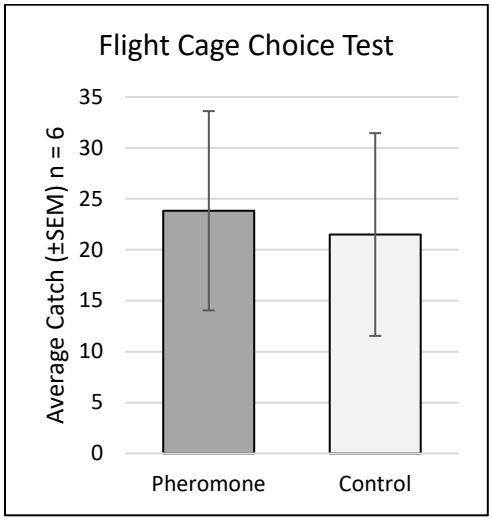

Figure 1. Cage study showing choice test of treated and control pear shoots with 100 winter form psylla. Bar chart shows average number of psylla found on shoots after 24 hours

Results and Discussion

2. Field trials were conducted in managed orchard with high population of pear psylla. Wood dowels were coated with 13-methylheptacosane, or left untreated, and then coated with tangle foot insect glue. Dowels were used to simulate pear shoots that male and female psylla might use to court, mate, and lay eggs. Ten trees were randomly selected with the block, each with a single paired trial. Trail ran for one week in Early February 2022.

Results

No significant difference was found between the two treatments. Total catch was much lower than expected as beat tray samples, taken the week before the trial was set up, found ten to twenty psylla per tap in most trees in this block. Catch was most likely due to random chance and not attraction.

Figure 2. Field trials showing wood dowels treated with 13-methylheptacosane, or a control, coated in tangle foot glue. Bar chart shows catch data of psylla found in tangle foot glue.

Executive summary

Pear psylla is a major pest of commercial pears in the PNW. Even moderate psylla populations are capable of producing enough honeydew to cause black sooty mold and russeting that lowers fruit value and creates sticky conditions that negatively affect workers harvesting fruit. While pesticides can provide good control, pear psylla has developed resistance to some key chemistries (Van De Bann and Croft 1991), and the future loss of chemistries due to insecticide resistance is always a concern. Pear psylla is best managed through careful, well-timed controls and IPM practices that minimize impacts to non-target organisms and promote natural enemies.

A key IPM tool that fundamentally changed codling moth (*Cydia pomonella*) management in apple, is the development and wide-spread adoption of pheromone mating disruption and attractive monitoring traps. Pheromone communication has been well documented in Lepidoptera (Allison and Carde 2016) and these highly active chemical attractants have been used to successfully suppress insect populations for several decades (Knipling 1976).

The two trails I ran did not produce positive results. This pheromone is a close contact cuticular pheromone that is a solid at room temperature and functions differently than volatile sex pheromones produced by female moths designed to float on the wind. There are technical challenges to making a molecule as large as 13-methylheptacosane volatile at room temperature, or at the outdoor

temperatures of early February. However, the potential for developing a new tool for monitoring or disruption of early season psylla makes this pheromone worth investigating further.

I am returning the funding form 2022 and putting this project on hold until I can recruit a chemical ecologist that can devote more time to experimentation on this product. I have a relationship with the company that is producing this compound and they donated several bottles to my lab. We feel that there may still be some useful applications for this product, so we will continue to look into novel ways to apply this chemical towards the management of pear psylla.

Project Title: What factors impact mite outbreaks in pear?

Report Type: Continuing Project Report,

Primary PI: Rebecca Schmidt-Jeffris
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Co-PI 2: Louis Nottingham/Robert Orpet
Organization: Washington State University
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Address: 1100 N Western Ave
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CO-PI 3: Chris Adams
Organization: Oregon State University
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Address: 3005 Experiment Station Dr.
City/State/Zip: Hood River, OR 97031

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $33,054
Total Project Request for Year 2 Funding: $33,782

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Rebecca Schmidt-Jeffris
Organization Name: USDA-ARS
Contract Administrator: Mara Guttman
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Contract administrator email address: mara.guttman@usda.gov
Supervisor: Rodney Cooper
Supervisor email address: rodney.cooper@usda.gov

Item	2022	2023
Salaries[1]	$9,297	$9,529
Benefits[1]	$744	$762
Wages	$0	$0
Benefits	$0	$0
Equipment	$0	$0
Supplies[2]	$9,000	$9,000
Travel[3]	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$19,041**	**$19,291**

Footnotes:
[1]GS-4 technician for 4 months per year, 100% FTE at 8% benefits, Year 2 includes 2.5% COLA increase. Technician would conduct sampling in the Yakima area, process/count samples, and slide mount mites for identification (Schmidt-Jeffris will identify). This technician will also conduct surface sterilization and PCR for gut content analysis for all samples (Yakima, Wenatchee, and Hood River).
[2]Molecular supplies for gut content analysis, sticky cards for field sampling – to be purchased for entire project team.
[3]Fuel to field sites will be provided by USDA base funds and is not requested.

Budget 2
Primary PI: Louis Nottingham
Organization Name: WSU
Contract Administrator: Shelli Tompkins
Telephone: 509-293-8803
Email address: shelli.tompkins@wsu.edu
Station Manager/Supervisor: Chad Kruger **Email Address:** cekruger@wsu.edu

Item	2022	2023
Salaries[1]	$1,827	$1,900
Benefits[2]	$553	$575
Wages[3]	$3,900	$4,056
Benefits[3]	$373	$388
Equipment	$0	$0
Supplies	$0	$0
Travel	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$6,653**	**$6,919**

Footnotes:
[1]Nottingham salary ($7,612.50/mo × 12 mo × 2% FTE = $1,827 Year 1, Year 2 reflects 4% COLA increase) Nottingham to supervise data collection efforts in the Wenatchee area.
[2] Benefits rate for Nottingham is 30.3%.
[3]Summer technician at $15/hr×13 hr/wk ×20 wks, 9.6% benefits rate, salary includes 4% COLA increase in Year 2

Budget 3
Primary PI: Chris Adams
Organization Name: OSU
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228
Email address: charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Steve Castagnoli
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Item	2022	2023
Salaries[1]	$2,187	$2,252
Benefits[2]	$875	$901
Wages[3]	$3,900	$4,017
Benefits[3]	$390	$402
Equipment	$0	$0
Supplies	$0	$0
Travel	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$7,352**	**$7,572**

Footnotes:
[1]Adams salary ($109,344/yr × 12 mo × 2% FTE = $2,187 Year 1, Year 2 reflects 4% COLA increase). Adams to supervise data collection efforts in pear in the Hood River area.
[2] Benefits rate for Adams is 40%.
[3]Technician at $31,200/yr × 5 mo × 40% FTE. 10% benefits rate. Includes 4% COLA increase in Year 2.

OBJECTIVES

1. Identify management practices that affect pest mite and natural enemy populations.

2. Identify which natural enemies are more frequently consuming pest mites.

3. Determine if there is an association between spider mite and pear psylla abundance.

SIGNIFICANT FINDINGS

- Wenatchee Valley had substantially higher twospotted spider mite populations than Yakima Valley or Hood River. Hood River locations had very few spider mites.
- Yakima Valley had much higher rust mite populations than the other two regions.
- Weed washes in alcohol were an effective method for detecting spider mites and phytoseiids in the ground cover.
- While phytoseiids ("typhs") were found in the survey, they were much less common than in apple orchards. This suggests that in pear orchards where pest mites do not flare, other natural enemies are responsible for biological control.

METHODS

Description of roles. Each PI will lead data collection efforts in their area: Schmidt-Jeffris (Yakima), Nottingham (Wenatchee), and Adams (Hood River). Schmidt-Jeffris will lead overall project efforts, summarize data, slide mount and identify predatory mites, and lead processing of all gut content analysis samples. Sample collection will be performed by one grant-funded technician at each location. Gut content analysis will be performed by a USDA based-funded technician, assisted by the USDA grant-funded technician.

This two-year (2022-2023) study will be conducted in 5-10 commercial pear orchards in each of three pear-growing regions: Wenatchee, Yakima, and Hood River (total of 15-30 orchards sampled). Orchards will be selected to represent a variety of management types (e.g., conventional, organic, soft IPM) and mite outbreak frequency and intensity. We will include orchards that regularly have serious mite problems, as well as those that rarely have mite issues. Each orchard will be sampled once weekly for four weeks, targeting the time of year when mite outbreaks are most likely to occur (late July to mid-August). Additional, less frequent sampling will be conducted earlier and later in the season. We will use existing contacts between the PIs and industry (e.g., Gilbert Fruit, G.S. Long) to identify sampling locations. Many locations will overlap with existing pear psylla monitoring locations so that historical data can be used as a reference point for overwintering pest mite densities.

At each sampling date, a 50-leaf sample will be collected from throughout the orchard block. Leaves will be brushed with a mite brushing machine and the resulting sample will be counted using a microscope. We will count eggs and motiles of twospotted spider mites, eggs and motiles of any other spider mite species, pear rust mites, pear psylla eggs and nymphs, and predatory mites. Any predatory mites found will be removed from the sample and stored in 70% ethanol for later slide-mounting and identification. Five sticky cards will also be placed throughout the orchard block. From these, we will count

Deraeocoris, anthocorids (to genus), *Stethorus*, *Campylomma*, *Geocoris*, and *Nabis*. We will also conduct beat samples on 5 trees spaced roughly evenly throughout the orchard block. Any small predatory insects (of the appropriate size to eat mites) will be directly placed in molecular grade ethanol for later counting and gut content analysis by PCR.

At this point, we will also assess herbicide strip weediness. We will measure the distance from the edge of the herbicide strip to the trunk for the five sample trees to determine the herbicide strip size. For the same set of trees, we will also estimate percent weeds in the space adjacent to the tree (0.5×0.5 m quadrat) and quantify percent composition of bare ground, grass, and other (weeds). Notes will be taken on dominant weed species. Weeds will be collected from within the quadrat, brought to the lab, and rinsed with ethanol to remove any arthropods. Spider mites within the sample will be counted. Landscape surrounding the orchard will be quantified using Cropscape and QGIS analysis procedures.

We will request pesticide records from growers for the two growing seasons in which the study occurs and limit our study to orchards where growers are able to share this information. We will also request that growers record the number of times per year and timing for row middle mowing and will ask them to indicate if they consider their orchard "dusty". We will use this management information and weather data (WSU AgWeatherNet) to determine through statistical modelling which factors most strongly impact spider and rust mite populations. Model building procedures will be similar to those used in Schmidt-Jeffris et al. 2015. This will allow us to (1) determine if "bad mite years" can be predicted, (2) identify which practices are associated with mite flareups so growers can avoid them, and (3) identify the most important natural enemies of spider and rust mites so appropriate conservation methods can be implemented. Our data will also allow us to determine which management factors most impact abundance of key pest mite natural enemies; this information can be used to better conserve these predators.

Expected Results and Timing
At the end of each field season, grower records will be obtained and natural enemy counts will be analyzed. PCR-based gut content analysis and identification of slide-mounted mites will be conducted in the Fall-Winter following each growing season. The final model will be built using both years of data in late winter 2023 and factors impacting pest mite populations will be identified.

For all objectives, project updates will be presented annually at a minimum of one grower meeting and at the Orchard Pest Management and Disease Conference. Completed project results will be summarized as an extension article (Fruit Matters) and a peer-reviewed publication to be submitted in Winter 2023-2024.

The results from this project will be used to identify practices that should be examined to improve control of pest mites (i.e. those factors that are found to influence pest mite abundance or help natural enemy populations). It will also determine which natural enemies should be the target of conservation efforts and the focus of grower scouting.

RESULTS AND DISCUSSION

In 2022, we monitored a total of 21 locations: ten in Yakima Valley, six in Wenatchee Valley, and five in Hood River. Data analysis is pending while we are in the process of obtaining grower spray records. We have completed DNA extraction from all predators collected in our tap samples and are currently designing and optimizing primers for detecting twospotted mite, pear rust mite, and pear psylla in predator gut contents.

Pest mites were nearly absent at all locations in Hood River. Wenatchee Valley sites had by far higher twospotted spider mite populations than the other two regions, with Yakima Valley intermediate. To compare sites and regions, we plotted each site's "mite peak" for both twospotted spider mite and pear rust mite (Fig. 1).

Across regions, the most common mite natural enemies in beat tray samples were *Deraeocoris*, spiders, *Stethorus*, and *Campylomma*. The majority of spiders were philodromids, likely *Philodromus cespitum*. This spider is known to be an important natural enemy of pear psylla in Europe. *Stethorus* and *Hippodamia convergens* were the most common ladybeetles collected. These natural enemies (except for spiders) were also collected in the sticky card samples.

Alcohol weed washes were effective at detecting spider mites in the ground cover. These were nearly always twospotted spider mite. In Yakima, a weed wash sample would typically contain 0-2 mites per sample date. In Wenatchee, as many as 63 twospotted spider mites were found in one sample. Phytoseiids were also found in the weed wash samples and are in the process of being identified to species. The most common weed species were mallow, dandelion, chickweed, clover, and field bindweed.

This spring, we will use grower spray records and natural enemy counts to build an initial model that determines which factors are most associated with pest mite flares.

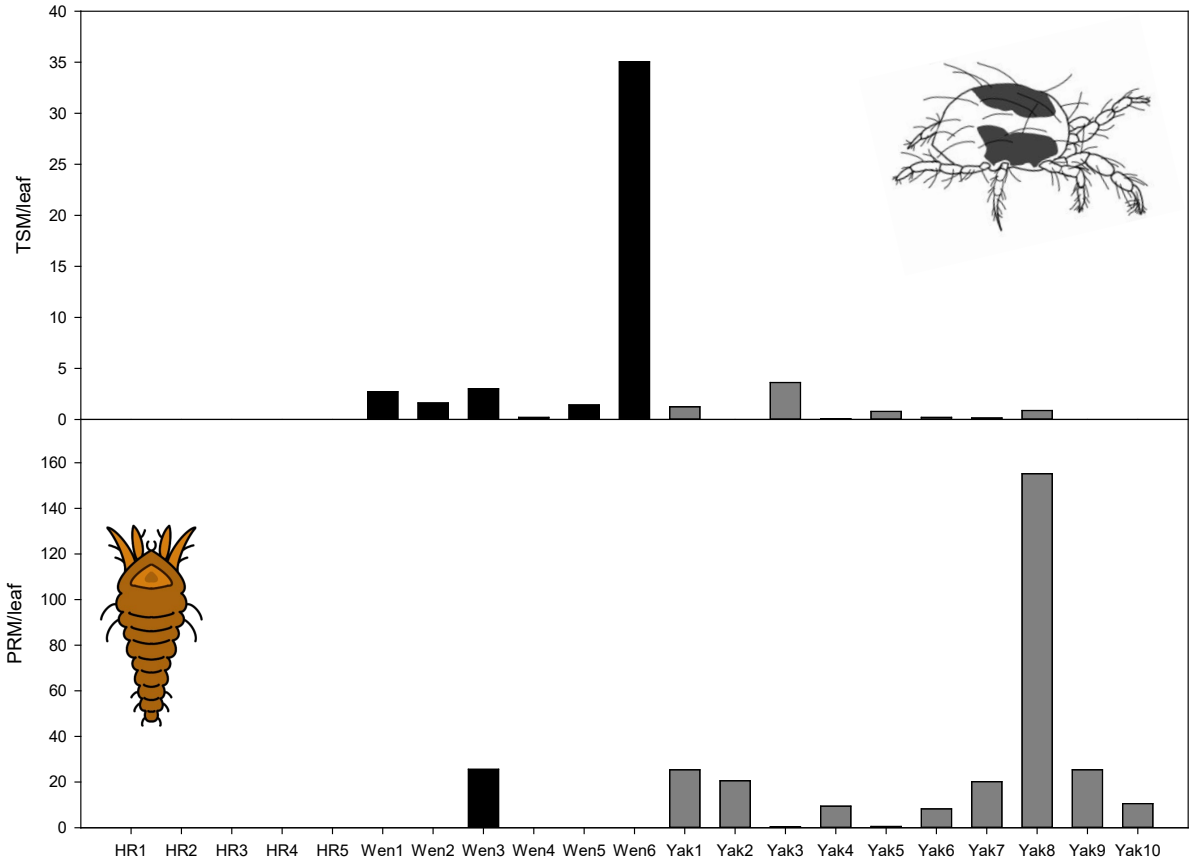

Fig. 1. Twospotted spider mite (TSM) and pear rust mites (PRM) per leaf collected at each site. Numbers shown are for each site's "worst" date.

CONTINUING PROJECT REPORT **YEAR**: Final
WTFRC Project Number: 9-20-p

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

PI: **Achala KC**
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City/State/Zip: Central Point/OR/97502

Co-PI: Achour Amiri
Organization: Washington State University
Telephone: 509-293-8752
Email: a.amiri@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA, 98801

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:** $100,000
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:** Josh Kvidt
Telephone: 541-737-4066 **Email address:** josh.kvidt@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 samples throughout the season from bloom to harvest at low and variable frequencies between locations in WA, Hood River, and Medford. Variabilities in inoculum size and dynamics throughout the season were observed among orchards located in different districts.
- In all locations, the size of *Botrytis* inoculum was greater in organic orchards compared to conventional orchards.
- *Botrytis* was detected in pear tissues from OR and WA fruit samples, including calyx, stem-bowl, cuticle, and flesh indicating latent (dormant) infections from previous infections in the orchard
- About 700 *Botrytis* spp. isolates were collected from WA and OR, respectively in 2019 and 2020.
- *B. cinerea* was the only species detected among 220 isolates screened from the 700 collected.
- In south OR 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.
- In WA, four seasonal field spray programs to improve gray mold management were tested in 2020 and 2021 field seasons. Results indicate that sprays conducted at petal fall, fruit set, and 7 to 0 days preharvest are critical to reduce gray mold in storage. A summer spray on green fruit, would optimize gray mold management in storage especially for fruit stored long-term (>6 months).

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 in 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.

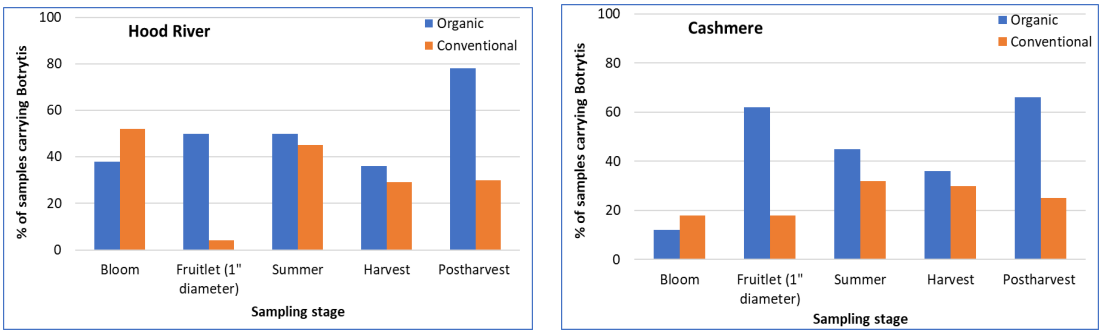

Figure 1. Evaluation of *Botrytis* incidence on organic and conventional Anjou pear in Hood River and Cashmere throughout the 2019-20 preharvest growing season and after 6 months of CA storage as detected by qPCR.

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.

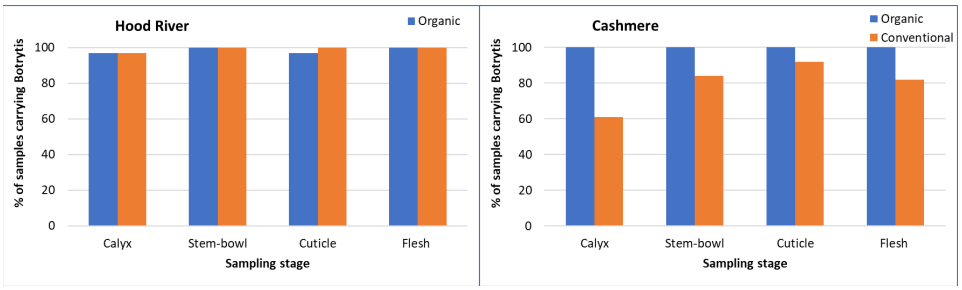

Figure 2. Incidence of *Botrytis cinerea* on different organs of the fruit at commercial maturity (harvest time) Anjou pear in organic and conventional orchards in 2019.

Trials at SO (Year 2)

Comice pears were collected in a commercial orchard in Southern Oregon starting in early April to late August of 2019 and 2020 from conventional and organic blocks in 5 stages. Based on qPCR detection of Botrytis on these samples, it was detected in all samples throughout the season with variable frequencies (Figure 3). Out of the collected pears that were grown conventionally, *Botrytis* was detected on average of 28, 9, 3, 13, and 31% 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 detected in 13, 36, 18, 20, and 14% respectively from full bloom, petal fall/fruit set, fruitlet, mid-summer, and commercial maturity. When the individual pear tissues were analyzed for Botrytis presence, we detected in all types of tissue types at commercial maturity (Figure 4). From conventional fruit samples, it was detected in 11, 13, 13, and 19% of the samples from calyx, stem-bowl, cuticle, and flesh tissues respectively. Whereas from organic fruit samples, it was detected in 5, 1, 2, and 4% of the samples from calyx, stem-bowl, cuticle, and flesh tissues respectively. The overall detection

percentages in southern Oregon samples were relatively low compared to Hood River and Cashmere samples.

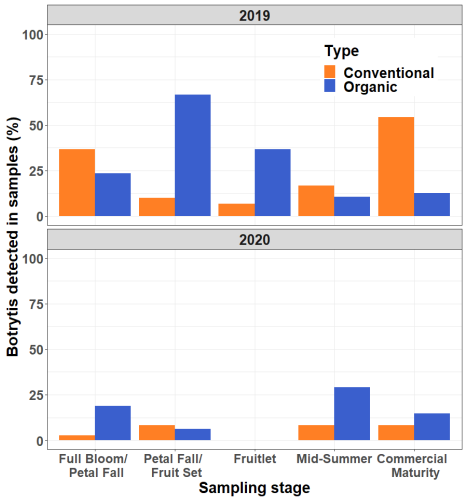

Figure 3: Percentage of *Botrytis cinerea* detected from pear samples collected in Medford organic and conventional orchards at different stages during their development in 2019 and 2020.

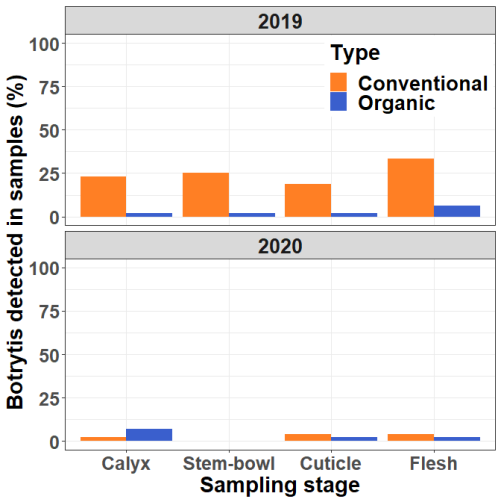

Figure 4: Percentage of *Botrytis cinerea* detected from pear tissues collected in Medford organic and conventional orchards at commercial maturity in 2019 and 2020.

Activity 1.2. **Investigate the causal species of gray mold in the PNW.**

220 isolates collected from multiple orchards WA (n = 140 isolates) and OR (n = 80 isolates) were subjected to species characterization to determine what *Botrytis* species is causing gray mold in the PNW. Molecular primers developed previously for *B. cinerea*, *B. pseudocinerea B. mali*, and *Botrytis group S*, were used to screen the 220 isolates. These species were reported to cause gray mold on several other hosts. Our investigation revealed that the 220 isolates were all *B. cinerea* (Table 1) confirming that this species is predominant in the region. The Postdoctoral Scientist leading this effort has left which has delayed the screening of the remaining isolates from nearly 700 isolates collected. Results will be shared with pear fruit stakeholders as soon as they available through extension meetings and publication.

Table 1. Characterization of species causing gray mold in the PNW to the species level

	WA	OR
Target species	**n = 140**	**n = 80**
B. cinerea	140	80
B. pseudocinerea	0	0
B. mali	0	0
B. group S	0	0

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 (mancozeb), Ziram 76DF (ziram), Ph-D (polyoxin-D), and Botran 5F (dicloran) respectively were tested for their effectiveness against 21 Botrytis isolates in plate assays. The effective concentration to reduce radial growth by 50% (EC_{50}) values for mancozeb, ziram, polyoxin D, and dicloran ranged from 21.65 µg/ml to 136.02 µg/ml, 25.33 µg/ml to 156.77 µg/ml, 4.05 µg/ml to 619.02 µg/ml, and from 4.08 µg/ml to 26.75 µg/ml respectively (Figure 5). Overall, Botran performed the best against Botrytis isolates at concentrations of 10 ug/ml and above in plate assays followed by Ph-D. However, Botran is not registered for pear in PNW. It is interesting to note that when the isolates were grouped by the orchards they were collected from, some trends in sensitivity emerged. For instance, polyoxin D was effective against isolates collected from orchard 1, but not against isolates collected from orchard 3. This suggests that where Botrytis isolates originate from may also have an effect on their resistance towards different fungicides.

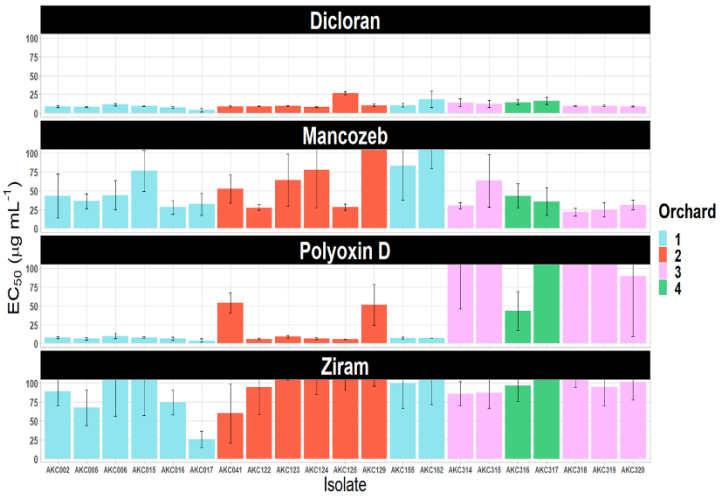

Figure 5: *In vitro* sensitivity of four fungicides against 21 Botrytis isolates in plate

In fruit assays, same fungicides 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 (Figure 5). 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 6). 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.

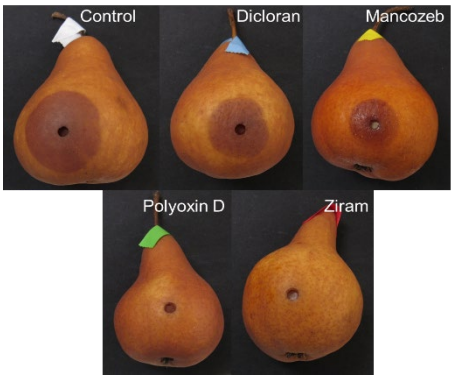

Figure 5: Wound inoculated fruit assays by four fungicides tested in this study.

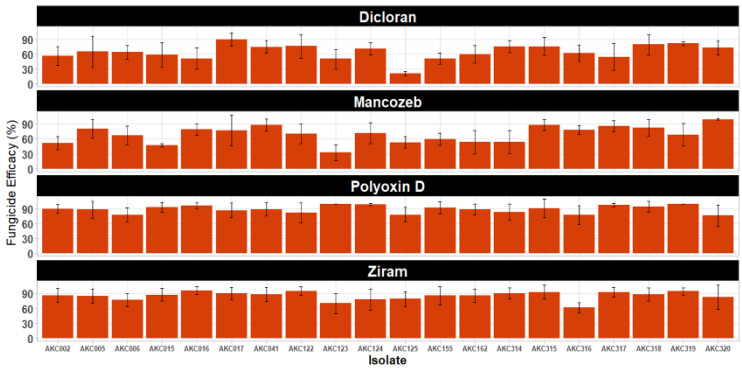

Figure 6: Efficacy of four fungicides on wound inoculated fruit trials.

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

Spray programs tested in WA: 2020 and 2021

Three types of sprays, i.e., a conservative spray (1 preharvest spray), a moderate program (2 preharvest sprays), and an intensive spray (3 preharvest sprays) were tested in WA state during 2 consecutive seasons (Table 2). Sprays were conducted at different phenological stages, using fungicides from different chemical FRAC groups, in relation with epidemiological knowledge gathered from Obj. 1. Overall, gray mold incidence was lower in treatment that included a spray at fruit set with the lowest gray mold incidence being recorded in the intensive spray consisting of 3 sprays at fruit set, green fruit (mid-summer) and 7 days preharvest. The efficacy of sprays somewhere between petal fall and fruit set relate to the epidemiology of *Botrytis* which cause latent infections that may be important at this phenological stage as some remaining parts of the blossoms that serve for fruit setting may be carrying botrytis infections that occur at bloom and during petal fall. The program consisting of one preharvest spray at 7 days preharvest, which may be a standard in the WA pear industry reduced gray mold significantly compared to the control but was 5% less effective than intensive spray. In WA, we did not test a posthavest spray as our aim was to assess preharvest sprays, but it will be important to assess the efficacy of these sprays in combination with postharvest sprays in the future.

Table 2. Overall decay and gray mold incidences in d 'Anjou pears treated with different spray programs in WA in 2020 and 2021 seasons.

						Decay incidence			
		Fungicide sprayed at				2020		2021	
Treatment type	Number of sprays	Petall fall	Fruit Set	Mid-summer	7 DPH	Overall	Gray mold	Overall	Gray mold
	Untreated control	-	-	-	-	40.0	17.0	47.0	23
Conservative	1 spray-early	-	Pri	-	-	16.0	10.0	18.0	11.0
	1 spray-early	-		Pri	-	13.0	7.0	27.0	13.0
	1 spray-late	-	-	-	Pri	22.0	12.0	27.0	13.0
Moderate-Low	2 sprays-early	TopM	Pri	-	-	23.0	16.0	26.0	12.0
	2 sprays-mid	-	TopM	Pri	-	12.0	8.0	14.0	8.0
	2 sprays-mid/late			TopM	Pri	13.0	8.0	15.0	7.0
Intensive	3 sprays-early/mid-late	-	LunaS	TopM	Pri	8.0	6.0	13.0	7.5

LunaS= Luna Sensation, Pri = Pristine, TopM = Topsin-M

Spray programs tested in South OR: 2020 and 2021

Similar to WA field trials, all three types of sprays, i.e., a conservative spray (1 preharvest spray), a moderate program (2 preharvest sprays), and an intensive spray (3 preharvest sprays) were tested in southern Oregon during two consecutive seasons (Table 3 and 4). We tested two programs with two different sets of fungicides in SO. Unlike WA trials, we also included postharvest application in all but one treatment (intensive spray program). In SO, gray mold incidence was lower in both years compared to WA trials. Due to low disease incidence, no significance differences among the treatments were observed in SO gray mold result. In addition to gray mold, we also collected data on overall rot incidence. In 2020, lower disease incidence was observed in treatments that involved extensive spray program during the growing season but without postharvest sprays. This was observed for both programs that involved Topsin M, Pristine, and Luna Sensation; Ziram, Ph-D, and Inspire Super (Table 3 and Table 4). In 2021, the overall rot incidence was significantly low compared to 2020 and no significant differences between the treatments were observed for both fungicide programs. We believe that the low precipitation during fruit growing stages (March through May) in 2021 (1.4 inches in 2021 vs. 3.7 inches in 2020) could have contributed to lower rot incidence in 2021.

Table 3. Overall decay and gray mold incidences in 'Bosc' pears treated with spray program (1) in southern Oregon in 2020 and 2021 seasons.

						Decay incidence			
		Fungicide sprayed at				2020		2021	
Treatment type	Number of sprays	Bloom	petal fall / fruit set	summer	7DPH	Overall	Gray mold	Overall	Gray mold
Control	0					18.1	0.6	6.5	0
	1-Early		Pristine			33.1	0	12.3	0
Conservative	1-Mid			Pristine		26.7	0	0.8	0.6
	1-Late				Pristine	36.3	0	0.8	0
	2-Early	TopsinM	Pristine			33.1	0	0	0
Moderate	2-Mid		TopsinM	Pristine		38.1	0	5.8	0
	2-Mid/Late			TopsinM	Pristine	38.8	0.6	2.5	1.3
	3-Early/Mid/Late-No postharvest		Luna Sensation	TopsinM	Pristine	22.5	0	3.8	0
Extensive	3-Early/Mid/Late		Luna Sensation	TopsinM	Pristine	56.9	0.7	0.8	0

Table 4. Overall decay and gray mold incidences in 'Bosc' pears treated with spray program (2) in southern Oregon in 2020 and 2021 seasons.

						Decay incidence			
		Fungicide sprayed at				2020		2021	
Treatment type	Number of sprays	Bloom	petal fall / fruit set	summer	7DPH	Overall	Gray mold	Overall	Gray mold
Control	0					28.1	0.6	9	0
	1-Early		Ph-D			46.9	0	5.8	0
Conservative	1-Mid			Ph-D		50.6	1.3	1.3	0
	1-Late				Ph-D	50.6	0.6	1.3	0
	2-Early	Ziram	Ph-D			21.3	0	2.5	0
Moderate	2-Mid		Ziram	Ph-D		22.5	0	4.5	0
	2-Mid/Late			Ziram	Ph-D	60.6	0.6	13.3	0.6
	3-Early/Mid/Late-No postharvest		Inspire Super	Ziram	Ph-D	12.5	0.6	5.8	0.6
Extensive	3-Early/Mid/Late		Inspire Super	Ziram	Ph-D	30.6	0.6	0.8	0

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

Outreach activities in WA State:

Dr. Amiri and his team have provided four talks in WA since 2020 on Botrytis epidemiology and gray mold management in pear and publish a factsheet on gray mold disease and management.

***Talks*:**

Amiri A. Management of Postharvest decays. Workshop on postharvest diseases in conventional systems. Wenatchee, CTC, March 2020

Amiri A. Management of Postharvest decays. Workshop on postharvest diseases in conventional systems. Wenatchee, CTC, March 2020

Acosta W., Amiri A. 2020. *Botrytis cinerea* in pome fruit systems of the Pacific Northwest. *Phytopathology* 111-11-S2:37.

Acosta W., Amiri A. Management of gray mold pre and postharvest. *Northwest Apple Day*. Jan 21st, 2020.

***Publications*:**

Amiri A. & Acosta W., 2021. Understanding the epidemiology of gray mold caused by Amiri A., Acosta W. 2020. Gray mold factsheet. http://treefruit.wsu.edu/crop-protection/disease-management/gray-mold/

Outreach activities in OR:

Dr. KC and her team presented six talks in WA and OR since 2020 on Botrytis epidemiology and gray mold management in pear and published three abstracts in American Phytopathological Society conferences. Dr. KC was invited to interview with Pacific Northwest AG Network through which two series on Focus on Fruit have been published. On these series, she concentrated her talk on pear storage decay management. In addition, a talk by Dr. KC has been approved to be included in Post-Harvest diseases concurrent session at International Congress of Plant Pathology, Lyon, France, August 20-25.

Talks

KC, A. N. 2023. Preharvest factors associated with gray mold development in European pears. International Congress of Plant Pathology, Lyon, France, August 2023.

KC, A. N. 2023. Preharvest management of postharvest pathogens - Insights from Oregon. NCW Pear Day. Virtual presentation, January, 2023.

KC, A. N. 2022. Postharvest rot on pears. Southern Oregon Pest Management Forum. August, 2022.

KC, A. N. 2021. Pear: managing gray mold and other major pear decays in the Pacific Northwest. Washington State Tree Fruit Association 117th Annual Meeting and NW Hort. Expo. Virtual meeting, December, 2021.

KC, A. N. 2021. Postharvest rot on pears. Southern Oregon Pest Management Forum. July, 2021.

Hernandez, M., and KC, A. N. 2021. *Botrytis cinerea* infection at different stages of pear fruit development. Southern Oregon Pest Management Forum. March, 2021.

Hernandez M., and KC, A. N. 2020. *Botrytis cinerea* infection at different stages of pear fruit development. Orchard Pest and Disease Management Conference, 2020.

Radio series published in Pacific Northwest AG Network

KC, A. N. 2022. Focus on Fruit: Pacific Northwest AG Network, December 2022. https://pnwag.net/focus-on-fruit-120822/

KC, A. N. 2022. Focus on Fruit: Pacific Northwest AG Network, October 2022. https://pnwag.net/focus-on-fruit-102022/

Abstracts published in scientific conferences and presentation

Hernandez, M., and KC, A. N. 2022. *Botrytis cinerea* colonization occurs early in pear fruit development. American Phytopathological Society, 2022.

Hernandez, M., and KC, A. N. 2022. *Botrytis cinerea* varies in its sensitivity towards common fungicides used in pear orchards. APS Pacific Division virtual meeting, 2022.

Hernandez, M., and KC, A. N. 2021. Evaluation of *Botrytis cinerea* sensitivity towards fungicides commonly used in pear orchards. APS Pacific Division virtual meeting, 2021.

Executive Summary
Project title: Epidemiology and management of pear gray mold in the PNW

Key words: Gray mold, Botrytis, Pear, preharvest, management

Abstract:

Based on ongoing studies on postharvest rots of pome fruits in WA and OR, gray mold caused by *Botrytis cinerea* was identified as one of the most prevalent diseases causing postharvest rot in pears. Gray mold was found in 85% to 90% of the grower lots surveyed in 2016 and 2017 across the region with incidences ranging from 5% to 75% of total decay per lot. In order to understand the gray mold disease development during fruit developmental stages and utilize that information in developing fungicide management programs, we conducted three years of study at three districts in the PNW, Cashmere- WA, Hood River-OR, and Medford-OR. In the first two years, samples collected from both organic and conventional pear orchards were analyzed for the presence of *B. cinerea* at bloom, fruit set, mid-summer, and commercial maturity. At all sites, *B. cinerea* was detected in samples collected throughout the season from bloom to harvest at low and variable frequencies between locations in WA, Hood River, and Medford. Variabilities in inoculum size and dynamics throughout the season were observed among orchards located in different districts. In all locations, the size of *Botrytis* inoculum was greater in organic orchards compared to conventional orchards. *Botrytis* was detected in pear tissues including calyx, stem-bowl, cuticle, and flesh indicating latent (dormant) infections from previous infections in the orchard. In a concurrent study, four preharvest fungicides, Manzate Pro-Stick (mancozeb), Ziram 76DF (ziram), Ph-D (polyoxin-D), and Botran 5F (dicloran) respectively were tested for their effectiveness against 21 Botrytis isolates in both plate and fruit assays for their efficacy against *B. cinerea*. Except for Botran 5F, other fungicides are registered for pear to control other preharvest diseases such as scab, powdery mildew and other postharvest rots. Among the registered fungicides, Ziram 76DF and Ph-D provided improved efficacy in either plate or fruit or both assays. Based on the information collected from this and the previous studies, two spray programs with different fungicide groups (1: Topsin M, Pristine, and Luna Sensation; 2: Ziram, Ph-D, and Inspire Super) were developed and tested in field trials. The first program was tested at Cashmere- WA, and both programs were tested at Medford-OR in 2020 and 2021. Within each program, three spray regimes, a conservative spray (1 preharvest spray), a moderate spray (2 preharvest sprays), and an intensive spray (3 preharvest sprays) were tested in both locations. In Medford, due to low disease pressure no significant differences in gray mold at storage were observed. Whereas, in WA results indicated that sprays conducted at petal fall, fruit set, and 7 to 0 days preharvest are critical to reduce gray mold in storage. A summer spray on green fruit, would optimize gray mold management in storage especially for fruit stored long-term (>6 months).

Additional Items:

Grants
Dr. KC has assembled a multidisciplinary team to collaborate on postharvest decay management project. The team with ten scientists from nationally renowned institutions representing both east and west coast pome fruit industries submitted a preproposal to USDA-NIFA-SCRI for 2023 funding cycle requesting $4.2 Million for the research project. If funded, the project is expected to cover various areas of postharvest decay research and data from this study will be instrumental in proving baseline information for some of the project activities.

Co-PI Amiri has leveraged funds from this grant to secure two extra-mural grants, one from the Specialty Crop Block, WA State Department of Agriculture (WSDA) and another from the USDA-

Crop Protection and Pest Management (CPPM) programs to continue research and extension efforts to better manage gray mold in the PNW.

1. Epidemiology-based tactics to abate gray mold of pome fruit in the Pacific Northwest. USDA-NIFA CPPM. $199,805. P.I.: A. Amiri, Co-PI.: Karina Gallardo.

2. Strategies to enhance pre- and postharvest management of gray mold in pome fruit. Specialty Crop Block Grant program (SCBG), WSDA-USDA. $230,155. P.I.: A. Amiri, Co-P.I.: T. Peever.

This is equivalent to $3 brought for each $1 invested by the FPPC in this project (Amiri Program).

Talks and Publications:

Talks:

Amiri A. Management of Postharvest decays. Workshop on postharvest diseases in conventional systems. Wenatchee, CTC, March 2020

Amiri A. Management of Postharvest decays. Workshop on postharvest diseases in conventional systems. Wenatchee, CTC, March 2020

Acosta W., Amiri A. 2020. *Botrytis cinerea* in pome fruit systems of the Pacific Northwest. *Phytopathology* 111-11-S2:37.

Acosta W., Amiri A. Management of gray mold pre and postharvest. *Northwest Apple Day*. Jan 21st, 2020.

KC, A. N. 2023. Preharvest factors associated with gray mold development in European pears. International Congress of Plant Pathology, Lyon, France, August 2023.

KC, A. N. 2023. Preharvest management of postharvest pathogens - Insights from Oregon. NCW Pear Day. Virtual presentation, January, 2023.

KC, A. N. 2022. Postharvest rot on pears. Southern Oregon Pest Management Forum. August, 2022.

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KC, A. N. 2021. Postharvest rot on pears. Southern Oregon Pest Management Forum. July, 2021.

Hernandez, M., and KC, A. N. 2021. *Botrytis cinerea* infection at different stages of pear fruit development. Southern Oregon Pest Management Forum. March, 2021.

Hernandez M., and KC, A. N. 2020. *Botrytis cinerea* infection at different stages of pear fruit development. Orchard Pest and Disease Management Conference, 2020.

Publications:

Amiri A. & Acosta W., 2021. Understanding the epidemiology of gray mold caused by Amiri A., Acosta W. 2020. Gray mold factsheet. http://treefruit.wsu.edu/crop-protection/disease-management/gray-mold/

Hernandez, M., and KC, A. N. 2022. *Botrytis cinerea* colonization occurs early in pear fruit development. *Abstract*: American Phytopathological Society, 2022.

Hernandez, M., and KC, A. N. 2022. *Botrytis cinerea* varies in its sensitivity towards common fungicides used in pear orchards. *Abstract*: APS Pacific Division virtual meeting, 2022.

Hernandez, M., and KC, A. N. 2021. Evaluation of *Botrytis cinerea* sensitivity towards fungicides commonly used in pear orchards. *Abstract*: APS Pacific Division virtual meeting, 2021.

Amiri A., Janis F., Hernandez, M., and KC, A. N. 2023. Epidemiology of *Botrytis* spp. in the pome fruit in the US Pacific Northwest. Plant Disease. In preparation.

Hernandez, M., and KC, A. N.3 2021. *In vitro* sensitivity of *Botrytis cinerea* isolates collected from European pears to fungicides with different modes of action. Plant Disease. In preparation.

Hernandez, M., Acosta, W., Amiri, A., and KC, A. N. 2022. Evaluating the seasonal fungicide programs for gray mold management in European pears. Plant Disease. In preparation.

Project Title: Fire Blight Product Testing for Effective Recommendations
Report Type: Final Project Report

PI: Tianna DuPont
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Telephone: (509) 293-8758
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Cooperator: Aina Baró Sabé, Washington State University

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $14,255
Total Project Request for Year 2 Funding: $14,686
Total Project Request for Year 3 Funding: $0 (reduced from $15,132)

Other funding sources: Awarded
Funding duration: 2020-21
Amount: $30,000
Agency Name: USDA NIFA IR4

Other funding sources: Awarded
Funding duration: 2020-22
Amount: $88,250
Agency Name: Gift Grants from Product Companies

Other funding sources: Awarded
Funding duration: 2020-23
Amount: $416,000
Agency Name: USDA Specialty Crop Research Initiative

Budget 1
Primary PI: Tianna DuPont
Organization Name: Washington State University
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Station Manager/Supervisor: Chad Kreuger
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Item	(Type year of project start date here)	(Type year start date of year 2 here if relevant)	(Type year start date of year 3 here if relevant)
Salaries	$7,800.00	$8,112.00	
Benefits	$2,955.00	$3,074.00	
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$500.00	$500.00	
Travel			
Plot Fees	$3,000.00	$3,000.00	
Miscellaneous			
Total	$14,255.00	$14,686.00	$0.00

Footnotes:

Salaries for a scientific assistant 2 month/ yr.

Benefits at 38% for scientific assistant.

Fire Blight Product Testing for Effective Recommendations

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

SIGNIFICANT FINDINGS
- Alum (potassium aluminum sulfate) provided good control similar to antibiotic checks as well as biological Blossom Protect (*A. pullulans*) and several copper products (Previsto, Mastercop, Instill).
- Several essential oil, and peracetic acid-peroxide products (Oxidate 5.0, Jet Ag, Thyme Guard, Thymox, Cinnerate) provided moderate disease suppression similar to some other biological and copper products (Serenade Opti, Cueva) and may be best incorporated as rotational products as part of an integrated program during lower risk periods.
- In order to minimize the risk of fruit marking managers should consider drying times for essential oils and peracetic acid-peroxide products as well as soluble coppers.

RESULTS and DISCUSSION

Alum
Potassium aluminum sulfate compounds are aseptic, astringent compounds known to inhibit the growth of bacteria, fungi and oomycetes potentially due to pH, cellular ionic imbalance or disruption of membranes (Kolaei et al. 2013; Mecteau et al. 2002).

Alum (Potassium aluminum sulfate) has been tested for six years in Washington. This compound is experimental (non-labeled). It has had generally consistent positive results with an average of 72% control relative to the untreated check in 2016 to 2022 trials when the product was applied at an 8 to 10 lb per 100 gal rate. This control is comparable to 74% in the oxytetracycline standard and 85% in the streptomycin standard (2013 to 2022 median). Marking from chemical russet was negligible in all WA trials (< 1 on a 0 to 15 scale). In 2022 relative control from alum was 88%, not significantly different than the streptomycin standard and significantly better than the water treated check (Table 1). In 2021 relative control was approximately 50%, but still significantly different from the water treated check and comparable to the relative control obtained using oxytetracycline check (56% relative control) and streptomycin check (58% relative control) (Table 2). However, in 2020 relative control from alum was 28% compared to the water treated check (Table 3).

Suppression of fire blight by alum was similar to trials in Germany where potassium-aluminum sulfate averaged 72% efficiency in eight trials (Kunz and Donat 2013). Alum also had high relative disease suppression in recent Oregon trials averaging 74% (*n*=8) (Johnson et al. 2022).

Table 1. Effect of mineral based biopesticides to pear, cv. Anjou on infection of *E. amylovora* in pear blossoms in Wenatchee, WA in 2022 [u]

Treatment	Rate per 100 gallons water	Application timings [z]	Infections per 100 clusters [y]				Fruit russet [v]
Streptomycin standard (Firewall 50WP) [x]	8 oz	3	4.4	±	1.2	c [w]	0.2
Oxytetracycline standard (Fireline 45WP) [x]	9 oz	3,6	15.7	±	4.8	b	0.2
Alum [t]	8 lb	3,4,6	3.9	±	1.4	c	0.5
Alum [t]	8 lb	3,4,6,8,9,10	4.1	±	0.4	c	1.8
Blossom Protect + Buffer Protect Alum [t]	1.25 lb + 5 lb 8 lb	1,2 3,6	6.8	±	1.6	bc	1.5

Blossom Protect + Buffer Protect	1.25 lb + 5 lb	1,3	15.5	±	4.4	b		0.3
Water treated check	NA	3,4,6	35.5	±	5.4	a		0.3

[z] Timings 1: 70% bloom, 2: 90% bloom, 3: morning before evening inoculation (full bloom), 4: morning after inoculation, 5: 2 days after inoculation, 6: 3 days after inoculation (petal fall), 7: 4 days after inoculation, 8: 6 days after inoculation, 9: 2 weeks after inoculation, 10: 3 weeks after inoculation

[u] Inoculation was conducted on the evening of 22 Apr 2021 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 17x10^6 CFU ml^{-1}).

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

[x] Amended with Regulaid: 16 fl. oz. per 100 gallons. Buffered to 5.6 pH.

[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[t] Amended with Regulaid: 16 fl. oz. per 100 gallons. pH verified at 4.0.

[v] Fruit marking is rated from an average of 25 fruit per tree. In 2022 less than 25 fruit were often present. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades. No statistical differences were observed between treatments.

Table 2. Effect of mineral based biopesticides on *E. amylovora* infection of apple blossoms cv. Red Delicious in Wenatchee, WA, in 2021[z]

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters[y]				Fruit russet[t]
Streptomycin (Firewall 17)[x]	8 oz	100% bloom	16.1	±	2.3	ab[w]	0.06
Oxytetracycline (Fireline 17)[x]	16 oz	100% bloom, petal fall	17.0	±	5.7	a	0.00
Organic standard apple Blossom Protect + Buffer Protect Previsto	1.24 lb+ 8.75 lb 3 qt	70% bloom, 100% bloom, 100% bloom + 1 day, petal fall	17.8	±	4.5	ab	0.69
Organic standard pear Blossom Protect + Buffer Protect Serenade Opti[u]	1.24 lb + 8.75 lb 20 oz	70% bloom, 100% bloom, 100% bloom + 1 day, petal fall	14.0	±	2.6	a	0.73
Alum[v]	8 lb	100% bloom, 100 bloom + 1 day, petal fall	19.3	±	2.4	ab	0.19
TDA-NC-1[u]	571 g	pink, 50% bloom, 100% bloom, petal fall	26.7	±	3.9	bc	0.05
Water-treated check	NA	100% bloom, petal fall, petal fall + 3 days	38.6	±	5.1	c	0.00

[z] Application dates were:18 Apr (70% bloom), 19 Apr (full bloom), 20 Apr (full bloom + 1 day), 23 Apr (petal fall), 26 April (petal fall + 3 days). Inoculation was conducted on the evening of 19 Apr 2021 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells and 50% live cells of *E. amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 40-94 x10^6 CFU ml^{-1}).

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

[x] Amended with Regulaid: 16 fl. oz. per 100 gallons. Buffered to 5.6 pH.

[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[v] Amended with Regulaid: 16 fl. oz. per 100 gallons.

[u] Amended with Swilet spreader sticker 23 fl. oz per 100 gallons.

[t] Fruit marking, average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

Table 3. Effect of Mineral Product Treatments on *E. amylovora* infection of apple blossoms in Wenatchee, WA, in 2020[‡]

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters				Fruit russet[t]
Streptomycin (Firewall 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	±	1.2	a	0
Oxytetracycline (Fireline 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2	b	0
Organic Standard Blossom Protect + Buffer Protect + Soluble Copper (Previsto)	1.24 lb + 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5	±	1.3	bc	0.02
Alum[y]	8 lb	100% bloom, petal fall	22	±	4.2	d	0.02
TDA-NC-1[x]	17.1 g	Tight cluster, 50% bloom, 100% bloom + 1 day, petal fall	13	±	2.3	bc	0
Water-treated check	NA	100% bloom, +1 day, petal fall	31	±	7.1	d	0

[‡]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 *E. amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.
[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.
[t]Fruit marking, average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

Oxidizers

Several new peroxide products with higher levels of peracetic acid have recently been released (e.g. Jet Ag, Oxidate 5.0). Peracetic acid denatures proteins, disrupts cell wall permeability, and oxidizes sulfhydryl and sulfur bonds in proteins, enzymes, and other metabolites. Peracetic acid and peroxide oxidizers generally have little residual activity.

Oxidizing agents (Jet Ag and Oxidate 5.0) produced median relative disease suppression of 53% and 62% with 2 to 3 applications post inoculation (4 trials: 2019-2022). In 2022, with applications the day after inoculation, petal fall and 6 days after inoculation (petal fall plus 3 days), oxidizers provided 53 and 62% relative control, but when applied at full bloom, day after inoculation and petal fall the relative control was 42.4% (Table 4). In 2021 control relative to the water treated check for peroxide + peracetic acid treatments was 63-67% with three applications (100% bloom + 1 day, petal fall and petal fall + 3 days), not significantly different than the organic standard (Table 5). In 2020 with two applications relative control for peroxide + peracetic acid treatments was not significantly different than the water treated check (Table 6). At these application timings no significant fruit marking was observed (less than 1 on a 0 to 15 scale). In comparison 2013 to 2022 Washington long term averages are 85% relative control for the streptomycin standard (N=35), 72% relative control Blossom Protect + Buffer Protect (N=25), 74% relative control oxytetracycline standard and 73% relative control Previsto. Enumeration of bacterial populations in the flower suggest that the 3-day post petal fall application in 2021 and 2022 was important to keep populations lower compared to in 2020 when 1 week post petal fall *Erwinia* numbers reached high levels in peroxide + peracetic acid treated trees (Fig 1-3).

In a previous study, peroxide + peracetic acid products were applied after antibiotics during the post petal fall period (Fireline at: 50% bloom, 100% bloom, PF peroxide/peracetic acid product at: 5, 7, 10, 14 days after full bloom). Multiple post petal fall applications resulted in significant fruit marking which would have resulted in culled fruit (average 8.2 on 0 to 15 scale). In order to limit fruit marking potential peroxide + peracetic acid products should be applied only in fast drying conditions and up until the early post-petal fall period.

Table 4. Effect of hydrogen peroxide, peracetic acid treatments applied to pear, cv. Anjou on infection from *E. amylovora* in pear blossoms in Wenatchee, WA in 2022[u]

Treatment	Rate per 100 gallons water	Application timings [z]	Infections per 100 clusters[y]			Fruit russet [v]
Streptomycin standard (Firewall 50WP) [x]	8 oz	3	4.4	± 1.2	c [w]	0.2
Oxytetracycline standard (Fireline 45WP) [x]	9 oz	3,6	15.7	± 4.8	b	0.2
Organic standard apple Blossom Protect + Buffer Protect, Previsto	1.25 lb + 5 lb 3 qt	1,2 3,6	11.1	± 4.0	bc	1.1
Organic standard pear Blossom Protect + Buffer Protect Serenade Aso	1.25 lb+ 5 lb 96 fl oz	1,3 4,6	16.9	± 2.6	ab	0.6
Blossom Protect + Buffer Protect	1.25 lb + 5 lb	1,3	15.5	± 4.4	b	0.3
hydrogen peroxide (26.5%), peracetic acid (4.9%) (Jet Ag)	128 fl oz	4,6,8	13.5	± 3.3	b	0.4
hydrogen peroxide (27%), peracetic acid (5%) (Oxidate 5.0)	128 fl oz	4,6,8	16.7	± 3.0	ab	0.6

hydrogen peroxide (27%), peracetic acid (5%) Oxidate 5.0	128 fl oz	3,4,6	20.4	±	5.7	ab	0.8
Blossom Protect + Buffer Protect hydrogen peroxide (26.5%), peracetic acid (4.9%) Jet Ag Stargus	1.25 lb + 5 lb 128 fl oz 2 qt	1,3 5 7	16.1	±	2.8	ab	1.3
Blossom Protect + Buffer Protect hydrogen peroxide (27%), peracetic acid (5%) (Oxidate 5.0)	1.25 lb + 5 lb 128 fl oz	1,3 5,7	17.2	±	2.2	ab	0.8
Water treated check	NA	3,4,6	35.5	±	5.4	a	0.3

[z] Timings 1: 70% bloom, 2: 90% bloom, 3: morning before evening inoculation (full bloom), 4: morning after inoculation, 5: 2 days after inoculation, 6: 3 days after inoculation (petal fall), 7: 4 days after inoculation, 8: 6 days after inoculation, 9: 2 weeks after inoculation, 10: 3 weeks after inoculation.

[u] Inoculation was conducted on the evening of 22 Apr 2021 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 17x10^6 CFU ml^{-1}).

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

[x] Amended with Regulaid: 16 fl. Oz. per 100 gallons. Buffered to 5.6 pH.

[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[v] Fruit marking is rated from an average of 25 fruit per tree. In 2022 less than 25 fruit were often present. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades. No statistical differences were observed between treatments.

Table 5. Effect of hydrogen peroxide, peracetic acid treatments applied to apple, cv. Red Delicious on infection from *E. amylovora* in apple blossoms in Wenatchee, WA, in 2021[z]

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters[y]				Fruit russet[v]
Streptomycin (Firewall 17) [x]	8 oz	100% bloom	16.1	±	2.3	a[w]	0.06
Oxytetracycline (Fireline 17) [x]	16 oz	100% bloom, petal fall	17.0	±	5.7	a	0.00
Organic standard apple Blossom Protect + Buffer Protect Previsto	1.24 lb + 8.75 lb 3 qt	70% bloom, 100% bloom, 100% bloom + 1 d, petal fall	17.8	±	4.5	a	0.69
Organic standard pear Blossom Protect + Buffer Protect Serenade Opti	1.24 lb + 8.75 lb 20 oz	70% bloom, 100% bloom, 100% bloom + 1 d, petal fall	13.9	±	2.6	a	0.73
hydrogen peroxide (26.5%), peracetic acid (4.9%) (Jet Ag)	128 oz	100% bloom + 1 day, petal fall, petal fall + 3 days	12.8	±	1.6	a	0.75
hydrogen peroxide (27%), peracetic acid (5%) (Oxidate 5.0)	128 oz	100% bloom + 1 day, petal fall, petal fall + 3 days	14.2	±	1.2	a	0.51
Blossom Protect + Buffer Protect hydrogen peroxide (26.5%), peracetic acid (4.9%) (Jet Ag) *Bacillus amyloliquefaciens* (Stargus)	1.24 lb + 8.75 lb 128 oz 2 qt	70% bloom, 100% bloom petal fall petal fall + 3 days	11.4	±	0.7	a	0.99
Water-treated check	NA	100% bloom, petal fall, petal fall + 3 days	38.6	±	5.1	b	0.00

[z] Application dates were: 18 Apr (70% bloom), 19 Apr (full bloom), 20 Apr (full bloom + 1 day), 23 Apr (petal fall), 26 April (petal fall + 3 days). Inoculation was conducted on the evening of 19 Apr 2021 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells and 50% live cells of *E. amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 40-94 x10^6 CFU ml^{-1}).

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

[x] Amended with Regulaid: 16 fl. Oz. per 100 gallons. Buffered to 5.6 pH.

[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[v] Fruit marking, average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

Table 6. Effect of hydrogen peroxide and peracetic acid treatments applied to Red delicious apple trees on infection from *E. amylovora* in apple blossoms in Orondo, WA, in 2020[‡]

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters [u]				Fruit russet[t]
Streptomycin standard (Firewall 17)[zy]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	±	1.2	a	0
Oxytetracycline standard (Fireline 17) [zy]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2	b	0

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	0.02
hydrogen peroxide (26.5%), peracetic acid (4.9%) (Jet Ag)	128 fl oz	Day after inoc and 3 days after inoc[v]	27.8	±	3.9	c	0
hydrogen peroxide (27%), peracetic acid (5%) (Oxidate 5.0)	128 fl oz	Day after inoc and 3 days after inoc	24.1	±	3.8	c	0.02
hydrogen peroxide (27%), peracetic acid (5%) (Oxidate 5.0)	50 fl oz	Day after inoc and 3 days after inoc	28	±	4.1	c	0.07
Untreated water check	----	100% bloom, +1 day, petal fall	30.7	±	7.1	c	0

[‡]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 *E. amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10^6 CFU per ml.

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

[z] Buffered to 5.6 pH.

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

[v] Note inoculation was done at dusk. Day after spray is done early morning next day. 3 days after inoculation coincided with petal fall sprays.

[t] Fruit marking, average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

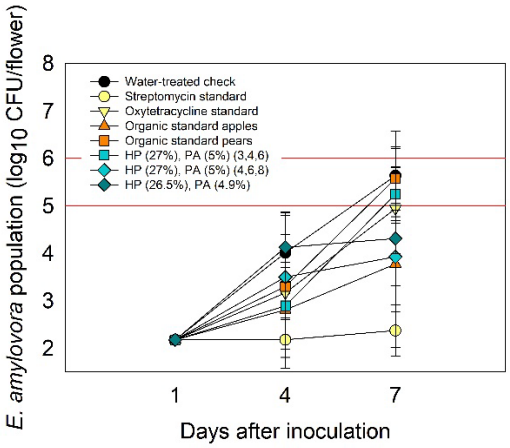

Figure 1. Effect of hydrogen peroxide (HP), peracetic acid (PA) treatments applied to pear cv. Anjou trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers 1, 4 and 7 days post-inoculation of the pathogen in Wenatchee, WA, in 2022.

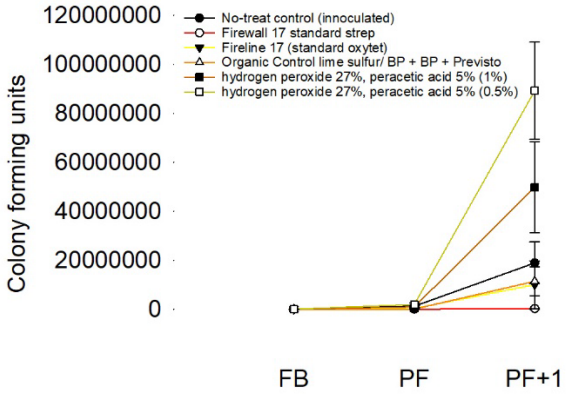

Figure 2. Effect of treatments applied to Red delicious apple trees to suppress fire blight on the

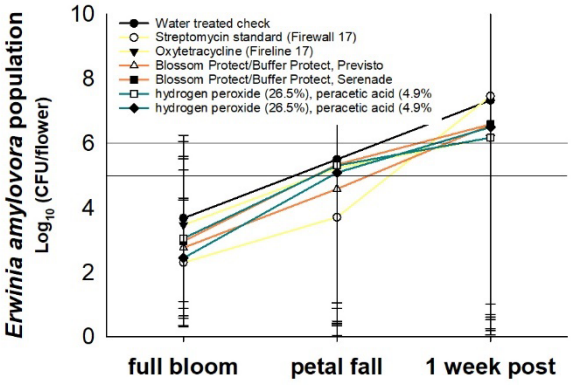

Figure 3. Effect of hydrogen peroxide and peracetic acid treatments applied to Red delicious

population size of *E. amylovora* strain 153N on flowers at Full Bloom (FB), Petal Fall (PF) and Petal Fall + 1 week (PF+1) in WA in 2020.

apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers at full bloom, petal fall and 1 week post petal fall in Wenatchee, WA, in 2021.

Essential Oils

Essential oils (e.g. from thyme, mint, cinnamon, oregano) have known antimicrobial activity. In one laboratory study, active compounds from *Origanum compactum* (oregano family) and *Thymus vulgaris (Thyme)* were most effective (Kokoskova *et al.*, 2011). In another study, Apium graveolens (celery seed) and Curcuma longa (turmeric) essential oils showed a reduction in *E. amylovora* virulence (Akhlaghi *et al.* 2017). These oils are rich in antioxidative phenolic compounds, which are believed to be responsible for their antimicrobial activity (Chizzola *et al.*, 2008). Several essential oil products are available commercially, which may be of interest including Thyme Guard, Thymox, and Cinnerate.

Essential oil plant extracts from thyme and cinnamon (Thyme Guard, Thymox, Cinnerate) resulted in median relative disease suppression of 49% (thyme oils 3 to 6 applications) and 45% (cinnamon oils 3 to 4 applications) between 2019 and 2022. Thyme oils (Thyme Guard and Thymox) had infection incidence significantly lower than water treated controls in 2021 and 2022 but not in 2019 and 2020 (Tables 7-10). Cinnamon oil compounds (Cinnerate) significantly reduced infection incidence compared to water treated controls in 2021 with four applications, but not in 2020 with three applications. Essential oil products with 3 to 4 applications resulted in low fruit marking but with 7 applications in 2019 the thyme oil product resulted in significant fruit marking (average of 4 on a 1 to 15 scale). In 2021 and 2022 the alternative organic program Blossom Protect + Buffer Protect at 50% and 100% bloom followed by Previsto at 100% bloom + 1 day and by thyme oil product at petal fall was not significantly different than organic apple and pear standard programs where Blossom Protect + Buffer Protect were followed by Previsto or Serenade Opti at 100% bloom and petal fall. Enumeration of bacterial populations in the flower showed no significant reduction of E. amylovora after the application of essential oils in any of the years (Fig. 4 - 8).

Table 7. Effect of essential oil/plant extract treatments applied to pear, cv. Anjou on infection of *E. amylovora* in pear blossoms in Wenatchee, WA in 2022 [u]

Treatment	Rate per 100 gallons water	Application timings [z]	Infections per 100 clusters [y]				Fruit russet [v]
Streptomycin standard (Firewall 50WP) [x]	8 oz	3	4.4	±	1.2	c [w]	0.2
Oxytetracycline standard (Fireline 45WP) [x]	9 oz	3,6	15.7	±	4.8	ab	0.2
Organic standard apple Blossom Protect + Buffer Protect Previsto	 1.25 lb + 5 lb 3 qt	 1,2 3,6	11.1	±	4.0	bc	1.1
Organic standard pear Blossom Protect + Buffer Protect Serenade Aso	 1.25 lb+ 5 lb 96 fl oz	 1,3 4,6	16.9	±	2.6	ab	0.6
Blossom Protect + Buffer Protect	1.25 lb + 5 lb	1,3	15.5	±	4.4	b	0.3
Thyme Guard [t]	2 qt	3,4,6	11.2	±	2.3	bc	0.9
Cinnerate	32 fl oz	3,4,6,8,9,10	16.1	±	4.0	ab	0.9
Cinnerate	32 fl oz	3,4,6	18.5	±	3.3	ab	0.5
Blossom Protect + Buffer Protect Previsto Thyme Guard [t]	1.25 lb + 5 lb 3 qt 2 qt	1,2 3 6	11.2	±	7.5	c	0.4
Blossom Protect + Buffer Protect Cinnerate	1.25 lb + 5 lb 32 fl oz	1,2 4,6	21.3	±	4.3	ab	0.4
Problad Verde [s] Cinnerate	40 fl oz 32 fl oz	1,3 2,6	15.3	±	3.1	ab	0.8

Water treated check	NA	3,4,6	35.5	±	5.4	a	0.3

[z] Timings 1: 70% bloom, 2: 90% bloom, 3: morning before evening inoculation (full bloom), 4: morning after inoculation, 5: 2 days after inoculation, 6: 3 days after inoculation (petal fall), 7: 4 days after inoculation, 8: 6 days after inoculation, 9: 2 weeks after inoculation, 10: 3 weeks after inoculation

[u] Inoculation was conducted on the evening of 22 Apr 2021 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 17x10^6 CFU ml^{-1}).

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

[x] Amended with Regulaid: 16 fl. oz. per 100 gallons. Buffered to 5.6 pH.

[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[t] Acidified to pH 4.

[s] Amended with NuFilm: 16 fl. oz. per 100 gallons.

[v] Fruit marking is rated from an average of 25 fruit per tree. In 2022 less than 25 fruit were often present. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades. No statistical differences were observed between treatments.

Table 8. Effect of essential oil/ plant extract treatments applied to apple, cv. Red Delicious on infection of *E. amylovora* in apple blossoms in Wenatchee, WA, in 2021[z]

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters[y]				Fruit russet[u]
Streptomycin standard (Firewall 17)[x]	8 oz	100% bloom	16.1	±	2.3	a[w]	0.06
Oxytetracycline standard[y] (Fireline 17)[x]	16 oz	100% bloom, petal fall	17.0	±	5.7	a	0.00
Organic standard apple Blossom Protect + Buffer Protect Previsto	1.24 lb + 8.75 lb 3 qt	70% bloom, 100% bloom, 100% bloom + 1 day, petal fall	17.8	±	4.5	a	0.69
Organic standard pear Blossom Protect + Buffer Protect Serenade Opti	1.24 lb + 8.75 lb 20 oz	70% bloom, 100% bloom, 100% bloom + 1 day, petal fall	13.9	±	2.6	a	0.73
Blossom Protect + Buffer Protect Previsto Thyme oil (23%) (Thyme Guard)[v]	1.24 lb + 8.75 lb 3 qt 2 qt	50% bloom, 100% bloom, 100% bloom + 1 day, petal fall	16.0	±	1.9	a	0.34
Thyme oil (23%) (Thyme Guard)[v]	2 qt	100% bloom, 100% bloom + 1 day, petal fall	21.4	±	3.9	ab	0.24
Thymol (23%) (Thymox)	2 qt	100% bloom, 100% bloom + 1 day, petal fall	22.9	±	5.7	ab	0.35
ET91[v]	640 oz	100% bloom, 100% bloom + 1 day, petal fall	21.7	±	5.3	ab	0.06
ET91[v]	320 oz	100% bloom, 100% bloom + 1 day, petal fall	21.9	±	3.7	ab	0.06
Cinnamon oil (60%) (Cinnerate) + Lupine[h]	32 oz + 40 oz	100% bloom, 100% bloom + 1 day, petal fall, petal fall + 3 days	17.6	±	3.2	ab	0.02
Cinnamon oil (60%) (Cinnerate)	32 oz	100% bloom, 100% bloom + 1 day, petal fall, petal fall + 3 days	20.8	±	3.7	ab	0.01
Thyme oil (3%) (G)	256 oz	100% bloom, 100% bloom + 1 day, petal fall	35.9	±	8.4	bc	0.00
Water-treated check	NA	100% bloom, petal fall, petal fall + 3 days	38.6	±	5.1	c	0.00

[z] Application dates were: 18 Apr (70% bloom), 19 Apr (full bloom), 20 Apr (full bloom + 1 day), 23 Apr (petal fall), 26 April (petal fall + 3 days). Inoculation was conducted on the evening of 19 Apr 2021 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells and 50% live cells of *E. amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 40-94 x10^6 CFU ml^{-1}).

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

[x] Amended with Regulaid: 16 fl. oz. per 100 gallons. Buffered to 5.6 pH.

[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[v] Acidified to pH 4.

[u] Fruit marking, average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

[h] Banda de Lupinus albus doce (20%).

Table 9. Effect of Essential Oil/ Plant Extract Treatments on infection of *E. amylovora* in apple blossoms in Orondo, WA, in 2020 [‡]

Treatment	Application timings	Infections per	Fruit

	Rate per 100 gallon water		100 clusters				russet
Streptomycin (Firewall 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	± 1.2	a		0
Oxytetracycline [y] (Fireline 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	± 2	b		0
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		0.2
Thyme oil (23%) (Thyme Guard)	2 qrt	80% bloom, 100% bloom +1, petal fall	17	± 2.3	cd		0
Thymol (23%) (Thymox)	2 qrt	80% bloom, 100% bloom, petal fall	22	3.5	d		0
Cinnamon oil (60%) (Cinnerate)	1 qt	50% bloom, morning after inoc, petal fall	19	± 3.5	d		0
TS28	21.9 ml	100% bloom, +1 day, petal fall	23	± 5.5	cd		0
TS108	25 ml	100% bloom, +1 day, petal fall	31	± 5.8	d		0
ET91	38.4 oz	100% bloom, +1 day, petal fall	10	± 6.6	b		1.9
Lupine[u]	40 oz	50% bloom, morning after inoc, petal fall	22.6	± 4.1	cd		0
Water-treated check	NA	100% bloom, +1 day, petal fall	31	± 7.1	d		0

[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 *E. 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%).

Table 10. Effect of Essential Oil/Plant Extract Treatments on infection of *E. amylovora* in apple blossoms in Wenatchee, WA, in 2019[‡]

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters**			Fruit russet
Streptomycin (Firewall 17)[yz]	28.8 oz	50% bloom, 100% bloom, petal fall	4.6	± 2.7	a	0
Oxytetracycline (Fireline 17) [yz]	24 oz	50% bloom, 100% bloom, petal fall	5.8	± 3.2	a	0
Organic standard (lime sulfur, Blossom Protect+ Buffer Protect, Previsto)	6 gal 1.24+8.75 lb 3 qt	LS: 70% bloom BP: 20% bloom, 80% bloom PR: 100% bloom, petal fall	6.1	± 1.2	a	0
Cueva/ Previsto	4qt/3qt	day before and day after 100% bloom, petal pall	9.7	± 2.7	a	0
Thyme oil (23%) (Thyme Guard)	2 qrt	50%, 100% bloom, petal fall, + 4 post petal fall apps	9.2	± 5.3	a	4.1 ± 0.9
Untreated, Inoculated check	NA	100% bloom	20.9	± 11.1	b	0

[z]Buffered to 5.6 pH.
[y] Amended with Regulaid: 32 fl. oz. per 100 gallons.
[‡]Application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019. Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blooms) using a suspension of freeze-dried cells of *E. amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10^6 CFU per ml and on May 1, 2019 using live culture prepared at 1x10^6 CFU ml^{-1}.

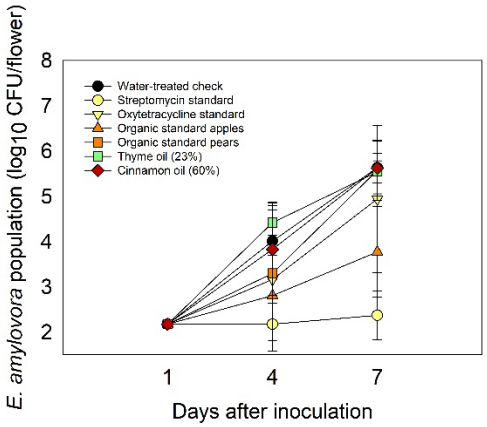

Figure 4. Effect of essential oil/plant extract treatments applied to pear cv. Anjou trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers 1, 4 and 7 days post-inoculation of the pathogen in Wenatchee, WA, in 2022.

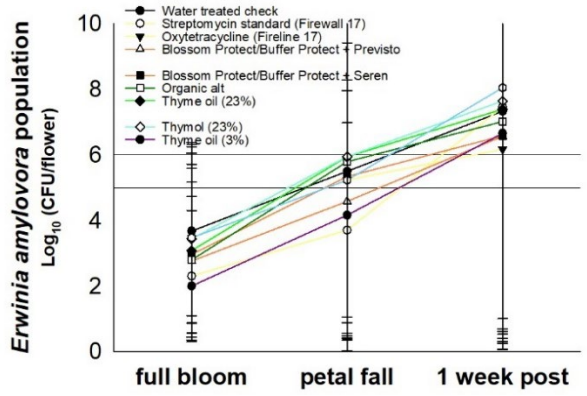

Figure 5. Effect of thyme treatments on the population size of *E. amylovora* strain 153N on flowers at full bloom, petal fall and 1 week post petal fall in Wenatchee, WA, in 2021.

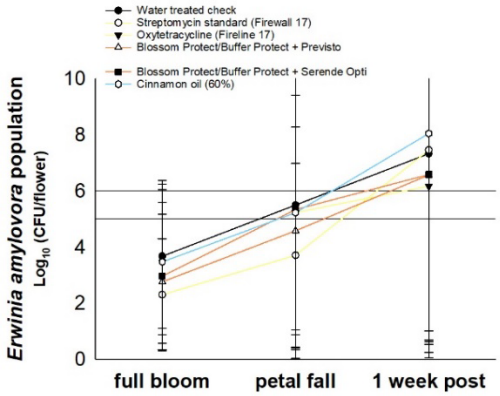

Figure 6. Effect of cinnamon oil products on the population size of *E. amylovora* strain 153N on flowers at full bloom, petal fall and 1 week post petal fall in Wenatchee, WA, in 2021.

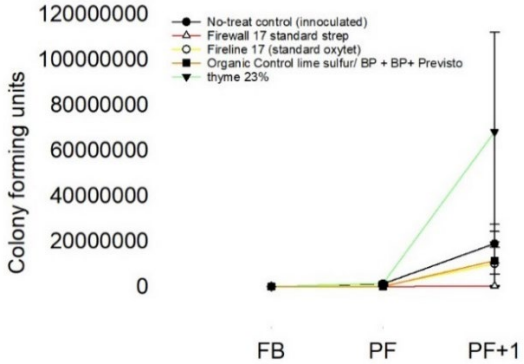

Figure 7. Effect of thyme oil treatments on the population size of *E. amylovora* strain 153N on flowers at full bloom (FB), petal fall (PF) and petal fall + 1 week (PF+1) in Orondo, WA, in 2020.

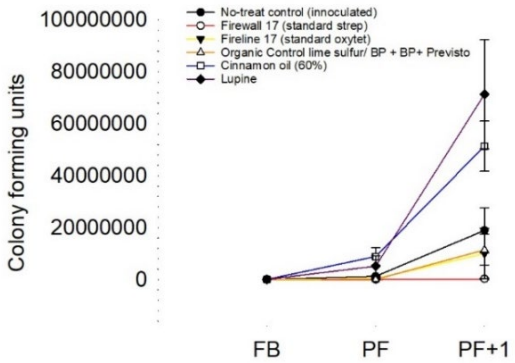

Figure 8. Effect of cinnamon oil treatments on blight on the population size of *E. amylovora* strain 153N on flowers at full bloom (FB), petal fall (PF) and petal fall + 1 week (PF+1) in Orondo, WA, in 2020.

Biological Control Products

There is interest in bacteriophage products for control of fire blight. A *bacteriophage* is a type of virus that infects bacteria. "*Bacteriophage*" literally means "bacteria eater," because *bacteriophage* destroy their host cells. *Bacteriophage* infect bacteria and multiply inside the host (lytic cycle), killing the host and releasing the progeny. *Bacteriophage* are composed of a nucleic acid molecule that is surrounded by a protein structure. *Bacteriophage* are very specific to a type of bacteria which make them an attractive option for IPM management. However, *bacteriophage* have some challenging features. *Bacteriophage* can only replicate in bacterial cells and are sensitive to environmental conditions. pH, UV, and precipitation can all reduce their ability to live on the leaf surface (Gill and Abedon 2003). Interestingly, there is some evidence that *bacteriophage* can be effective when they penetrate and translocate through the plant (Nagy et al. 2015). For example, *bacteriophage* have been effective for bacterial wilt of tomato in greenhouse trials (Fujiwara et al. Vol. 77, No. 12; Iriarte et al. 2012).

In 2020 and preliminary trials in 2019 *bacteriophage* products performed no better than the water treated check (Tables 13,14). In 2022 the *bacteriophage* product provided 58% relative control not significantly lower than oxytetracycline standards. Based on work by Sundin (Michigan State University) it was hypothesized that the addition of a particle film sun protectant would reduce *bacteriophage* die-off due to UV and enhance control potential. In 2020 addition of kaolin clay (Surround) did not improve control (Table 12).

Bacteriophage active against *E. amylovora* have had variable results. For example in Michigan OmniLytics phage tested in 2018 with 74% relative disease suppression and Fire Quencher phage with 42% relative disease suppression while in 2019 AgriPhage had 35 to 39% relative disease suppression (Outwater et al. 2019; Sundin et al. 2018). This variation has also been observed in Washington, where a relative disease suppresison of *bacteriophage* products below 20% was observed in 2019 and 2020 (Tables 13,14), while in 2022 the application of Agriphage at full bloom, one day after full bloom and petal fall provided 58% relative disease suppression (Table 11). One factor explaining this variation could be UV light from the sun, as it has been reported that *bacteriophage* of *E. amylovora* are sensitive to it (Buttimer et al. 2017). In fact, the total solar radiation in 2019 and 2020 was above 20 MJ/m^2 more days than in 2022.

Table 11. Effect of biological treatments applied to pear, cv. Anjou on infection of *E. amylovora* in pear blossoms in Wenatchee, WA in 2022 [u]

Treatment	Rate per 100 gallons water	Application timings [z]	Infections per 100 clusters [y]				Fruit russet [v]
Streptomycin standard (Firewall 50WP) [x]	8 oz	3	4.4	±	1.2	d [w]	0.2
Oxytetracycline standard (Fireline 45WP) [x]	9 oz	3,6	15.7	±	4.8	bc	0.2
Blossom Protect + Buffer Protect	1.25 lb + 5 lb	1,3	15.5	±	4.4	c	0.3
Bacillus Subtilis (Serenade Aso)	96 fl oz	3,4,6	16.7	±	2.8	bc	0.6
Phage 7 (Agriphage)	2 qt	3,4,6	14.9	±	1.2	bc	0.2
Citric acid (F)	1.4 gal	3,4,6	15.9	±	3.2	bc	0.4
PSU1	200 g	2,4,6	25.5	±	3.2	ab	0.2
PSU2	1.7 kg	2,4,6	18.3	±	6.5	bc	0.3
PSU3	500 g	2,4,6	15.0	±	3.5	bc	0.2
Water treated check	NA	3,4,6	35.5	±	5.4	a	0.3

[z] Timings 1: 70% bloom, 2: 90% bloom, 3: morning before evening inoculation (full bloom), 4: morning after inoculation, 5: 2 days after inoculation, 6: 3 days after inoculation (petal fall), 7: 4 days after inoculation, 8: 6 days after inoculation, 9: 2 weeks after inoculation, 10: 3 weeks after inoculation

[u] Inoculation was conducted on the evening of 22 Apr 2021 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 17x10^6 CFU ml^{-1}).

[y] Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.
[x] Amended with Regulaid: 16 fl. oz. per 100 gallons. Buffered to 5.6 pH.
[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).
Fruit marking is rated from an average of 25 fruit per tree. In 2022 less than 25 fruit were often present. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades. No statistical differences were observed between treatments.

Table 12. Effect of biological treatments applied to apple, cv. Red Delicious on infection of *E. amylovora* in apple blossoms in Wenatchee, WA, in 2021[z]

Treatment	Rate per 100 gallons water	Timing	Infections per 100 clusters[y]				Fruit russet[s]
Streptomycin standard (Firewall 17)[x]	8 oz	100% bloom	16.1	±	2.3	ab[w]	0.06
Oxytetracycline standard (Fireline 17)[x]	16 oz	100% bloom, petal fall	17.0	±	5.7	a	0.00
Organic standard apple Blossom Protect + Buffer Protect Previsto	1.24 lb+ 8.75 lb 3 qt	70% bloom, 100% bloom, 100% bloom + 1 day, petal fall	17.8	±	4.5	a	0.69
Organic standard pear Blossom Protect + Buffer Protect Serenade Opti	1.24 lb + 8.75 lb 20 oz	70% bloom, 100% bloom, 100% bloom + 1 day, petal fall	13.9	±	2.6	a	0.73
RejuGro[u]	15.1 g	100% bloom, 100 bloom + 1 day,	19.1	±	1.8	ab	0.00
UW37_4RLE	400 ml	100% bloom, 100% bloom + 1 day, petal fall	30.4	±	4.5	bc	0.00
UW58_4DLA	400 ml	100% bloom, 100% bloom + 1 day, petal fall	17.0	±	4.4	a	0.05
UW29_2ALA1	400 ml	100% bloom, 100% bloom + 1 day, petal fall	23.4	±	3.5	abc	0.00
PSU1[t]	1x10^9 CFU ml^{-1}	100% bloom, 100% bloom + 1 day	14.5	±	4.3	a	0.05
Water-treated check	NA	100% bloom, petal fall, petal fall + 3 days	38.6	±	5.1	c	0.00

[z] Application dates were:18 Apr (70% bloom), 19 Apr (full bloom), 20 Apr (full bloom + 1 day), 23 Apr (petal fall), 26 April (petal fall + 3 days). Inoculation was conducted on the evening of 19 Apr 2021 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells and 50% live cells of *E. amylovora* strain Ea153 (streptomycin and oxytetracycline sensitive strain) prepared at 1 x10^6 CFU ml^{-1} (verified at 40-94 x10^6 CFU ml^{-1}).
[y] Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.
[x] Amended with Regulaid: 16 fl. oz. per 100 gallons. Buffered to 5.6 pH.
[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).
[u] Amended with PEG4000 and Regulaid: 16 fl. oz. per 100 gallons.
[s] Fruit marking, average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

Table 13. Effect of Biological Control Product Treatments on *E. amylovora* infection of apple blossoms in Wenatchee, WA, in 2020.[‡]

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters**				Fruit Russet
Untreated, Inoculated Check	water	100% bloom, +1 day, petal fall	31	±	7.1	c	0
Streptomycin standard (Firewall 17)[zy]	28.8 oz	50% bloom, 100% bloom, petal fall	2.8	±	1.2	a	0
Oxytetracycline standard (Fireline 17)[zy]	28.8 oz	50% bloom, 100% bloom, petal fall	8.2	±	2.0	b	0
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	0.02
Phage7 (Agriphage)	2 qt	100% bloom 12hr before ap, +1 day, +3 days	24	±	4.8	c	0

Phage7 + Surround (Agriphage)	2 qt + 0.1 lb	100% bloom 12hr before ap, +1 day, +3 days	31	±	3.7	c	0

** 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.

Table 14. Effect of Biological Control Product Treatments on *E. amylovora* infection of apple blossoms in Wenatchee, WA, in 2019‡

Treatment	Rate per 100 gallons water	Application timings	Infections per 100 clusters			
Streptomycin standard (Firewall 17)[zy]	28.8 oz	50% bloom, 100% bloom, petal fall	4.6	±	2.7	a
Oxytetracycline standard (Fireline 17) [zy]	24 oz	50% bloom, 100% bloom, petal fall	5.8	±	3.2	ab
Organic standard (lime sulfur, Blossom Protect+ Buffer Protect/ Previsto)	6 gal 1.24 lb/8.75 lb 3 qt	LS: 70% bloom BP: 20% bloom, 80% bloom PR: 100% bloom, petal fall	6.1	±	1.1	ab
Cueva/ Previsto	4qt/3qt	day before and day after 100% bloom, petal pall	9.7	±	2.7	abc
Phage7[y]	1 qt	50% bloom, 100% bloom, petal fall	17.3	±	3.6	bc
Phage7 + oxytet (Fireline)[y]	1 qt + 0.1 lb	50% bloom, 100% bloom, petal fall	12.4	±	3.4	abc
Bacillus Subtilis (A)	30 oz	50% bloom, 100% bloom, petal fall	22.5	±	7.1	c
Bacillus Subtilis QST 713 strain (Serenade Opti)	20 oz	day before and day after 100% bloom, petal fall	16.0	±	3.2	abc
Untreated, Inoculated Check	water	100% bloom	20.9	±	11.1	c

[y]Amended with Regulaid: 32 fl. oz. per 100 gallons.

[Z]Buffered to 5.6 pH.

‡Application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019. Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blooms) using a suspension of freeze-dried cells of *E. amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10^6 CFU per ml and on May 1, 2019 using live culture prepared at 1x10^6 CFU ml^{-1}.

Buttimer, C., McAuliffe, O., Ross, R. P., Hill, C., O'Mahony, J., and Coffey, A. (2017). Bacteriophages and bacterial plant diseases. Frontiers in Microbial Biology 8.

Fujiwara, A., Fujisawa, M., Hamasaki, R., Kawasaki, T., Fujie, M., and Yamada, T. (Vol. 77, No. 12). Biocontrol of ralstonia solanacearum by treatment with lytic bacteriophages. Appl. Environ. Microbiol. 77.

Gill, J., and Abedon, S. T. (2003). Bacteriophage ecology and plants. APS Net Feature doi: 10.1094/APSnetFeature-2003-1103.

Iriarte, F. B., Obradović, A., Wernsing, M. H., Jackson, L. E., Balogh, B., Hong, J. A., Momol, M. T., Jones, J. B., and Vallad, G. E. (2012). Soil-based systemic delivery and phyllosphere in vivo propagation of bacteriophages. Bacteriophage 2:e23530.

Johnson, K. B., Temple, T. N., Kc, A., and Elkins, R. B. (2022). Refinement of nonantibiotic spray programs for fire blight control in organic pome fruit. Plant Dis. 106:623-633.

Kolaei, E. A., Cenatus, C., Tweddell, R. J., and Avis, T. J. (2013). Antifungal activity of aluminium-containing salts against the development of carrot cavity spot and potato dry rot. Ann. Appl. Biol. 163:311-317.

Kunz, S., and Donat, C. (2013). Field results for the efficacy of fire blight control agents in the last fifteen years in germany. Pages 101-106 in: 13th International Workshop on Fire Blight, Zurich, Switzerland.

Mecteau, M. R., Arul, J., and Tweddell, R. J. (2002). Effect of organic and inorganic salts on the growth and development of fusarium sambucinum, a causal agent of potato dry rot. Mycological Research 106:688-696.

Nagy, J. K., Schwarczinger, I., Kunstler, A., Pogany, M., and Kiraly, L. (2015). Penetration and translocation of erwinia amylovora-specific bacteriophages in apple - a possibility of enhanced control of fire blight. European Journal of Plant Pathology 142:815-827.

Outwater, C. A., Proffer, T. J., Slack, S. M., and Sundin, G. W. (2019). Evaluation of new and existing biological control materials for the control of fire blight on gala apples, 2019. Plant Disease Management Reports 14:1.

Sundin, G. W., Outwater, C. A., Slack, S. M., and Dobbins, M. R. (2018). Evaluation of biological control materials for the control of fire blight on buckeye gala apples in michigan, 2018. Plant Disease Management Reports 13:1.

Executive Summary

Fire Blight Product Testing for Effective Recommendations

Keywords: fire blight, apple, pear, biopesticides, essential oils, biologicals

Abstract:

The efficacy of several alternatives to antibiotics for the control of fire blight were tested in four Washington trials. Alum (potassium aluminum sulfate) provided good control similar to antibiotic checks as well as biological Blossom Protect (*A. pullulans*) and several copper products (Previsto, Mastercop, Instill). Alum provided a median of 72% control relative to the water treated check in 2016 to 2022 trials when the product was applied at an 8 to 10 lb per 100 gal rate. This control is comparable to comparable to 74% in the oxytetracycline standard and 85% in the streptomycin standard (2013 to 2022 median). Marking from chemical russet was negligible in all WA trials (< 1 on a 0 to 15 scale). Several essential oil, and peracetic acid-peroxide products (Oxidate 5.0, Jet Ag, Thyme Guard, Thymox, Cinnerate) provided moderate disease suppression similar to some other biological and copper products (Serenade Opti, Cueva) and may be best incorporated as rotational products as part of an integrated program during lower risk periods. Oxidizing agents (Jet Ag and Oxidate 5.0) produced median relative disease suppression of 53% to 62% with 2 to 3 applications post inoculation (4 trials: 2019-2022). Essential oil plant extracts from thyme and cinnamon (Thyme Guard, Thymox, Cinnerate) resulted in median relative disease suppression of 49% (thyme oils 3 to 6 applications) and 45% (cinnamon oils 3 to 4 applications) between 2019 and 2022. In 2020 and preliminary trials in 2019 bacteriophage products performed no better than the water treated check. In 2022 the bacteriophage product provided 58% relative control not significantly lower than oxytetracycline standards. In order to minimize the risk of fruit marking managers should consider drying times for essential oils and peracetic acid-peroxide products as well as soluble coppers.

Project title: Pear Consumer Preference Testing

Report type: Final Project Report

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Project Duration: 1-Year with a 1-year no-cost extension
Total Project Request for Year 1 Funding: $50,000.
Other related/associated funding sources: None.
WTFRC Collaborative Costs: None.
Budget 1
Organization Name: WSU **Contract Administrator: Carolyn Ross**
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Item	**(type current year here)**	**(type additional year if relevant)**
Salaries		
Benefits		
Supplies: Pears, Pear transport, pear conditioning, Trained panel profiling of pears, chemical analyses	$20,000	
Travel		
Total	$20,000	Total year 2

Budget 2
Organization Name: OSU **Contract Administrator:** Ann Colonna
Telephone: 503-872-6677 **Email address:** ann.colonna@oregonstate.edu

Item	(type current year here)	(type additional year if relevant)
Salaries	$11,455	
Benefits	$5,125	
Wages		
Benefits		
Equipment		
Supplies	$300	
Travel	$900	
Miscellaneous – Panelist payment	$7,200	
Total	$24,980	Total year 2

Budget 3
Organization Name: WSU **Contract Administrator:** R. Karina Gallardo
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Station Manager/Supervisor: Todd Murray Email Address: tmurray@wsu.edu

Item	(type current year here)	(type additional year if relevant)
Salaries		
Benefits		
Wages - for data analysis	$5,000	
Total	$5,000	Total year 2

Objectives
The objective of this project was to identify the pear sensory characteristics considered to be desirable by consumers in the Pacific Northwest (PNW). Previous research has provided information regarding the traits that make a well-liked pear, but this current research project proposed testing new varieties, and seeking to understand what sparks consumer interest in pears in current Pacific Northwest consumers. We were able to fully accomplish the objectives of this project.

Significant findings
- Twenty-three pear' varieties with varied sensory properties were profiled by a trained sensory panel (n=10) and important differences among those properties were identified between them.
- Consumer (n=219) testing of 12 pear varieties determined the sensory attributes (pear flavor, sweetness and juiciness) that mostly influenced their acceptability and willingness to pay.
- Relationships among sensory properties (from the trained sensory panel) and the consumer liking of the pears were determined and allowed to identify consumer preferences.
- Consumer preferences of 12 pear varieties (Bartlett and Seckel in the summer set and Paragon, Green Anjou, Concorde and Comice in the winter set) were determined.
- Willingness to pay showed different tiers, for Summer varieties, first Bartlett, followed by the second tier 573 and Seckel, and the third tier 642, 417, and 720. For Winter varieties there are two tiers, the first one composed by Paragon, Concorde, and Green Anjou and the second one composed by Comice, Gem (not ripened), and Bosc.

Results and Discussion

Pears used in the research

One key objective of this research was to source a large and diverse array of pears for both the descriptive analysis and consumer sensory evaluation portions of the study. Many growers, researchers and other stakeholders were interviewed for advice and pear sourcing suggestions in the months leading up to the trials to ensure a large and diverse sample of fruit was available for evaluation. A large sample set of **23 pears** (11 summer and 12 winter varieties) were obtained for descriptive analysis and instrumental measures. Pears were evaluated at two time points, October, and December, depending on their seasonality. Based on the findings obtained through the descriptive analysis procedure, a diverse set of six pears per trial were selected for consumer sensory evaluation at the Oregon State University Food Innovation Center (OSU FIC) in Portland, OR. Each set of six pears represented a range of seasonal pear sensory attributes on offer within the U.S. Varieties tested are listed in **Table 1.** Codes were used to identify proprietary varieties.

Instrumental measurements of the pears evaluated by the trained panel

For both summer and winter season, there were significant differences ($p<0.05$) in most of the physicochemical measurements conducted in twelve of the varieties profiled by the trained panel (see **Table 2** and **3**). The selection of these set of pears reflected those that were tested by the consumers. The **summer varieties** presented highly significant ($p<0.0001$) differences on the means of all the physicochemical measurements conducted (**Table 2).** For weight, the values ranged between 126.0-310.9g. 720 presented the highest weight of the six varieties, and Seckel the lowest. The other four varieties presented more similar weights that ranged between 186.1-230.6g. Hunter et al. (2009) reported mean fruit weights of approximate 231g for 720 and 135g for Bartlett. In our study the weights for these two varieties were higher.

Firmness was the measurement that presented more differences among the six varieties. Typically, the firmness of pears is between 6-7kg when harvested, and between 2-3kg or less when ready for consumption (S. Musacchi, personal communication). Based on this fact all summer varieties had an optimum for consumption except for 720 (5.6kg) and 642 (5.3kg). Bartlett presented the lowest firmness (0.8kg). Seckel and 573 had very similar firmness. The soluble solids content (SSC), ranged between 12.3 and 16.3°Brix. There were not significant differences on the SSC of most of the tested varieties, specifically Bartlett, 573, 720 and 417. The most distinct pear was Seckel that presented the highest content of SSC.

The results of the physicochemical characterization of the winter varieties are shown in **Table 3**. The mean weight of the six selected varieties ranged from 186.5 to 262.4g. Concorde presented the highest mean weight (262.4g) and was significantly different from the mean weight of Paragon (186.5g), Comice (196.5g) and Green Anjou (204.8g). The firmness means of the six varieties were among the range consider optimum for consumption. The two varieties with the highest firmness were Gem (not ripened) (3.3kg) and Green Anjou (3.1kg). The firmness of the other four varieties was around 1kg or less. Vaysse et al. (2005) reported a firmness of 1.1kg for Comice before consumption. Jaeger et al. (2003) reported a firmness of 0.6 kg for ripe Comice and 1.7kg for ripe Bosc. The findings of these two studies align with the results we obtained for these two varieties. The mean SSC ranged from 10.2 to 18.6°Brix. Bosc presented the lowest SSC (10.2°Brix). Jaeger et al. (2003) reported an SSC of 12.3 g for ripe Bosc. The variety with the highest content of soluble solids was Paragon (18.6°Brix).

Trained Panel Descriptive Analysis

A total of 10 (80% female) panelists within an age range of 24-60 years old and with previous experience in conducting descriptive analysis underwent a training period of 15 hours. The training

was divided in 10 sessions of 1.5h each within a period of 3 weeks and one day. Over a series of training sessions, the panelists were familiarized with the sensory characteristics, terms and reference standards that have been previously used for the sensory profiling of pears for pears (Jaeger et al., 2003). The final list of attributes comprised 18 attributes, of which eight were related to aroma/ flavor, three to taste, one to mouthfeel and six to texture.

Summer Pears: The PCA of the significant attributes ($p<0.05$), explained 65.40% of the variation among the summer pears, with 49.86% and 15.54% explained by PC1 and PC2, respectively (**Figure 1**). PC1 was defined by the positively loaded attributes *pear flavor, pear aroma*, *grassy/green aroma* and *flavor*, *floral aroma* and *flavor*, and *sour* in contrast to the negatively loaded attributes of *fruity flavor*, *apple aroma* and *flavor,* and *astringent*. PC2 was associated with the contrasting relationship of *apple flavor,* and *fruity flavor* with *vanilla flavor*, *stemmy/woody aroma* and *juicy*. Pear varieties such as 573, Sylvania had higher associations with positively loaded attributes on PC1 while varieties like 720 and 391 had higher association with negatively loaded attributes.

Winter Pears*:* The PCA of the significant attributes ($p<0.05$), explained 69.62% of the variation among the winter pears, with 50.52.86% and 19.10% explained by PC1 and PC2, respectively (**Figure 2**). PC1 was defined by the positively loaded attributes *pear flavor* and *aroma*, *juicy, sweet*, f*ruity aroma* and *flavor*, and *vanilla aroma* and fl*avor* in contrast to the negatively loaded attributes of *grassy/green flavor*, *apple flavor, sour* and *astringent*. PC2 was associated with the contrasting relationship of *grassy/ green aroma and flavor* with *bitter*, *stemmy/woody flavor* and *other flavor*. Pear varieties such as Comice, Paragon and Concorde had higher associations with positively loaded attributes on PC1 while varieties like Green Anjou and Gem (not ripened) had higher association with negatively loaded attributes.

Consumer Sensory Evaluation

Two large-scale consumer sensory evaluation tests were conducted at the OSU FIC in Portland, OR. Consumer sensory evaluations were conducted to assess the quality of 12 pear varieties (six summer varieties and 6 winter varieties) to understand the effect of appearance, flavor and texture on consumer acceptability, willingness to pay and purchase intent.

Over 100 consumers were used for each sensory study (ie. Summer pears tested in October and winter pears tested in December). Consumers were recruited from the Portland Metro Area through the OSU FIC database and pre-screened for pear purchase behavior, consumption habits and demographics. Consumers who participated were given a $40 incentive to participate in the one-hour sensory test. Pears for the sensory evaluations were sliced just prior to each session. Sensory data were collected with Compusense® software. Consumer sensory evaluation of appearance, aroma, color, flavor, texture, firmness, juiciness, crunchiness, sweetness, tartness, and aftertaste was conducted utilizing 9-point hedonic scale ratings, just about right (JAR) scales, open ended questions, and willingness to pay. Sensory ratings for each attribute were analyzed using analysis of variance.

Summer pears: Consumers rated the overall appearance of pears 417 (small squat pear shape with orangish red color), 573 (mid-sized yellow green pear with some blush), Bartlett and 720 (larger blocky shaped yellowish green pear with occasional red blush) significantly higher ($p=0.00$) than the Seckel and 642 (a small, apple shaped Asian European cross that is yellow with a red spotty blush) varieties **(Table 4)**. The color of the skin of 417 (orangish red color) was more liked than 642 (yellow with a red, spotty blush) or Seckel, which were less well liked for skin color.

For the overall liking, results showed Bartlett, 573 and Seckel were liked significantly more ($p=0.00$) than pears 642, 417 and 720. The Bartlett also received the highest mean score for pear flavor liking (7.62) and was liked significantly more in this attribute than all other summer pears tested except for

the Seckel (7.02) (p=0.00). The Bartlett and Seckel varieties were rated by the most consumers as just about right for pear flavor (76% and 64% respectively). When regarding sweetness preferences, the Bartlett and Seckel were the two varieties in this study among the summer pear varieties that most exemplified the sweetness consumers prefer in a pear. These pears scored highest in sweetness liking (7.55 and 6.93 respectively) and were rated just about right in sweetness by the most consumers (80% and 73% respectively).

The Bartlett was liked significantly more (p=0.00) than all other summer pears tested for juiciness (7.75) and was rated by 86% of all consumers tested as just about right in juiciness, whereas the next highest rated pear in this attribute was the Seckel at 68%. Very few consumers rated any of the pears as having too much pear flavor, sweetness or juiciness as these were highly desirable attributes and were qualities linked to varieties with the highest overall liking such as the Bartlett (7.33) and Seckel (6.75). Pear 417, which was described by many consumers in open ended comments as "bland" or "lacking flavor," was rated by 90% of consumers as having too little pear flavor. It was also rated by 73% as not sweet enough and 52% as not tart enough. Pear 417 was rated significantly lower than the highest rated pears (Bartlett, 573 and Seckel) in overall liking, even though it had the highest mean score in overall appearance liking (7.45) and skin color liking (7.68). The pears with the lowest overall liking scores (720, 642 and 417) had the fewest number of consumers rating just about right for pear flavor and sweetness and the most consumers rating the pears as too firm, too crisp/crunchy and too dry/mealy. The three varieties that were rated as just about right in firmness by the most consumers were the Bartlett (66%), 573 (64%) and Seckel (59%).

Winter pears: The appearance of the Gem (not ripened) (7.71), Concorde (7.27), Green Anjou (7.21) and Comice (7.13) were liked significantly (p=0.00) more than the Paragon (Comice x Bartlett cross, smaller pear, thin skin ripens from green to yellow, slightly misshapen) and Bosc (**Table 5**). The skin color of the Gem (not ripened) (7.90), with its light green color that turns yellow when ripe and up to 35% red blush was liked significantly (p=0.00) more than all the other pears tested except the Comice (7.46). The aroma of the Concorde (6.73) and Comice (6.66), both known to be highly aromatic were liked significantly (p=0.00) more than all other pears tested except the Paragon (6.53) and Green Anjou (6.13).

The Paragon had the highest mean score for overall liking (7.46) and was rated between like moderately and like very much on the 9-point hedonic scale. The Green Anjou (6.99), Concorde (6.98) and Comice (6.80) were rated statistically similarly (p=0.00) to the Paragon in overall liking, while the not ripened Gem (not ripened) (6.24) and Bosc (5.86) were significantly (p=0.00) lower in this attribute. The same pears that were rated highest in overall liking also were rated highest in pear flavor, with Paragon at the top (7.54), followed by Comice (7.13), Green Anjou (6.97) and Concorde (6.96). These four pears were liked significantly (p=0.00) more in pear flavor than the Bosc (5.92) and not ripened Gem (5.86). The Paragon was rated by 73% of consumers as just about right in flavor (Figure X). The liking responses for sweetness also showed preferences for the same four varieties, Paragon, Green Anjou, Concorde and Comice (Table X). These varieties were rated by most consumers as just about right in sweetness, where the Bosc and not ripened Gem (not ripened) were both rated by over 50% of consumers as not sweet enough. The Bosc was rated statistically (p=0.00) lower in tartness/acidity liking than the other five winter pear varieties with a mean score near neither like nor dislike on the 9-point hedonic scale (5.28).

In overall texture liking, the Concorde (7.29) and Paragon (7.10) were scored significantly (p=0.00) higher than the other varieties tested except the Green Anjou (6.90). The firmness liking of these three varieties were rated significantly (p=0.00) higher than the Comice and not ripened Gem. The Gem (not ripened) was rated by 54% of consumers as too firm, while the Comice was rated by 49% of consumers as too soft. The Paragon (7.60), Concorde (7.56), Green Anjou (7.41) and Comice

(7.16) were all well liked in juiciness with mean scores at or above like moderately; over 80% of consumers rated these four varieties as just about right in juiciness. The not ripened Gem and Bosc were rated significantly (p=0.00) lower in juiciness liking; 44% rated the Gem as too dry/mealy, while 45% rated it as too crisp/crunchy. The skin texture of the Paragon and Gem (not ripened) were rated by over 75% of consumers as just about right, whereas the skin texture of the Comice was rated by 47% as too thick/tough.

Preference mapping (Descriptive analysis + Consumer Sensory Evaluation)
Summer (S) pears: As shown in **Figure 3**, five clusters were identified based on the consumers' (n=107) liking of the six summer varieties, 71.8% of the variance within consumers accounted for. Bartlett and Seckel were the most preferred varieties and 80% of the consumers were satisfied or liked these two varieties the most. Bartlett is one of the major cultivars grown in North America (Westwood, 1993), so this result is unsurprising given that consumers are likely to be very familiarized with this variety and have a particular preference for it.

Consumers in **Cluster S1** (n= 33) liked Seckel the most. Seckel pear was mostly characterized for its juicy texture. The second variety most liked by the consumers in this cluster was Bartlett, followed by 417. Consumers in Cluster 1 (73% women) were characterized as having consumed mostly Bartlett pears in the last year. Participants in **Clusters S2** (n=29) and **S4** (n=27) liked Bartlett the most. Bartlett pear was characterized with positive attributes such as *pear aroma, grassy/ green aroma, pear flavor, sweet* taste, and *juicy* texture. Consumers in **Cluster S2** (69% women) responded that Bartlett was the type of pear that they had eaten the most in the last year. Consumers in **Cluster S4** (63% women) consumed Bartlett the most in the last year. These consumers also indicated that their favorite pear variety is Bartlett, mostly because of its *sweetness, juiciness,* and *texture (crispness).*

Consumers in **Cluster S3** (n=8) presented the highest preference for the red pear varieties 642 and 417 and the lowest liking for Bartlett. The red pears, which were liked by 20% of the consumers overall, were characterized by attributes such as *stemmy/woody aroma, fermented aroma, stemmy/woody flavor, fermented flavor, bitter taste, astringent,* and *grainy/gritty texture.* This finding might be an indicator that there is a potential niche group that prefers the red varieties. These pears have a very different sensory profile compared to the profiles of more traditional and well-known varieties such as Bartlett. In the last year, 80% of the consumers in cluster 3 (100% women) consumed Asian pears. They indicated this variety as their favorite because of the *texture* (e.g., *crunchy, crispness*), *juiciness* and *flavor* (e.g., *apple flavor*).

Consumers in **Cluster S5** formed also a small cluster (n=10) of 50% women. They mostly consumed Bosc, followed by Bartlett in the last year. This cluster presented a profile of consumers open and willing to explore new varieties of pear. 573 was not presented as one of the possible options to select. However, 573 was preferred by 60% of the consumers in the study and was the most liked variety for Cluster 5. This pear was mainly characterized by attributes such as *floral aroma*, *green/grassy flavor*, *floral flavor* and *sour taste.* This pear has been recently released in North America and has been described as *firm* with *sweet, juicy flavor* and rosy, yellow-green skin (Vineland Research and Innovation Centre, 2022). 30 % of these consumers expressed having *tried some of the newer varieties and liked them too,* or they made comments such as: *no particular favorite; I like the unique differences,* and *I like ripe pears that have a complex sweetness, some tartness and juicy.* These comments may be indicative of these consumers being more willing or open to try or appreciate newer varieties such as 573.

***Winter(W) pears*:** Four clusters were identified based on the consumers' (n=112) liking of the six winter pear varieties. 81.4% of the variance within consumers preferences was accounted for (see **Figure 4**). Comice and Paragon were the varieties most liked by the consumers; 75% were satisfied

with the sensory profile of these pears. Both varieties were characterized by attributes such as *pear aroma, fruity aroma, pear flavor, fruity flavor, sweet taste* and *juicy texture.* Consumers in **Cluster W2** (n=12), **Cluster W3** (n=45), and **Cluster W4** (n=25) expressed the highest preference for Comice and the lowest for Bosc (Cluster W2 and W3) and Green Anjou (Cluster W4).

Fifty percent of the consumers (n=112) were satisfied with Green Anjou, Gem (not ripened) and Concorde. The Green Anjou sensory profile was characterized with the following attributes: *grassy/green flavor, apple flavor, sour taste* and *astringent.* Gem (not ripened) was mostly characterized with texture-related attributes such as *crispy*, *crunchy, skin toughness* and *firm*. Concorde was profiled as having a *vanilla aroma*, *vanilla flavor* and *bitter taste.*

Consumers in **Cluster W1** (n=29) liked Bosc the most. Overall, this variety satisfied the liking/preference of 25% of the total consumers. Bosc was mainly described as having a *stemmy/woody flavor*. When asked about the pear varieties consumed in the past year, 79% of consumers in Cluster W1 (41% women) indicated Bosc (79%) as their most consumed variety, followed by Bartlett (76%) and Asian pears (65%). For consumers in **Cluster W2** (n=12) (58% women), Comice was the most liked and Green Anjou was the second most liked variety. Green Anjou was described with attributes such as *apple flavor*, *grassy/green flavor*, *sour taste*, and *crispy texture.* Consumers in this cluster expressed that their most frequently consumed pears in the last year were Bartlett (100%), Bosc (75%), and Comice (67%). The favorite varieties for these consumers were Bartlett (42%) and Asian pears (25%).

Based on the preference mapping results, consumers in **Cluster W3** (n=45) (62% women) preferred Comice the most, followed by Paragon and Green Anjou. Comice and Paragon were mostly described by *pear aroma*, *pear flavor* and *sweet taste.* When characterizing the consumers in Cluster 3, the pear varieties most commonly consumed in the last year were Bartlett (89%) and Green Anjou (71%), followed by Red Anjou (62%) and Asian pears (62%). **Cluster W4** (n=25) (56% women) gathered consumers who had also had the highest preference for Comice. Clusters 3 and 4 shared some characteristics. As in Cluster 3, Paragon was also the second most liked variety followed by Concorde. When asked about the most consumed pears in the last years, the most commonly mentioned varieties in Cluster 4 were Bartlett (96%), Green Anjou (68%), Bosc (68%), and Asian Pear (68%). Comice was consumed by 36% of the consumers in this cluster and Concorde by 24%. The favorite pear for the consumers in Cluster 4 was Bartlett (44%) because of its *size, color, taste overall, perfect pear flavor, right amount of sweetness, juiciness,* and *classic pear [type]*.

Willingness to Pay

A questionnaire tool to estimate the willingness to pay was developed. The tool included questions to elicit the willingness to pay following the contingent valuation methodology. Grocery store prices for fresh pears in the Portland area were collected and used in the questionnaire. The bids for different pear samples allowed us to estimate the WTP for each pear sample, and the marginal value of the salient pear quality characteristics. The average respondent, based on the self-reported sociodemographic responses, was on average of White ethnicity, female, older than 35, college educated, self-reported healthy, had a household with at least two members, the household had one child less 18 years old, and the household income was greater than $60,000/year.

Summer Pears: Results are presented in **Table 6** and **7** The highest WTP was for Bartlett. This variety had the highest overall liking for flavor with 7.33. The second highest WTP was for Happy with 1.92, this variety had the second highest overall liking for flavor with 6.76. The third highest WTP was for Seckel with 1.89, and this variety had the third highest overall liking for flavor with 6.75. When estimating the pairwise statistically significant differences, the WTP for Bartlett was higher compared to each of the other varieties. There were significant differences Happy and the

other varieties (642, 417, and 720). There were statistically significant differences between Seckel and 642, 417 and 720. This result indicates the presence of three tiers of varieties, in terms of WTP, first Bartlett, followed by the second tier 573 and Seckel, and the third tier 642, 417, and 720.

***Winter Pears*:** Results are presented in **Table 8** and **9**. The highest WTP value was for Paragon at $2.19/lb. This variety also had the highest mean liking score for overall flavor at 7.46 (on a 1-9 scale, 1=dislike extremely, 9=like extremely; hereafter, all liking scores will consider this scale), despite a lower appearance liking score (6.62). The second highest WTP is for Concorde with $2.09/lb, with a mean liking of overall flavor at 6.98 and overall appearance at 7.27. The third highest mean WTP was for Green Anjou with $2.05/lb, with a mean liking of the overall flavor of 6.99 and an overall appearance of 7.21. Importantly when estimating the statistically significant differences across WTP values, we note that there are no differences between Paragon and Concorde but were between Paragon and Green Anjou. These results show that there are two tiers of Winter pears in terms of the WTP, the first one composed by Paragon, Concorde, and Green Anjou and the second one composed by Comice, Gem (not ripened), and Bosc.

Tables and Figures

Table 1. Pear varieties, harvest season and inclusion into the consumer evaluations.

Pear Variety	**Season**	**Consumer Trials**
Bartlett	Summer	**x**
720- Cross between Bartlett and a numbered U.S. selection, large fruit, yellowish-green skin with red blush, white flesh, good storage variety, https://www.ontario.ca/page/pear-production-ontario	Summer	**x**
573- Bartlett heritage, yellow green pear with some blush, denser texture, sweet, juicy	Summer	**x**
804- Early season pear, mild sweet flavor, red blush over smooth yellow skin with grit-free white flesh, slightly firm, https://www.ontario.ca/page/pear-production-ontario	Summer	
391- Related to Bartlett, late season pear with small to medium sized fruit, yellow with a red blush, sweet and juicy, https://www.ontario.ca/page/pear-production-ontario	Summer	
417- Asian/European hybrid, bright red color, crisp, juicy, low acid pear, slightly sweet flavor	Summer	**x**
642- Red-skinned/blush, yellow fleshed Asian pear that is apple-like in taste, juicy and crisp texture	Summer	**x**
Seckel	Summer	**x**
Starkrimson	Summer	
Summer Blood Birne	Summer	
Sylvania	Summer	
Pear Variety	**Season**	**Consumer Trials**
Abate Fetel	Winter	
Bosc	Winter	**x**
Comice	Winter	**x**
Concorde	Winter	**x**
Forelle	Winter	
Gem, not ripened (nr)	Winter	**x**
Green Anjou	Winter	**x**
Packham's Triumph	Winter	

Paragon	Winter	**x**
Red Anjou	Winter	
OHUS-US783012-022	Winter	
US79453-007	Winter	

Table 2. Trained panel physicochemical measurements, **summer pear varieties** (n=10).

Variety	**Weight (g)**	**Firmness (kg)**	**Soluble solids (°Brix)**
Bartlett	198.5±11.0 ab	0.8±0.1 a	12.4±1.2 a
573	221.0±11.2 ab	2.4±0.3 b	12.3±1.9 a
720	310.9± 27.1 c	5.6±0.6 c	13.0±0.4 ab
417	186.1±28.8 b	3.5±0.4 d	12.3±0.6 a
642	230.6±25.7 a	5.3±0.4 c	15.0±0.9 bc
Seckel	126.0±5.1 d	2.1±0.3 b	16.3±1.2 c
p-value	**<0.0001**	**<0.0001**	**<0.0001**

Table 3. Trained panel physicochemical measurements, **winter pear varieties** (n=10).

Variety	**Weight (g)**	**Firmness (kg)**	**Soluble solids (°Brix)**
Bosc	224.8±13.6 abc	1.1±0.1 a	10.2±0.5 a
Comice	196.5±8.2 bc	0.7±0.2 a	16.1±1.1 b
Concorde	262.4±37.3 a	1.5±0.3 a	14.2±0.5 c
Gem (nr)	234.2±21.0 ab	3.3±0.9 b	13.3±0.4 cd
Green Anjou	204.8±15.8 bc	3.1±1.0 b	12.8±0.9 d
Paragon	186.5±20.6 c	0.89±0.1 a	18.6±2.8 e
p-value	**<0.0001**	**<0.0001**	**<0.0001**

Table 4. Consumer liking responses for varietals tested in October (summer pears), n=107

Summer Pears	**Overall Liking**	**Appearance Liking**	**Color of Skin**	**Aroma**	**Pear Flavor**	**Sweetness**	**Tartness/ Acidity**
Bartlett	7.33 a	7.32 a	7.12 ab	6.86 a	7.62 a	7.55 a	6.58 a
573	6.76 a	7.33 a	7.31 ab	6.99 a	6.81 b	6.75 b	6.30 ab
Seckel	6.75 a	6.26 b	6.46 b	5.06 c	7.02 ab	6.93 ab	5.93 b
642	5.45 b	6.11 b	6.83 bc	5.18 c	5.14 c	5.70 c	5.28 c
417	5.32 b	7.45 a	7.68 a	6.71 a	4.79 c	4.92 d	4.74 c
720	5.13 b	6.94 a	7.12 ab	6.03 b	5.24 c	4.93 d	5.28 c
HSD value	0.68	0.61	0.56	0.54	0.66	0.66	0.62
p-value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Summer Pears	**Overall Texture**	**Firmness**	**Juiciness**	**Crispiness/ Crunchiness**	**Aftertaste**
Bartlett	6.85 a	6.44 a	7.75 a	6.01 a	6.89 a
573	6.49 ab	6.70 a	6.13 b	6.26 a	6.28 a
Seckel	6.30 ab	6.59 a	6.74 b	6.03 a	6.40 a
642	5.87 b	6.17 a	6.74 b	6.39 a	5.31 b
417	5.79 b	6.09 a	6.23 b	6.24 a	5.03 b

720	4.64 c	4.70 b	4.07 c	4.93 b	5.09 b
HSD value	0.80	0.80	0.66	0.81	0.66
p-value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Table 5. Consumer liking responses for varietals tested in December (winter pears), n=112

Winter Pears	**Overall Liking**	**Appearance Liking**	**Color of Skin**	**Aroma**	**Pear Flavor**	**Sweetness**	**Tartness/ Acidity**
Paragon	7.46 a	6.62 bc	6.69 c	6.53 ab	7.54 a	7.35 a	6.46 a
Green Anjou	6.99 a	7.21 ab	7.06 bc	6.13 ab	6.97 a	6.94 a	6.28 a
Concorde	6.98 a	7.27 a	7.17 bc	6.73 a	6.96 a	6.91 a	6.06 a
Comice	6.80 ab	7.13 ab	7.46 ab	6.66 a	7.13 a	6.88 a	6.16 a
Gem (nr)	6.24 bc	7.71 a	7.90 a	6.05 bc	5.86 b	5.99 b	6.06 a
Bosc	5.86 c	6.22 c	5.98 d	5.46 c	5.92 b	5.74 b	5.28 b
HSD value	0.70	0.64	0.64	0.60	0.71	0.71	0.68
p-value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Winter Pears	**Overall Texture**	**Firmness**	**Juiciness**	**Crispiness/ Crunchiness**	**Aftertaste**
Paragon	7.10 a	7.04 a	7.60 a	6.38 ab	6.65 a
Green Anjou	6.90 ab	6.96 ab	7.41 a	6.29 abc	6.77 a
Concorde	7.29 a	7.16 a	7.56 a	6.89 a	6.47 a
Comice	6.25 bc	6.19 c	7.16 a	5.65 c	6.24 ab
Gem (nr)	6.20 bc	6.30 bc	6.08 b	6.47 ab	6.28 a
Bosc	6.13 c	6.53 abc	6.51 b	6.04 bc	5.52 b
HSD value	0.73	0.71	0.62	0.73	0.74
p-value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Table 6. Willingness to pay (WTP) mean, WTP pairwise comparison between summer pear varieties.

Varieties	WTP mean	Standard error	WTP-Pairwise comparison between varieties		
			Varieties		t-value
Bartlett	2.10	0.066	Bartlett	573	2.83**
573	1.92	0.067	Bartlett	Seckel	3.28***
Seckel	1.89	0.066	Bartlett	642	6.84***
642	1.62	0.072	Bartlett	417	7.64***
417	1.55	0.075	Bartlett	720	7.80***
720	1.54	0.075	573	Seckel	0.43
			573	642	4.19***
			573	417	5.08***
			573	720	5.25***
			Seckel	642	3.81***
			Seckel	417	4.72***
			Seckel	720	4.88***
			642	417	0.97

			642	720	1.13
			417	720	0.15
Single, double, and triple asterisks (*, **, ***) indicate statistical significance at the 10%, 5%, and 1% levels					

Table 7. Overall appearance and overall flavor rating score and pairwise comparison between summer pear varieties

Varieties	Rating score		WTP-Pairwise comparison between varieties		
	Mean (Std. dev)		Varieties	t-value	
	Overall appearance	Overall flavor		Overall appearance	Overall flavor
Bartlett	7.32	7.33	Bartlett-573	-0.05	2.52**
	(1.25)	(1.64)	Bartlett-Seckel	4.41***	2.55**
573	7.33	6.76	Bartlett-642	5.14***	7.16***
	(1.34)	(1.67)	Bartlett-417	-0.67	8.03***
Seckel	6.26	6.75	Bartlett-720	1.875*	8.39***
	(2.14)	(1.68)	573-Seckel	4.37***	0.04
642	6.11	5.45	573-642	5.08***	4.96***
	(2.08)	(2.16)	573-417	-0.60	5.72***
417	7.45	5.32	573-720	1.87*	6.18***
	(1.60)	(2.00)	Seckel-642	0.52	4.91***
720	6.94	5.13	Seckel-417	-4.60***	-5.66***
	(1.64)	(2.16)	Seckel-720	-2.62***	6.12***
			642-720	-3.25***	1.08
			642-417	-5.27***	0.46
			417-720	2.28**	0.66
Single, double, and triple asterisks (*, **, ***) indicate significance at 10%, 5%, and 1% levels					

Table 8. Willingness to pay (WTP) mean, WTP pairwise comparison between winter pear varieties.

Varieties	WTP mean	Standard error	WTP-Pairwise comparison between varieties		
			Varieties		t-value
Paragon	2.19	0.067	Paragon	Concorde	1.46
Concorde	2.09	0.067	Paragon	Green Anjou	2.07**
Green Anjou	2.05	0.067	Paragon	Comice	3.55***
Comice	1.96	0.066	Paragon	Gem (nr)	5.58***
Gem (nr)	1.81	0.067	Paragon	Bosc	7.14***
Bosc	1.69	0.070	Concorde	Green Anjou	0.6
			Concorde	Comice	2.05**
			Concorde	Gem (nr)	4.11***
			Concorde	Bosc	5.71***
			Green Anjou	Comice	1.45
			Green Anjou	Gem (nr)	3.54***

			Green Anjou	Bosc	5.16***
			Comice	Gem (nr)	2.14**
			Comice	Bosc	3.83***
			Gem (nr)	Bosc	1.72*
Single, double, and triple asterisks (*, **, ***) indicate statistical significance at the 10%, 5%, and 1% levels					

Table 9. Winter pear varieties-Overall appearance and overall flavor rating score and pairwise comparison between varieties.

Varieties	Rating score		Rating score-Pairwise comparison between varieties		
	Mean (Std. dev)		Varieties	t-value	
	Overall appearance	Overall flavor		Overall appearance	Overall flavor
Paragon	6.62	7.46	Paragon-Green Anjou	-2.72***	2.33***
	(1.70)	(1.42)	Paragon-Concorde	-3.07***	2.13***
Green Anjou	7.21	6.99	Paragon-Comice	-2.23**	2.94***
	(1.59)	(1.61)	Paragon-Gem	-5.38***	5.36***
Concorde	7.27	6.98	Paragon-Bosc	1.51	6.80***
	(1.47)	(1.93)	Green Anjou-Concorde	-0.26	0.04
Comice	7.13	6.80	Green Anjou-Comice	0.41	0.80**
	(1.71)	(1.91)	Green Anjou-Gem	-2.53**	3.14***
Gem (nr)	7.71	6.24	Green Anjou-Bosc	3.91***	4.60***
	(1.31)	(1.96)	Concorde-Comice	0.67	0.70**
Bosc	6.22	5.86	Concorde-Gem	-2.35**	2.86***
	(2.17)	(2.06)	Concorde-Bosc	4.22***	4.22***
			Comice-Gem	-2.86***	2.18***
			Bosc-Comice	-3.46***	-3.57***
			Gem-Bosc	6.20***	1.43***
Single, double, and triple asterisks (*, **, ***) indicate significance at the 10%, 5%, and 1% levels					

Figures

Figures 1 & 2. PCA of all significant attributes of the **summer and winter season pears** as determined by the trained panel (n=10). The aroma and flavor-related attributes are presented in green. The aroma-related attributes are represented as -A and the flavor-related attributes are represented as -F. The basic taste attributes are presented in yellow, and the texture-related attributes are presented in red. Pears varieties highlighted in yellow were evaluated by consumers at OSU FIC.

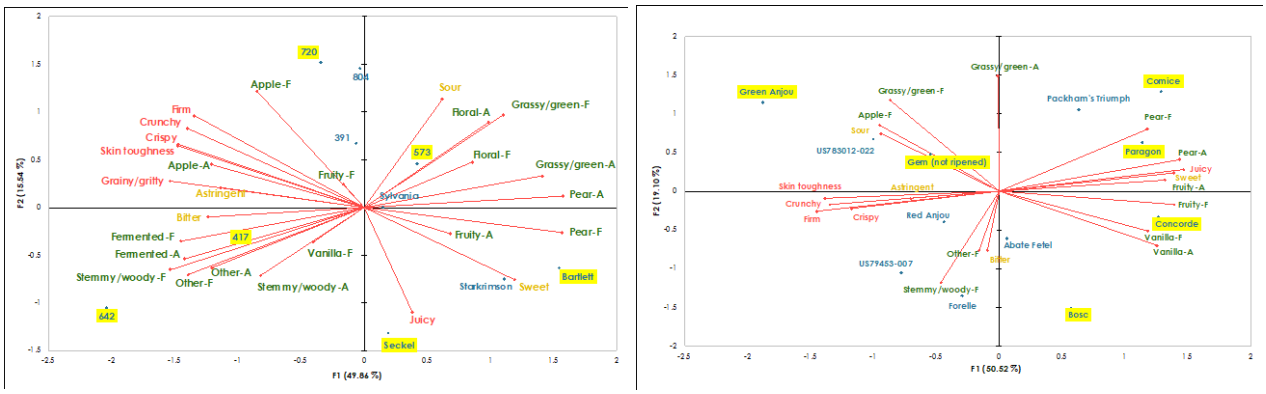


Figure 3. Preference map of sensory profiling data for six **summer pear varieties** explaining 71.8% of the total variance overlaid by consumer (n=107) liking data via a Vector model. Percentages represent regions whereby the given percentage of consumers have a preference above the mean.

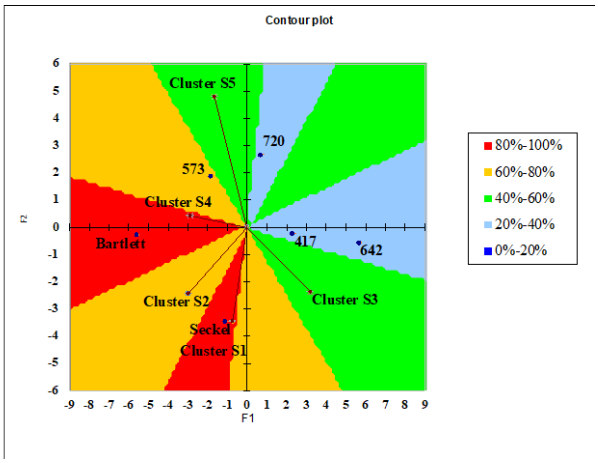
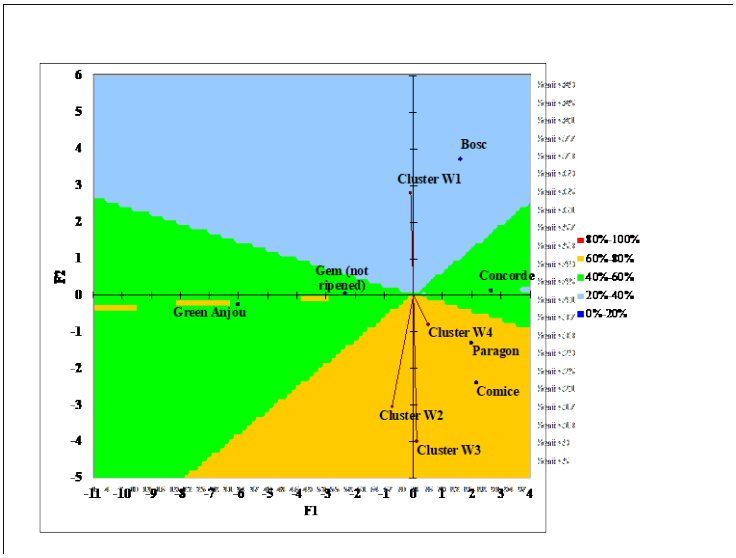


Figure 4. Preference map of sensory profiling data for six **winter pear varieties** explaining 81.4% of the total variance overlaid by consumer (n=112) liking data via a Vector model. Percentages represent regions whereby the given percentage of consumers have a preference above the mean.



Executive summary

Project title: Pear Consumer Preference Testing

Keywords: pears, consumer acceptance, purchasing, preference map, descriptive analysis

Abstract:

The objective of this study was to better understand pear consumers in the Pacific Northwest region of the United States, specifically the sensory attributes that they desired in a pear. To accomplish this, descriptive analysis (DA), consumer acceptance data and preference mapping were combined to determine the sensory profile of pear varieties from the summer and winter season. The willingness to pay (WTP) of the pears evaluated by consumers was calculated using a contingent valuation approach. A trained sensory panel (n=10) evaluated multiple sensory attributes (appearance, aroma/ flavor, taste, mouthfeel and texture) of 23 pear varieties grown in the PNW. A selection of twelve pears, six from summer and six from winter season, were evaluated by consumers (n=219) for their liking of different attributes of the pears. Results showed that the trained panelists significantly discriminated the summer and winter pears on most of the sensory modalities. To identify the attributes driving consumer acceptability, external preference mapping was applied. Attributes such as pear aroma, pear flavor, sweet, sour and juicy were identified as most contributing attributes to the liking of the summer pears. Conversely, *fermented aroma, stemmy-woody aroma*, *fermented flavor, stemmy-woody flavor, grainy-gritty* attributes were associated with a reduction in consumer liking. Based on preferences for specific sensory attributes, different clusters of consumers were identified. For the summer varieties, 573, Bartlett and Seckel were identified as having the broadest appeal, satisfying between 60% and 80% of the consumers. For the winter varieties, 75% of the consumers identified Comice and Paragon as the most appealing. Pear consumers (n=107) rated the overall flavor liking of the summer pears Bartlett, 573 and Seckel significantly higher ($p<0.5$) than 642, 417 and 720. For the winter varieties, consumers (n=112) rated the overall flavor liking of Paragon, Green Anjou, Concorde, and Comice as significantly higher ($p<0.5$) than not ripened Gem and Bosc. For both sets of varieties, the WTP values were consistent with the overall flavor scores. Willingness to pay showed different tiers, for summer varieties, first Bartlett, followed by the second tier 573 and Seckel, and the third tier 642, 417, and 720. For winter varieties, there were two tiers, the first one composed of Paragon, Concorde, and Green Anjou and the second one composed by Comice, not ripened Gem, and Bosc.

The introduction of these cultivars should satisfy t14he largest group of consumers in the Pacific Northwest market. Attributes such as crispness, firmness, juiciness, flavor, aroma, tartness, sweetness, sugar/acid balance were rated by over 77% of both sets of panelists (n=219) as important or very important in their purchase intent. Second to eating quality were shelf life attributes (freshness, ripeness, and shelf life), followed by appearance attributes (attractive and uniform external color, pear size, free of defects, uniform shape). Taste and flavor appeal and health and nutrition were the highest ranked factors in influencing overall food choices and eating patterns. The most important resources to help increase consumer interest in eating pears at home were in-store sampling and tasting with a recipe. Individual farmers and universities were rated as the most trusted sources of information of those listed on how food is produced, while the media, food manufacturers and social media were the least trusted.

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

Project Title: Survey of pear packers on storage and handling of Anjou Pears

PI: Carolina Torres
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Cooperators: PNW packers

Budget: $15,975 Year 1: $15,975 Year 2: $0 Year 3: $0

Other funding sources

Cost-sharing: $24,360
Notes: Funds for 0.3 FTE (Co-PI) ($16,560/yr) and 0.05 FTE (P.I) from the Tree Fruit Endowment funds to WSU.

Budget 1
Organization Name: Washington State University Contract Administrator 1: Katy Roberts
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Contract Administrator 2 (TFREC): Shelli Tompkins
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Item	2021
Salaries	$8,640
Benefits	$835
Supplies	
Travel	$6,500
Miscellaneous	
Total	$15,975

Footnotes:
Salaries: Temporary personnel to assist in fruit evaluations.
Benefits: $835 are requested for benefits tied to the temporary personnel.
Travel: $6,500 for mileage and associated travel costs at a rate of $0.535/mi and adhering to all university policies for per diem associated with overnight travel.

OBJECTIVES

1. Obtain information about varied storage and handling practices of Anjou pears from multiple warehouses.
2. Correlate different storage and handling practices with fruit quality.

SIGNIFICANT FINDINGS

- Overall quality including appearance and texture was uniform and optimum in all sampled lots across warehouses.

Objective 1. Obtain information about varied storage and handling practices of Anjou pears from multiple warehouses.

Activities:

Five commercial pear packinghouses were surveyed selected from 3 distinct growing regions NCW (1), Mid-Columbia (3), and Yakima (1). Storage information requested included: Harvest date, receiving firmness, receiving defects, bin type, bin drenching chemical, storage unit (bin, boxes), packing date, storage type, storage temperature, gases levels in CA, storage duration, postharvest chemical treatments, packing defects

This information will be mapped to illustrate logistical differences between warehouses.

RESULTS

We received the completed survey from 4 warehouses. In each of them they individualized each sampled lot throughout the storage season. Flow charts of different packing procedures are shown in Fig. 1.

Only 1 out of 4 packers still use wood bins. None of the surveyed lots were treated with drenched chemicals. Hundred-percent of them were thermofogged with ethoxyquin and fungicide (pyrimethanil or fludioxonil). Three out of five packers stored in bins and boxes. Before shipping four out of 5 packers conditioned their fruit in a room using forced air or fans, one did not respond. None of of the packers released their O_2 and CO_2 concentration on their controlled atmosphere storage.

None of the surveyed packers used 1-MCP on their sampled lots. Nevertheless, some indicated that they could use it for late stored fruit for certain markets.

Regarding decision-making about postharvest storage and handling, packers can use firmness and orchard history. Quality control makes the storage decision.

Passive cooling less 7 days…

My understanding is that fruit are conditioned post-storage (e.g. warmed and treated with ethylene post-storage) only when requested, it's not a standard practice; but the packinghouses I spoke will do the conditioning in a room, not a trailer, which is good

All use ethoxyquin for scald control as an insurance policy; whether it's thermofogged or a line spray varies with handling practices and storage duration

Pre-size lines can sort fruit to remove major defects, damaged fruit, and learn exact size distributions going into storage (although not all fruit going into storage goes over the pre-size line, some goes into storage as field run); Commit-to-pack lines that store field run fruit prior to packing handle the fruit less – so both work for different reasons

Some years fruit finish can be an issue; I'm new enough it's hard for me to gauge but I believe this year there was more marking on some fruit than would be preferred due to untimely wind/storms, and psylla ended up being more of an issue than expected

Objective 2. Correlate different storage and handling practices with fruit quality.

Activities:
Anjou pears from 5 diferent warehouse and lots were collected and fruit quality recorded at sampling day 1, 7 and 14 days after at 68°F. Fruit maturity (weight, flesh firmness, soluble solids content (SSC) and chlorophyll degradation (DA meter-Sintelia, Italy; I_{AD} units), and visual color rating (green-yellow scale; 1-4) and defects were ealuated

RESULTS
In general, fruit had good eating quality since the first sampling period (throughout this period. Table 1 shows the averages for flesh firmness, soluble solids, chlorophyll degradation (I_{AD} index), and visual color assessment. The latest can also be observed in Figure 1's pictures from some of the lots sampled.

Table 1. Average quality parameters for Anjou pear fruit, from five different warehouses (A,B, C, D and, E) and lots, after 1, 7 and, 14 days at 68F. Color scale used for visual evaluation is showed in fig 1.

Table 1. Fruit maturity in d'Anjou pears from different commercial lots and packers sampled in October thru December 2021.

Warehouse	Lot	Weight (g)			Firmness (lb)			SSC (°Brix)			I_{AD} (0-2)			
		Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	
A (MC)	1517	179.9±6.0	179.7±7.8	174.8±5.3	5.4±0.6	2.1±0.4	0.9±0.3	17.4±1.6	16.8±1.3	16.7±1.1	1.5±0.2	1.5±0.2	0.8±0.3	2
	2134	181.6±5.6	181.7±5.0	174.9±5.1	5.1±0.7	1.6±0.5	0.5±0.1	13.9±1.0	13.5±1.3	13.8±1.2	1.6±0.1	1.4±0.1	0.7±0.2	2
B (MC)	49	234.4±9.9	236.0±7.3	220.2±8.6	4.2±0.5	0.7±0.1	0.4±0.0	14.3±0.9	14.1±0.8	14.6±0.9	N/A	1.0±0.2	0.4±0.2	2
	466	240.1±8.7	229.1±10.9	226.9±7.6	4.8±0.6	0.6±0.2	0.5±0.1	15.1±0.9	14.6±0.9	14.7±1.4	1.5±0.2	1.1±0.2	0.4±0.2	2
C (YV)	741	201.9±10.7	200.9±10.5	194.5±7.2	4.6±0.5	0.6±0.1	0.5±0.1	14.7±0.7	15.0±0.7	14.5±0.7	1.5±0.1	0.7±0.2	0.3±0.2	2
	5303	205.9±9.1	201.9±7.3	188.8±27.7	5.1±0.6	0.6±0.1	0.6±0.1	14.0±0.7	14.6±0.9	14.2±1.2	1.6±0.1	0.9±0.2	0.5±0.3	1
	5405	205.7±11.3	200.2±8.5	197.0±9.6	4.7±0.5	0.8±0.4	0.5±0.2	13.0±0.9	13.4±1.0	13.2±0.9	1.5±0.1	0.9±0.4	0.3±0.2	1
D (MC)	111	266.9±43.0	278.4±10.6	271.1±13.5	5.3±0.4	1.1±0.2	0.7±0.1	13.7±0.8	13.6±0.9	13.0±0.7	1.6±0.1	0.8±0.3	0.3±0.2	2
	653	279.6±17.9	286.2±17.0	274.5±16.3	4.9±0.8	1.2±0.3	0.7±0.1	13.6±0.8	13.8±0.6	13.2±0.8	1.3±0.3	1.0±0.3	0.4±0.2	2
	663	285.0±15.4	274.3±15.7	272.5±13.6	1.6±0.4	0.9±0.1	0.7±0.1	13.6±0.7	13.5±0.7	12.7±0.5	0.9±0.4	0.5±0.2	0.3±0.1	2
E (NCW)	7260	229.5±26.7	240.1±13.2	240.2±12.6	4.3±1.0	0.9±0.2	0.6±0.1	13.1±0.6	13.4±0.9	13.4±0.5	1.2±0.3	0.7±0.3	0.3±0.2	2
	7650	255.2±17.8	242.9±12.8	230.1±41.6	3.0±0.8	1.0±0.1	0.8±0.2	14.2±0.9	13.7±0.6	13.5±0.8	0.7±0.3	0.3±0.2	0.0±0.0	2
	7056	238.5±17.2	229.4±15.9	226.8±12.4	3.7±0.6	1.2±0.2	0.8±0.2	13.9±1.4	15.3±1.2	14.8±1.2	1.0±0.3	0.8±0.3	0.3±0.2	2

Table 2. Fruit maturity in d'Anjou pears from different commercial lots and packers sampled in February 2022.

Warehouse	Lot	Weight (g)			Firmness (lb)			SSC (°Brix)			I_{AD} (0-2)			
		Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	
A (MC)	8259	199.9±7.4	196.5±6.9	194.3±5.8	10.8±0.6	2.3±0.6	2.2±0.4	14.7±1.3	13.9±1.0	12.3±0.7	1.5±0.2	1.4±0.2	0.4±0.3	
	2134	199.9±7.9	198.4±7.3	199.0±12.2	11.4±1.2	1.9±0.5	1.9±0.7	14.6±0.7	14.4±1.1	13.9±1.3	1.5±0.2	1.0±0.3	0.3±0.2	
	1661	197.7±7.1	193.0±19.3	201.4±16.4	10.2±0.6	2.2±0.4	1.7±0.5	13.8±1.0	13.9±1.1	14.6±1.4	1.2±0.4	1.1±0.2	0.3±0.2	
B (MC)	8109	232.9±9.9	227.2±11.3	224.3±11.9	10.9±0.8	1.5±0.2	1.2±0.2	14.8±1.0	14.6±0.8	15.0±1.2	1.5±0.2	0.9±0.2	0.2±0.2	
	2134	242.1±10.0	238.0±13.1	231.6±10.8	4.4±0.3	4.4±0.3	1.6±0.4	13.2±0.5	13.5±0.9	13.9±1.0	1.6±0.1	1.2±0.2	0.3±0.2	
	221	242.0±12.8	238.4±10.5	240.7±11.6	9.7±0.9	2.2±0.4	1.6±0.4	13.5±0.6	14.2±1.2	13.7±0.9	1.6±0.1	0.8±0.2	0.3±0.2	
C (YV)	741	203.3±10.9	196.4±9.1	191.3±9.0	5.2±0.8	N/A	1.5±0.3	16.2±1.3	15.4±0.8	14.6±0.7	0.7±0.3	0.7±0.2	0.2±0.2	
	5101	201.1±7.5	199.0±10.0	192.2±6.7	6.1±1.4	N/A	1.0±0.3	14.4±0.9	15.2±0.7	13.2±1.0	0.7±0.3	0.8±0.2	0.1±0.2	
	852	199.0±10.1	193.5±7.8	192.7±10.7	4.9±0.8	N/A	1.0±0.2	14.4±1.1	14.4±0.7	14.1±0.8	1.0±0.5	0.9±0.1	0.1±0.1	

D (MC)	2020	176.0±7.3	177.3±4.2	176.5±7.1	5.4±0.4	3.2±0.8	1.2±0.2	13.9±0.8	12.9±0.9	13.6±0.9	1.7±0.1	1.3±0.3	0.1±0
	6611	253.5±25.2	276.4±21.3	282.6±19.9	5.6±0.2	2.6±0.3	1.5±0.2	15.6±1.0	15.4±0.9	15.6±1.0	1.7±0.1	1.1±0.4	0.3±0
	6610	277.9±23.5	250.9±12.4	253.4±11.1	5.6±0.2	2.1±0.4	1.3±0.2	13.2±1.0	13.1±0.9	12.7±0.8	1.6±0.3	1.4±0.2	0.2±0
	0059	197.2±8.2	198.3±6.6	N/A	12.7±0.7	2.5±0.7	1.2±0.3	12.4±0.5	12.8±0.9	13.5±1.0	1.6±0.2	0.8±0.4	0.2±0

Table 3. Fruit maturity in d'Anjou pears from different commercial lots and packers sampled in April thru May 2022.

Warehouse	Lot	Weight (g)			Firmness (lb)			SSC (°Brix)			I_{AD} (0-2)		
		Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day
A (MC)	2552	198.0±7.5	186.4±42.3	186.6±42.4	12.2±0.9	2.7±0.5	1.5±0.3	14.6±0.9	14.4±0.8	14.8±0.7	1.6±0.1	N/A	0.4±
	1110	198.0±7.7	196.3±8.5	187.4±42.5	12.6±0.7	2.3±0.3	1.7±0.5	14.1±0.8	14.9±1.0	14.2±0.8	1.7±0.1	N/A	0.3±
	2125	198.7±7.6	195.6±8.2	196.0±8.9	11.7±1.1	2.6±0.7	1.6±0.3	14.6±1.0	14.9±0.6	14.1±1.0	1.6±0.2	N/A	0.5±
B (MC)	49	237.2±7.5	236.9±10.1	237.2±10.3	7.1±1.1	1.7±0.4	1.6±0.4	13.8±0.7	13.2±0.8	12.7±2.4	1.4±0.2	0.6±0.2	0.3±
	30	239.4±13.1	236.3±10.0	236.2±8.8	8.3±0.8	1.8±0.2	1.9±0.6	14.4±0.6	14.8±0.6	13.9±0.5	1.6±0.3	1.2±0.3	0.5±
	369	235.9±10.0	235.1±10.0	231.8±10.2	7.5±0.7	1.6±0.3	1.5±0.4	20.0±28.5	13.2±0.5	13.3±0.6	1.4±0.2	0.7±0.2	0.3±
D (MC)	6610	225.8±7.3	212.5±48.7	228.0±8.5	12.2±0.9	2.1±0.7	1.8±0.4	13.2±0.7	13.2±0.5	13.1±0.8	1.7±0.1	1.1±0.2	0.5±
	6611	285.5±15.4	281.2±11.4	279.7±16.1	9.9±0.8	2.1±0.4	1.6±0.4	14.9±0.9	14.9±0.5	14.3±0.6	1.5±0.2	0.8±0.2	0.3±
	0168	196.7±4.9	197.6±6.1	196.2±6.1	12.2±0.7	2.5±0.5	1.3±0.2	14.4±1.0	15.0±0.9	14.3±0.7	1.7±0.1	1.3±0.2	0.5±

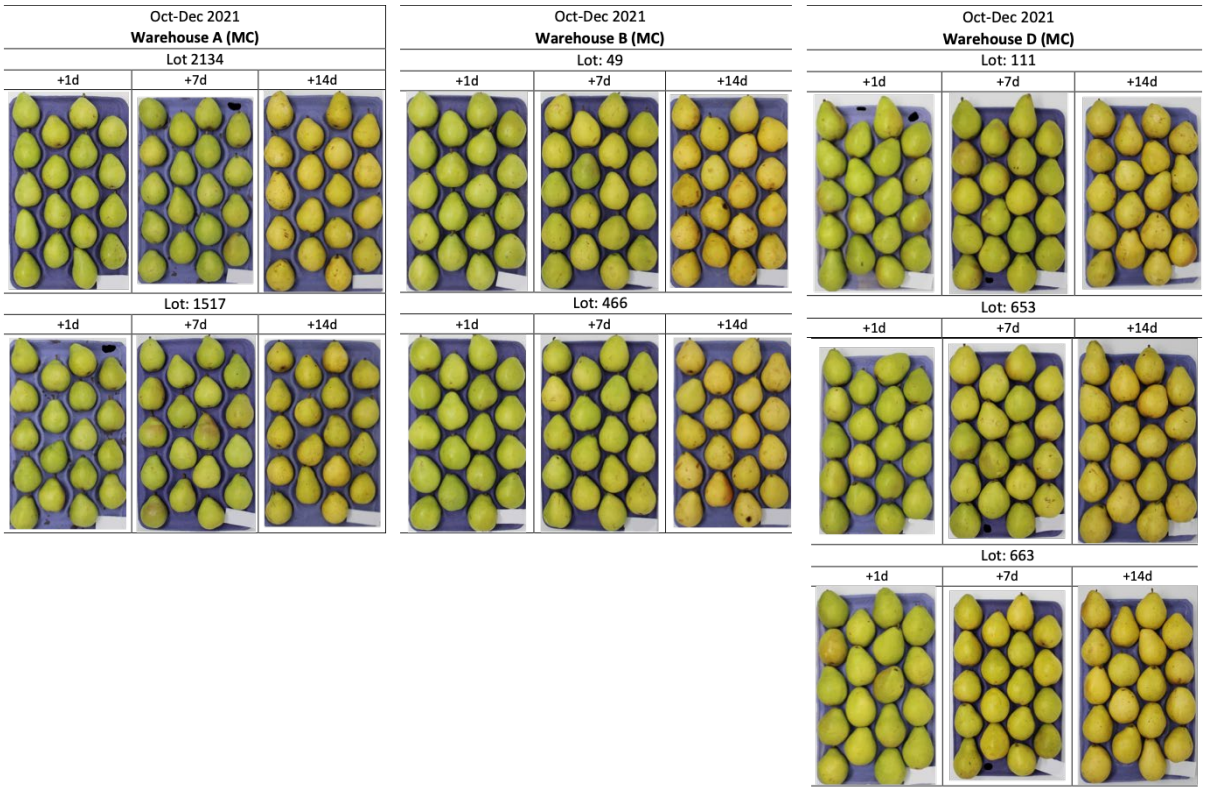

Fig 1. Anjou pear samples from five different warehouses (A, B, C, D and, E) after 1, 7 and 14 days at 68F. Color scale for visual evaluation is showed at the bottom.

Project Title: Germplasm evaluation for fruit quality and post-harvest traits

Report Type: Continuing Project Report.

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Cooperators: None

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 33,000
Total Project Request for Year 2 Funding: $ 12,000
Total Project Request for Year 3 Funding: $ 10,000

Other related/associated funding sources: Requested
Funding Duration: 2023 - 2027
Amount: $ 4,122,169
Agency Name: USDA SCRI
Notes: Title: Integrating multidisciplinary and translational approaches to manage postharvest rots on apples and pears in major U.S. pome fruit growing regions. All three PIs are listed as co-PIs on this project.

Item	2022	2023	2024
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping	$6,000.00	$6,000.00	$6,000.00
Supplies	$4,000.00	$4,000.00	$2,000.00
Travel	$3,000.00	$2,000.00	$2,000.00
Plot Fees			
Miscellaneous			
Equipment	$20,000.00		
Total	$33,000.00	$12,000.00	$10,000.00

Footnotes:

Budget 1
Primary PI: Dr. Christopher Gottschalk
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Station Manager/Supervisor: Dr. Tracy Leskey
Station manager/supervisor email address: tracy.leskey@usda.gov

Item	2022	2023	2024
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$1,700.00	$3,400.00	$1,700.00
Travel	$3,000.00	$2,000.00	$2,000.00
Plot Fees			
Miscellaneous			
Equipment	$18,500.00		
Total	$23,200.00	$5,400.00	$3,700.00

Footnotes:

If project duration is only 1 year, delete Year 2 and Year 3 columns.

(*Complete the following budget tables if funding is split between organizations,* ***otherwise delete*** *extra tables.)*

Budget 2
Co PI 2: Dr. Lauri Reinhold
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Station Manager/Supervisor: Carolyn Scagel
Station manager/supervisor email address: carolyn.scagel@usda.gov

Item	2022	2023	2024
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping	$6,000.00	$6,000.00	$6,000.00
Supplies	$2,300.00	$600.00	$300.00
Travel			
Plot Fees			
Miscellaneous			
Equipment	$1,500.00		
Total	$9,800.00	$6,600.00	$6,300.00

Footnotes:

Objectives
Our proposed project had four objectives that complementarily address the evaluation of pear germplasm for post-harvest traits. **The first objective is to evaluate the USDA Pear Collection for optimal harvest and storage time for 50 high-value genotypes.** We proposed using two germplasm sources to acquire 50 genotypes: 1) USDA Pear Collection at the USDA ARS National Clonal Germplasm Repository (NCGR) in Corvallis, OR, which contains nearly 2,300 unique pear cultivars, breeding lines, and hybrids that represent 36 species and 2) as a backup the USDA ARS Appalachian Fruit Research Station (AFRS) breeding program in Kearneysville, WV. This objective aims to evaluate the lines for harvest dates, storage requirements, and the presence/absence of post-harvest diseases. We are approaching the disease evaluations in a two-step process. First, evaluate the fruit for natural infections and the classification of pathogens present. Second, conduct resistance testing by inoculating the genotypes found to be free of natural infection for resistance to the identified pathogens. **The second objective is to characterize the 50 high-value genotypes for fruit quality, attributes including total soluble solids, acidity, polyphenolic content, texture, peel and flesh**

color, and grade. This objective aims to characterize fruit quality traits using two approaches, destructive and non-destructive, correlate their measures, and develop models used to predict the destructive trait measurements using the non-destructive equipment. **The third objective is to challenge the 50 high-value genotypes in simulated supply-chain stress to document resistance to bruising, scuffing, and puncturing.** This objective aims to identify germplasm that can withstand the intense forces that are exerted on the fruit during the supply-chain process. The approach here is to simulate the shipping conditions on fruit that is at a consumer-ripe condition and document the degree of or absence of damage. **The fourth objective is to document and distribute findings through publications and presentations regarding the resistance of the 50 high-value genotypes to storage disorders and diseases.** The aim here is to provide communication with the stakeholders and provide any products developed from the analyzes as impactful tools for evaluation of post-harvest traits in pear.

Significant Findings

Objective 1:

- Germplasm is available at AFRS with a full range of harvest dates
- Germplasm is available at AFRS with desirable ranges of cold condition requirements (< 21 days and > 60 days)
- Identified four genotypes with low natural post-harvest disease incidence
- Collected 855 fungal isolates from fruit
- Identified a potential bio-control microbe that reduced pathogen growth *in vitro*

Objective 2:

- Identified genotypes associated with large fruit size
- Prospective processing pear genotype that has a large fruit size and can yield high juice amounts when processed

Methods

Objective 1: We identified high-value germplasm from historical texts, the USDA GRIN database, and recommendations from germplasm curators and previous breeders. The terms that were used as queries in the literature search for desirable genotypes included disease-resistance (fire blight, *Monolinia*, and post-harvest pathogens), ships well, excellent flavor, keeps well, fruit quality, acidic, phenolic (non-perry), early ripening, late-ripening, and tree-ripe. Following bloom and prior to the fruit ripening period, crop load was estimated from each tree to determine if the minimal fruit number need for all analyses was available.

For harvest timing, five randomly selected fruit from each tree were collected weekly. Each fruit was cataloged for color development and underwent firmness testing using a penetrometer with a measurement taken from the sun-exposed and shaded side of the fruit following removal of the peel. A genotype will be determined as harvest-ready when firmness decreases to an average of 20 lbf, and color development has reached its peak. We additionally found that the simple approach of lifting the pear(s) on a branch from the bottom of the fruit, with a minimal force that resulted in release, the pear was determined as harvest ripe. Several of the AFRS breeding lines correlated with known harvest dates using that approach as opposed to decreases in firmness. Potentially, this result is due to the hybrid (*Pyrus* spp.*)* origins of many of the breeding lines. First-year results were obtained in the Fall of 2022.

Each genotype then had 75 fruits harvested and packed into 40 lbs fruit boxes and stored at USDA AFRS in a new cold storage unit. The boxes of fruit were kept in cold storage at 30 ˚F and 90-98% relative humidity. At biweekly intervals, starting at two weeks in storage to 12 weeks or until ripe, three randomly selected fruit will be taken out of storage and rested at room temperature for 48 hours. Following the acclimation period, the selected fruit was tested for firmness using a

penetrometer. The genotypes will be considered ripe when average firmness reaches 3 lbf or less. First-year results were obtained in the Fall and Winter of 2022.

For post-harvest disease evaluations, 24 fruit were selected and remained in cold storage and evaluated/rated weekly for the development of soft scald and the presence or absence of *Botrytis cinerea* (Gray mold), *Penicillium expansum* (Blue mold), *Mucor piriformis* (Mucor rot), and *Colletotrichum* spp. (bitter rot). When a disease was identified, pathogens were sampled and plated for identification of pathogen species and/or complex based on morphology and DNA sequence using universal fungal primers *ITS1* and *ITS4*. Data collection and analysis are ongoing from fruit collected in the Fall of 2022.

Objective 2: We originally proposed using twelve randomly selected pears from each genotype, that are identified as at an optimal eating quality following storage, to be used to evaluate fruit quality traits. However, limited crop loads, higher soft scald incidence, an outbreak of *Fabraea* leaf spot at AFRS, and longer cold condition sampling time points than anticipated required the decrease of the number of replicates to five for this objective. The five fruits first underwent size (length, diameter, and mass) and shape (qualitative) measures. Following non-destructive measurements, all five of the replicate fruit per genotype were analyzed using Near-infrared (NIR) Produce Quality Meter (Felix Instruments). After NIR measurement, each replicate pear was processed to extract juice using a Good Nature M-1 Fruit Grinder and Press. The extracted juice was frozen and will undergo measurements for TSS (ATAGO PAL-1), TA and pH (Orion Star T910 Autotitrator), and total polyphenolic content (Folin-Cointreau; absorbance using a spectrometer) using industry-standard measurement methods in Spring 2023.

The data obtained from the NIR meter and industry-standard methods will be inputted into Felix Instrument's model-building software to develop and validate models for the NIR meter for future use. In years two and three, the NIR meter will be the sole instrument used to determine all fruit quality metrics except for a juice extraction to determine polyphenolic content. Due to the limited replicate fruit, we were unable to conduct a sensory evaluation using a trained three-person panel consisting of staff at AFRS or sent to the USDA ARS Fruit Quality Lab in Beltsville, MD. Data collection and analysis are ongoing from fruit collected in the Fall of 2022. Results for year one are anticipated by late Spring 2023.

Objective 3: We will evaluate each genotype for resilience to stress associated with the supply chain including bruising, scuffing, and puncturing. This objective will begin during the 2023 season due to the limited fruit available during the 2022 season and the need to identify the cold conditioning requirements for each genotype. Fruit used in this objective will need to be at or near consumer-ripeness for evaluation, a typical time for pears to exit commercial storage and transit through the supply chain. For each of the three injury tests, five replicate pear fruits – at optimal fruit maturity – will be removed from storage and subjected to stress tests. For evaluation of resistance to bruising, we will utilize a penetrometer to apply pressure to the fruit at a marked location on the fruit's surface. The penetrometer will apply an even pressure of 7 lbf to the fruit (the peel is not removed during this test). The fruit will then be rested at room temperature for 5 days. Following the rest period, the fruit will be dissected across the marked bruising site. The injury, if present, will then be documented for color (oxidation) and depth of bruising.

An alternate approach will utilize a robot arm to simulate container loading and unloading which would cause bruising. However, the robot arm is currently unavailable due to equipment failure and COVID-19 disruptions to the supply chain for replacement parts. We hope to fix and make this machine available for use during the upcoming years of the project. The robotic stress will be applied by having the robot's arm traverse the lower ¼ quadrant of a circle at a speed setting that mimics truck movement on the roadway and a drop treatment that covers a distance of 600 mm in < 1 sec. The robotic-associated testing will occur at AFRS under the guidance of Dr. Amy Tabb who has performed similar simulations (Nixon et al., 2019). To evaluate scuffing, a simulated conveyor belt will be

constructed that consists of a rectangular box outfitted with fruit conveyor belt material. The box containing five replicate fruits will then be placed onto a shaker table that will operate at 100 RPMs for five mins. Following the stress, the fruit will be rested for 5 days at room temperature and then evaluated for presence/absence of scuffing and scuffing severity. The final evaluation test will be a puncture test where five replicate fruits will be subjected to a penetrometer outfitted with a 4 mm plug. The pressure it takes for the plug on the penetrometer to puncture the peel of the fruit will then be recorded.

Objective 4: The results gained from Objectives 1-3 will be presented and distributed to the research community and stakeholders through three different channels. First, following the conclusion of the project in year three, a poster and/or oral presentation will be made at the WTFRC Pear Research Review by PI Gottschalk. Second, the results gained from this study will be published in a horticultural-focused journal(s) such as HortScience or the Journal of American Society of Horticultural Science. In conjunction with the publication(s), the data generated and analyzed will be indexed into the USDA GRIN database for public accessibility. Lastly, the validated models developed for TSS, TA, and other fruit quality metrics using Felix Instrument's F-750 NIR Fruit Quality Meter will be made publicly available through supplemental information accompanying the publication(s) and/or through a digital repository such as GitHub. Results for this objective are anticipated after year three of the project and, thus, are ongoing.

Results and Discussion

Objective 1: The identification of 50 high-value varieties was successful. 14 were found in the historical texts *Book of Pears* (2015) – J. Morgan and six from *Pears of NY* (1913) – Hendrick. The remaining 30 were found through description/observation searches in GRIN or from recommendations by J. Postmen (USDA Pear curator – retired). We additionally, were able to properly re-identify 60+ genotypes in the historic AFRS germplasm that had returned to a feral condition during the period of dormancy following R. Bell's retirement and PI Gottschalk's onboarding. Of those lines, four were noted as having potential post-harvest desirable traits: NJ 15 for storage resilience/post-harvest disease resistance, US 79439-004 for high fruit quality and long cold conditioning requirement, Shenandoah for post-harvest disease resistance, and US 78302-022 for unique fruit quality attributes (tropical flavor and aroma).

Unfortunately, a minor frost in the spring of 2022 and biennial bearing habits extremely limited the fruit available for the NCGR. 44 of the 50 genotypes were below the threshold of fruit required and as a result attention was focused on the germplasm available at AFRS. The AFRS germplasm had 38 lines with enough fruit to evaluate and determine harvest date and cold conditioning requirements (**Table 1**), natural disease presence/absence, and measurements for Obj. 2 fruit quality traits. Notable results related to harvest date and cold condition requirements are: 1) AFRS germplasm spans much of the pear harvest season, including several genotypes with late harvest dates (early- to mid-September). These genotypes are of interest for the breeding program to further extend the harvest season, to not compete with the Fall pear (*e.g.,* 'Bartlett') market. 2) Regarding cold conditioning requirements, AFRS germplasm was also found to exhibit a wide range of requirements of 14 to 84 days. Additionally, eight genotypes were found to have an extended period in which their cold conditioning requirements were met. Within the distribution of the conditioning period, the genotypes that required < 21 days and > 60 days are of interest for the breeding program. Breeding for ripening times that are outside the normality will allow for the extension of the distribution season by packinghouses and direct markets. For example, developing a variety that requires > 60 days of cold conditioning could be associated with extending the viable storage time (in controlled atmospheric conditions). Presenting distributors with an opportunity to market pears that are high-quality during periods in which domestic stocks typically diminish and imports increase in the marketplace. The genotypes with inconsistent cold conditioning requirements are also of concern, as their use as breeding parents is limited due to the difficulty in managing variable ripening fruit.

Table 1. AFRS breeding lines evaluated for harvest and cold conditioning requirements.

AFRS Line	Harvest Date	Date Conditioning Requirements Met	Cold Requirement (Minimum Days)
Gem	8/24/2022	10/3/2022	40
US 83825-223	8/11/2022	on-going trials	-
US 69426-038	8/11/2022	8/22/2022	11
US 84907-078	8/22/2022	9/5/2022	14
NJ 15	8/22/2022	9/5/2022	14
US 83825-020	8/22/2022	on-going trials	-
NJ Rock R18 T227	8/22/2022	9/5/2022	14
84907-069	8/24/2022	9/19/2022	26
84907-166	8/24/2022	on-going trials	-
Bell	8/25/2022	9/5/2022	11
69442-060	8/25/2022	10/3/2022	39
81606-044	8/25/2022	10/14/2022	50
US 68309-106	8/29/2022	10/14/2022	46
US 84909-184	8/29/2022	11/18/2022	81
US 83825-261	8/30/2022	on-going trials	-
US 84905-017	8/30/2022	10/14/2022	45
US 70537-006	9/1/2022	9/19/2022	18
US 80524-024	9/1/2022	10/21/2022	50
US 82728-016	9/6/2022	11/7/2022	62
US-309	9/6/2022	10/21/2022	45
US 99422-202	9/6/2022	on-going trials	-
US 82728-028	9/7/2022	11/7/2022	61
US 78302-022	9/7/2022	on-going trials	-
NJ B9 R1 T117	9/7/2022	10/21/2022	44
US 79453-007	9/7/2022	11/30/2022	84
NJ ROCK R18 T227	9/7/2022	on-going trials	-
US 79439-004	9/8/2022	11/7/2022	60
82728-015	9/13/2022	11/18/2022	66
78307-045	9/13/2022	11/7/2022	55
70526-075	9/14/2022	10/21/2022	37
NJ Rock R25 T238	9/13/2022	on-going trials	-
Ill-2ON-028	9/14/2022	11/7/2022	54
78302-018	9/13/2022	10/14/2022	31
NJ 487601193	9/13/2022	on-going trials	-
Shenandoah	9/16/2022	11/7/2022	52
Comice	9/16/2022	on-going trials	-
NJ Rock R25 T65	9/16/2022	on-going trials	-

24 fruits harvested from each genotype were divided into three replicates of eight fruits and were evaluated weekly for the presence or absence of disease during cold storage. If disease was present, pears were removed from cold storage and fungal species were isolated from the fruit surface. A total of 885 fungal isolates have been collected from pear fruit harvested in 2022. Fungal isolates were grown on potato dextrose agarose and preliminary identification was made based on morphology. *Colletotrichum* spp., *Mucor piriformis*, *Botrytis cinera*, and *Penicillium expansum* were all observed, but the majority of isolates were preliminarily identified as *Colletotrichum sp.* based on morphology. We also observed *Diaporthe sp.* and *Fusarium sp.* which were first reported to cause rot in European pears in the United States in 2019. Genetic confirmation of fungal isolate identities is underway. After 12 weeks in cold storage all the pear genotypes had developed some disease, although the percent incidence of rot ranged from 8.3% to 100% (**Fig. 1**). High disease pressure is expected, as fruit were not treated prior to cold storage. We anticipate that genotypes that perform well under high disease pressure will perform even better under commercial storage conditions. We identified four genotypes with low disease incidence (<15%) which included US 83825-223, US 84907-166, US 83825-261, and Comice. Low natural disease incidence could indicate these genotypes have some level of genetic resistance. The identified genotypes will be used for wound-inoculation experiments in year 2. Alternatively, fruit with low natural disease incidence could also have beneficial microorganisms on the fruit surface that either induce resistance or have antagonistic interactions with plant pathogens. We isolated a bacterium from the surface of US 78302-018 that displayed antagonistic activity against *Diaporthe sp.* on three types of growth media (**Fig. 2**). Efforts to test for antagonistic activity against additional pear pathogens is underway. We expect that identification of beneficial microorganisms and understanding their interaction with pathogens will lead to the development of new consortia of microorganisms for biocontrol opportunities.

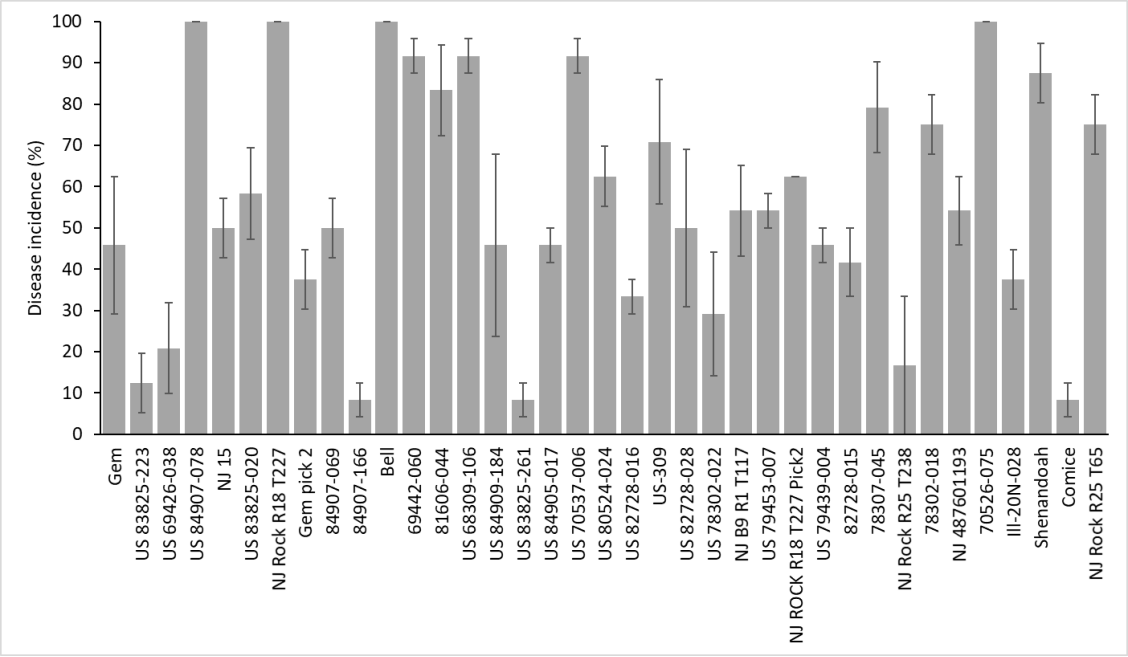

Figure 1. Disease incidence in AFRS pear germplasm after 12 weeks of storage under high disease pressure. Bars represent the mean of three replicates comprised of eight fruits ± standard error. Genotypes are ordered on the X-axis by harvest date.

Figure 2. A bacteria isolated from pear surface preliminarily identified as *Streptomyces sp.* that has antagonistic activity against *Diaporthe sp.* which is a pathogen that causes fruit rot of European pears. The isolates were grown on three different media, potato dextrose agar (PDA), nutrient yeast glucose agar (NYDA) and Luria-Bertani (LB) agar.

Objective 2: We are currently analyzing the fruit quality traits from the 2022 season. The results presented below represent preliminary data analyzed thus far. We measured length, diameter, mass, and juice yield for 26 genotypes that persisted through storage and reached consumer ripeness (**Fig. 3**). Of note, many breeding lines (containing "US" identifier) were larger than many of the previously released/named genotypes evaluated (*e.g.,* 'Bell', 'Gem', 'Shenandoah'). This result suggests previous breeding efforts resulted in the selection of larger fruit sizes. The genotypes US 84905-017, US 78453-007, and US 68309-106 were characterized as the largest fruit based on average lengths, diameter, and mass. These lines represent suitable future parent selections to breed for larger fruit size. However, each line exhibited a wide variation in those measurements suggesting less uniformity in fruit size. In comparison to the name genotypes, they were very uniform in fruit size which reflects their outcome of being named and released. In the process of destructive sampling for measurement of brix, pH, acidity, and phenolic content (awaiting results), we documented the amount of juice produced per replicate fruit. Although an abstract measure for fruit quality, this trait provides desirable information for potential identification of traits for the processing industry (*i.e.,* fresh juice, perry [fermented pear juice/cider]). The juiciest genotypes were found to be US 84905-017, US 78453-007, US 70537-006, and NJ Rock R18 T227 with average yields of 135-155 mL/fruit. Of those four, US 78453-007 is of particular interest for future use in breeding for processing traits. During the initial evaluation in 2021, PI Gottschalk noted US 78453-007 as having substantial phenolic characteristics, a highly desirable trait for processors that ferment juice but are absent in improved pear cultivars. Historically, producers have relied on perry pears to acquire phenolic contents, but those varieties are hundreds of years old and were never bred for traits suitable for modern production systems.

Objective 3 and 4: Results have not been obtained yet for the final two objectives. We anticipate collecting results for Objective 3 in Fall of 2023. Objective 4 results are anticipated to begin during the final year of the project.

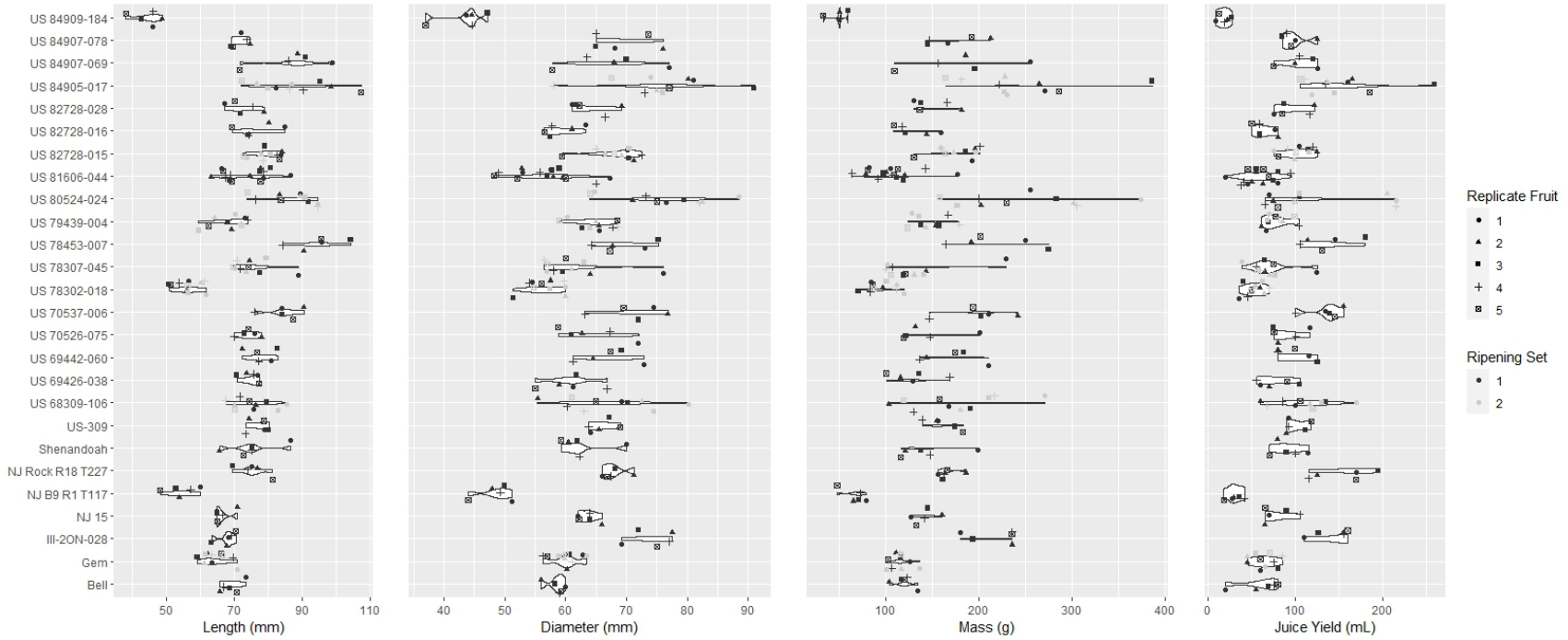

Figure 3. Distributions of fruit size and juice yield of AFRS pear germplasm. Each genotype had five replicated fruits measured and, in several cases, two sets of five replicate fruits when non-uniform cold condition requirements were identified (ripening sets).

Project Title: Development of a Rapid-Cycle Breeding Tool for Pear

Report Type: Continuing Project Report,

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Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 32,915
Total Project Request for Year 2 Funding: $ 33,737
Total Project Request for Year 3 Funding: $ 68,825

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2023
Amount: $62,241.50/3 yrs.
Agency Name: USDA-ARS, In-house project
Notes: In-house project with complimentary objectives. Half funding for 100% FTE (salary+benefits) technician for years 1 and 2 ($30,705 and $31,536.50, respectively).

WTFRC Collaborative Costs: none

Budget 1
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Item	2021	2022	2023
Salaries	22,250	22,850	48,279
Benefits	8,455	8,686.50	18,346
Wages			
Benefits			
Equipment			
Supplies	2,210	2,200	2,200
Travel			
Miscellaneous			

Plot Fees			
Total	32,915	33,737	68,825

Footnotes:

1Biological Science Technician = Half funding for 100% FTE (salary+benefits) technician for years 1 and 2, and full funding for year 3.

2RNA/DNA extraction, tissue culture, greenhouse, molecular supplies and consumables.

Objectives

1. **Transform pear rootstock germplasm with a flowering-activating, chemically-induced system.** Introduce flowering genes into fire-blight resistant pear rootstock germplasm whose expression can be induced by an inexpensive agrochemical, allowing early flowering for rapid breeding without the negative phenotypes seen in other Rapid-Cycle Breeding (RCB) systems.
2. **Early molecular and phenotypic characterization of transformants.** Confirm the presence and location of the inducible flower genes. Test lines for flowering response.
3. **In-depth characterization and optimization of RCB plants.** Characterize flowering gene expression and flowering response to agrochemical in detail. Determine optimal dose and delivery of chemical induction. Test viability of flowers to be pollinated and begin crossing with germplasm containing additional traits of interest.

Significant Findings

- Successful transformation of Arabidopsis with the RCB construct containing the flowering gene CiFT demonstrated that the Kanamycin gene we introduced is functional.
- Successful transformation of pear callus tissue with the CiFT RCB construct was indicated by a red fluorescent marker.
- Development of a Hygromycin-resistant version of the CiFT RCB construct may aid in potential issues with Kanamycin resistance.
- Optimization of transformation and plant regeneration protocols was undertaken for the purpose of obtaining transformants containing the CiFT RCB construct.

Methods

Objective 1. Transform pear rootstock germplasm with flowering-activating, chemically-induced system (Years 1-2)

1a. Selection of germplasm to be transformed

In Year 1, we were able to obtain OHxF 87, 97 (recently confirmed to actually be Old Home x Bartlett crosses by (*1*)), and Bartlett tissue and initiated these into tissue culture. Successful micropropagation has continued. In Year 2, we have begun optimizing transformation and regeneration protocols, for which we have focused largely on Bartlett, due to it's predictability and established responses to micropropagation. What we have learned with Bartlett will be applied to OHxF87 and 97 in the coming year.

1b. Use developed transgenic flower-inducing constructs and develop additional versions

The RCB construct developed in Year 1 and used in transformation trials this year (Year 2) contains an antibiotic resistance gene (NptII, resistance to Kanamycin) and a flowering gene (either CiFT or BpMADS4), respectively (Fig. 1). Kanamycin is an effective antibiotic for transformant selection in plants, however there are reports that sensitivity to kanamycin varies between plant species (*2-4*). We have also found reports that Kanamycin efficacy varies dependent on the gelling agent used in the transformation process (*5*). To avoid potential difficulties of using Kanamycin selection, we constructed a modified version of the RCB construct using a methods called Gibson cloning (*6*), replacing the Kanamycin-resistance gene with a Hygromycin-resistance gene (Fig. 1). Sequencing is currently underway to verify correct insertion of the Hygromycin-resistance gene, and viability of resistance will be tested quickly by transforming Arabidopsis (see below).

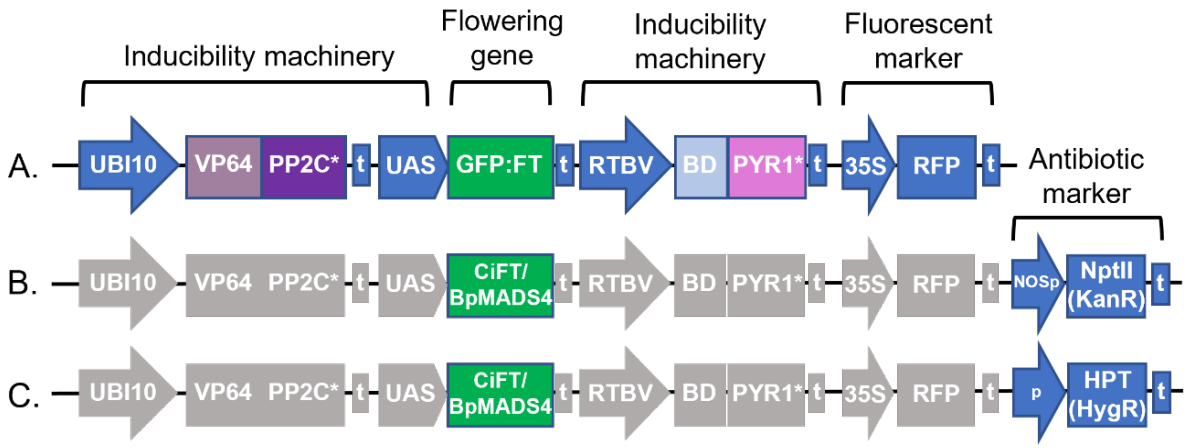

Figure 1. Construct development. A. Original construct received from Cutler lab. B. Construct developed in Year 1, containing flowering genes for pear and a Kanamycin-resistance gene (NptII). C. Construct developed in Year 2 containing a Hygromycin-resistance gene (HPT), replacing KanR.

1c. Transform germplasm

Our initial transformation attempts in Year 1 did not result in regenerated, transformed plants. In Obj. 1b, the Kanamycin-resistant versions of the construct we developed gave us an additional way to select for transformed plants. To quickly test whether this Kanamycin-resistance gene was functional and inserted correctly, we transformed Arabidopsis with the RCB construct containing the Kanamycin-resistance gene and the flowering gene (NptII and CiFT/BpMADS4 respectively, Fig. 1). Arabidopsis was dipped in a culture of Agrobacterium containing the construct (this method is called the floral dip method (*7*)), allowed to set seed, and seed was collected. Seeds were sterilized and sown on agar plates containing Kanamycin to select for transformed seedlings. Seedlings were confirmed both by survival on Kanamycin and by red fluorescence under green light, using an Xite Fluorescence Flashlight (NIGHTSEA, nightsea.com/products/xite-flashlights/) (Fig. 2). Seedlings were transferred to soil after ~2 weeks and grown up to collect the next generation of seed.

In our previous report, we outlined the major steps in the transformation process: removal of leaves, inoculation with Agrobacterium containing the RCB construct, co-culturing the leaves with the Agrobacterium, washing away Agrobacterium, growing leaves on antibiotic-selection in the dark (during which callus should form and leaf tissue should begin to regenerate from it), and growth in the light (to continue regeneration). After our initial attempts resulted in no transformants, we sought to optimize transformation rates by comparing many of these parameters, including: Agrobacterium concentration during transformation (concentrations of OD600= 0.1, 0.3, and 0.6), co-cultivation times and methods (liquid versus solid media, 3 days versus 6 days), inoculation methods (vacuum infiltration versus soaking and wounding), nutrient bases (MS-based media versus NN69-based media), inclusion of Betaine and Acetosyringone at different steps (throughout Agrobacterium growth versus immediately before inoculation), and different leaf tissue wounding approaches to trigger callus formation (leaf discs vs slicing whole leaves). Further, we developed an Agrobacterium growth curve to determine the rate and timing of the cell culture using our lab equipment (Fig. 3). This allowed us to determine the optimized timing for growing the inoculation culture, with and without Acetosyringone in the media – a chemical reported to activate virulence of Agrobacterium (*8*).

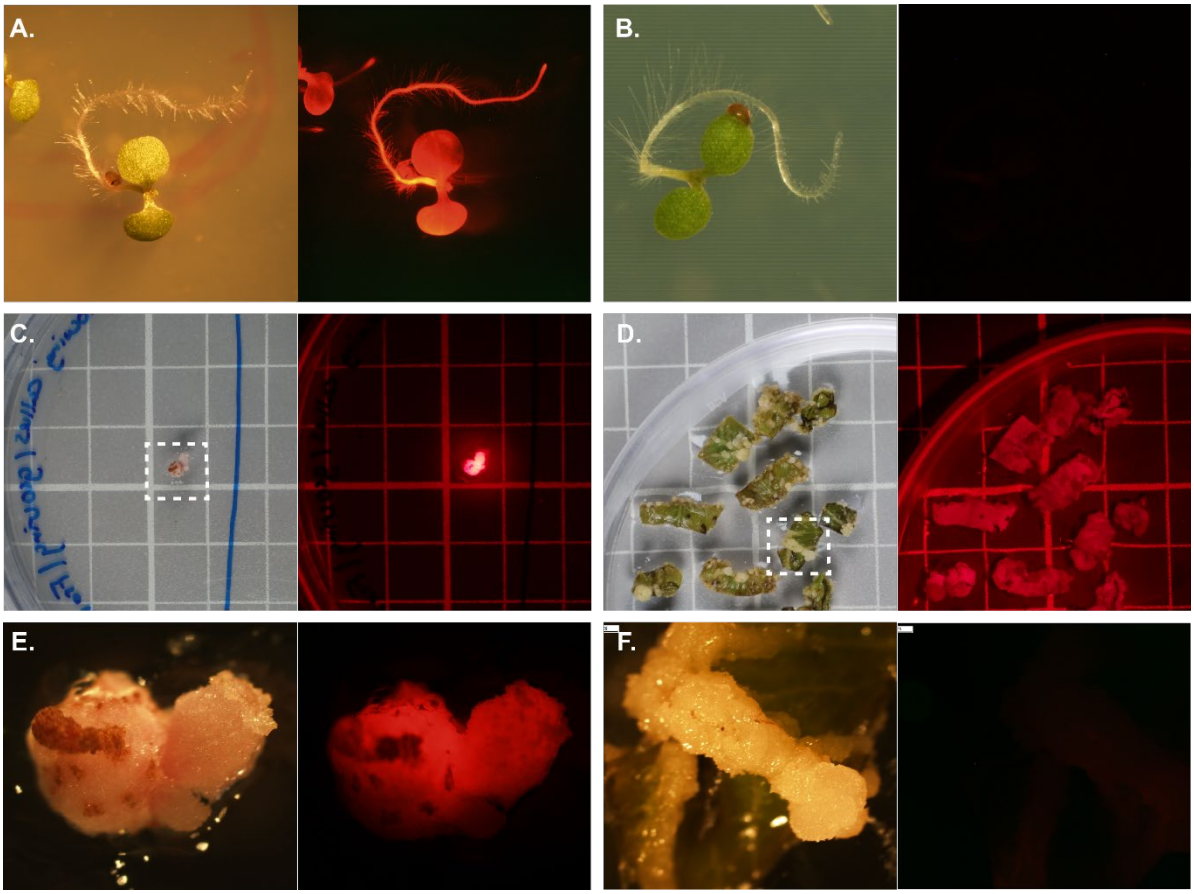

Figure 2. Red fluorescent marker indicates transformation of Arabidopsis and pear callus tissue. A. Arabidopsis seedlings that have been successfully transformed with the RCB construct and selected on Kanamycin, in white light (left) and green light to excite the red fluorescence (right). B. Arabidopsis seedlings that have not been transformed, for reference. Chlorophyll fluoresces to a low level, but the bright red of the fluorescent marker is absent. C. Transformed pear callus that has been isolated from a leaf, in white light (left) and green light (right). D. Non-transformed (control), pear leaf squares growing callus, not showing the bright red of the marker. E-F. Zoomed images of the calli highlighted by the dotted boxes in C and D. The bright red marker can be seen in E, but not F.

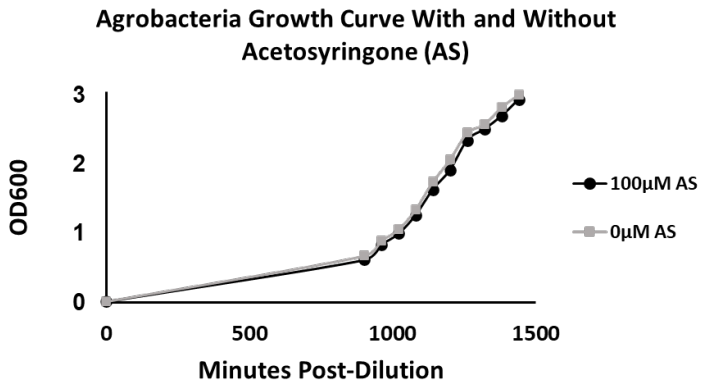

Figure 3: Agrobacterium growth curves, with and without Acetosyringone (AS). 4mL LB Rif+Kan cultures were inoculated with 1 EHA105 colony containing our construct. They were diluted to OD600 0.05 and 0.008 in media containing 100uM AS or no AS and grown 15 hours before measurements began.

See below for a table of experimental parameters (Table 1). Initially, very little callus was generated and the leaves died after several months, due to necrosis. However, transformation of some callus tissue was confirmed by red fluorescence under green light (Fig. 2). As we have found more fluorescing callus cells, we remove them from the dead leaf tissue and transfer to new plates to allow them to grow more and attempt regeneration directly from these cells (Fig. 2C as example). Callus transformation rates for experiments where we had access to the green light can be found in Table 1.

As discussed below, two potential bottlenecks preventing regeneration of transformed tissue were identified: adventitious shoot regeneration and callus production. Control plates from initial transformation attempts (in which no Agrobacterium inoculation took place, but leaves were otherwise treated similarly) yielded remarkably low adventitious shoot regeneration (0-4%). An in-depth literature review of adventitious shoot regeneration was conducted and we identified several parameters we could alter to improve regeneration rates. We tested several of these, including: differing shoot regeneration hormone concentrations (1nM versus 5nM NAA, and 5.7nM versus 22.7nM TDZ), length of dark treatments (3-4 weeks versus 17 days), pre-soaking leaf material in liquid (water versus nutrient media, 5 minutes versus 1 hour), wounding methods (vacuum infiltration, slicing, or leaf discs), using leaf tissue from plants grown on different hormones (meta-Topolin versus BA), and media nutrient bases (PM2, Regeneration Media (REM), or NN69). Treatments that showed major differences or improvements are summarized in Table 2. We are currently planning experiments to determine optimal parameters for callus production including: concentration and types of hormones in the media, media nutrient bases, as well as time series experiments to determine differences in callus formation between cultivars.

Objective 2: Early molecular and phenotypic characterization of transformants (Year 2-3)

<u>2a. Rescue transformants, confirm presence of construct</u>

In the coming year (February 2023-January 2024) we will focus on regenerating plant tissue from the callus that has been transformed. Following this, we will rescue transformed plants growing on antibiotic selection and containing the red fluorescent marker, both indicating that they contain the RCB construct. Additionally, we will be able to check insertion of the construct into plant DNA using PCR-based genotyping. Finally, to confirm the location of the transgene within the genome, we will sequence confirmed lines. Confirmed plants that reach sufficient size will be rooted, acclimated, and moved to soil before moving on to characterization. While we were previously concerned about ability to root these cultivars, this year we have tested rooting protocols and seen success for Bartlett, OHxF 87, and OHxF 97 (Table 3 shows results from one experiment comparing responses of Bartlett, OHxF 97, and a hybrid variety to different rooting treatments).

<u>2b. Test flowering-induction in response to chemical induction and select clones to move forward</u>
Among transformed plants, we want to initially determine clones that are responsive to chemical induction of flowering. Plants will be sprayed with Mandipropamid and flowering will be observed. These initial flowers will also be analyzed for morphology. Results will be used to determine which transformed lines to move forward with in-depth characterization. Lines will also be replicated/propagated to ensure we have sufficient material for analysis. We expect that this subobjective will begin to be addressed in year 3.

Exp. Code	Vector	Agro concentration	Leaf tissue	Inoculation method	Acetosyringone addition	Co-cultivation parameters	Dark treatment	Agro removal	Media nutrient base	Callus transformation rate
220329	RCB (CiFT and KanR)	OD600 = 0.3 or 0.6	Excised whole, young lvs	Soak and cut	Immeditely before inoculation	3 days @ 25C in dark, solid	3 weeks	1x Tim wash, growth on Tim	MS-based	nd
220519	RCB (CiFT and KanR)	0.1 or 0.3	Excised whole, young lvs	Soak and cut	Immeditely before inoculation	6 days @ 25C in dark, solid	3 weeks	1x Tim wash, growth on Tim	MS-based	nd
220614	RCB (CiFT and KanR)	0.3	Excised whole, young lvs	Soak and cut vs. Vacuum and cut	Immeditely before inoculation	6 days @ 25C in dark, solid	3 weeks	1x Tim wash, growth on Tim	MS-based	nd
220615	RCB (CiFT and KanR)	0.3	Excised whole, young lvs	Soak and cut vs. Vac and cut	In Agro media throughout growth	6 days @ 25C in dark, solid	3 weeks	3x Tim washes, growth on Tim	MS-based	nd
220803	RCB (CiFT and KanR)	0.3	Leaf squares, scalpel	Soak and cut vs. Vac and cut	Throughout growth	6 days @ 25C in dark, solid	3 weeks	3x Tim washes, growth on Tim	MS-based	18/100 for vac/soak combined*
221028	Known functional vector with KanR	0.6	Leaf squares, scalpel	Soak and cut	Throughout growth	4 days @ 20C in dark, liquid	17 days	3x Tim+Cef washes, growth on Tim+Cef	NN69-based	n/a**
221110	Known functional vector with KanR	0.8	Leaf squares, scalpel	Soak and cut	Throughout growth	4 days @ 20C in dark, liquid	17 days	3x Tim+Cef washes, growth on Tim+Cef	NN69-based	n/a**
221205	RCB (CiFT and KanR)	0.8	Leaf disks, biopsy punch	Soak and cut	Throughout growth	4 days @ 20C in dark, liquid	17 days	16hr soak in Tim+Cef, select 3 days Tim+Kan, growth on Tim+Cef	NN69-based	65 transformed spots on calli / 150 leaf discs [36 dpi]
221215	RCB (CiFT and KanR)	0.8	Leaf disks, biopsy punch	Soak and cut	Throughout growth	4 days @ 20C in dark, liquid	17 days	16hr soak in Tim+Cef, select 3 days Tim+Kan, growth on Tim+Cef	NN69-based	35 transformed spots on calli / 173 leaf discs [26 dpi]

Table 1. Experimental parameters for transformation attempts. Highlighted in yellow are changes from the previous experiment. "Soak and cut" refers to the method of excising leaves, inoculating in agrobacterium for 10-20 minutes and making slices with a scalpel across the midrib. "Leaf" squares refers to cutting squares containing midrib tissue out of young, excised leaves with a scalpel. Tim = timentin and Cef = Cefotaxime, both antibiotics against Agrobacterium in general, but safe for plant growth. Acetosyringone is a chemical known to activate Agrobacterium virulence. Gelzan is a gelling agent that has been suggested to induce callus production. nd = not determined (these are for experiments for which we didn't have access to the green fluorescent light). *can't determine exact numbers or treatment efficiency due to agro overgrowth/leaf death (for this experiment we did not have the green light until later, after many leaves had died). **The vector used in these trials did not contain a red fluorescent marker, as we were testing for Kan-resistance with a previously used vector.

Experiment purpose	Parameters compared/tested	Parameters held constant	Outcomes
Combinatorial experiment to test multiple parameters for improving regeneration rate from excised leaves	Growth media: PM2 vs. Regeneration Media Media Nitrogen ratio: 1:2 vs. 1:3 Dark Treatment: 3 weeks vs. 2 months Cytokinin concentration: 2.5uM vs. 22.7uM TDZ Auxin concentration: 5.3uM vs. 1uM NAA Wounding methods: vacuum infiltration prior to slices across midrib vs. slices only	Prior media: PM2 + 4.4uM BA (as cytokinin) Soaking prior to wounding: Excised leaves rest in water for a few minutes prior to wounding Mock "Co-cultivation" period: No agro, but 6 days in the dark on Gelzan plates, then switched to plates containing Sigma and Phytotech agar. Cytokinin type: TDZ Auxin type: NAA Leaf material: whole excised leaves	Low regeneration for all: only up to 4% in a few treatments, with no correlations. Most leaf tissue was lost to necrosis. A repeat of experiment is needed, considering results from necrosis experiment below.
Effect of soaking time and leaf material on necrosis	Soaking time prior to wounding: 5 minutes vs. 60 minutes Soaking media prior to wounding: water vs. liquid Regeneration Media with TDZ (cytokinin)	Prior media: PM2 + 4.4uM BA (as cytokinin) Growth media: Regeneration Media Media Nitrogen ratio: 1:3 Mock "Co-cultivation" period: None - growth on plates containing Sigma and Phytotech agar. Dark Treatment: 3 weeks Cytokinin type and concentration: 22.7uM TDZ Auxin type and concentration: 1uM NAA Leaf material: leaf squares cut from excised leaves, containing midvein	Soaking for a longer time improved regeneration rate, soaking with media may have also improved, but needs more study. Regeneration Rates: 5min in water - 7% 5min in media - 4% 60min in water - 19% 60min in media - 23%
Effect of prior growth media on regeneration rate	Prior media cytokinin concentration (for plants from which leaves were excised): PM2 with 4.4uM BA vs. PM2 with 5uM meta-Topolin	Growth media: NN69 Media Nitrogen ratio: 1:2.3 Mock "Co-cultivation" period: None - growth on plates containing Sigma and Phytotech agar. Dark Treatment: 3 weeks Cytokinin type and concentration: 22.7uM TDZ Auxin type and concentration: 1uM NAA Leaf material: leaf squares cut from excised leaves, containing midvein	There appears to be no difference in regeneration rates, however shoot quality may differ between treatments. A larger sample size and more replicates will be needed. Regeneration rates (avg of two reps): PM2 with BA: 11.1% +/- 7.9 SEM PM2 with mT: 10.8% +/- 6.3 SEM
Effect of leaf material type and nutrient base on regeneration rate	Leaf material: whole excised leaves vs. leaf squares or punches Growth media: Regeneration Media vs. NN69	n/a	Not a formal experimental comparison. Switching from whole leaves to leaf squares or punches, and switching from Regeneration Media to NN69, has not only saved a great deal of time, but appears to improve access of leaf material to nutrients in media (improved surface area), and may improve callus formation and regeneration.

Table 2. Parameters tested for improvement of regenerations rates.

Cultivar	Auxin	Conc.	% Rooted @ 4 weeks	Average # roots
Bartlett	IBA	10mM	90	6.8
	IBA + CA	10mM + 5uM	90	7.8
	NAA	10mM	100	6.22
	DMSO (ctrl)	n/a	0	n/a
OHxF 97	IBA	10mM	40	8.25
	IBA + CA	10mM + 5uM	40	4.75
	NAA	10mM	80	5.6
	DMSO (ctrl)	n/a	0	n/a
Hybrid	IBA	10mM	10	1
	IBA + CA	10mM + 5uM	10	1
	NAA	10mM	10	2
	DMSO (ctrl)	n/a	0	n/a

Table 3: Three Pyrus communis cultivars differ in rooting efficiency in response to different auxin-based rooting treatments. Average root number is averaged across plants that successfully rooted. CA: cinnamic acid, IBA: Indole-3-butyric acid, NAA: 1-Naphthaleneacetic acid. N=10 plants per treatment.

Objective 3: In-depth characterization and optimization of RCB plants (Year 3+)

3a. Determine gene expression and flowering responses to chemical-induction

Confirmed transformed plants will be allowed to grow until branches can support fruit weight. At this point we will characterize flowering gene expression and flowering responses to chemical induction in more detail. After spraying leaves with Mandipropamid, we will collect leaf and bud tissue and use quantitative PCR to determine gene expression levels compared with control genes and control tissues. We will observe timing of flowering as well as inflorescence and flower morphology. In citrus, the Cutler lab and collaborators have seen high levels of gene expression in response to chemical induction, as well as flowering occurring in the axillary bud associated with leaves sprayed after about 2-3 weeks. We will perform experiments to determine the optimal chemical doses (varying concentrations), the best way to deliver the chemical (varying addition of surfactant/wetting agents), and how timing of flowering and flower morphology respond to these different factors. Given difficulties in regenerating plants from transformed tissue (Obj. 1c), this work may begin towards the end of Year 3.

3b. Test the ability of induced flowers to be pollinated, develop fruit

In other RCB systems, continuous flowering often led to abnormal flower morphology, however in most cases flowers were still able to develop fruit and viable seed. While we hope to avoid these abnormal phenotypes with an inducible system, it will be important to test transformed germplasm to determine whether flowers are able to be pollinated, as well as phenotype fruit and seed development. We will induce multiple flowers per plant and observe stages of pollination, fruit set, fruit and seed development, and seed viability. In citrus, these tests were able to be performed in 1 year old transformed trees. This work will take place once we induce and characterize flowers, in Obj. 3a.

3c. Begin crossing with germplasm containing other desirable traits.

Once stable lines have been optimized and characterized, we will begin performing crosses with desirable germplasm. Initially, we will cross with fire-blight resistant germplasm identified in Objective 1a, containing additional sources of resistance to OHxF backgrounds. Because there are multiple sources of fire-blight resistance (*9-11*), we can perform multiple crosses to introgress fire-blight resistant traits. Future crosses include germplasm identified by the breeding program to show dwarfing traits, or accessions exhibiting resistance to other key pathogens or pests. This tool may also be of use to quickly generate mapping populations for identifying unknown genetic sources of desirable traits.

Future steps beyond the length of this proposal will be phenotyping for fire blight resistance, as well as other traits we may be crossing for. Whenever possible, we will used developed markers to assist in more rapid assessment of traits.

Results and Discussion

After our initial transformation attempts failed to produce transformed plant tissue, we wanted to test several hypotheses about what was missing or could be improved. One hypothesis was that when we added the Kanamycin-resistance gene to the RCB construct, something went wrong in the cloning process and it might not function properly. To test this, we chose to transform Arabidopsis with the RCB construct, as it would lead to a quick answer (only ~6-8 weeks to transform, collect seed, and test seedlings). This test resulted in multiple seedlings that were both resistant to Kanamycin, and contained the red fluorescent marker, signifying that our RCB construct was indeed functional.

A second hypothesis was that using Kanamycin is either too harsh or ineffective for pear tissue from these cultivars during the regeneration process. To address this second hypothesis, we developed a version of the RCB construct replacing the Kanamycin-resistance gene with the Hygromycin-resistance gene (*HPT*). Studies using pear callus tissue found it difficult to select with Kanamycin, while having success with Hygromycin (*4*). These same studies found that Hygromycin was associated with a higher rate of transformed pear callus, with highly reduced non-transformed tissue when compared with selection using Kanamycin (*4*). Further, Hygromycin is not known to interact adversely with any gelling agents, unlike Kanamycin (*5*). An alternative approach we will try with our Kanamycin constructs is to test a range of Kanamycin concentrations, as has been done in other pear cultivars and other tree crops (*2, 3*). We expect to begin transformations with the Hygromycin-resistant version in early 2023.

A third hypothesis was that we had not yet found the ideal conditions or parameters for transforming these cultivars. Only a few pear transformation protocols are published (*3, 12-15*), and these have largely focused on other cultivars. Further, all published protocols are quite lacking in detail, missing many key pieces of information to reliably reproduce them. The initial protocol we used was developed by our cooperators at the USDA-ARS Appalachian Fruit Research Station in Kearneysville, however while it is quite detailed, it focused on the Conference cultivar, which may help explain why we have not had success with it. Thus, we began to test different parameters present in all available protocols to determine optimized conditions, initially for Bartlett, but also in OHxF 87 97. While there is more work to be done, we found that the method of harvesting leaf material and wounding (using leaf discs harvested with a biopsy punch), method of agrobacterium removal post-inoculation (using both Timentin and Cefotaxime, adding them to the liquid co-culture), pre-soaking the leaf material, and the nutrient base for media (NN69) had the strongest effect on the number of transformed cells in callus tissue (Table 1). Future work will focus on regenerating tissue from these cells (see below).
Initially, we relied solely on the Kanamycin marker to select for transformed tissue, as none of the microscopes at our USDA location had the proper light setup to look for red fluorescence without removing plants from plates. However, part way through Year 2, we were able to find and purchase an affordable solution: an Xite Flourescence Flashlight emitting the proper wavelength to show the red fluorescence (510-540nm excitation), and a filter set to allow us to visualize and image the plants (600nm longpass filter). This allowed us to see that we were indeed transforming tissue, but it was remaining in the form of callus and not regenerating into plant organs (Fig. 2). We have transferred this callus onto new media with the intent to expand the amount of transformed callus that we have (Fig. 2). Once we have enough, we will attempt to regenerate shoots from the transformed callus. While the transformed callus is expanding, we will continue to experiment with callus induction and

regeneration in Bartlett, OHxF 87 and 97 leaf tissue in order to determine ideal hormone and media concentrations.

Our fourth hypothesis was that we had not yet found the ideal conditions for regeneration. This hypothesis is supported by the presence of red fluorescent callus but no shoot regeneration from the transformed tissue. Further, we found that in many of our transformation attempts, we were seeing leaf tissue become brown or necrotic and die. Both of these led us to test different regeneration parameters, including: different hormone concentrations of auxin and cytokinin, wounding methods such as leaf disks or slices across the leaf midrib, different soaking times in media rich solution or water to determine if extended soaking was causing necrosis, and type of cytokinin used for propagation prior to regeneration. These small-scale experiments have improved regeneration in control (non-transformed) tissue, from initial rates between 0-4% to now achieving rates up to 23% (Table 2). An exhaustive literature search suggests this can be further improved. A higher regeneration rate and better understanding of adventitious shoot regeneration should dramatically improve our ability to regenerate shoots from transformed callus.

References

1. S. Montanari, J. Postman, N. V. Bassil, D. B. Neale, Reconstruction of the Largest Pedigree Network for Pear Cultivars and Evaluation of the Genetic Diversity of the USDA-ARS National Pyrus Collection. *G3 (Bethesda)* **10**, 3285-3297 (2020).
2. Q. T. Fu *et al.*, An efficient protocol for Agrobacterium-mediated transformation of the biofuel plant Jatropha curcas by optimizing kanamycin concentration and duration of delayed selection. *Plant Biotechnol Rep* **9**, 405-416 (2015).
3. N. Matsuda, M. Gao, K. Isuzugawa, T. Takashina, K. Nishimura, Development of an Agrobacterium-mediated transformation method for pear (Pyrus communis L.) with leaf-section and axillary shoot-meristem explants. *Plant Cell Rep* **24**, 45-51 (2005).
4. M. L. Ming *et al.*, Highly efficient CRISPR systems for loss-of-function and gain-of-function research in pear calli. *Hortic Res-England* **9**, (2022).
5. E. Laine *et al.*, Gelling agent influences the detrimental effect of kanamycin on adventitious budding in flax. *Plant Cell Tiss Org* **63**, 77-80 (2000).
6. D. G. Gibson *et al.*, Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature Methods* **6**, 343-U341 (2009).
7. S. J. Clough, A. F. Bent, Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant Journal* **16**, 735-743 (1998).
8. S. E. Stachel, E. Messens, M. Vanmontagu, P. Zambryski, Identification of the Signal Molecules Produced by Wounded Plant-Cells That Activate T-DNA Transfer in Agrobacterium-Tumefaciens. *Nature* **318**, 624-629 (1985).
9. S. Montanari *et al.*, A QTL detected in an interspecific pear population confers stable fire blight resistance across different environments and genetic backgrounds. *Molecular Breeding* **36**, (2016).
10. A. Peil *et al.*, Improvement of Fire Blight Resistance in Apple and Pear. *International Journal of Plant Breeding* **3**, 1-27 (2009).
11. J. D. Zurn, J. L. Norelli, S. Montanari, R. Bell, N. V. Bassil, Dissecting Genetic Resistance to Fire Blight in Three Pear Populations. *Phytopathology* **110**, 1305-1311 (2020).
12. L. H. Zhu, M. Welander, in *Transgenic Crops of the World,* I. S. Curtis, Ed. (Kluwer Academic Publishers, 2004), chap. 16, pp. 217-228.
13. E. Chevreau *et al.*, Agroinfiltration is a key factor to improve the efficiency of apple and pear transformation. *Scientia Horticulturae* **251**, 150-154 (2019).

14. F. Mourgues, E. Chevreau, C. Lambert, A. de Bondt, Efficient Agrobacterium-mediated transformation and recovery of transgenic plants from pear (Pyrus communis L.). *Plant Cell Rep* **16**, 5 (1996).
15. Q. R. Sun *et al.*, High-efficiency and stable genetic transformation of pear (Pyrus communis L.) leaf segments and regeneration of transgenic plants. *Acta Physiologiae Plantarum* **33**, 383-390 (2011).

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

Project Title: Field evaluation and propagation of novel cold-hardy quince rootstocks

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Total Project Request: **Year 1:** $89,508 **Year 2:**$93,636 **Year 3:** $97,684

Other funding sources: **None**

WTFRC Budget: ***None***

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator: Dan Arp**
Telephone: 541-737-4866 **Email address: dan.j.arp@oregonstate.edu**
Station Manager/Supervisor: **Email Address:**

Item	2021	2022	2023
Salaries	8,000	8,400	8,820
Benefits[1]	6,800	7,140	7,497
Wages[2]	2,850	2,993	3,142
Benefits	285	299	314
Equipment			
Supplies	500	500	500
Travel[3]	2,172	2,192	2,213
Cold storage fees[4]	375	386	398
Plot Fees[5]	5,000	5,000	5,000
Total	25,982	26,910	27,884

Footnotes:
[1] Benefits were calculated from actual OPE rates (20% of OSU technician). An annual increase of 5% was applied to years 2 and 3.
[2] Wages are for part-time employee to help with general maintenance during the season; 190 hours at $15/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 (total $1,500) to travel to plots to perform pruning and training tasks and meet with K. Galimba and S. Musacchi and grower

collaborators (airfare was estimated at $750 roundtrip, three nights hotel ($100/night), car rental ($400) and per diem ($60/day).
[4] Cold storage fees are for 3 months at $125 per month with 3% annual increase.
[5] Plot fees are to compensate growers for land, resources and fruit.

Budget 2

Organization Name: WSU **Contract Administrator:** Kathy Roberts, Shelli Tompkins
Telephone: (509) 293-8803 **Email:** katy.roberts@wsu.edu, shelli.tompkins@wsu.edu
Station Manager/Supervisor: **Email Address:**

Item	2021	2022	2023
Salaries	$ 25,133	$ 27,339	$ 29,445
Benefits	$ 9,048	$ 9,842	$ 10,600
Wages	$ 6,000	$ 6,000	$ 6,000
Benefits	$ 1,345	$ 1,345	$ 1,345
Equipment			
Supplies	$ 9,000	$ 9,200	$ 9,410
Travel	$ 3,000	$ 3,000	$ 3,000
Plot Fees			
Miscellaneous			
Total	$ 53,526	$ 56,726	$59,800

Footnotes:
1 Salary for a 6 months of a Research assistant ($4,000/month) (Musacchi)
2 Benefit on salary at 36%
3 One non-student temporary for 10 wks: 40hrs/wk at $15/hr (Musacchi).
4 Benefits on temporary at 22.4%
5 Labware/consumable, fruit sample reimbursement (Musacchi)
6 5,217 miles/year for domestic travel (0.575$/mile) to go to the orchard.

Budget 3

Organization Name: North American Plants, Inc. **Contract Administrator:Yongjian Chang**
Telephone: 503-474-1852 **Email address:** ychang@naplants.com
Station Manager/Supervisor: **Email Address:**

Item	2021	2022	2023
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies[1]	$10,000	$10,000	$10,000
Travel			
Plot Fees			
Miscellaneous			
Total	$10,000	$10,000	$10,000

Footnotes:
[1]Consumables, reagents, nutrients, hormones, storage of cultures, pots, substrate, etc.

Significant Findings:

Objective 1: Continue to evaluate vegetative and fruiting performance of Bartlett and d'Anjou pear trees on nine quince rootstocks in current field performance trials (WA and OR).

- **Based on growth habit, vigor, canopy balance, precocity and production during the first three cropping years (2022 is the third crop), the vast proportion of these rootstocks continue to perform extremely well; four of these, which have produced highly uniform trees with excellent productivity, may in fact be of similar origin based on a 2022 finger printing analysis.**
- **Notable observations of the varying growth and balance of individual combinations suggest future examination of diverse interstem selections to improve compatibility. In the existing trials, Comice serves as an interstem given its generally good compatibility with quince; however, pear scions do differ in their relative compatibility with quince. Thus, the poor performance of a few rootstocks may in fact be attributed to interstem issues (i.e., with Comice). This is further supported by their differential behavior when direct-grafted to either Bartlett or Anjou.**
- **Our site selection facilitates a comparative analysis of the environmental effects on tree development. The marked climate and soil differences between sites resulted in a 50-100% larger tree (for any rootstock combination) in OR compared to WA.**
- **Tree pruning and training performed in spring 2022 produced narrow canopies of abundant fruiting limbs. Short-pruning of Bartlett trees in OR during 2021 and 2022 has corrected the canopy fruiting habit, previously compromised by omitting pruning in 2020 due to COVID; the fruiting canopy of these trees is now within the allotted space in the orchard. D'Anjou trees, surprisingly, are not exceeding their in-row spacing of 3 ft. A combination of short and long pruning has been applied to 'D'Anjou to accomplish this goal.**
- **Significant frost events and very poor pollination conditions occurred in both OR and WA in spring of 2022. Collectively, these had a negative effect on fruit size and set; however, the very high flower density on these dwarf trees (between 100 and 200 clusters per tree) likely facilitated relatively high yields (all things considered).**
- **High-performing 'D'Anjou' trees on size controlling quince rootstocks had 20 lbs of fruit per tree in WA, equating to ~ 50 fruit per tree or ~20 bins per acre at the tree density of the planting (1210 trees/acre). Fruit size for these combinations was quite good (box counts of 90 to 100), averaging 200g per fruit. OR data were still being processed at the time of this report.**
- **The yields of high-performing Bartlett trees on size controlling quince were higher than D'Anjou at 30 lbs per tree in WA, representing per acre yields of 36 bins. Fruit size, however, was very small (150 g per fruit) and was likely attributed to non-lethal but injurious weather events. OR data were still being processed at the time of this report.**

Objective 2: Determine the propagation potential of the remaining 11 cold-hardy quince clones that could not be tissue-cultured and successfully micropropagate them for new field performance trials

- **All cold hardy quince selections that were not previously tissue-cultured in 2021 (roughly half of the remaining 11) were successfully micropropagated from shoot tips in 2022. These represent diverse germplasm of cold hardy and plausibly dwarfing pear rootstocks and include the three hardiest quince taxa of the entire germplasm collection. We will proceed to rooting a sufficient number of each selection to facilitate new tree production for future field-performance trials.**

Results and Discussion:

Objective 1: Continue to evaluate vegetative and fruiting performance of Bartlett and d'Anjou pear trees on nine quince rootstocks in current field performance trials (WA and OR), and successfully micropropagate the remaining 11 cold-hardy quince selections for establishment in new field performance trials.

Given the similar performance of several rootstocks, leaves were collected from rootstocks suckers in WA and from all tissue culture jars at NAP. Material was sent to an external molecular laboratory specialized in fingerprint by SSR markers. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported to have some level of genetic similarity that must be investigated further in 2023. Until we do have confirmation, we will present the data for all 9 accessions since they have been assumed to be independent and all data have been collected accordingly. These accessions are identified in Tables 1 and 2.

Mortality

Mortality has been reported in previous reports as the average percent survival for each combination in which differentiated alive and struggling trees. 68.002 had the highest proportion of dead trees with both scions after approximately 4 years from planting (~50%). For high-performing combinations, significant changes in mortality between 2021 and 2022 were not observed at either site. Regarding combinations without an interstem, Anjou/99.002 (direct graft) had the highest incidence of tree failure (83%), while Bartlett/99.002 (direct graft) had 0% mortality in WA (data not shown). These data support a future evaluation of compatibility in order to determine the best interstem pear scions for these rootstocks.

Pruning

Dormant pruning of the Entiat, WA and Parkdale, OR plots was conducted in March and April 2022, respectively. The same methodology as reported in the previous years was executed in each plot. For Anjou, some significant differences emerged when comparing the average pruning weights (as kg per tree) among the 9 combinations in trial with Comice as interstem; Anjou/Comice/99.002 had greater than 2 kg per tree of pruned wood, which was significantly higher than all other combinations and agrees with trunk measurements. At the other extreme, Anjou/Comice/68.002 produced approximately half the pruning weights and also aligned with the tree size (as measured by trunks). In OR, pruning weights and trunk size were also the lowest for this combination. For Bartlett, no differences among combinations were observed for average pruning weight in 2022, but clear differences emerged for cumulative pruning weights over 5 consecutive years. Bartlett/Comice/65.001 was the most vigorous combination with nearly four-fold the pruning weights than the least vigorous combination, Bartlett/Comice/118.001 (Table 1). These extremes were also observed in OR, suggesting that despite vast differences in climate, the genotypes are performing similarly.

In OR, two years of corrective pruning was able to return the fruiting close to the central leader in Bartlett (Photo 1). Despite the characteristic vigor of Anjou, canopies have been maintained in a planar configuration with ample fruiting wood and do not exceed their allotted 3 ft. of in-row space (Photo 1).

Photo 1. Bartlett (left) and Anjou trees (right) in OR after April 2022 pruning.

Table 1: 2022 dormant and cumulative pruning weights (kg per tree) from 2018-2022 in Entiat (WA) for Anjou and Bartlett with Comice interstem on 9 different quince accessions (table sorted by cv and CYD acc.=rootstock). Combinations without interstem were excluded from statistical analysis

Cultivar	Rootstock	Interstem	Count of reps 2022	Pruned Weight (kg/tree) 2022		Pruned weight in 5 years 2018-2022 (kg/tree)	
d'Anjou	*22.001*	Comice	3	0.76	B	2.67	AB
	23.001	Comice	3	0.73	B	2.96	AB
	57.001	Comice	4	1.03	B	3.21	AB
	65.001	Comice	3	0.77	B	3.21	AB
	67.001	Comice	3	0.48	B	2.23	AB
	68.002	Comice	3	0.30	B	1.01	B
	70.001	Comice	7	0.86	B	2.14	AB
	99.002	Comice	3	2.07	A	3.92	A
	118.001	Comice	3	0.35	B	1.32	B
Significance				**		**	
Bartlett	*22.001*	Comice	7	0.79		3.19	ABC
	23.001	Comice	3	0.88		3.11	ABC
	57.001	Comice	3	1.06		3.82	AB
	65.001	Comice	3	1.20		4.29	A
	67.001	Comice	4	0.56		2.09	ABC
	68.002	Comice	3	0.46		1.28	BC
	70.001	Comice	4	1.12		3.12	ABC
	99.002	Comice	8	0.94		3.26	ABC
	118.001	Comice	6	0.25		0.99	C
Significance				NS		**	

The 4 rootstocks reported in italics font and shaded in the table are under investigation for fingerprinting analysis having shown in 2022 some level of similarities. Combinations on direct graft (interstem= none) have been excluded from statistical analysis. Significance: *, p<0.05, **, p<0.01, NS= not signifcant. Letters separate means for combination with interstem by SNK for (alpha=0.05).

Bloom

The number of flower clusters per tree counted in spring 2022 was considered excellent, with most combinations having between 100 and 200 clusters per tree in WA and 100 to 300 clusters in OR. No significant differences emerged among the 9 combinations, irrespective of cultivar, for bloom. Anjou produced, on average, 150 clusters per tree, which was slightly higher than Bartlett having, on average, 131 clusters per tree (Table 2).

Table 2: Primary and secondary bloom of Anjou and Bartlett trees grafted on 9 different quince accessions each with a Comice interstem, counted on April 18th May 20th, respectively in Entiat (WA). Combinations without interstem are not reported in this table.

Cultivar	Rootstock (CYD acc.)	Interstem	Count of reps 2022	N flower cluster/tree 4/18/22	N secondary bloom clusters/tree 5/20/22
d'Anjou	*22.001*	Comice	3	**226**	13
	23.001	Comice	3	**162**	6
	57.001	Comice	3	**116**	3
	65.001	Comice	3	**163**	8
	67.001	Comice	3	**148**	3
	68.002	Comice	3	**135**	3
	70.001	Comice	3	**103**	2
	99.002	Comice	3	**166**	6
	118.001	Comice	3	**133**	7
Significance				**NS**	**NS**
Bartlett	*22.001*	Comice	3	**169**	11
	23.001	Comice	3	**140**	8
	57.001	Comice	3	**108**	2
	65.001	Comice	3	**149**	6
	67.001	Comice	3	**135**	5
	68.002	Comice	3	**107**	3
	70.001	Comice	3	**91**	5
	99.002	Comice	3	**111**	9
	118.001	Comice	3	**169**	8
Significance				**NS**	**NS**

The 4 rootstocks reported in italics font and shaded in the table are under investigation for fingerprinting analysis having shown in 2022 some level of similarities. Combinations on direct graft (interstem= none) have been excluded from statistical analysis. Significance: *, p<0.05, **, p<0.01, NS= not signifcant. Letters separate means for combination with interstem by SNK for (alpha=0.05).

In the third week of April 2022, a severe cold event occurred accompanied by snow, a minimum temperature of -1.5 °C (Figure 1A) measured at 3 m from the ground for 5-hour duration. Likely, temperatures were even lower in the canopy. Several days later, on April 18th, when flower clusters were counted, phenology was between tight and loose cluster and necrosis could already be observed. (Figure 1C). On May 25th, the incidence of secondary bloom was assessed by counting the late bloom clusters on representative trees. The secondary bloom clusters were then pinched off trees to limit fire blight infection. As visible in Figure 1D, Anjou fruitlets displayed significant browning symptoms. Low temperatures of (1.5° F) in December 2021 would not likely have contributed to this injury; with respect to roots, whether analyzing temperatures for rootstock shank hardiness (ambient) or root systems (soil), there should also have been no injury as these accessions are capable of tolerating -22 F during endodormancy (Figure 1B).

Considering the spring frost events, visible injury to clusters, and relatively low temperatures during pollination (especially in OR), thinning (chemical or hand) was not applied to either orchard. Full bloom dates for Anjou and Bartlett were 24-April and 27-April in Entiat and 29-April and 1-May in Parkdale.

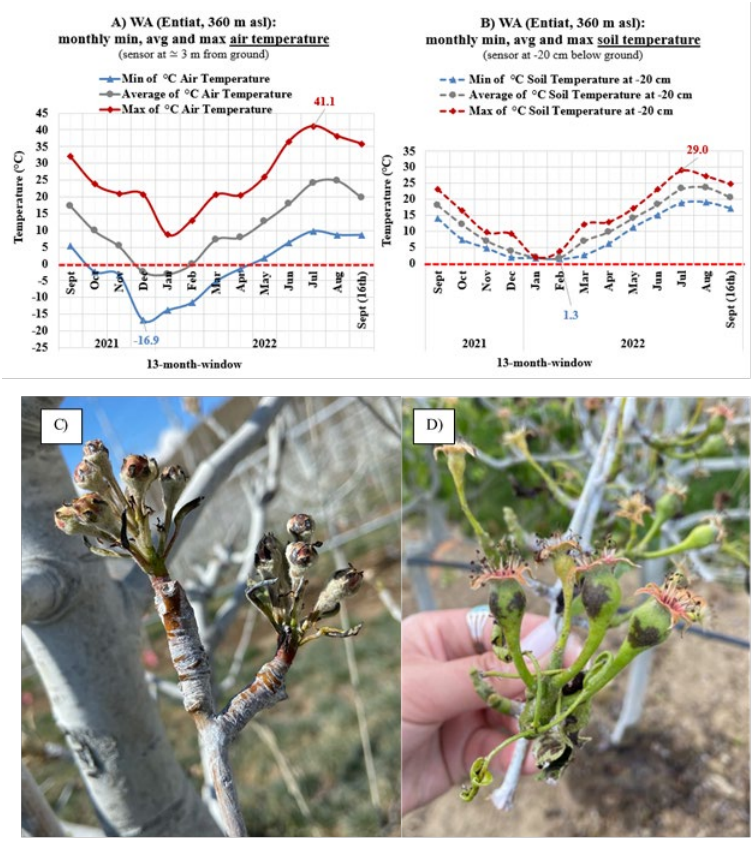
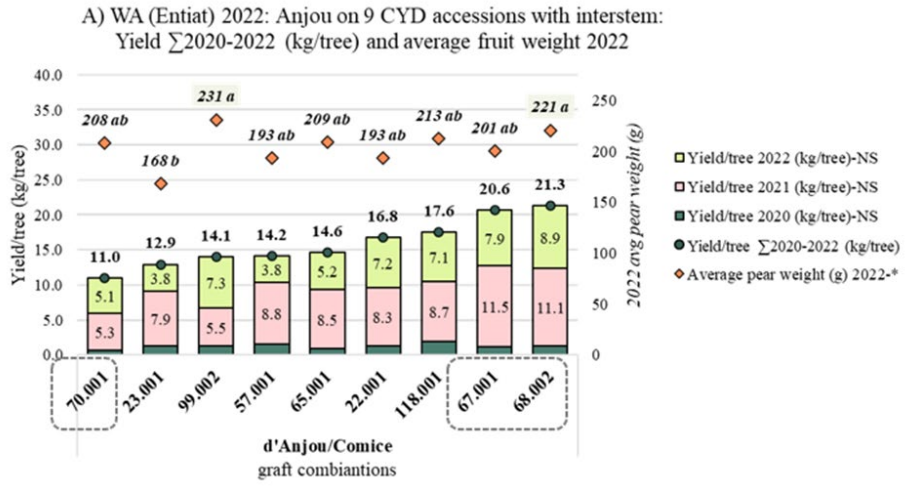


Figure 1. Air and soil temperature data at 3 m above ground and 20 cm below ground, respectively (A and B) in Entiat, WA. Cold injury on Bartlett (C)and Anjou (D) flowers and developing fruitlets on 15-April and 25-May, respectively.

Productivity

2022 was the third cropping year from orchard establishment. Anjou was harvested on 9/16/22 in WA with relatively low production per tree (avg. 31 pears/tree and 6.3 kg/tree) though the highest yielding combination produced 8.9 kg/tree (Figure 3 A), which equates to roughly 22 bins per acre. The only harvest parameter with statistical significance across combinations was the average weight of individual fruit; Anjou/Comice/99.002 and Anjou/Comice/68.002, had the greatest mass (231 and 221 g, respectively). Anjou/Comice/23.001 had low yield and fruit weight. Over the three cropping years, Anjou/Comice/68.002 and Anjou/Comice/67.001 accumulated the highest yields and Anjou/Comice/70.001 the least (Figure 3A). OR yield data is being prepared and will be presented at the review.

Bartlett was harvested on 9/9/2022 (135 DAFB). There were no statistical differences among combinations for any of the harvest parameters (Figure 3B). The average fruit weight was quite low for Bartlett (150 g; 135 box size) with approximately 85 pears per tree. Despite the lack of significance, Bartlett/Comice/70.001 tended to have the fewest number of pears per tree at harvest, and the highest average fruit mass (180 g, Figure 3B). For Bartlett, Comice/57.001 and Comice/68.002 were the most productive combinations and Comice/70.001 was the least, as similarly observed in 2021 (Figure 3 B). OR yield data is being prepared and will be presented at the review.



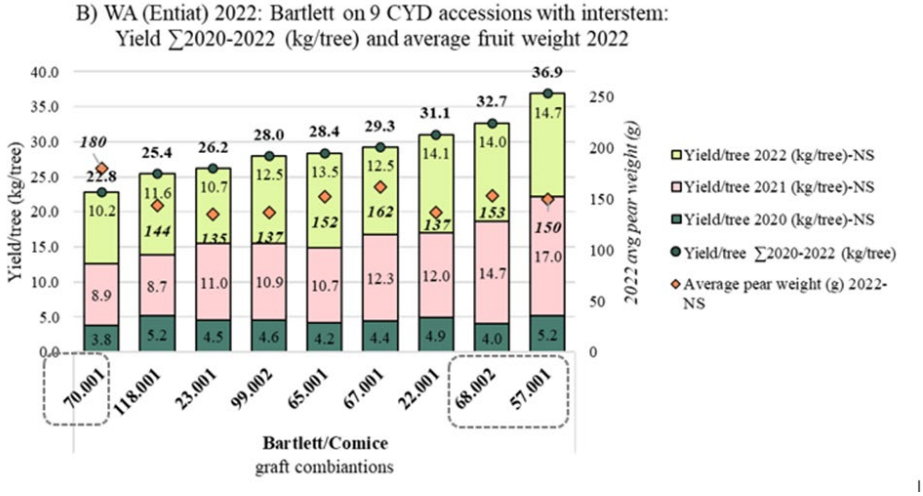


Figure 2*: Yield data in 2022 expressed as kg per tree and average fruit weight (g) for Anjou (A) and Bartlett (B) grafted on 9 different quince accessions in Entiat (WA). The chart is sorted by ascending cumulated yield/tree for each variety. Combinations without interstem (direct graft) were excluded from statistical analysis and not displayed here. NS= not significant differences emerged between the combinations for the indicated parameters (see legend).*

Fruit quality

Fruit quality data were collected at both sites but space limitations do not allow discussion or presentation of these data; any notable findings will be discussed at the 2023 Pear Review

Objective 2: Determine the propagation potential of previously identified cold-hardy quince clones not included in the field trial described above (a total of 11 accessions).

After several attempts (2021 and 2022) to establish cultures, NAP has successfully cultured all of the missing accessions where material still exists at the NCGR in Corvallis, OR (10 of 11 original accessions) in sufficient numbers to begin generating trees for future rootstock trials (Table 3). These include the top three cold hardy accessions previously not propagated due to challenges with media/material. Objective 2 is on schedule and tree production will begin spring of 2023.

CONTINUING PROJECT REPORT
WTFRC Project Number: PR-22-102

Project Title: Pear Rootstock Breeding

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Cooperators: Amit Dhingra (Texas A&M University), Jessica Waite (USDA-ARS Wenatchee, WA), Lauri Reinhold (USDA-ARS Corvallis, OR), Nahla Bassil (USDA-ARS Corvallis, OR), Stefano Musacchi (WSU-TFREC)

Total Project Request: **Year 1**: $100,592 **Year 2**: $101,401 **Year 3**: $101,025

Other Funding Sources

Agency Name: USDA-SCRI Coordinated Agricultural Project
Amount Pending: $5.6 million (2023-2027)
Notes: "Adaptation of the U.S. pome fruit industry to climate change" (PD: Lee Kalcsits; Co-PIs: Evans, Galimba, Einhorn, Moran, Rajagopalan)
Synergistic project to improve low temperature stress tolerance of pear rootstock during acclimation, dormancy, and de-acclimation.

Agency Name: Program Royalties
Amount Awarded: Ph.D. Research Assistantship (2019-2022) Zara York
Notes: "Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock breeding populations." (PI: Evans)

Agency Name: USDA-NIFA AFRI
Amount Awarded: Summer intern (2022) Edwin Polanco
Notes: "FACT: Research Experience for Undergraduates on Phenomics Big Data Management." (PI: Sankaran)

Budget
Organization Name: WSU-TFREC
Contact Administrator: Anastasia (Stacy) Mondy
Telephone: 509-335-4563
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Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@wsu.edu

Item	2022	2023	2024
Salaries[1]	$53,144	$55,270	$57,481
Benefits[1]	$17,507	$18,207	$18,936
Wages[2]	$6,955	$7,233	$7,522
Benefits[2]	$4,365	$4,539	$4,721
Equipment & Supplies (TFREC)	$12,890	$9,890	$5,890
Travel[3]	$3,080	$3,080	$3,080
Plot Fees	$2,651	$3,182	$3,395
Total	$100,592	**$101,401**	$101,025

[1]Salaries for research assistant professor (Teh) 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. Conduct marker-trait association for rootstock-conferred traits in seedling populations
3. Validate stability/repeatability of preliminary dwarfing locus
4. Maintain a relevant pear rootstock parent germplasm
5. Evaluate B × A and B × C selections

SIGNIFICANT FINDINGS

- Approximately 2,000 *Pyrus* seedlings were evaluated for scion and rootstock vigor traits in winter 2022/2023.
- 130 rootstock seedlings were rebudded with d'Anjou scions.
- Ten precocious seedlings that were previously micropropagated (10 replicates per seedlings) were transferred to the WSU TFREC for overwintering.
- All 37 B × A and B × C trees produced flowers in spring 2022; however, significant inflorescence damage from spring/summer frost resulted in limited fruit set (i.e., 31 fruit from 5 trees).
- Genetic maps were improved by incorporating additional genotypic data, resolving previous challenges of sizeable gaps.
- A previously identified dwarfing genomic region on chromosome 15 was validated using 2022 phenotypic data.

METHODS

Objective 1: Develop seedling populations to produce new rootstocks

Approximately 2,000 seedlings (budded with d'Anjou) segregating for vigor, precocity and other horticultural traits were established at the WSU Columbia View orchard in 2018, 2020, and 2021. Vigor/dwarfing potential of rootstock seedlings and scion traits were collected annually, as shown in **Table 1**. The most precocious individuals bloomed in spring 2021.

Many of these traits need to be evaluated for up to three more years (the timeframe of this proposal) to enable accurate selection.

Cross year	Number of seedlings	Existing data collection	
		Rootstock traits	Scion (d'Anjou) traits
2016	~600	Branch angle (2019) Presence of spine (2019) Trunk diameter (2020-2022)	Branch angle (2020-2022) Floral bud count (2021) Internode length (2020-2022) Scion growth (2020-2022) Trunk diameter (2020-2022)
2017	~320	Branch angle (2020) Presence of spine (2020)	Scion growth (2022) Trunk diameter (2022)
2019	~1,000	Branch angle (2022) Presence of spine (2022)	

Table 1: Existing data collection of various rootstock seedling and scion (d'Anjou) traits for breeding and selection.

We expect to be able to select seedlings with superior dwarfing potential and precocity to advance to 'Phase 2' in the final year of this proposal. These selections will be propagated and further tested in replicated plantings beyond the timeframe of this proposal. A final round of evaluation of elite selections is envisaged before final decisions are taken for wide-scale propagation (Figure 1). Selections will also be considered for inclusion in Rapid Cycle System, which is currently being built by Dr. Waite (USDA-ARS, Wenatchee).

In addition, these seedling populations are being leveraged through collaborations with Dr. Sindhuja Sankaran (WSU Department of Biological Systems Engineering) and Dr. Lee Kalcsits (WSU Department of Horticulture) to develop more efficient, reliable and accurate phenotyping of vigor/dwarfing traits.

Objective 2: Conduct marker-trait association for rootstock-conferred traits in seedling populations

This objective goes in tandem with the phenotypic traits from *Objective 1*, and builds on the existing groundwork accomplished. Previously, a pear genomic/genotyping tool (PI: Neale; "Development of marker-based breeding technologies"; PR-14-111) was utilized to develop high-resolution genetic maps (PI: Evans; "Pear Rootstock Breeding"; PR-19-108). These maps enabled marker-trait association analysis, which identified a novel preliminary dwarfing locus (i.e., genetic determinant) on chromosome 15. Continued close collaboration within the U.S. and international pear genomics community was fostered to facilitate cost efficiencies in genotyping analysis.

In this project, as additional years of more robust phenotypic data are collected, they will be analyzed on the completed genetic maps to identify other novel genetic determinants for dwarfing and/or precocity. Additional phenotypic data collected through collaborations with Dr. Sankaran and Dr. Kalcsits will be analyzed to uncover associated genetic determinants/loci. Identification of dwarfing determinants would facilitate more efficient future selection of dwarfing parental and seedling rootstocks.

This objective will be accomplished through continuing collaboration with national and international pear researchers to: (1) identify cost-effective measures for genotyping services, and (2) communicate standard operating procedures in preliminary steps of data curation – reducing duplication of efforts.

Objective 3: Validate stability/repeatability of preliminary dwarfing locus

In the previous project (PI: Evans; "Pear Rootstock Breeding"; PR-19-108), a preliminary dwarfing locus/determinant was mapped on chromosome 15 using one year of phenotypic data (i.e., total scion branch length). Building on the existing genotypic framework, additional years of more robust phenotypic data (as seedling trees age and mature) will be analyzed to validate the presence of this dwarfing locus. Phenotypes of more mature trees are needed to validate the stability/repeatability of the preliminary dwarfing locus. This analysis will also be validated in other populations. Furthermore, digital phenotypes from remote sensing tools will be analyzed to determine if a genetic locus was mapped to the similar position on chromosome 15.

Confirmation of dwarfing determinants would facilitate future development of DNA-based tools to select dwarfing parental and seedling rootstocks. In addition, we will continue to liaise with Dr. Waite (USDA-ARS, Wenatchee) regarding outputs from related transcriptomics studies and monitor new published relevant (i.e., dwarfing, precocity) markers to be tested in our parental germplasm and/or seedling populations.

Objective 4: Maintain a relevant pear rootstock parent germplasm

This objective builds upon the previous project (PI: Evans; "Pear Rootstock Breeding"; PR-19-108), where ten precocious seedling candidates were identified, selected, and micropropagated. In spring 2022, these individuals will be added to the pear rootstock parent germplasm at the WSU Sunrise orchard for future use as crossing parents.

In addition, we will continue monitoring partner programs (e.g., USDA National Clonal Repository Program, Corvallis, OR) and published literature for relevant germplasm to be added to (or removed from) our current parent collection.

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

Previously, seedlings from crosses of 'Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice' that exhibited dwarf seedling stature in the greenhouse were selected and replicated (PI: Dhingra; "Establishing NW-acclimated *Pyrus* Rootstock Breeding Material; PR-14-107). In 2017, a total of 14 selections in triplicate (approximately 45 trees) were planted at the WSU Columbia View orchard. (PI: Evans; "Pear Rootstock Breeding"; PR-15-105). These trees were budded with d'Anjou. Evaluation for dwarfing potential and precocity is ongoing. Trees are just starting to fruit with six accessions bearing fruit in fall 2021. Ten of the 14 accessions did not bloom in spring 2021.

In the next three years, more information on dwarfing and precocity will be collected to determine which rootstocks would be discarded based on low dwarfing potential and non-precocious bearing. In addition, yield, fruit size, texture and skin finish will be evaluated, as relevant.

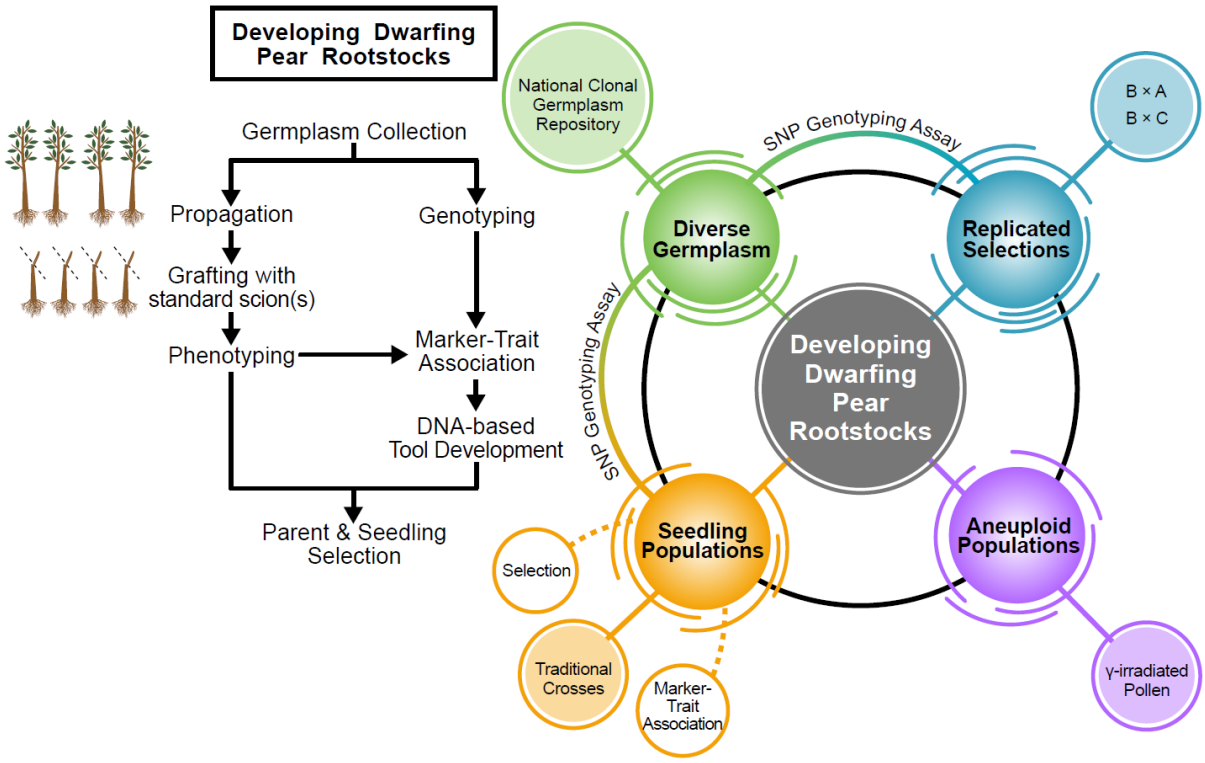

Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. Proposed endeavors include (a) expansion of existing seedling populations, (b) propagation of rootstock seedlings with 'd'Anjou', (c) collection of scion and rootstock phenotypic data, (d) DNA genotyping/sequencing, (e) construction of genetic maps, and (f) marker-trait association to identify DNA regions associated with dwarfing potential. Replicated aneuploid populations will be transferred from the Dhingra lab to the Waite USDA lab in 2022 (outside the scope of this proposal).

RESULTS AND DISCUSSION

Objective 1: Develop seedling populations to produce new rootstocks

Previously (PI: Evans; PR-19-108), 1,000 pear seedlings planted in spring 2021 were evaluated for rootstock traits during the 2021 growing season. They were budded with d'Anjou in fall 2021. Seedlings that failed to bud (about 10%) were rebudded in fall 2022. All successfully budded seedlings were evaluated for scion and rootstock vigor traits in winter 2022/2023 (**Table 2**).

In addition, ~600 and ~320 seedlings, planted in 2018 and 2020 respectively, were evaluated for scion and rootstock vigor traits in winter 2022/2023. We currently have 3 years of robust vigor data for the ~600 seedlings (oldest seedlings in the ground) to make selections by the end of 2024 for replicated evaluation in Phase Two, which is beyond the timeframe of this project.

Cross year	Number of seedlings	Phenotypic data collected in winter 2022/2023
2016	~600	Rootstock trunk diameter Scion growth Scion trunk diameter Tree height
2017	~320	Rootstock trunk diameter Scion growth Scion trunk diameter Tree height
2019	~1,000	Internode length Rootstock trunk diameter Scion growth Scion trunk diameter Tree height

Table 2: Phenotypic data of rootstock and scion (i.e., d'Anjou) traits collected for *Pyrus* seedling populations in winter 2022/2023.

Objective 2: Conduct marker-trait association for rootstock-conferred traits in seedling populations

Genetic maps provide the foundation for identifying genetic determinants of vigor/dwarfing traits. Previously (PI: Evans; PR-19-108), we developed genetic maps for two sub-populations. However, the maps in one sub-population contained sizable gaps that were problematic for marker-trait association. In October 2021, additional DNA samples were genotyped with the SNP array to improve these problematic maps. This year, we incorporated the genotyping data and developed revised genetic maps, thereby resolving the sizeable gaps. This improvement is critical to the breeding program, enabling us to identify genetic determinants of vigor/dwarfing and potentially improve breeding efficiency.

Objective 3: Validate stability/repeatability of preliminary dwarfing locus

In our previous preliminary work (PI: Evans; PR-19-108), we detected a dwarfing locus on chromosome 15 that needed additional years of phenotypic data for validation. With improved maps from Objective 2 and additional phenotypic data from winter 2022, we have validated the presence of the chromosome 15 dwarfing locus. We are liaising with Dr. Waite (USDA-ARS, Wenatchee) to add precision to the DNA region associated with dwarfing, with the potential of developing markers for breeding use.

Objective 4: Maintain a relevant pear rootstock parent germplasm

In our previous project (PI: Evans; PR-19-108), ten precocious seedling candidates were identified, selected and micropropagated (10 replicates per seedling). In summer 2022, replicated rooted seedlings were received at WSU TFREC. As most of the replicated seedlings were tiny and lacked chilling requirements, they are currently overwintering in the WSU TFREC hoop house, protected with straw mulch.

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

In 2017, seedlings from crosses of 'Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice' of short rootstock stature in the greenhouse were selected, replicated, and planted at WSU Columbia View orchard. In our previous project, we determined that rootstock stature (i.e., dwarf) was not correlated with vigor (or dwarfing). Beginning spring 2021, precocity data were collected, and basic yield information was collected (limited fruit in 2021 fall).

In spring 2022, all 37 B × A and B × C trees produced flowers, with about 40% having over 30 flower clusters. However, significant inflorescence damage from spring/summer frost was observed, resulting in limited fruit set (i.e., 31 fruit from 5 trees) in fall 2022. These trees will continue to be evaluated for precocity and fruit set. By the end of 2024, rootstocks of low dwarfing potential and non-precocious bearing will be discarded. In 2023 and 2024, accessions with precocious bearing and dwarfing capacity will be evaluated for yield, fruit size, texture, and skin finish, as relevant.

OUTREACH

- Soon Li Teh presented "WSU pome fruit breeding program" for Crop and Soil Sciences Graduate Student Statewide Tour at Wenatchee, WA on May 11, 2022.
- Kate Evans presented "WSU pome fruit breeding program" for WSU Research & Extension Experience Undergraduate Introductory Symposium, on-line on June 8, 2022.
- Edwin Polanco presented a poster entitled "Automated image processing of pear rootstock seedling vigor using PlantCV" at the WSU Research & Extension Experience Undergraduate Final Symposium, Pullman, WA on July 29, 2022.
- Soon Li Teh gave an "Overview of WSU pear rootstock breeding program" during the International New Variety Network nursery group field visit at WSU Columbia View orchard, Wenatchee, WA on September 2, 2022.
- Soon Li Teh gave an "Introduction to WSU pear rootstock breeding" to the Sunrise Rotary Club at Wenatchee, WA on January 3, 2023.
- Soon Li Teh presented "Pear rootstock breeding in the U.S. Pacific Northwest" at the XIV International Pear Symposium at Stellenbosch, South Africa on January 26, 2023.
- Soon Li Teh presented "Updates and progress of WSU pear rootstock breeding" during the Northwest Wholesale Cashmere grower meeting at Cashmere, WA on January 31, 2023.