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

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Report Type: Continuing Project Report

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: Pays research assistant professor (Nottingham) salary

WTFRC Budget: none

Budget 1:
Primary PI: Louis Nottingham
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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:

- **Obj. 1. Pesticide Database:** Results from past research and new experiments conducted in our lab have been compiled into a database and used to build a phenology spray program based on life-stage efficacy and non-target effects. Top candidate materials include Surround (kaolin), Esteem (pyriproxyfen), Ultor (spirotetramat), Centaur (buprofezin), Dimilin (diflubenzuron), AzaDirect (azadirachtin), and Cinnerate (cinnamon oil) due to demonstrated efficacy when targeting adults and eggs, while having minimal impacts on natural enemies.
- **Obj. 2. Surround Timing:** The most effective timings for Surround applications were delayed dormant and budburst . Fall surround sprays provided intermediate efficacy and petal fall sprays had no efficacy on 1st and 2nd generation.
- **Obj. 2. Honeydew Washing:** A honeydew washing threshold was established: 30% of leaves with visible honeydew droplets. The sample size to accurately monitor for honeydew threshold was 7 shoots, with at least 10 leaves per shoot, per orchard zone (2-4 ac. approximately).
- **Obj. 3. Psylla Phenology IPM program development:** A spray program was devised involving selective insecticides Surround, Esteem, Centaur, Dimilin, Ultor, AzaDirect, and Cinnerate at optimal timings (some timed to psylla degree days, others to tree phenology).
- **Obj. 3. Psylla Phenology IPM program testing**. The phenology program was tested in four orchards in 2021. Phenology orchards had significant fewer psylla and more natural enemies compared with conventional programs. The phenology program will be tested in least six commercial orchards in 2022.
- **Obj. 3. Extension:** The psylla phenology model and spray recommendations were broadcasted to growers across the northwest throughout the 2021 season via the pear IPM listserv (134 stakeholders) and the Fruit Matters Extension Newsletter. In 2022, the model and recommendations will be available on DAS (https://decisionaid.systems/) and via the Tree Fruit Extension Pear IPM website.

METHODS AND RESULTS:

Obj. 1. Build a pesticide effects database.

Methods: Pesticide effects of various materials have been made into an Endnote/Mendeley database. The primary purpose of this was to compile sufficient data on available pesticides to understand their effects on specific life stages of pear psylla, and if non-target effects on natural enemies may be a concern. Sources used include peer reviewed publications, editor reviewed publications (i.e., the journal, Arthropod Management Tests), and unpublished works such as reports

to the Washington Tree Fruit Research Commission and reports from private research firms. We have also conducted new tests over the past two years to confirm effects on pear psylla and natural enemies for insecticide candidates of high interest. Primary insecticides include Surround (kaolin), Ultor (spirotetramat), Esteem, (pyriproxyfen), Dimilin (diflubenzuron), AzaDirect/Neemix (azadirachtin), and Cinnerate (cinnamon oil).

Results: *Surround:* Literature on Surround suggests that it is best used prior to adult colonization and again within 25% of egg-lay. While it can reduce nymph development as well, it is most effective at preventing egg-lay. Our studies also confirmed this, showing that pre-oviposition sprays lead to 80-100% control, while post oviposition sprays provide 30-50% control. Therefore, surround timing will target pre-oviposition and early oviposition timings.

Pesticides: Past research suggests that each of the candidate insecticide materials named above have modest suppression of pear psylla life-stages with no impacts on natural enemies, which is optimal for season-long pear psylla management (Burts 1983, DuPont et al. 2021). Some insecticides such as Dimilin and Esteem also suppress other pests like codling moth, scale, mealybug, and leafrollers. In 2021, we conducted additional experiments to examine the impact of materials on natural enemies to confirm their low risk to biocontrol. Our experiments confirmed that the insecticide materials above had no impacts on the predators tested (lad beetles and earwigs). Esteem was shown to work best on adult psylla, causing them to lay sterile eggs (Higbee et al. 1995). Dimilin (Burts 1992) and Ultor (Beers and Greenfield 2015; PC Fruit, unpublished) both are thought to effect young nymphs; however, studies suggest that spray timings should aim for early egg developmental, possibly so the materials are present when nymphs hatch. From our own work, AzaDirect and Cinnerate are effective on all life stages, but provide the best control when timed with adults or eggs. Because most of these materials are best timed at early egg development, leniency in choosing among these materials is acceptable, as long as timings and labels are followed. <u>The detailed spray program and model are shown in Objective 3.</u>

Obj. 2 Enhancing the management program with cultural techniques.

2a. Surround Timings:

Methods. Surround was applied to small replicated plots at various timings in the fall of 2020 and spring of 2021 to determine optimal timings. Each timing was considered a treatment, and received five replicate 4-tree plots at either the Wenatchee (TFREC) or Rock Island (Sunrise) orchard (10 replicates, 40 trees per treatment timing, total). Treatment timings were fall (10 Nov.), budbreak (4 Mar.), budburst (30 Mar.), 60% petal fall (21 Apr.), and an untreated check. Psylla adults, eggs and nymphs were sampled throughout this timeframe when each life-stage was present.

Results. Results for the effect of each Surround timing on psylla life stages are shown in Fig. 1. Surround applied during the fall did not show a noticeable effect on psylla adults at the TFREC, and had a modest reduction in adults compared with the check at Rock Island (Sunrise). Fall Surround significantly reduced eggs and nymphs compared with untreated checks at both sites. Surround at budbreak ("Spring") reduced adults, eggs, and nymphs substantially at both sites. Surround at budburst was too late to reduce winterform adults and did not reduce summerform adults, but it did significantly reduce eggs and nymphs similar to the budbreak timing. Surround applied at petal fall was also too late to reduce winterform adults and eggs, and did not reduce nymphs or summerform adults.

Overall, early spring (budbreak) and budburst timings were most effective at suppressing the first generation of pear psylla. Fall timings could help improve outcomes if the early spring spray is not realistic, such as on steep and wet terrain. Two timings will provide optimal control, preferably early spring and budburst , or Fall and budburst when early sprays are not possible.

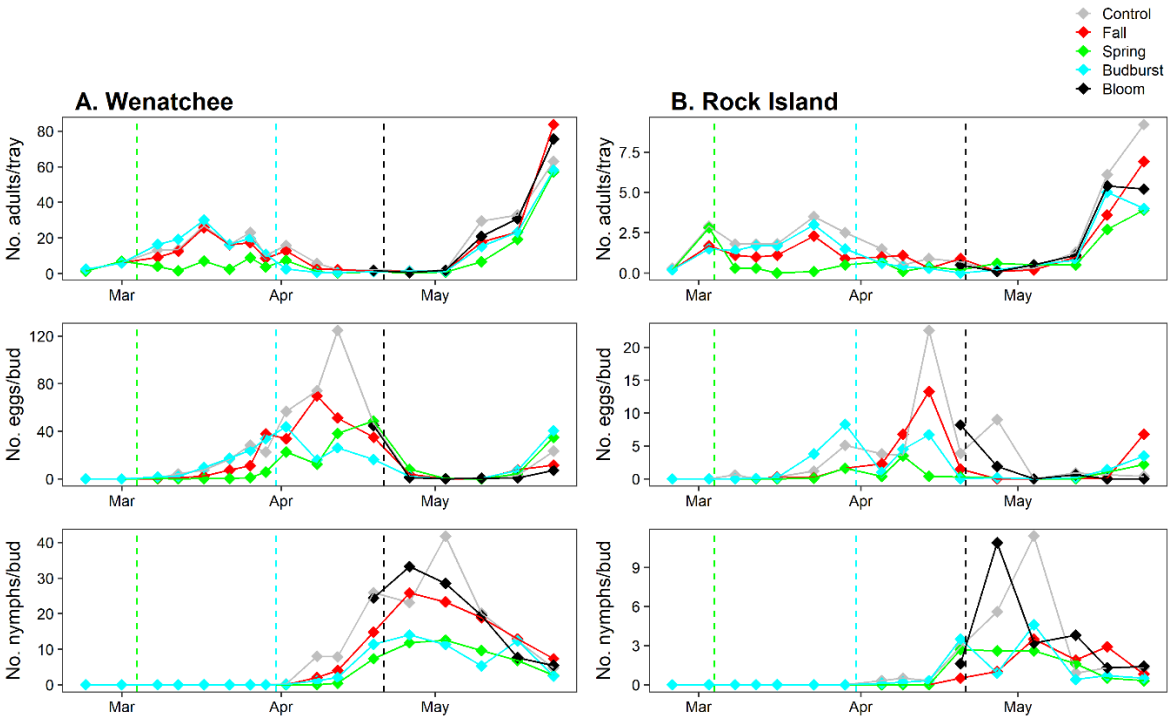

Fig. 1. Pear psylla adults/tray, eggs/bud, and nymphs/bud in Surround timing treatments: Fall (10 Nov.), Spring (budbreak, 7 Mar.), budburst (30 Mar), bloom (60% petal fall, 21 Apr.). Dashed vertical lines represent spray timings (Fall not shown), and colored lines are psylla average densities.

2b. Honeydew Washing Timing:

Methods: An experiment was conducted to establish honeydew injury thresholds to determine washing timings, based on leaves with visible honeydew droplets. The % 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 selected, on which 10 leaves and 20 fruit were sampled for presence or absence of honeydew. The percentage of honeydew affected leaves/100 leaves and fruit/200 fruit was determined. In orchards where honeydew reached high levels on fruit (greater than 5%) the honeydew % on leaves the previous week was considered for threshold.

A second experiment was conducted to determine the appropriate number of shoots and leaves to be visually sampled for threshold monitoring (% honeydew affected leaves). One shoot with at least 10 leaves was collected for 100 trees at 6 orchards (600 shoot and trees). Tree within and orchards were chosen randomly and spaced evenly, spanning orchards zones of 2-4 acres. 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 # 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 % honeydew affected leaves and error as sampling 100 shoots).

Results. Percentage of honeydew affected fruit rose in conventional orchards in week 8, hitting 20% followed buy over 30% in week 9. IPM and organic orchards maintain low levels, below 5% of honeydew affected fruit throughout the summer. 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 37%, suggesting that the visual threshold is between 20 and 37%. Therefore, our honeydew washing threshold is 30% of leaves with visible honeydew droplets. This study will be repeated in 2022 to challenge our findings.

Between five and 10 shoots per orchard area provided the same results as sampling 100 shoots, therefore, 7 was established as the minimum # 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".

To summarize, 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.

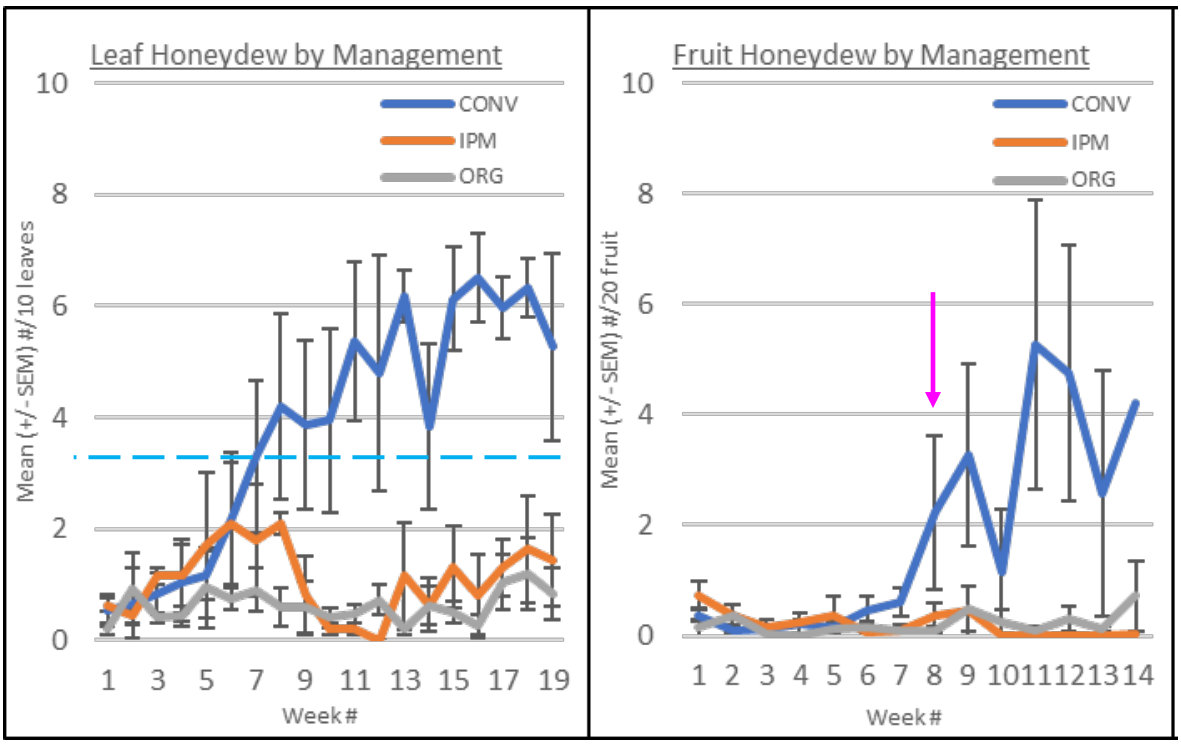

Fig. 2. Left: Mean (+/- SEM) # of leaves with visible honeydew bubbles/10 leaves from 10 trees/orchard/week. Right: Mean (+/- SEM) # fruit with visible honeydew/20 fruit from 10 trees/orchard/week. Pink arrows show where fruit injury signficantly increases (week 8). Blue dashed line shows the level of honeydew on leaves (measured in # 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, Dimilin, Esteem, Ultor, azadirachtin, Cinnerate and/or oil at strategic timings. The program was developed using a wholistic approach that not only aligned materials with their best life stage target, but also considered elements like costs savings, potential non-target effects, vulnerable tree stages, convenience (i.e., grouping materials into single sprays when possible), logical constraints (avoiding bloom or use of particle films too late in the season) and label restrictions (minimum intervals between spray and seasonal timing restrictions).

Timings for tree washing and pruning were not examined in 2021 experiments, however, PDD timings were devised based on logical understanding of the system, and washing can now be proscribed based on the scouting threshold information described in objective 2b.

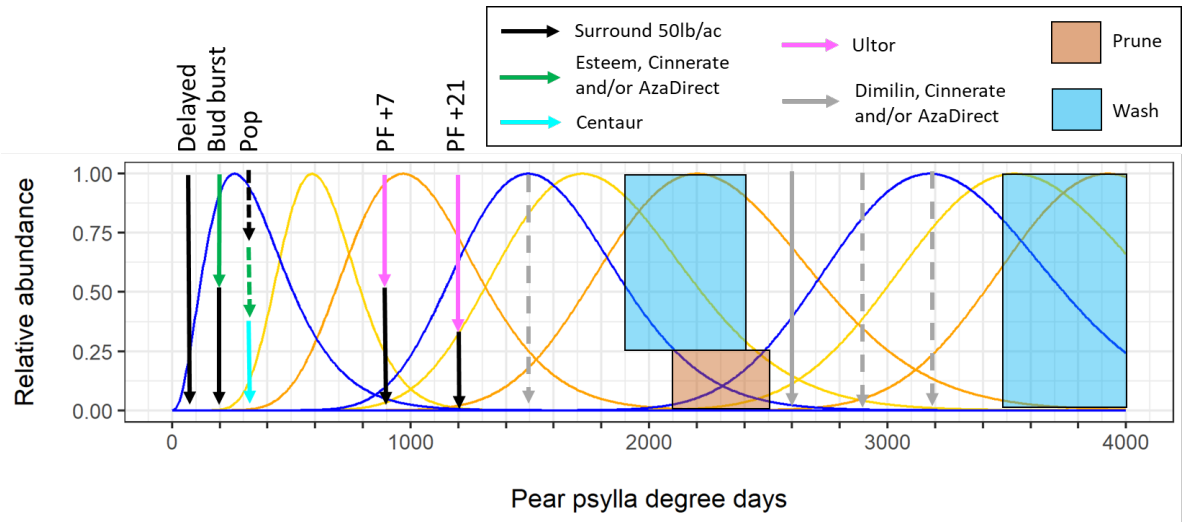

Fig. 3. Pear psylla degree day (PDD) model with overlayed management recommendations. Solid line arrows indicate "mandatory" sprays (recommended timings regardless of psylla pressure), dashed lines are for high pressure areas and/or years, and blocks are timeframes for cultural techniques. Closest phenological times are shown above the first five timings: delayed dormant, budburst , popcorn, petal fall + 7 days and petal fall + 21 days. *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.

Results: The psylla degree day model is displayed in Fig 3 with recommendations overlayed. The phenology model-based recommendations involve 6 "mandatory" sprays with 3 optional sprays (necessary if pressure is high). Most of the sprays for psylla are prophylactic, targeting adult and eggs to prevent outbreaks ahead of time. If timing cannot be hit perfectly, spraying early is better than spraying late.

Prebloom: The first two Surround sprays occur at 75-100 psylla degree days (PDD) and pre-budburst (approx. 200 PDD). The second spray should be timed with the tree's phenology at just before budburst. Psylla egg lay will rapidly increase once buds open, since adults target the new plant tissue. Surround will repel adults from the trees and Esteem will sterilize any adults that are not repelled (Higbee et al. 1995). While not preferred, Bexar can be used in high pressure situation at the pre-budburst time if pressure is very high. One spray of this material is unlikely to cause season-long disruption of natural enemies, and this timing will provide exceptional control of the first generation of psylla due to high efficacy on adults and eggs. A third Surround is optional if pressure is high, but is likely unnecessary if thorough coverage is achieved with the first two sprays. Centaur (buprofezin) should be used to control mealybug and pear psylla just before bloom (popcorn).

Postbloom: Two more Surround sprays should be applied after bloom, at 900 and 1200 PDD, to prevent colonization and oviposition of the second adult generation (summerforms). Even if adequate control of the first generation is achieved, adults will recolonize from other areas and natural enemies are not yet robust enough to provide full control, so measures are necessary. The 900 and 1200 PDD sprays should also include Ultor. This systemic insecticide is most effective on young nymphs as they hatch. Unlike Esteem and Dimilin, it has more restrictions on timings and therefore needs to be timed with the second nymph generation (Ultor cannot be used before petal fall and because it moves systemically through the whole tree, it works best before excessive vegetation is present). Bioassays from our lab and others' show that Ultor is best timed with first eggs (900 PDD), then again after 14 days (minimum allowed interval between sprays).

Summer: While there is less information Dimilin timings, studies show that is likely to effect adults, eggs, and young nymphs, so it can be used at similar manner as Esteem and Ultor (Burts 1983, 1992). We have placed it as a possible spray on the second nymph generation around first codling moth cover, and in the third generation to keep psylla suppressed while also aiding control of codling moth. Codling moth resistant management suggests that materials should not be used across generations, so products should be chosen with this in mind (i.e. if Dimilin is used at 1500, Cinnerate or AzaDirect should be used for the 3rd generation of psylla). It should also be noted that Altacor is very effective on codling moth and soft on natural enemies. Mating disruption and one or two Altacor sprays may be sufficient to control codling moth.

Organic options: AzaDirect and Cinnerate have been shown to affect both adults and immature psylla, reducing survival by approximately 40-60% when more than one spray is applied on a 1-2 week interval. AzaDirect and Cinnerate can either precede, proceed, or be combined with the Esteem and or Ultor timings to improve efficacy. Organic orchards will use these materials in place of Esteem and Ultor, and will need at least three sprays per the first two psylla generations if pressure is high.

Cultural controls: Honeydew washing via overheads or airblast should occur on the 2nd and/or 3rd psylla nymph generation; the first generation may be too early, resulting in fire blight outbreaks. However, if honeydew is exceptionally high during the 1st generation and a wash would help, washing may be performed more safely if temperatures are low and if a fungicide/bactericide labeled for fire blight is used prior to washing. Washing should occur between 1900 and 2400 PDD for the 2nd generation, and 3500 and 4000 PDD for the 3rd generation. These timings are when psylla are transitioning from young and old nymphs, so honeydew is accumulating rapidly. It is important to scout through this period to ensure that a wash is necessary: if 30% of leaves have visible honeydew bubble, a wash should be performed (see Obj. 2b).

Pruning should be timed between 40-70% of the old nymph development curve (2100-2500 PDD). Much of the psylla population can be eliminated by pruning at this time because they are almost all immatures attached to the vegetative shoots which will removed. Few adults that could avoid pruning are present. Washing ahead of pruning (at 1900 PDD) will reduce the chance of honeydew from earlier nymphs causing damage, and will make the pruning process more pleasant (less sticky) for workers.

Costs: Costs of insecticides and other products were obtained from distribution warehouses and verified by local crop advisors. Spray records from commercial growers in Wenatchee were obtained from T. DuPont, and a medium cost program (one not using mating disruption) was used for comparison to the devised phenology model program (table 1). The phenology model program used to compare costs was also in the middle of the cost spectrum, using sprays for multiple "optional" times, and included other pest controls such as mite sprays, codling moth sprays, and mating disruption. The phenology model program was $230/ac less expensive than the conventional program.

3b. Model Recommendation Trials in Commercial Orchards:

Methods: Testing the phenology model program in commercial blocks began a year earlier than expected, due our collaboration with Tianna DuPont's Extension program and a USDA NIFA Crop Protection and Pest Management Grant. DuPont was in the fifth year comparing IPM, conventional, and organic pear management programs for control of pear psylla and natural enemies. Because the phenology model program fit the criteria for IPM (avoiding broad spectrum insecticides) she allowed us to guide her IPM sites with model-based recommendations.

Each orchard block was approximately 4-6 acres. The phenology model program was tested on 4 blocks, and compared with 3 organic and 3 conventional blocks. The model program followed the recommendations listed above (at least 5 of 6 mandatory sprays were used), while conventional block programs were supplied by local crop advisors and used upwards of 15 broad spectrum insecticides for pear psylla. Organic orchards used only organic insecticides. Sampling of pear psylla, other pests, and natural enemies was performed weekly from April through Nov 1, 2021. Honeydew on leaves and fruit was rated weekly beginning in May and continued until harvest.

Results: The phenology model program (IPM) resulted in consistent control of pear psylla, keeping populations below the treatment threshold of 0.3 nymphs/leaf throughout the season (Fig. 4). Natural enemies in the phenology model program were conserved similar to organic plots, and were significantly greater than conventional plots throughout the season. Overall, the program proved highly effective in this first year, and will be tested again in at least six plots, with equal number of conventional comparison plots.

Table 1. Cost summary example phenology management and conventional pear programs, 2021.

PDD	Phenology program	Cost/ ac
75-100	Surround	$60
	Lime sulfur (mites)	$40
200	Surround	$60
	Esteem	$50
pre-Budburst	Centaur	
(350)	(psylla/mealybug)	$65
bloom	mating disruption	$120
	(codling moth)	
900	Surround	$60
	Ultor	$60
	Altacor (codling moth)	$60
1200	Surround	$60
	Ultor	$60
	Dimilin (codling moth)	$60
2600	Dimilin	$60
2900	Dimilin	$60
	Nealta, Vendex, or	
if necessary	Envidor (mites)	$50
	Phenology total cost/acre:	**$925**

Date	2021 Conventional Program	Cost/ ac
3/25/21	Surround	60
	Lime Sulfur	40
	Lorsban	15
	Malathion	15
4/15/21	Surround	60
	Cormoran	75
5/3/21	Manzate Pro-Stick	15
	Cormoran	75
	Ultor	60
	Agri-Mek	15
	Bexar	70
5/27/21	Bexar	70
	Ultor	60
	Actara	30
6/15/21	Centaur	65
7/7/21	Assail	50
	FujiMite	50
	Centaur	65
7/20/21	Delegate WG	75
	Nealta	50
	Vendex	55
8/2/21	Delegate	75
	Macho	10
	Conventional total cost/acre:	**$1,155**

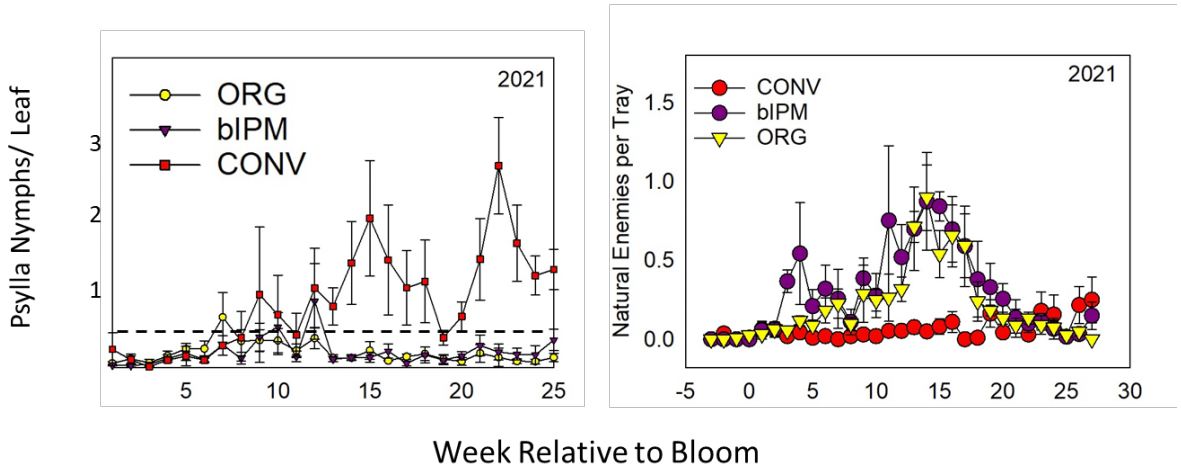

Fig 4. Pear psylla and combined natural enemy densities in commercial orchard blocks following either the phenology model-based program ("bIPM"), conventional, or organic management. The dotted line is the treatment threshold of 0.3 psylla nymphs per leaf.

REFERENCES CITED:

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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.

Project Title: Improving pear pest management with integrated approaches

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Report Type: Final Project Report

Project Duration: 3-Year

Total Project Request for Year 1 Funding: $110,363
Total Project Request for Year 2 Funding: $114,718
Total Project Request for Year 3 Funding: $119,247

Other funding sources: Awarded

Funding Duration: 2020-2022
Amount: $323,622
Agency Name: USDA NIFA CPPM

Funding Duration: 2020-2021
Amount: $23,652
Agency Name: Washington State Commission on Pesticide Registration

Funding Duration: 2020-2023
Amount: $249,926
Agency Name: WSDA Specialty Crop Block Grant

Funding Duration: 2020-2023
Amount: $348,733
Agency Name: Western SARE

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Station manager/supervisor email address: cekruger@wsu.edu

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

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

Objectives:

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

Significant Findings and Accomplishments (2021-2022):

- Numerous organic, selective conventional, and broad-spectrum conventional insecticides were examined for direct mortality and sublethal effects on natural enemies.
    - Altacor, AzaDirect, Celite, Centaur, Cinnerate, Esteem, Surround, Ultor had no mortal effects on natural enemies.
    - Actara, Admire, AgriMek, Assail, Bexar, Delegate, and Rimon caused moderate to high mortality in most, but not all assays.
    - Malathion caused high (near 100%) predator mortality in all assays.
    - Bexar consistently reduced activity (distance traveled) in individuals that survived sprays. IGRs and AgriMek affected activity in some assays, but results were not consistent. *Not all materials were tested for sublethal effects.
- Surround, Celite, Microna, and Cocoon did not significantly reduce survival of pear psylla young nymphs when sprayed over young nymphs (however, all did in 2020). In 2020, Surround and Celite provided 95-100% reductions of psylla oviposition, relative to checks, which was more effective than Microna (60% reductions).
- Earwig releases in conventional orchards using broad spectrum insecticides did not establish; however, in selective (soft) conventional orchards, earwigs increased significantly following releases. This may have occurred regardless of releases, as control sites (no releases) within selective conventional orchards experienced similar increases in earwigs.
- Mylar and Extenday suppressed pear psylla population by approximately 50% in organic commercial orchards. Extenday's effects were longer lived than mylar, likely due to durability and placement in the center of drive rows where it is not shaded. Both appeared to increase pear yield according to the grower (not officially measured).
- Bexar LC_{50}s for psylla nymphs and adults were determined for five colonies established in 2020. The average LC_{50}s of all colonizes were 129 mg (AI) /liter (H_2O) for young nymphs and 102 mg (AI) /liter (H_2O) for adults. The field rate (27 fl oz/ acre) at 100 gpa equals 339 mg (AI) /liter (H_2O), for comparison.
- **<u>Funding Leveraged Using Data from this Project</u>**:
    - USDA NIFA Crop Protection and Pest Management Grant, "Expanding the Pear IPM Toolbox", 2020-2022: **$323,622**.
    - WA State Commission on Pesticide Registration, "Pear psylla baseline toxicity to Bexar (tolfenpyrad) and non-target effects", 2020-2021: **$23,652**
    - WSDA Specialty Crop Block Grant, "Developing a phenology-based management program for pear psylla", 2020-2023: **$249,926**
    - Western SARE, "Wigging out, then wigging in: Earwig capture and augmentation for biocontrol in pears and apples", 2020-2023: **$348,733**

<u>Obj. 1</u>. Determine lethal and sublethal effects of common insecticides to psylla natural enemies.

Methods:

Lady beetles and earwigs were collected from unsprayed Bartlett and Anjou trees at the WSU Tree Fruit Research and Extension Center near Wenatchee, WA from June 2021-September 2021. Field collected natural enemies were exposed to pear leaves with pesticide residues individually at the maximum allowable field concentrations or a control treated with pure water in clean 1 oz solo cups (Fig 1). For each treatment and predator, there were at least 6 replications each with 5 individuals per rep (30 individual/treatment/experiment). A damp cotton wick was added to cups with survivors after 24 hours of exposure to prevent desiccation. Pesticide exposures consisted of either 1) direct spray, 2) high load residues on soaked and dried filter paper, or 3) a pear leaf dipped into the pesticide mixture and cut into five ≤1 ¼-inch diameter leaf discs. Leaf disk methods were also used to test residues at increasing time intervals following treatment. Mortality was documented in direct spray trials after 48 hours, and in residue exposures every 24 hours until hour 144. Insect species that exhibited mortality above controls from direct exposure at 0 hours of pesticide aging were subsequently exposed to aged residues to determine susceptibility to insecticides. If no mortality occurred from a material following direct contact, aged residues were not tested.

Fig 1. Predator bioassay arena. Dead earwig, dipped pear leaf, and cotton wick are pictured.

Sublethal effects (EthoVision): For most experiments, surviving individuals were tested for sublethal effects on activity using EthoVision. The primary response variable examined thus far is *distance traveled*, as this provided data with the least variability. Analysis and further examination are still being conducted.

Results:

Mortality: Mortality of earwigs and lady beetles from direct exposure is presented in Fig. 2, and fresh residues in Fig. 3. Due to the large number of treatments, exposure types, and residues for two insects, not all data are ready for presentation in this report. We are currently creating a webpage to go on the WSU Tree Fruit Pear IPM website for the comprehensive dataset.

Sublethal Effects: Insect growth regulators (IGRs) (Ultor, Esteem, Centaur, and Rimon) and Altacor had consistently negligible effects on mobility of earwigs and lady beetles. Actara, Assail, and Bexar had mixed outcomes, but each significantly reduced mobility in at least one assay. This suggests that IGRs are unlike to affect behavior or mobility of surviving insects, while mid-spectrum contact materials may result in impair mobility.

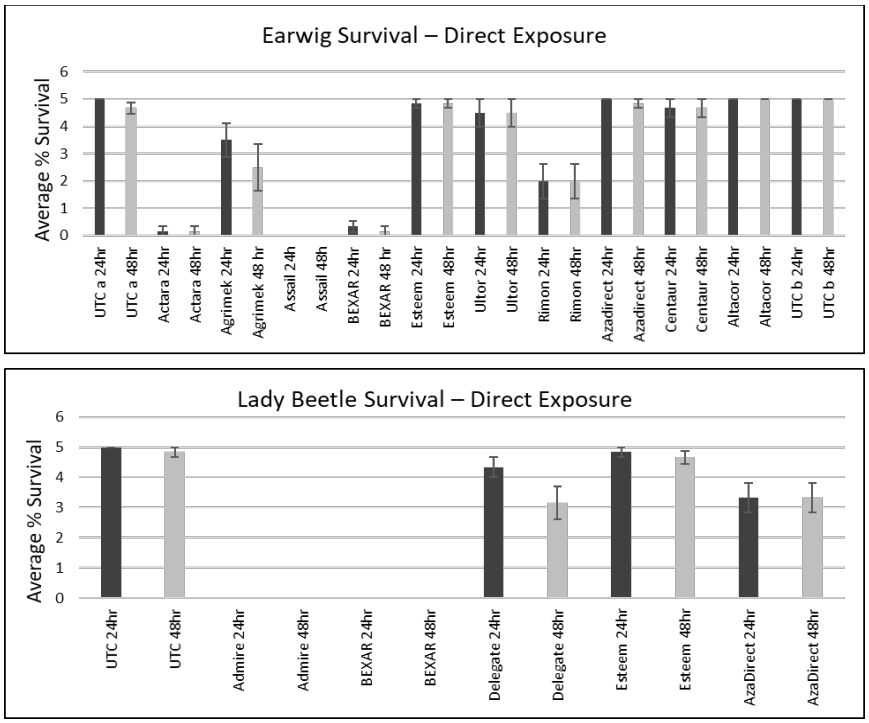

Fig. 2. Non-target effects. Survival of earwigs (top) and lady beetles (bottom) following exposure to insecticides as direct sprays, evaluated 24 and 48 hours after exposure.

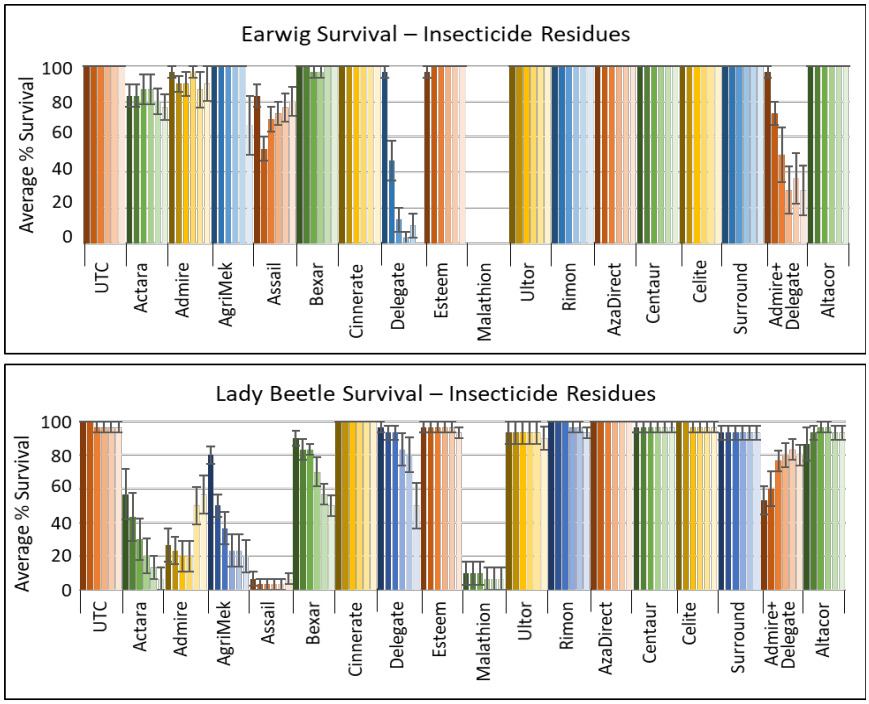

Fig. 3. Non-target effects. Survival of earwigs (top) and lady beetles (bottom) following Exposure to insecticides residues. Bars above a treatment moving left to right depict evaluations at increasing times after treatment.

<u>Obj 2</u>. Compare effects of particle films on pear psylla and natural enemies.

Methods:
Most of the work on this objective was completed in years 1 and 2. A final experiment was performed in 2021 to gain a second test on the effect of particles films on psylla nymphs (particle films sprayed over psylla as nymph instars 1-2). This experiment was conducted in May 2021 in an untreated pear orchard at the WSU TFREC. Leaves with at least 20 psylla eggs were found and tagged with a treatment and rep ID. Surrounding leaves were removed and tanglefoot was placed around the petiole to prevent nymphs from leaving or being attacked by predators. Each leaf was sprayed with one of each particle film (Surround, Celite, Microna, or Cocoon), at a concentration equivalent to 50 lb/ac. At 100 GPA (i.e., 60 g/L). Leaved remained attached to tree, and after 14 days, leaves were removed and brought back to the lab so surviving nymphs could be counted under a stereoscope.

Results:
Surround, Celite, Microna, and Cocoon did not significantly reduced survival of pear psylla young nymphs compared with the untreated check, but variability in nymph numbers per leaf was very high (Fig. 4). Surround and Celite resulted in the lowest averages, followed by Microna and Cocoon, respectively.

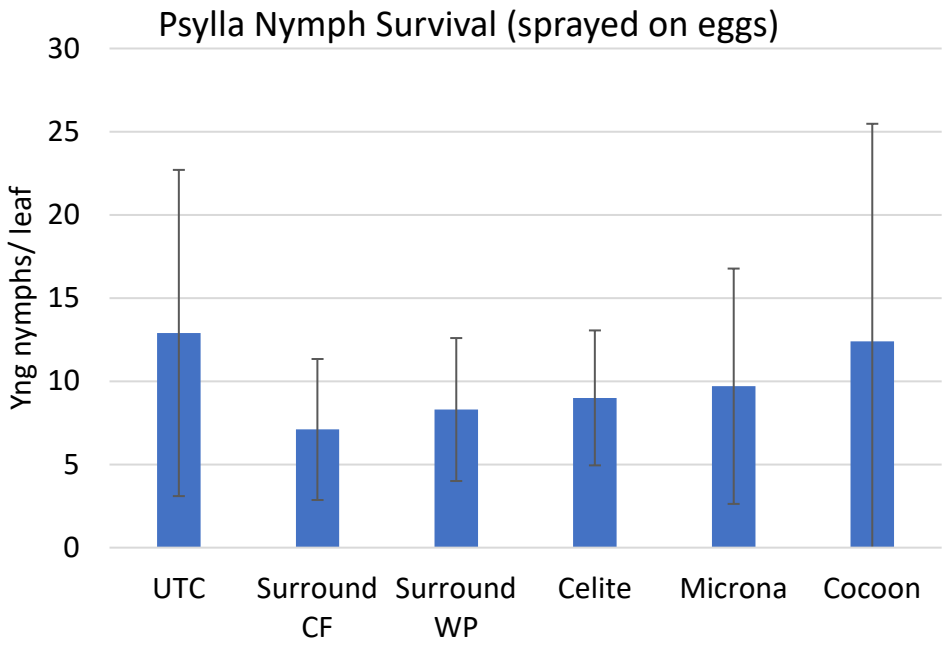

Fig 4. Survival of psylla nymphs 11 day after being sprayed by each particle film (eggs at time of spray). Treatments were not significantly different according to ANOVA.

<u>Obj. 3</u>. Evaluate potential for augmentative releases of earwigs for psylla control.

New field sites were established for earwig release experiments in 2020 to prevent carryover from the previous year affecting 2020 plots. 2019 field sites were monitored in 2020, however low recaptures in all but one site caused us to attempt a new set of methods to measure inoculation success and dispersal in smaller plots with more replications.

Methods:
From July to August 2021, earwigs were monitored in cardboard shelters placed in 4 “release” and 4 “control” plots in each of three pear orchards with low or no earwigs previously. Orchards were

located in Cashmere, Peshastin, and Rock Island. All treatment plots consisted of five adjacent pear trees, each receiving one corrugated cardboard shelter on its trunk just below the first major limb. Variable numbers of earwigs were released in release plots from July to August (143 per tree at Rock Island, 155 at Peshastin, 275 at Cashmere) with the goal of boosting earwig counts to over five per shelter per visit. All plots were over 30 m (98 ft) distant from each other, and shelters were placed in intermediate trees between each release and control plots to assess movement of earwigs.

Results:
No earwigs were found in the conventionally managed orchards in 2021 until August, during and after which a maximum of only 0.22 earwigs per shelter were found per day (Fig. 5). There was no relationship between numbers of earwigs found and release vs. control plot type. In contrast, at the WSU Rock Island research orchard (Sunrise) earwigs were first found in June and reached a maximum of about 25 per shelter in mid-July (this site had 1-2 earwigs per shelter prior to this experiment) (Fig. 5). High numbers of earwigs were found at Sunrise in both the control and release plots. Because of this, and the lack of earwigs at the two conventional sites, we could not assess whether the presence vs. absence of earwigs within a site affected pear psylla populations. The increase in earwigs from a maximum of 1–2 per shelter in 2018 and 2019, to ca. 10 per shelter in 2020 (when earwigs were released for this project), to ca. 30 per shelter in 2021 suggests that earwigs can persist and grow populations when spray programs are compatible with them. In contrast, the two conventional sites used many broad-spectrum sprays including Bexar, Rimon, and neonicotinoids known to harm earwigs. Further research should focus on orchards transitioning to soft programs (conventional or organic), and determine if earwig releases are necessary, or if populations are likely to increase naturally when less disruptive spray programs are employed.

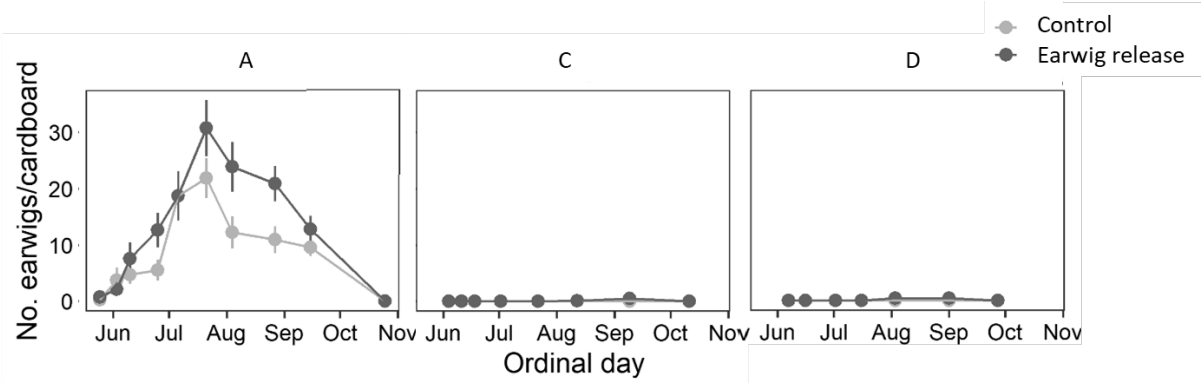

Fig. 5. Year 2021 mean earwigs per shelter (with standard error) at each of three orchards (A = Sunrise, C = Cashmere, D = Peshastin) in N = 4 release and control plots per orchard, with each plot consisting of five trees monitored with cardboard shelters. Earwig releases were conducted in 2020.

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

Methods:
In 2020, we monitored pear psylla at five commercial pear orchards trialing mylar and Extenday. Each site had each of three treatments, mylar, Extenday and UTC, used in addition to the growers' standard conventional program. Because the conventional programs controlled psylla through the early season, when reflective mulches are supposed to be most effective, there was little room for improvement. In 2021, we redesigned to the experiment to be tested in organic orchard systems, where early season control is more difficult. The experiment was conducted in an organic pear orchard in Dryden, WA with very high psylla pressure. Four replicated plots of each treatment (Extenday, mylar, and control) were established in a randomized complete block design. Each

treatment plot was about 0.25 acre (0.1 ha), with five drive rows, 25 m (82 ft) long. Cultivars at the site were a mix of Anjou, Bartlett, and Bosc. Mylar was placed in weed strips underneath trees, while Extenday covered the grassy drive rows. Groundcovers were installed on 17 March and removed 8 August. Sampling of insects was conducted each either weekly or biweekly throughout the season. At the end of the season, fruit were evaluated for horticultural defects and size using an AWETA fruit packing line and cup scanning grader (particularly to evaluate sunburn and blush that may occur from increased light intensity).

Results:
Extenday suppressed pear psylla adults by ca. 50% during spring and summer, and mylar suppressed pear psylla adults to a similar extent in spring only (Table 1). After ground covers were removed there were no effects on pear psylla eggs, nymphs, or spider mites, except there were more nymphs found in Extenday plots during fall. We found no evidence that pear weight, length, width, or sunburn was affected by reflective groundcovers (Table 2). The participating grower subjectively observed more fruit per tree in Extenday plots, and mylar plots to a lesser extent, so is planning to increase the scale of their trials in 2022 outside of this research project.

Table 1. Pest monitoring data from 2021 reflective groundcover trial: mean and SEM (N = 4 plots per treatment) of pear psylla life stages (adults per tray, eggs per bud in spring, eggs per leaf in summer and fall, nymphs per leaf) and pest mites (*Tetranychus* spp per leaf) by treatment across four sampling time ranges (precount, 2 March; spring, 18 March to 14 May; summer, 21 May to 6 Aug; fall, 18 August to 18 October). Values are mean seasonal averages within a time range. Mylar and Extenday were present during spring and summer. Different letters within a column for a life stage indicate significant differences (Tukey test, $\alpha = 0.05$).

		Precount		Spring		Summer		Fall	
Insect	Treatment	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
Psylla	Control	10.5	± 0.91	**0.90 a**	± 0.04	**0.24 a**	± 0.06	1.81	± 0.31
Adult	Mylar	11.2	± 0.47	**0.44 b**	± 0.03	**0.30 a**	± 0.02	1.83	± 0.22
	Extenday	11.3	± 1.5	**0.39 b**	± 0.04	**0.10 b**	± 0.02	1.69	± 0.27
Psylla	Control			2.62	± 0.84	1.23	± 0.40	4.65	± 2.6
Egg	Mylar			0.97	± 0.23	1.18	± 0.46	4.26	± 1.7
	Extenday			1.28	± 0.15	0.31	± 0.10	8.54	± 3.9
Psylla	Control			0.46	± 0.16	2.71	± 0.55	**4.69 a**	± 2.0
Nymph	Mylar			0.35	± 0.25	3.73	± 1.3	**5.41 a**	± 2.0
	Extenday			0.12	± 0.05	2.02	± 0.89	**12.0 b**	± 2.6
Spider	Control			0	n/a	6.74	± 2.3	12.1	± 6.3
mites	Mylar			0	n/a	7.66	± 2.5	3.90	± 1.8
	Extenday			0	n/a	4.24	± 1.2	15.0	± 7.3

Table 2. Mean and SEM of weight, length, width, and percentage sunburn on 80 Bartlett pears from each of N = 5 plots per treatment collected on 18 Aug for the 2021 reflective groundcover experiment.

	Weight (g)		Length (mm)		Width (mm)		Sunburn (% area)	
Treatment	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
Control	185.5	± 3.7	105.9	± 0.8	69.6	± 0.8	6.6	± 1.3
Mylar	188.7	± 5.1	104.7	± 0.8	70.3	± 0.7	8.8	± 0.8
Extenday	186.0	± 4.7	103.3	± 1.5	70.3	± 0.6	7.0	± 0.7

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

Methods:
Baseline toxicity assays for Bexar on psylla adults and nymphs were conducted in the spring of 2020. Five populations from Wenatchee, Yakima, and Okanogan, WA, and Hood River and Medford, OR were used to start colonies in a greenhouse at the WSU TFREC. Colonies were kept in mesh cages (two per region) with potted Anjou trees. For each population we conducted lethal concentration probit bioassays to determine LC10, 50 and 90 values for summerform adults and first-second instar nymphs. Adults were tested using the standard slide dip method in which adult psylla are adhered via their wings to double-sided tape on a microscope slide, then dipped into a solution of pesticide. Nymph assays were conducted by collecting leaves from psylla colony pear trees with at least three young nymphs, then leaves were dipping in a pesticide solution. Six concentrations of Bexar were used for treatments along with an untreated check. Mortality was rated at 24 hours after exposure, and not later due to rapid degradation of checks. The resulting LC values are shown below in Table 1.

Results.
Probit results for pear psylla nymphs and adults are shown in Table 2. Numbers of insects (n; sb = treated subjects, ctrl = untreated controls) are given with LC_{50} and 95% confidence intervals (CI) along with X^2 and slopes from R probit analysis. Hood River adults were more susceptible to Bexar than Wenatchee and Medford; Omak and Wapato were intermediate. Overall, the average LC50 value for nymphs was 109 mg (AI) /liter (H_2O), and for adults was 74.1 mg (AI) /liter (H_2O). When comparing regions, there was no difference in susceptibility for nymphs. These data can be used in the future as comparison if resistances in expected. We will publish the raw dataset in addition to summarized results to aid future comparisons.

Table 1. Results of LC50 analysis for Bexar used on C. pyricola nymphs and adults from populations in pear growing regions of Oregon and Washington.

Colony[a]	n (treated, ctrl)	LC_{50} (95% CI)[b]	Slope (SE)	X^2 (*P* value)	Heterogeneity factor
Nymphs					
HR	559, 86	99.9 (64.6 – 166.0)a	1.2 (0.09)	124*	3.1
MED	633, 115	67.0(40.5 – 123.9)a	1.1 (0.08)	167*	4.1
OMA	520, 131	135 .5(68.4 – 407.0)a	0.9 (0.09)	168*	4.2
WAP	877, 147	73.0 (50.6 – 111.7)a	1.2 (0.08)	167*	3.6
WEN	1173, 150	174 (98.0 – 425.1)a	0.9 (0.06)	292*	6.4
Adults					
HR	192, 33	24.6 (9.6-50.5)b	1.0 (0.13)	75*	2.2
MED	411, 64	101.5 (54.4-207.0)a	0.71 (0.08)	146*	2.1
OMA	216, 36	49.0 (26.1 -86.9)ab	0.99 (0.13)	51*	1.5
WAP	213, 35	56.5 (36.7 – 85.9)ab	1.1 (0.13)	42	
WEN	216, 36	138.9 (79.0 – 273.3)a	0.99 (0.13)	50*	1.5

[a]HR= Hood River, OR; MED = Medford, OR; OMA = Omak, WA; WAP = Wapato, WA; WEN = Wenatchee, WA
[b]Heterogeneity factor used in calculating 95% CI when $P < 0.05$ for X^2 goodness-of-fit, Locations followed by the same letter have overlapping 95% CI
* $P < 0.05$

Executive Summary

Title: Improving pear pest management with integrated approaches

Keywords: Pear Psylla, earwigs, particle films, reflective mulch, Bexar

Abstract. The goal of this project was to test multiple strategies and contributing factors to improve IPM programs for pear pests, mainly pear psylla. This project examined strategies such as reflective mulches, particle films, earwig releases for biological control, chemical insecticide efficacy, non-target effects of insecticides on natural enemies, and established baseline toxicities of a new insecticide, Bexar (tolfenpyrad) against pear psylla adults and nymphs, to aid resistance testing in the future. Many of the objectives provided preliminary information that allowed us to leverage additional funding from state, regional, and federal agencies to conduct more thorough investigations.

Summary. *Objective 1* examined non-target effects of pesticides on natural enemies. Findings demonstrated that many insecticides used in pears fall in between "selective" and "broad-spectrum"; i.e., causing mortality of 40-60% of individuals tested (natural enemies or psylla), or have high mortality on one species and little mortality on another. These "mid-spectrum" chemicals make up much of our conventional programs, such as imidacloprid, thiamethoxam, acetamiprid, novaluron, abamectin, spinetoram, and tolfenpyrad. However, some materials proved to be consistently selective or broad-spectrum. Selective chemistries included horticultural oils, pyriproxyfen, spirotetramat, chlorantraniliprole, azadirachtin, cinnamon oil, kaolin, and diatomaceous earth; broad spectrums included malathion. It would be useful to examine more product mixes in future tests. Mixing "mid-spectrum" chemistries is common, and this is likely to create a much more toxic outcome for natural enemies.

Objective 2 compared different particle films against pear psylla, with the primary focus on determining the relative efficacy of products formulated for psylla control (Surround [kaolin] and Celite [diatomaceous earth]) vs other particle films intended for non-insecticidal uses (Microna [calcium carbonate] and Cocoon [kaolin]). Overall, Surround and Celite provided the best and most consistent control of pear psylla, primarily by repelling adults from colonizing and ovipositing on pear trees. All products had variable abilities suppress nymph development, though often significant. Overall, Surround and Celite were the most reliable products, and sprays timings should precede adult colonization and egg lay.

Objective 3 examined the potential to release earwigs to reestablish populations in locations with low densities, providing control of pear psylla. Our results demonstrate that aggressive conventional programs prevented earwigs from establishing, so there is little merit in releasing earwigs into conventional orchards unless they are transitioning to soft or organic. In situations where soft or organic programs are being used, earwigs seem to re-establish naturally, and faster than expected, but more work is needed in transitional orchards to confirm. Earwig trapping and releases can be performed with little effort, and other works suggest that releases can increase biocontrol in tree fruit; so, this tactic may still have merit in organic, IPM, or transitional orchards.

Objective 4 trialed novel strategies to control pear psylla including reflective ground covers (Mylar and Extenday), soil applied systemic insecticides, and insecticidal netting. Insecticidal netting was ineffective at controlling psylla, likely due to the chemistry infused, the pyrethroid deltamethrin, which is not an effective mode of action against psylla. Reflective ground covers used in an organic program provided and addition 50% reduction pear psylla. Reflective ground covers did not provide added control to conventional programs, because broad-spectrum sprays provide nearly complete control of psylla at this point in the season. Further studies should examine the overall economic cost and benefits to determine if reflective ground covers are cost effective. The primary grower trialing

these products claimed that he would continue to use them due to increased yields and added control of psylla. The soil-applied insecticide, Platinum (thiamethoxam), resulted in about an 80% reduction in psylla nymph survival. Movento (spirotetramat) did not reduce pear psylla survival. Although the soil drench method was effective for Platinum, the material is not currently registered for use in pears, and in the current regulatory climate around thiamethoxam it is not likely to be. Registered materials for soil drenches in pears, like Admire Pro, could be tested; however, Admire Pro is also under regulatory pressure for pollinator and other environmental concerns.

Objective 5 established Bexar LC_{50}s for pear psylla adults and nymphs from five regional populations of pear psylla, including Washington, Oregon, and New York. LC_{50}s combined across regions were 109 mg (AI) /liter (H_2O) for young nymphs and 102 mg (AI) /liter (H_2O) for adults. The field rate (27 fl oz/ acre) at 100 gpa equals 339 mg (AI) /liter (H_2O), for comparison. Other insecticides were tested in various independent bioassays and field trials.

Most insecticide trials have been published in the open-access journal Arthropod Management Tests (some are still in review or in prep). We are currently preparing a webpage for the Tree Fruit Extension Pear IPM website that will house all insecticide trials and other trial results from this project.

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.

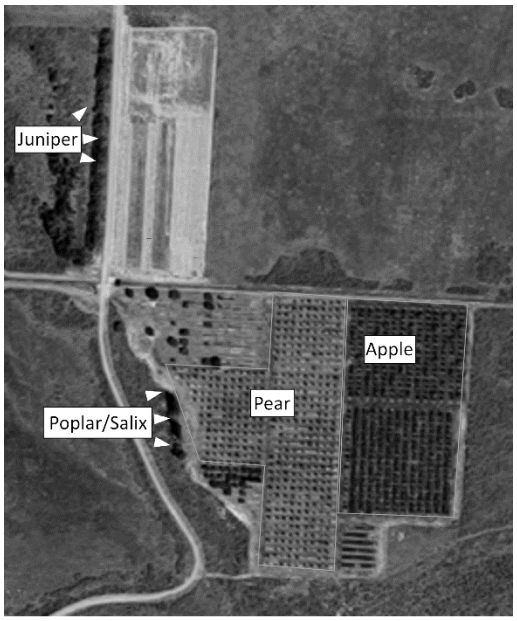

Figure 1. Layout of pear orchard at the USDA experimental farm in Moxee, where winterform psylla will be collected and where volatile collections will take place.

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.

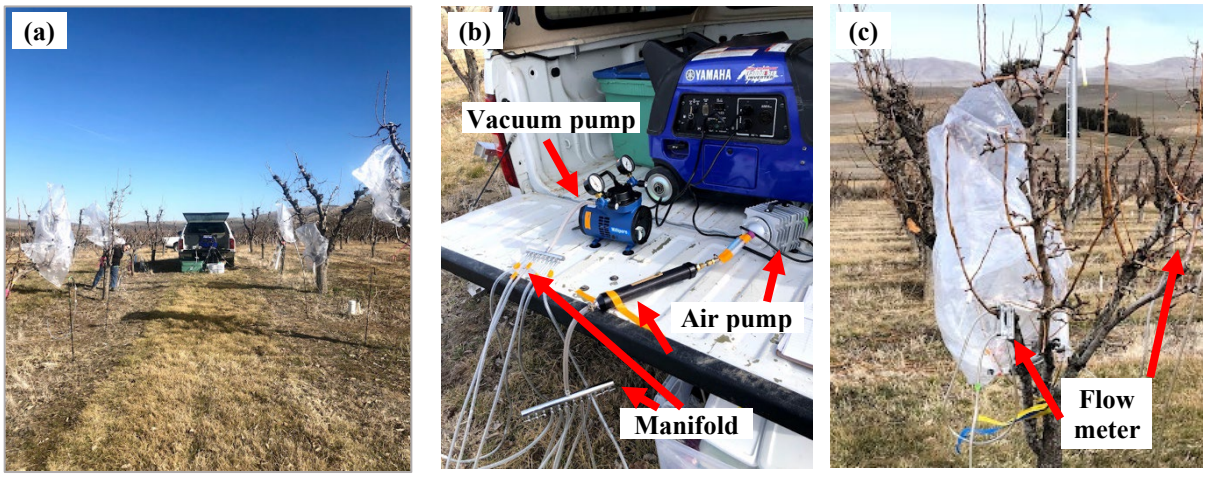

Figure 2. Example of volatile collection set up: (a) Volatiles being collected from 5 Bartlett pear trees at the USDA experimental farm in Moxee; (b) air pump, vacuum pump, and tubing set up; (c) up close image of volatile collection set up on pear tree.

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.

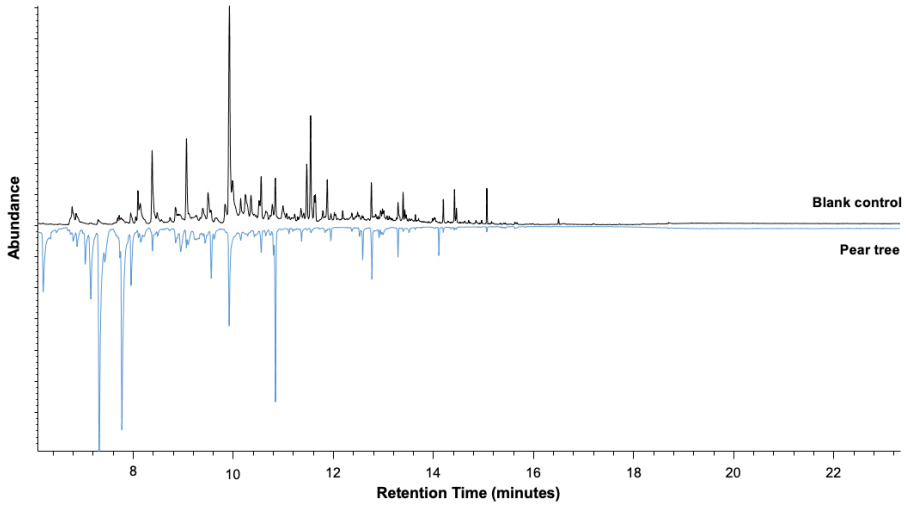

Figure 3. Representative GC analysis of volatiles sampled from an empty sampling bag (control), and a sampling bag that contained portions of a pear tree. Top trace: GC chromatogram of blank control. Inverted trace: GC chromatogram of sampled pear tree.

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).

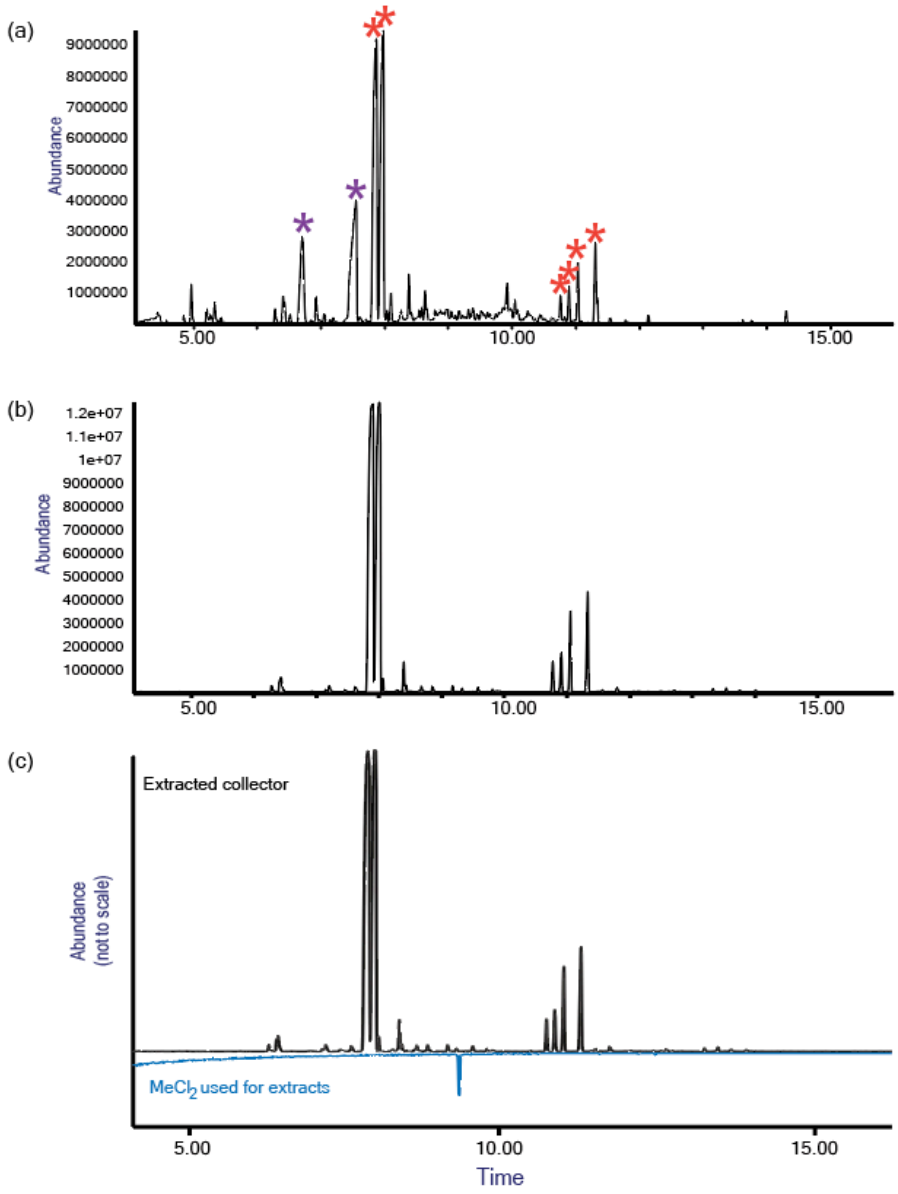

Figure 4. Representative GC-MS analyses of: (a) extracts of volatiles from a Bartlett pear tree collected in early April (the first two asterisks represent compounds identified in all extracts of volatiles, including the control and the remaining asterisks represent compounds that were identified as 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).

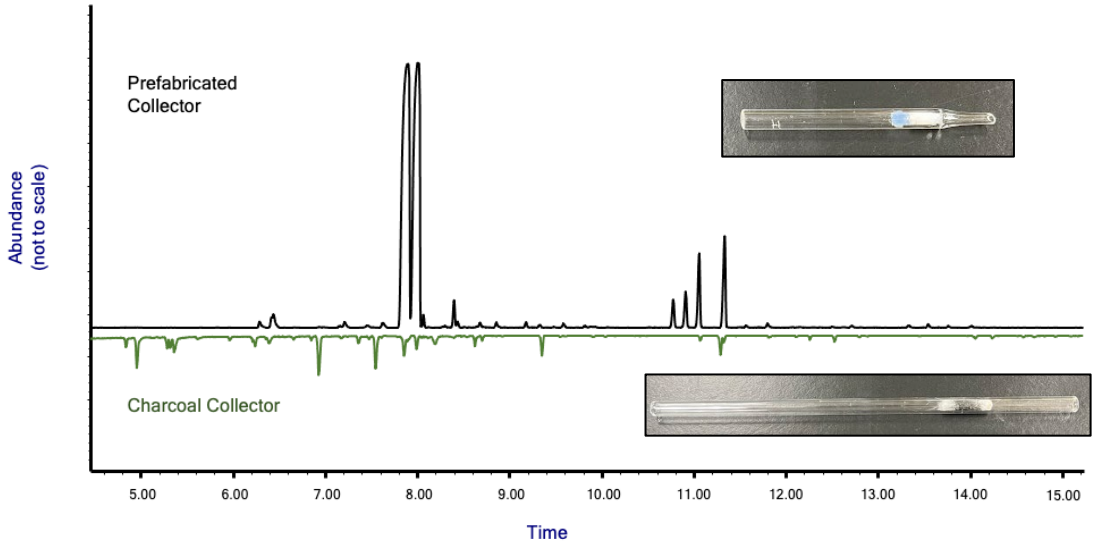

Figure 5. Representative chromatograms of an extract from an unused collector (top trace) and the extract from the new charcoal collectors (inverted trace). The trace representing the extract of the charcoal was scaled up for demonstration purposes.

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.

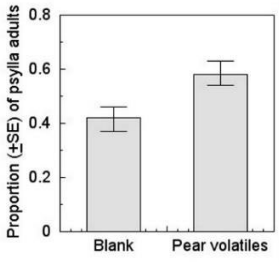

Figure 6. Mean (±SE) number of pear psylla caught in traps baited with a nontreated piece of filter paper ("Blank") and pear volatiles.

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

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), Mike Schmitten, Ben Smithson
[note: apple grower cooperators will be specified in apple report]

Report Type: Continuing Project 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 funding sources:	**Awarded**
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.

Other funding sources:	**Awarded**
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.

Other funding sources:	**Awarded**
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.

Other funding sources: **Awarded**
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: arcgrants@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.

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.

SIGNIFICANT FINDINGS

- We were unable to hire a qualified postdoc in time to start this work in 2021 due to multiple reasons: timing of receipt of Fresh and Processed Pear funding, delays in WSU grant processing, USDA COVID restrictions (we are currently unable to have non-citizens cleared to work in the building, so all non-citizen applicants were unable to qualify), and a limited number of qualified applicants, which seems to be a general trend for hiring postdocs at this time.

- To address this issue, we expanded our search to include an associate in research position to manage this project, which would only require a M.S., expanding our candidate pool.

- Because of funding through Western IPM Center (see "Other funding sources") we were able to still collect some data which will inform how we conduct this project starting in 2022. All results in this report are the result of the related WIPMC project.

- We were able to conduct a second year of work in 2021 on mealybug destroyer releases in large, one-acre apple plots, comparing drone versus ground releases of 1,000 mealybug destroyers per acre to a no-release control. Although some mealybugs were detected in the fruit at harvest, during the growing season, there were <0.04 mealybugs/trap. We also found very few mealybug destroyers immediately after release (1 day) and no mealybug destroyers 8 days after release; they likely dispersed due to lack of prey. This indicates that mealybug numbers must be fairly substantial for releases to work.

- This contrasted with our 2020 results, where releases of mealybug destroyers decreased mealybug populations. However, in that study, there were up to 6 mealybugs/trap during the growing season.

- In 2021, we also tested releases in apple of two species of lacewings as eggs or larvae: *Chrysoperla rufilabris* and *Chrysoperla carnea*. Through examining the materials, 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 were also effective (20,000/acre). Seasonal counts of aphid colonies were reduced by 57% and 43%, respectively.

- As part of the WIPMC project, we also collected survey data on apple and pear grower perspectives of releasing natural enemies in tree fruit. To date, the survey has collected 127 responses. Four stakeholder input sessions have been conducted (Omak, Wenatchee, Yakima, and Hood River) and a final session will be conducted in Medford on January 7th. Feedback from the survey will be used to determine future research directions and to obtain federal funding to expand the work in this project.

METHODS

This work will now initiate in 2022 due to hiring difficulties in 2021. All work will be conducted by the associate in research, supervised by Schmidt-Jeffris. The associate will also conduct the pear portion of the work, under the guidance of Nottingham. The summer technician (WSU) will assist with plot set up and data collection in all Wenatchee-area trials. The USDA technician will assist with data collection, plot set up, and processing of PCR/lab samples. The pear work will mirror the work conducted in apple, with pear psylla as the target pest, with some changes to the natural enemies released. Details will be provided in the pear grant, the work described here is for the apple portion of the study. BioBee (Steve Arthurs) will supply the natural enemies for releases and Chuck Weaver (Parabug) will pilot the drone in Obj. 2.

1. Improve retention of released natural enemies.

This two-year (2022-2023) study will be conducted in a commercial pear orchard in Cashmere, WA (Schmitten Orchards). The release day will target when early season pear nymph populations begin to rise, approximately bloom. There will be a total of six treatments made of combinations of lure use and food supplements: use of a methyl salicylate lure (Predalure) – yes/no × commercially available food supplements – Nutrimite (pollen), Artemac (brine shrimp cysts), or none. Each combination will be replicated in the orchard five times for a total of thirty plots. Each plot will consist of 0.5 acres. There will be a minimum of 5 rows or 1,000 feet between plots. One week prior to release, we will conduct precounts of pear psylla by collecting a 50-leaf sample from each plot and conducting 10 tap samples (one each on ten center trees) per plot, to count eggs/nymphs and adults, respectively. Treatments will be assigned to plots using pre-release levels of pear psylla to ensure initial pest abundance does not differ between treatments for a fair comparison. At this point, one methyl salicylate lure will be added to four trees 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 Nutrimite and Artemac to the 10 center trees of each plot at the insectary recommended rate. Artemac will be applied by tying tape with attached cysts to trees and Nutrimite will be applied by using the insectary-recommended handheld blower. Then, we will release two natural enemy species across the entire trial at insectary recommended release rates: 10,000 *Chrysoperla carnea* larvae per acre (green lacewing, BioBee) and 5,000 *Orius insidiosus* per acre (minute pirate bug, BioBee), using typical ground-releases by ATV. Post-release sampling will occur at 3, 7, and 14 days after release, with additional sampling on alternating weeks if treatment differences continue to be observed. Pear psylla will be sampled using leaf samples and tap counts as previously described. Beat tray samples will also be used to count natural enemies. 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 and all other natural enemies collected 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 three sticky cards on trees within the center of each plot to count key natural enemies to species (released *O. insidiosus* and *C. carnea*, but also resident lacewings, other anthocorids, *Campylomma*, *Deraeocoris*, and ladybeetles).

2. 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 (Smithson Ranch). 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 five one-acre replicates per treatment (25 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 10,000 lacewings/acre and 1,000 *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. Fruit damage and infestation will be assessed prior to harvest by examining five fruit from the ten center trees of each plot. We will compare cost of release by drone versus by ground for each species, accounting for time spent on releases/labor, and use pest control levels to determine which release method has the best combination of cost effectiveness and efficacy.

Methods used in the 2021 field studies

These methods have some overlap with the Obj. 2 methods described above and will allow us to make modifications to improve these trials. Because of the preliminary work we conducted in 2021 while unable to hire a postdoc for this project, we have identified some key areas for improvement. First, we will also examine differences in releases of *C. carnea* versus *C. rufilabris* for pear psylla management. Any releases of *C. carnea* will be confirmed to the correct species prior to release, due to our determination in 2021 that some insectaries use the incorrect species. We determined that sampling for adult lacewings will need to be more intensive (more traps, use of lures) to ascertain if released juveniles establish.

Mealybug destroyer releases

In 2021, we conducted releases of mealybug destroyers in an organic, commercial apple orchard in Pateros, WA. Plots were 1 acre, separated by at least 208 feet, and each treatment was replicated 5 times. The treatments were 1) ground release of 1,000 mealybug destroyers per acre, 2) drone release at dusk of 1,000 mealybug destroyers per acre, and 3) a no-release control. Releases were scheduled to be conducted on 12 May 2021, but were delayed due to shipping issues and were instead conducted on 25 May 2021. Mealybugs were sampled by tying one burlap strip trap to 20 center trees in each plot and by collecting one shoot sample from each of these trees. Mealybug destroyers were sampled by tap sampling 10 trees from the center of each plot (3 taps per tree). Samples were conducted once weekly, with the final sample collected on 7 July 2021.

Lacewing releases

In 2021, we conducted releases of lacewings in an organic, commercial apple orchard in Pateros, WA. Plots were 0.25 acres, separated by at least 104 feet, and each treatment was replicated 5 times. The treatments were single releases of 1) 100,000 *Chrysoperla rufilabris* eggs/acre, 2) 20,000 *C.*

rufilabris larvae/acre, 3) 100,000 *C. carnea* eggs/acre, 4) 20,000 *C. carnea* larvae/acre, and 5) no-release control. Releases were conducted on 5 May 2021. Aphids were sampled by counting the number of colonies (WAA) or infested leaves (green apple aphid) on 3 1-foot shoots per tree, for 12 trees located in the center of each plot. Rosy apple aphid was not present. Lacewings were sampled by performing tap counts on one limb from each of the 12 trees (3 taps per limb). Collected adults and larvae were placed in vials with ethanol for later identification. Adult lacewings were also sampled by hanging two sticky cards in each plot. Counts were conducted once weekly, with the final sample collected on 30 June 2021.

RESULTS AND DISCUSSION

These results are from the WIPMC grant conducted in 2021, which will be used to inform the work done on the project objectives initiated in 2022. An excellent benefit of conducting these initial trials is that we have identified more suitable locations to conduct this research in apples, which have very large populations of both aphids and mealybugs, especially compared to our previous research sites. We also identified potential issues with *C. carnea* shipments, which can be mitigated by early ordering and species confirmation.

Mealybug destroyer trial. Neither release treatment lowered mealybug counts compared to the control. This is likely because mealybug populations were very low (<0.04 mealybugs per trap, compared to up to 6 mealybugs per trap in the 2020 trial). Low mealybug populations likely caused the low establishment of mealybug destroyers. Although mealybug destroyers were found in low numbers through most of the growing season in the preliminary 2020 trial, in the 2021 trial we were unable to find any mealybug destroyers the week after release or any weeks following. Our results indicate that mealybug destroyers are only effective predators when mealybug populations are higher and therefore may only be useful in orchards where there is a serious, reoccurring issue with this pest.

Lacewing trial. The *C. rufilabris* were the species that were advertised. The *C. carnea* eggs came from an insectary in Mexico, whereas the larvae came from an insectary in Canada. The *C. carnea* eggs were indeed a species in the *carnea* species group (molecular work will be needed to determine exactly which species). However, the *C. carnea* larvae were *C. externa*, a species not in the *carnea* species group (Fig. 1). This is a known issue with insectaries, as lacewings within the genus

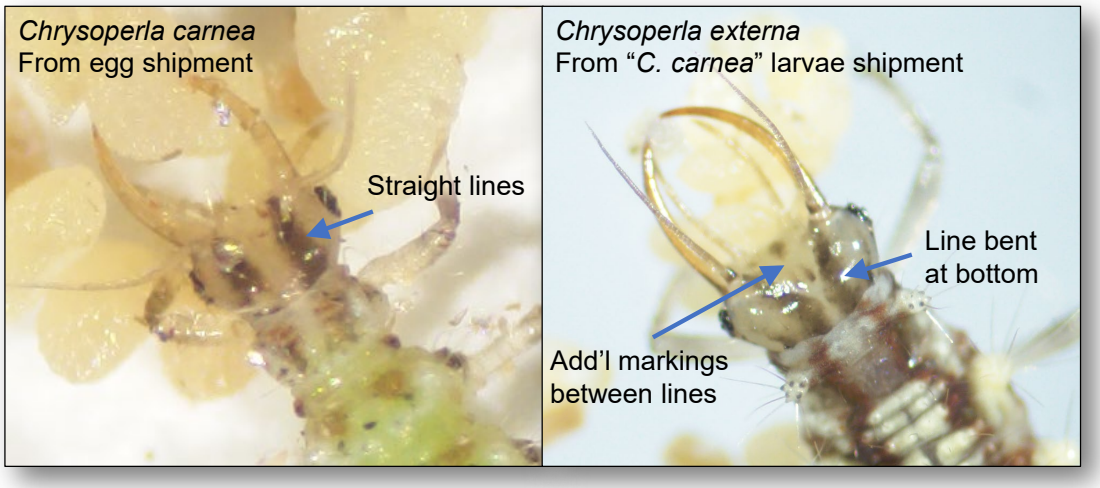

Fig. 1. Distinguishing between lacewing species received in shipments from insectaries. Head capsule markings are the most common way to distinguish between species in *Chrysoperla*.

Chrysoperla, especially in the *C. carnea* species group, are very difficult to identify without the expertise of a specialized taxonomist.

The *C. carnea* eggs and *C. rufilabris* larvae resulted in lower aphid populations compared to the control, whereas the other two treatments did not ("*C. carnea*" larvae and *C. rufilabris* eggs) (Fig. 2-4). More trial work will be needed to determine if this is a consistent pattern; there is always the possibility that a particular order of insects is going to be better or worse than another, due to either changes in health of a colony or differences in how the package is stored during shipping. Because the larvae were refrigerated overnight (to prevent cannibalism), while eggs were stored at room temperature (to encourage egg hatch) differences in treatments may also be due to variation in storage requirements.

Lacewing larvae from the releases were found up to a month after the release occurred. We recovered *C. rufilabris* from the "*C. rufilabris* larvae" treatment the most often, but never found any *C. rufilabris* larvae in the treatment where eggs were released. We found larvae of the correct species in all other plots. We also found several species of native, non-released *Chrysopa* lacewings, which appear to have a healthy population in the orchard. *Chrysopa* larvae were not found until three weeks after our releases and then in lower numbers than our released lacewings. This indicates that our treatments gave this orchard a headstart in aphid management compared to the no-release control. All adult lacewings that were found during the course of the trial were *Chrysopa*, therefore we do not yet have evidence that the juvenile lacewings released ever fully developed. However, recovery of lacewings in general was low, so they many have been present and not found. We will use these results to inform our sampling efforts for 2022-2023. In particular, adult sampling will be more intensive (we kept this minimal in 2021 to avoid removing too many released lacewings from plots) and we will bait at least one trap per plot with lacewing lures.

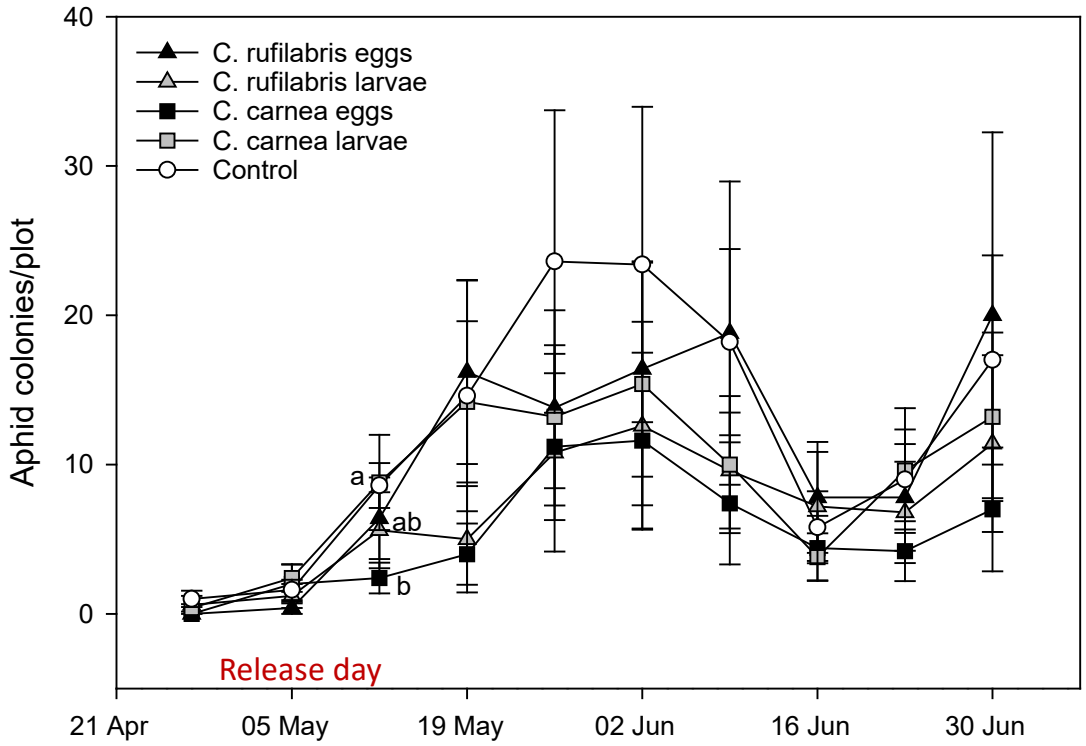

Fig. 2. Aphid colonies (WAA and green) per plot in lacewing release trial.

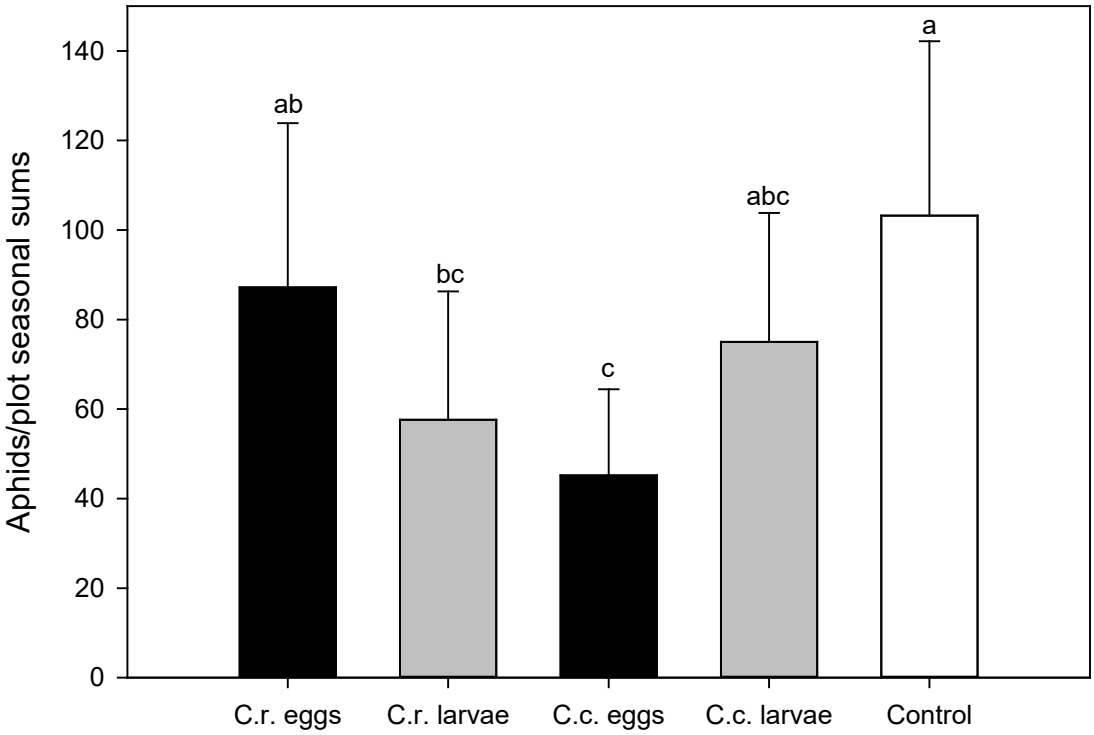

Fig. 3. Aphid colonies (WAA and green) per plot summed across the season in lacewing release trial.

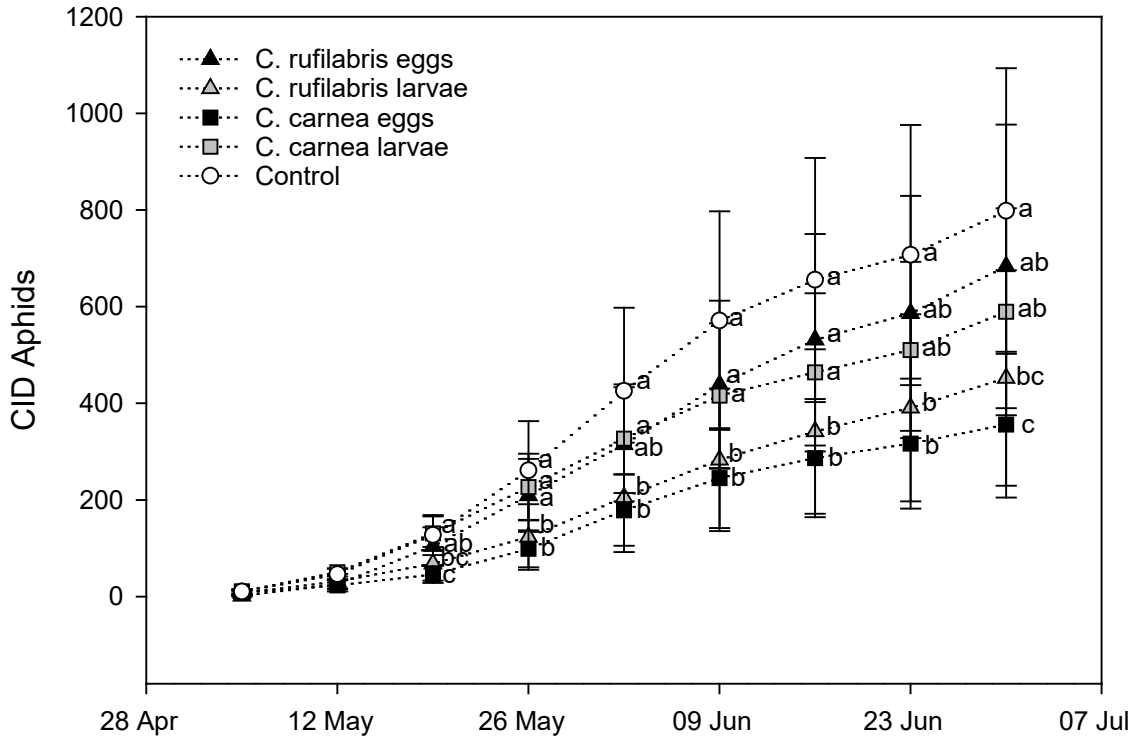

Fig. 4. Cumulative insect days (CID) for aphids in the lacewing release trial.

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

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

PI: Rebecca Schmidt-Jeffris
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Co-PI (5): Louis Nottingham
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City/State/Zip: Wapato, WA 98951

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

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

Other funding sources

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

Agency Name: WSCPR
Amt. requested: $20,596
Notes: This grant was submitted using data collected in Year 3 as preliminary data.

Budget 1

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

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

Item	2019	2020	2021
Salaries[1]	$17,404[2,3,4]	$17,839[2,3,4]	$18,286[2,3,4]
Benefits	$4,529[2,3,4]	$4,642[2,3,4]	$4,759[2,3,4]
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	2021
Salaries[1]	$2,510[2,3]	$2,572[2,3]	$2,638[2,3]
Benefits	$2,046[2,3]	2,096[2,3]	$2,150[2,3]
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 2022. The most efficient method for trapping *Trechnites* and which trap best reflects percent parasitism will be determined at conclusion of Year 3.

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

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

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 this winter to provide mummies for pesticide assays in early spring 2022.

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

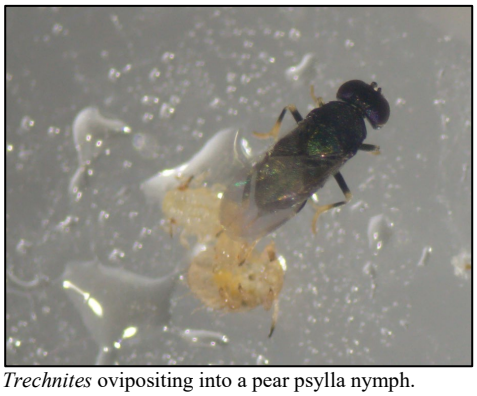
4. Examine native psyllids from multiple locations for *Trechnites*.

We will continue examining native 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 and will examine the wasps emerging from pear psylla mummies to determine if *T. sadkai* also attacks pear psylla. 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 were 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.

- 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.
- Funds from the Fresh and Processed Pear Committee was used to leverage additional funds from the WSDA Specialty Crop Block Grant Program (PI Schmidt; $245,974) and from the Washington State Committee for Pesticide Registration (PI, Zilnik; $20,596). These new funds will expand research on *Trechnites* and pear psylla distribution within orchards.

METHODS (updates included)

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

Adult Trechnites. At each of the four locations, five plots will be laid out in an orchard. Collection of all data will occur from April-late September at all locations. We will discontinue this sampling in the two Oregon research orchards, as *Trechnites* populations remain low. We will expand the use of traps in Oregon, but remove the random leaf/targeted nymph samples described below.

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

Percent parasitism. In Year 2, we attempted to use emergence cages to monitor percent parasitism instead of dissection. Ten psylla from each plot at a location were placed inside a cage on a detached pear leaf and monitored for emergence of parasitoids. Survival was poor using this method and was discontinued. In year 3, percent parasitism was determined solely by PCR detection of *Trechnites.*

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

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

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

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

We were unable to obtain an adequate number of *Trechnites spp.* adults in 2021. We adjusted our banding procedure to placing bands in three orchards with high *Trechnites spp.* counts. We placed bands in the second week of August 2021. The following week we took one band per tree from each of the three orchards for 12 weeks. Following this we were able to obtain 454 mummies and plan to conduct the pesticide screening in early Spring of 2022.

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

Each year, we will locate *Salix scouleriana*, *Salix prolixa*, and *Ribes* patches in early spring and *Salix exigua*, *Purshia tridentata,* and *Cercocarpus ledifolius* (Medford only) in spring and summer. We completed this work in the Yakima area in years 1-2, in year 3 we expanded this work as part of the new WSDA grant (2021-2023). These plant taxa host native psyllids that are related to pear psylla, and thus could be sources of parasites (including *Trechnites*) that attack pear psylla. Beat tray samples will be used to determine if adult psyllids are present. When adults are found, shoots infested with immature psyllids will be collected and shipped to USDA-ARS. From these samples, psyllid mummies will be isolated and the emerging parasites and psyllid host will be identified. Collection will occur 2-3 times per season, with the timing focused on life cycles of known psyllid species that feed on these plants. We will also record any hyperparasites of *Trechnites* that are found in collected psyllids.

RESULTS AND DISCUSSION

Obj. 1. We continued to sample orchards at four locations. Full analysis and tool building is in process, we present preliminary results here. Fig. 1 shows the percent parasitism in contrast with capture method at the Moxee farm. Both 3D-printed tube traps and sticky cards continue to collect high numbers of *T. insidiosus*. However, to obtain an accurate measure of psylla we need to model the relationship between sticky cards and tube traps. 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.

Obj. 2. We determined that additional information is needed to adequately model the relationship between *Trechnites* adult capture and levels of psylla parasitism. In Year 2, we collected random leaf samples to determine pear psylla age distribution. We successfully obtained funding from the WSDA to expand this work and hired a postdoc (Zilnik) with expertise in modelling. Zilnik is currently preparing the full model to predict parasitism based on trap capture from all three years. Fig 1. shows the summary of trap capture and detection of parasitism by PCR.

Obj. 3. We were unable to rear *Trechnites* in sufficient numbers to begin this objective in Year 1. In Year 1 (Oct 2019), we placed cardboard bands in the research orchards in Moxee and Wenatchee. We determined that parasitized psylla nymphs used these bands as overwintering sites and form mummies within the bands. In Feb 2020, we assessed emergence from these bands. At the Wenatchee site, we placed 115 bands in Bartlett trees and 99 bands in Anjou trees. There were 1.1 mummies per band in Bartlett and 0.5 mummies per band in Anjou. Differences may be due to bark texture – there are more alternative locations for shelter in Anjou trees. From the 186 mummies we collected, 73% had a wasp emerge, most of which were *T. insidiosus*. Other wasps (n=5) were *Dilyta* spp., a hyperparasitoid. Nearly all emergence occurred within 13-14 days of removing the mummies from the cold. 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 1200 bands placed (37.8% of bands contained at least 1 mummy).

In 2021 we adjusted our banding procedure and found evidence of *Trechnites* overwintering phenology (Fig 2). We will complete the bioassays by March 2022 using the methods we reported in 2021.

Obj. 4. In 2019, we found *Trechnites* emergence from *Cacopsylla americana* and *C. alba* collected from *Salix rigida/prolixa* and *S. exigua*. 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.

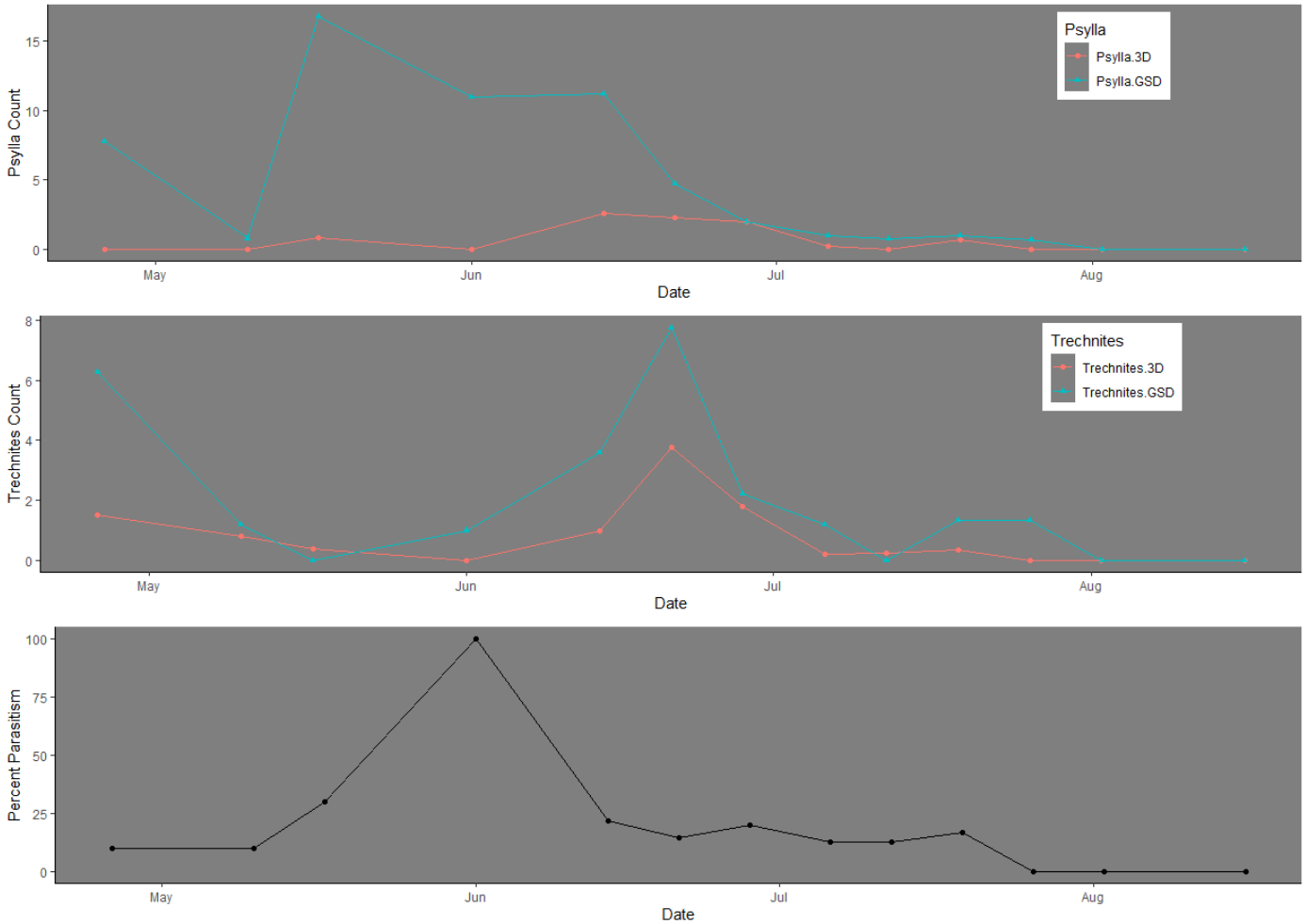


Fig 1. Comparison of 3D-printed tube traps (Trechnites.3D and Psylla.3D), sticky cards (Trechnites.GSD and Psylla.GSD) and parasitism levels at the research farm in Moxee, WA. The tube traps appear to be more effective at detecting *Trechnites* at low population levels than pear psylla, but they provide an accurate reflection of population peaks as a whole.

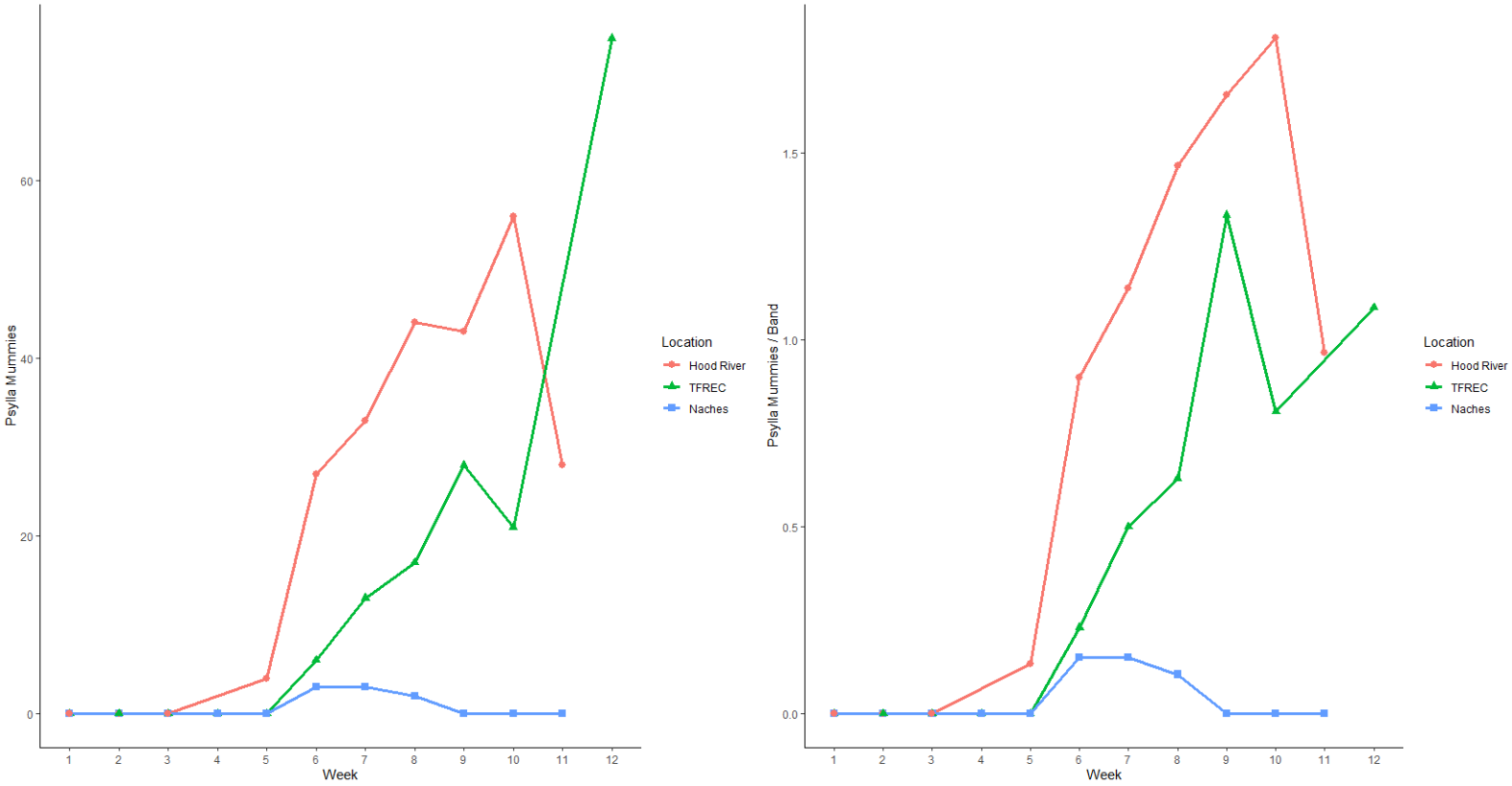


Fig. 2 Psylla mummy recovery total (left) and per band (right) from overwintering bands placed in August 2021. Week 1 collection begins September 1, 2021 and continues weekly for 12 weeks. We collected no mummies prior to the week of September 29, 2021. The majority of mummies were collected in October and the first week of November.

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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Email: Charlene.wilkinson@oregonstate.edu
Address: 1600 SW Western Blvd.,suite 320
City/State/Zip: Corvallis, OR 97333

Co-PI (1): Rebecca Schmidt-Jefferies

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Email: Rebecca.schmidt@usda.gov
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City/State/Zip: Wapato, WA 98951

Co-PI (2): Louis Nottingham
Organization: WSU
Telephone: 509-293-8756
Email: louis.nottingham@msu.edu
Address: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

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~~ **19,000** **Year 2:** $45,000 **Year 3:** $45,000

Other funding sources

Agency Name:

Amt. requested/awarded:

Notes:

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 (NEs) 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). However action thresholds have not been established for these important NE. 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 using lure baited yellow sticky cards.

1. **Use plant volatile baited monitoring traps to describe NE communities in orchard ecosystems.**
   Traps were placed at 20 pear orchards throughout Hood River County and were checked weekly for natural enemies. These traps were maintained from April 14th – September 9th.

2. **Compare capture of several key species of NEs in lure-baited traps with numbers measured from standard scouting techniques.**
   Compiled natural enemy data was sent to our collaborative crop consultants. We are currently waiting to receive their data on pest populations found in the orchard and if and when they made spray recommendations to the owners of these orchards.

3. **Establish action (or in-action) thresholds for key NEs.**
   We will begin to establish target thresholds for key natural enemy species that indicate that populations are building at a rate sufficient to control pests such as pear psylla after collecting NE data in 2022 and compiling it with the 2021 data.

Significant Findings

- ❖ 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 every week. This data is now part of our weekly updates to the stakeholders. 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.
- **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. These data suggest that lure baited monitoring traps can be used to gauge NE populations.
- **Objective 1 (33% 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 (15% complete):** Our data shows distinct increases and decreases in key natural enemy populations throughout the growing season.
- **Objective 3 (0% complete):** We will begin to establish target thresholds for key natural enemy species after collecting NE data in 2022 and compiling it with the 2021 data.

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 14th to September 9th, 2021. 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.

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. Scouting for NE gives a snap shot in time of both pest and predator populations, which makes it difficult to know if you have an accurate picture of the insect community. Traps have the advantage of continually collecting data. Catch data will be 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 will be 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 decision and scouting counts with trap capture for that same period of time.

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.
The traps placed at 20 pear orchards in Hood River Co (Fig 1) yielded a total of 5,037 natural enemies. Of these the most common insects found were green lacewings (1,680), Dereaocoris (1,836),

Yellow Jacket's (809), and earwigs (232) (Fig 2). Weekly averages of NE species showed when populations of the various natural enemy species were growing or decreasing (Fig 3).

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

We are currently waiting to get data from our collaborative crop consultants (who are all hunting now). However, we can see distinct increases and decreases in key natural enemy populations throughout the growing season. Once we obtain spray records from the crop consultants, we will be able to look for correlation with pest populations, fruit injury and NE numbers. Dates of pesticide spray are expected to correlate with a drop in NE populations.

Next year we will begin to take our own measurements of orchard pests (e.g. pear psylla) using standard scouting techniques at each of the orchards we place traps in. Gathering this data on our own will provide a finer measurement of the pest populations in the trees surrounding our NE traps. Our cooperative crop consultants may not be sampling this part of the orchard during each of their routine scouting trips.

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

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.

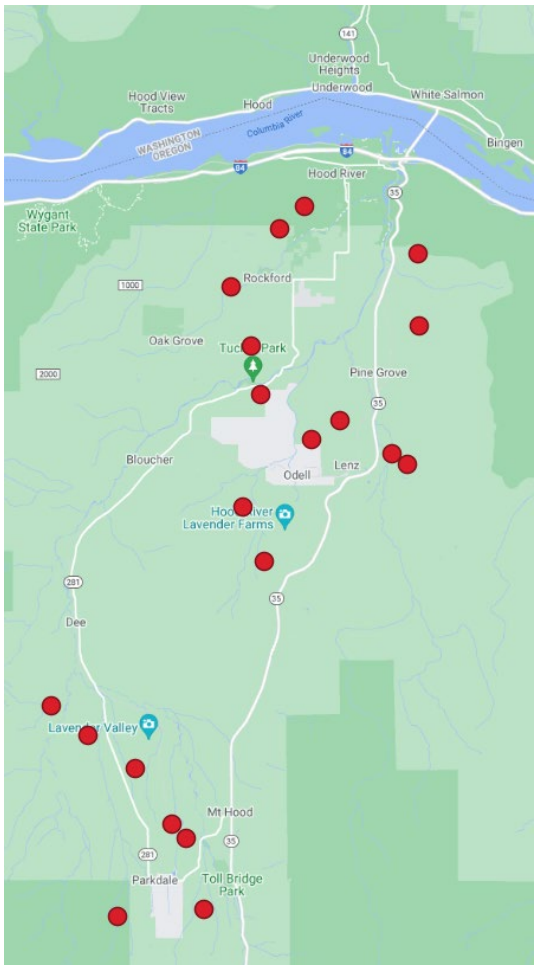

Figure 1. Map showing the sites where traps were placed in 2021.

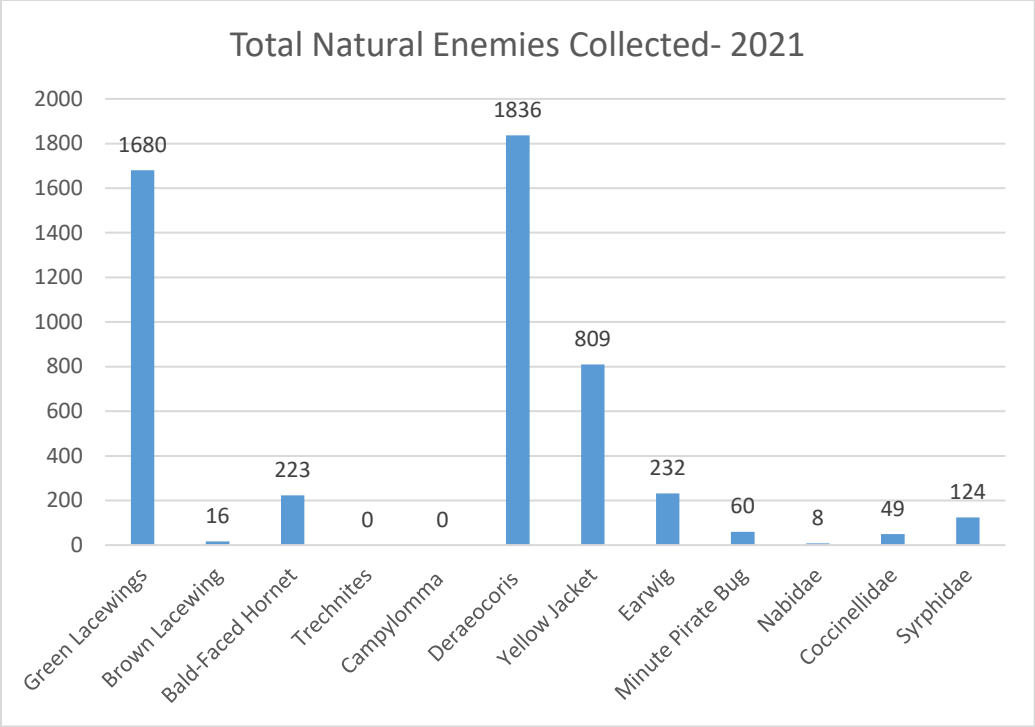

Figure 2. Total number of natural enemies collected and identified on the traps in 2021.

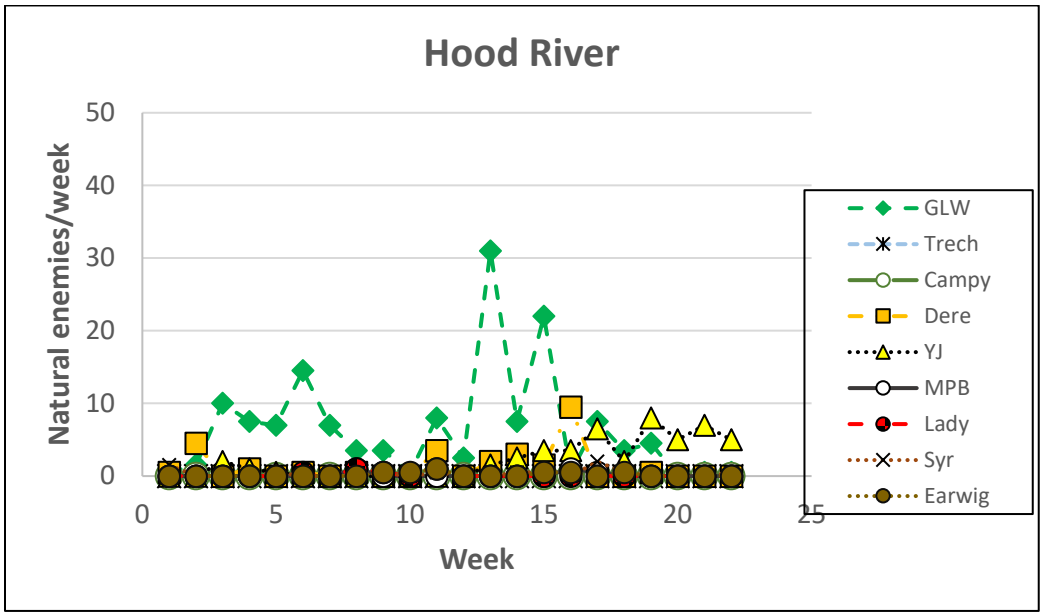

Figure 3. The average Natural Enemies found in the Hood River zone over the 22 weeks of trapping. GLW=green lacewing; Trech=Trechnites insidiosus; Campy=Campylomma verbasci; Dere=Deraeocoris brevis; YJ=yellow jackets; MPB= Minute Pirate Bug; Lady= Lady Beetle; Syr= Syrphidae.

Literature Reviewed.

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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.

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 (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 wasp egg-parasitoid 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, and new wasps were reared from BMSB eggs collected from the MCAREC lab colony. Releases of the wasps occurred weekly from August 12th- October 7th at 14 sites.
2. **Measure establishment using sentinel egg masses and yellow sticky traps (years 2 & 3)**
   We will begin to measure Tj establishment in 2022
3. **Describe the habitats where wasp establishment is most successful (years 2 & 3)**
   We will begin to describe the habitats where wasps successfully established in 2022.
4. **Measure the effectiveness of Tj biocontrol for preventing fruit damage (years 2 & 3)**
   We will begin to measure Tj effectiveness for preventing fruit damage in 2022.

Significant findings / outcomes

- **Other:** This project prompted conversations with other researchers in the PNW working on releasing this wasp. Recognizing that we should all be communicating in a more organized forum, I formed a **PNW Tj working group** consisting of researchers in Washington & Oregon, state agencies (ODA & WSDA) and federal agencies (USDA). We will coordinate on mapping release sites and recording establishment across the PNW.
- **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 (33% complete):** A total of 7,859 Tj were reared at the MCAREC insectary, and released at 14 orchards (13 pear and 1 peach) located throughout Hood River County.
- **Objective 1 (33% complete):** The Oregon Department of Agriculture donated 1,400 Tj from their colony for release in Hood River after learning of our release program.
- **Objective 1 (33% complete):** A total of 602 adult BMSB and 279 nymphs were collected from traps set at the 14 release sites. The traps helped locate orchards with large populations of BMSB, which should be beneficial for Tj establishment. This data was additionally used to initiate a regional BMSB trapping network where weekly reports of trap catch was shared with pear growers in the region.
- **Objective 2 (0% complete):** Research activities will begin in 2022**.**
- **Objective 3 (0% complete):** Research activities will begin in 2022**.**
- **Objective 4 (15% complete):** Most research activities will begin in 2022. The 602 adult BMSB and 279 nymphs collected from traps set at the 14 release sites, provided a year zero stink bug population measurement.

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 4). Stink bugs require fresh food and water daily and colony

maintenance and egg collection requires 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 eggs (Figure 5). Releases occurred weekly (Figure 6) from August 12th through October 7th at 14 sites (Figure 1). Weekly release numbers varied with the amount of wasps available. Sites with sprayer activity on release days were skipped and wasp for that site were released at other locations. Off-season egg production that exceeds what is needed to maintain our current colony will be frozen for later use.

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

Expected outcomes: We expect to exceed this year's release numbers in the coming field seasons.

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

We will begin to measure Tj establishment in 2022 using yellow sticky cards and sentinel egg masses at each of this year's release sites (Figure 1). Cards and sentinel eggs will be placed at sites where Tj was previously released and checked weekly. Sentinel eggs will be brought back to the lab and held in cages until stink bugs or wasps emerge. Parasitism by Tj in subsequent years will be considered evidence of establishment. Yellow sticky cards will be examined under microscope for presence of Tj wasps. 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 she released wasps at 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.

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 population 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. Direct inspection of randomly selected fruit will be conducted to measure changes in fruit injury over time.

Expected outcome: Year zero population numbers were so low that damage was undetectable. It may be necessary to coordinate with packing houses to collect possible feeding damage.

Results and Discussion

In this first year, 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 over 7,000 wasps at 14 locations across the Hood River growing region from this colony. We have also established a collaboration with ODA to assist with the distribution of Tj wasps from their state-wide program. This collaboration resulted in another 1,400 wasps released in the Hood River area. In addition, we are assisting Dr. Nik Wiman's PhD

student with her Tj wasp release in our area. Her project added another 1,200 wasps to the pear orchards in Hood River. Starting next year, we will begin trapping efforts to look for establishment of the wasp in these locations. Wasp releases will continue in new locations in subsequent years. Establishment of Tj will help control BMSB, and reduce the dependence on broad-spectrum insecticides. Once successfully established this biological control agent will help provide area-wide management of BMSB that is

selective (will not harm beneficial insects in the orchard), requires no inputs from growers, and is free.

Challenges: This past year, 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. As we now have an established colony, we will be starting the 2022 field season in a much better position.

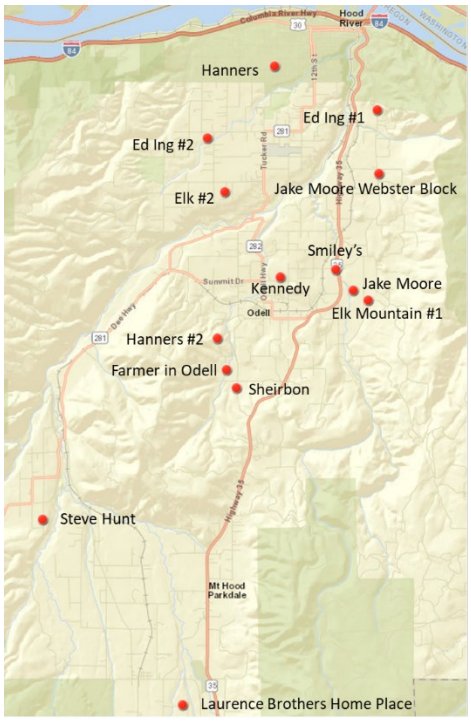

Figure 1: Sites *Trissolcus japonicus* were released in 2021.

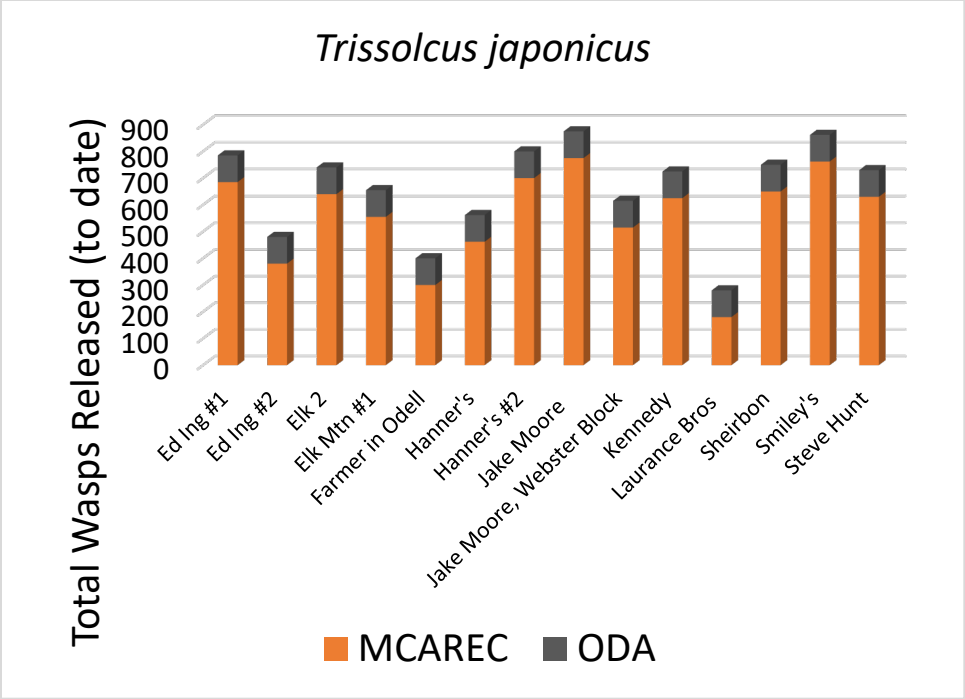

Figure 2. Number of *T. japonicus* released at each site reared by MCAREC and ODA.

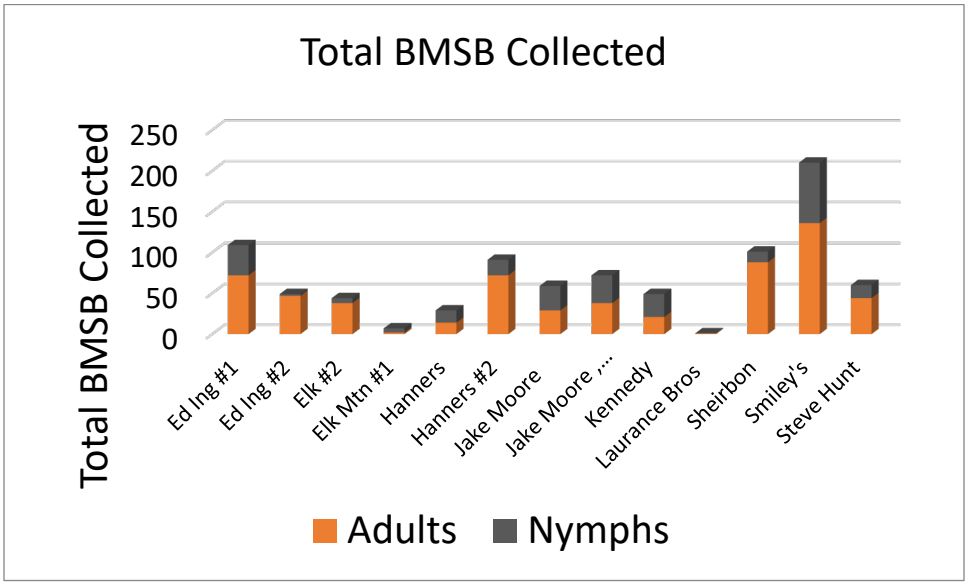

Figure 3. Seasonal total of BMSB adults and nymphs collected at each release site.

Figure 4. BMSB colony cages.

Figure 5. *Trissolcus japonicus* containers.

Figure 6. *Trissolcus japonicus* being released into the field.

Project Title: Enhancing pear psylla biological control through predator recruitment

Primary PI: Tobin Northfield
Organization: WSU TFREC Wenatchee
Telephone: 509-415-9751
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Address 2:
City/State/Zip: Wenatchee, WA 98801

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

Report Type: Final Project Report

Project Duration: 1-Year

Total Project Request for Year 1 Funding: $ 51,325

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Tobin Northfield
Organization Name: WSU TFREC
Contract Administrator: Anastasia Mondy
Telephone: 916-897-1960
Contract administrator email address: arcgrants@wsu.edu
Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@wsu.edu

Item	2019	2020 (NCE)	2021 (NCE)
Salaries[1]	23,750	0	0
Benefits[2]	8,723	0	0
Wages	5,760	0	0
Benefits[3]	92	0	0
Equipment	0	0	0
Supplies[4]	8,000	0	0
Travel[5]	5,000	0	0
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	51,325	0	0

Footnotes:
[1] Postdoctoral associate 50% FTE (Y1 -12 months, Y2 – 12 months)
[2] Postdoctoral associate (36.73%)
[3] 1.6%

[4] Includes lab and field supplies.
[5] In state travel.

OBJECTIVE

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

SIGNIFICANT FINDINGS

- In the 2021 field experiments *Orius insidiosus* provided weak and insignificant effects on reducing pear psylla abundance.
- In the 2021 field experiments thrips did not directly alter psylla abundance, presumably either through chemical induction, or predation.
- In the 2021 field experiment thrips did significantly not alter predation rates, negatively or positively by serving as alternative prey for *O. insidiosus.*
- In-field variability in the effectiveness chemical defense induction on pear psylla in previous experiments does not appear to be due to variability in thrips feeding, since thrips did not significantly alter psylla abundance.

METHODS

2019

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

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

The most common predator species were *Deraeocoris* sp. bugs (D), *Harmonia axyridis* ladybeetles (H), and *Adalia bincutata* lady beetles. Spiders were present too, but there were not enough of the same species to include in an experiment. Thrips were not abundant at this time.

We next designed an experiment to determine which combination of these predators provided the best control of psylla. Each cage included two individuals of either a single predator species, or a pairing of one individual from each of the three species listed above. We also included no-predator controls, and each treatment was replicated 4 times. Predators were introduced on July 26th 2019, and psylla abundances were estimated by peering through mesh sleeve cages, to avoid disruption of psylla treatments by opening cages. We introduced predators immediately after time zero psylla counts. Then, we broke down the experiment on August 12th and counted all psylla and predators.

2020

On March 1, 2020 prior to leaf growth, we set up 40 exclusion sleeve cages on pear trees at the Wenatchee WSU Tree Fruit Research and Extension center in the pear orchard (Fig. 1). To set up the cages, we first removed any overwintering pear psylla from the trees and put the sleeve cages on branches to ensure that all branches were free from psylla and thrips. This would allow us to introduce to the cages four treatments: 1) pear psylla only, 2) thrips and pear psylla, 3) anthocorids and pear psylla, and 4) thrips, anthocorids, and pear psylla. Our plan was to collect anthocorids from surrounding vegetation during bloom and use the most commonly collected anthocorid species for experiment. However, COVID restrictions occurred in March before the trial could be initiated, shutting down the experiment before it could begin. Later, in early summer we were able to develop lab protocols that allowed for methods to conduct research but reduce potential for COVID transmission and began planting pear trees for a similar experiment in growth rooms. However, the employee funded by the project needed to go on family medical leave, and we were not able to hire a new employee. To account for this, we kept 20 trees in a cold room so that we could plant them and grow them in a greenhouse with supplemental light when we were able to restart the experiments. In the fall we established pear psylla colonies, seeded from a colony at USDA Wapato that we kept on potted pear trees, and in November, we planted the pear trees from the cold room to prepare for an experiment (Fig. 2). However, the trees never sprouted, potentially due to either an issue in the cold room, or from the shock of being transplanted to a warmer environment. Therefore, we plan to restart the experiment in Spring 2021.

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

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

2021

On March 10, 2021, prior to leaf growth, we set up 40 exclusion sleeve cages on 10 pear trees (4 cages/tree) at the Wenatchee WSU Tree Fruit Research and Extension center in the pear orchard (Fig. 3). To set up the cages, we first removed any overwintering pear psylla from the trees and put the sleave cages on branches to ensure that all branches were free from psylla and other insects. These cages remained empty on trees from 10 March until 12 April, when the experiment was initiated. Because densities of each, thrips and anthocorids in orchards were low at the start of the experiment, we purchased *Orius insidiosus* from Arbico Organics, and collected western flower thrips (*Frankliniella occidentalis*) from a patch of dandelions growing at the WSU Tree Fruit Research and Extension Center. To each tree we set up four cages, each with a different treatment: 1) pear psylla only, 2) thrips and pear psylla, 3) *O. insidiosus* and pear psylla, and 4) thrips, *O. insidiosus*, and pear psylla. To each cage we introduced 10 female pear psylla on the evening of 12 April 2021 (cages 1-27) or the following morning (cages 28-40). Then, in the afternoon of 13 April 2021 we added 20 adult *Frankliniella occidentalis* thrips per cage to the cages with thrips treatments. Herbivores were allowed at least 72 hours to acclimate, and *O. insidiosus* was introduced to cages on 16 April, which we refer to as day one of the experiment. At day 20, (5 May), we cut all branches with sleeve cages off the trees, leaving the sleeve cage intact, and moved all branches to a refrigerator during sorting. We then visually observed and counted all insects on the branches. This method was effective for adult psylla and thrips, as well as *O. insidiosus*, but was not effective at counting immature psylla or thrips. Therefore, to count nymphs and thrips we also used a leaf brush to remove all insects off 20 randomly selected leaves per sleeve cage. We also counted the leaves within the sleeve cage to use the random leaf sample to estimate the abundance of immature psylla and thrips across the entire cage. To analyze the data, we used a generalized linear mixed model, using a negative binomial error distribution (typical for count data), a log-link function (assumes only positive numbers of insects), and a random effect of tree to account for variability between trees. Because the number of psylla nymphs were estimated, rather than discrete counts we used a gamma distribution to model the error distribution (the negative binomial is only suitable for discrete count data).

Figure 3. Sleeve cages on pear trees March 10, 2021, waiting for insect addition.

RESULTS & DISCUSSION

2019. In our 2019 experiment, we found that in July the most abundant predators were *Deraeocoris* sp. bugs and two species of lady beetles. While adult psyllas were abundant, we observed very few thrips. The experimental approach worked well, except we found very little reproduction. The four no-predator controls had very few psylla in cages, suggesting that reproduction was very low (mean of 3.5 psylla/cage). Numbers of psylla in other cages were highly variable, ranging from 0 to 18 psylla in the predator treatments. Discussion with Louis Nottingham suggested that this was due to a combination of aging adults from the previous

generation that were not reproducing, and newly emerged adults from the next generation. This solidified the benefit of studies early in the season when there is a single generation of psylla, such that psylla reproduction is similar across treatments.

2020. Our spring 2020 experiment was disrupted by the COVID pandemic and needed to be postponed until 2021.

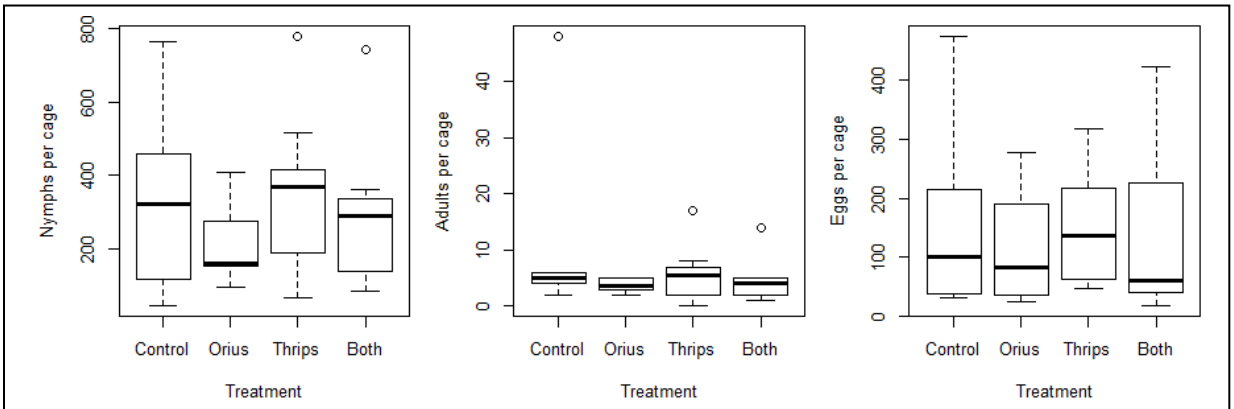

Figure 4. Total number of nymphs (left), adults (middle), or eggs (right) per cage in the presence of Orius insidiosus *(Orius), western flower thrips (Thrips), both* O. insidiosus *and thrips (Both), or none (Control). Boxplots are particularly useful to represent data when the error distribution is not symmetrical, as is often the case in count data. The bold horizontal line inside each box represents the median number of the insects for the treatment. The top and bottom of the box represents the 75th and 25th percentiles of the data, and the whiskers represent the theoretical maximum and minimum of the data. Dots represent outliers.*

2021. In our 2021 cage experiment, we found that *O. insidiosus* slightly reduced numbers of pear psylla nymphs, but the results were not statistically significant (Figure 4, generalized linear mixed model likelihood ratio test: $\chi^2 = 2.63$, $P = 0.105$). Thrips did not significantly alter pear psylla nymph density (Figure 4, generalized linear mixed model likelihood ratio test: $\chi^2 = 0.36$, $P = 0.5055$), or significantly alter the effects of *O. insidiosus* (Figure 4, generalized linear mixed model likelihood ratio test: $\chi^2 = 2.18$, $P = 0.14$). Similarly, none of the treatments affected the number of adult or egg pear psylla in the cages (Figure 4, generalized linear mixed model likelihood ratio tests: all $P > 0.05$).

The results from the experiment are interesting in light of experiments on the use of chemical defense elicitors that serve to promote particular defensive compounds in plants that reduce the ability of pear psylla to grow (Cooper and Horton 2015, 2017). Previous research suggests that chemical elicitors work to reduce pear psylla in the laboratory (Cooper and Horton 2015), but have variable results in the field (Cooper and Horton 2017, Orpet et al. 2021). Here, given the ability for western flower thrips to also influence chemical induction pathways (Steenbergen et al. 2018), we evaluated the potential for thrips to induce defense in the field to see if the variability is driven by inductions by other insects. We found no evidence that the variability in thrips abundances is altering psylla abundance, whether driven by chemical elicitation of defenses, by serving as alternative prey, or through direct predation (Hall 2014), at least within the confinements of cages. Previous research in Europe suggests that pear psylla (*Cacopsylla pyricolla* and *C. pyri*) can induce volatiles that recruit anthocorid predators (Scutareanu et al. 1997). The proposed evaluation of this effect in year 2 was not funded, but the relatively low impact of *O. insidiosus* in this experiment suggest the end result of these impacts

may be minimal as well. Another potential finding that may be worth exploring further is that *C. pyricola* has been shown to induce defenses in nearby pear trees in Europe as well (Scutareanu et al. 1996). If pear trees are communicating, the use of chemical elicitors may not be readily apparent on a tree scale, because control trees are also induced (through communication). Furthermore, if trees are indeed communicating, chemical elicitors may only need to be applied to a subset of trees, with the rest of the trees inducing defense through tree-to-tree communication. Further research may identify whether this mechanism is a way forward to promote defense induction while reducing application costs.

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Executive Summary

Project Title: Enhancing pear psylla biological control through predator recruitment

Key words: Pear psylla, biological control, induced defense

Abstract:
Recent research suggests induced defenses can reduce pear psylla growth, potentially improving control in the field. However, while previous lab results were promising, results have been highly variable in the field. This begs the question of whether variability in defense induction is driven by other herbivores feeding on pear trees, altering the hormonal pathways governing chemical defenses. Thrips are often found in pear orchards during bloom, and often induce chemical changes in a range of plant species that make them less palatable to pests. Furthermore, thrips commonly serve as alternative prey for anthocorid bugs that can attack psyllids and have even been observed eating a related herbivore, Asian citrus psyllid. Although thrips are present in pear orchards throughout the year, they generally do not cause economic damage to pears and therefore may provide 3 indirect benefits: *i*) inducing chemical defenses in the plant, *ii*) serving as alternative prey for predators to boost predator reproduction, and *iii*) attracting predators through inducing plant volatiles. Here, we evaluated pathways *i* and *ii* by conducting a field experiment, factorially manipulated thrips abundance and a shared predator (*Orius insidiosus*), known to respond to thrips abundances in other cropping systems. We conducted the experiments within sleeve cages on pear trees and initiated the experiment at bloom. While *O. insidiosus* slightly reduced psylla abundance, the finding was not significant, and thrips provided no impact on either, psylla abundance, or predation by *O. insidiosus*. These findings suggest that variability in chemical defense elicitation in the field is not driven by variation in thrips densities. An avenue of further research would be to evaluate tree to tree communication, to see if chemical defenses induced in one tree promotes defenses in nearby trees.

FINAL PROJECT REPORT

Project Title: Using transcriptomics to target key behaviors of pear psylla

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

Significant Findings

- Transcriptome libraries of summerform, diapausing winterform, and post-diapause winterform pear psylla were created and analyzed, substantially improving knowledge of seasonal biology of pear psylla.
- Differentially expressed genes that are likely related to changes in seasonal biology, including those involved in reproduction, immunity/defense, olfaction, sight, and muscle development, were identified and analyzed. This dataset will aid future studies on the overwinter biology and control of pear psylla.
- Funds for this project were used to leverage additional funds to sequence the pear psylla genome and to obtain long-read transcriptome sequences.
- Adding the transcriptomic and genomic data to the open access AgriVectors.org database will allow streamlined comparisons with transcriptomes and genomes of citrus psyllid and potato psyllid, enabling researchers to adapt gene-based therapies developed for other psyllids and related insect pests.

Results and Discussion
Background

Pear psylla occurs as two distinct seasonal morphotypes - summerform and winterform - that differ with respect to diapause, feeding behavior, plant attraction, and association with bacterial endosymbionts (Figure 1) (Ullman and McLean 1988, Krysan and Higbee 1990, Krysan and Horton 1991, Horton et al. 1998, Civolani et al. 2011, Cooper et al. 2017). Summerforms undergo several overlapping generations each year. The nymphs develop exclusively on pear, and summerform adults

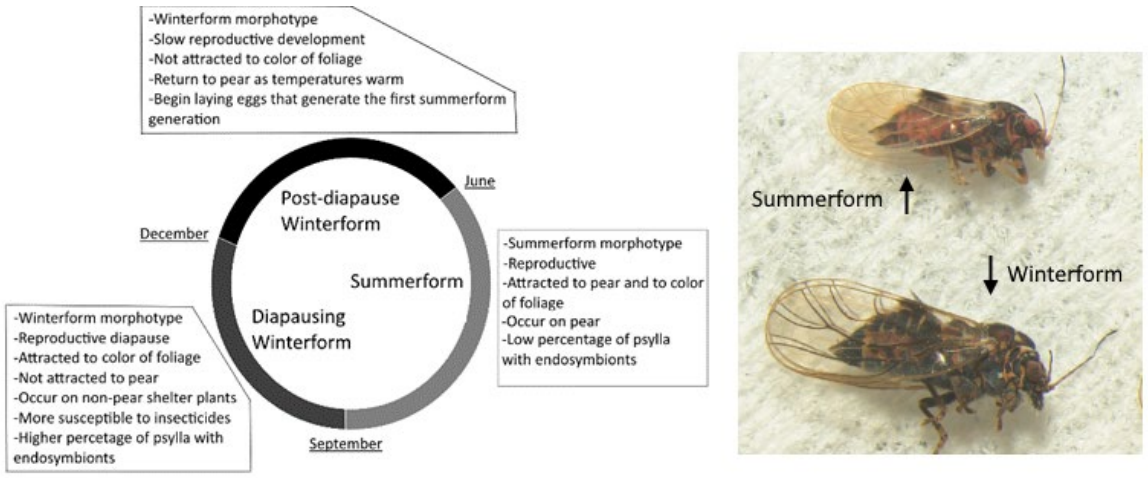

Figure 1. Annual occurrence and phenological traits associated with summerform (light grey), diapausing winterform (dark grey), and post-diapause winterform (black) pear psylla.

are rarely found on other plants (McMullen and Jong 1967). Nymphs develop into winterform in response to shortening photoperiods of early autumn. Winterform are larger and darker compared with summerform and occur as a single overwintering generation (Horton et al. 1998, Mustafa and Hodgson 2984). The winterforms begin autumn and winter in reproductive diapause characterized by a lack of mating and ovarian development. Reproductive diapause seems to be associated with reduced tolerance to certain insecticides (Unruh and Krysan 1994), and winterforms are more likely than summerforms to harbor certain bacterial endosymbionts (Cooper et al. 2017). Autumn leaf drop displaces winterforms from pear trees prompting many psylla to disperse from orchards (Horton et al. 1994). Diapausing winterforms remain attracted to the color of foliage, and often visit or settle upon evergreen trees and shrubs, or deciduous trees with leaf drop occurring later than in pear (Kaloostian 1970). Winterforms break diapause in late December, but reproductive development remains slow due to cold temperatures (McMullen and Jong 1967). As temperatures warm in February and March, post-diapause winterforms return to pear and begin laying eggs destined to become the first summerform generation.

Although changes in behaviors and phenotypes associated with summerform, diapausing winterform, and post-diapause winterform psylla are well-documented, the timing for these behavioral changes and mechanisms controlling behaviors are not currently understood. Comparative transcriptomics has proven highly useful to examine the seasonal or other life cycle shifts in behavior or physiology by other insect pests. The goal of our study was to use complete transcriptomes to compare gene expression among summerform, diapausing winterform, and post diapause winterform, which will allow us to pinpoint the exact timing for these changes and to develop gene-based insecticides to control pear psylla.

Methods

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

lifecycles of pear psylla. In 2020, quantitative PCR was used to confirm differential expression of a subset of genes involved in reproduction, immunity, defense, muscle function, and sensory.

Results

We first looked at overall numbers of differentially expressed genes among summerform, diapausing winterform, and post-diapausing winterform pear psylla. A higher number of differentially expressed genes indicates larger differences in physiology. Generally, there was a high degree of similarity in gene expression profiles among replications of each stage (Figure 2; like shading/fill = similar gene expression profiles). The exception was rep 3 of post-diapausing winterform, which exhibited a more similar gene expression profile to summerforms collected several months later (Figure 2B and C). However, the major differences in gene expression were observed between summerform and diapausing winterform (Figure 2A). Post-diapausing winterform exhibited gene expression profiles that were intermediate to those of diapausing winterform and summerforms (Figure 2). Overall, these results suggest that pear psylla undergo substantial physiological changes in autumn, but winterforms that are present in spring are more similar to summerforms than to autumn winterforms.

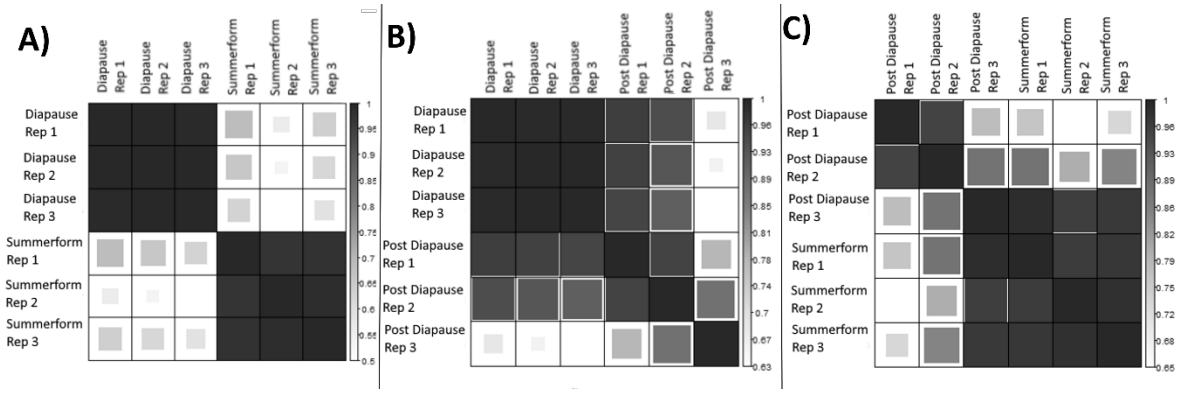

Figure 2. Relative differences in overall gene expression between summerform and diapausing winterform (A)), diapausing and post diapausing winterform (B), and post-diapause and summerform (C). Similar shading and filles represent greater similarity in gene expression among specimens.

BLAST2GO analysis was used to assign gene ontologies to differentially expressed genes. Results of that analysis are too broad for the scope of this report but will be included in a forthcoming manuscript submitted to a peer-reviewed journal. From the ~15,000 differentially expressed genes, we identified a subset of genes that are involved in reproduction, defense, immunity, photoreception, olfactory, and muscle structure and function (Figure 3). Consistent with the results presented in Figure 2, the largest variations in selected differentially expressed genes was observed in diapausing winterforms (Figure 3). A large number of genes were up- and down-regulated in diapausing winterforms relative to summerforms. The largest number of differentially expressed genes were related to muscle structure and function (Figure 3A). Winterforms are larger than summerforms (Figure 1) and likely have more muscle mass for long-distance dispersal. The differences in expression of genes between summerforms and winterforms may be related to this behavior.

We previously found that pear psylla collected in spring are more likely than those collected in summer to harbor the plant pathogen that causes pear decline, *Phytoplasma pyri* (Cooper et al. 2017), and winterforms are more susceptible to certain classes of insecticides compared with summerform (Unruh and Krysan 1994). The largest variation in differentially expressed genes associated with immunity and defense was observed between summerform and diapausing winterform (Figure 3), which may alter pear psylla's susceptibility to infection or pesticides. Further

research is needed to examine whether pear psylla are more susceptible to entomopathogens during the orchard re-entry phase in early spring.

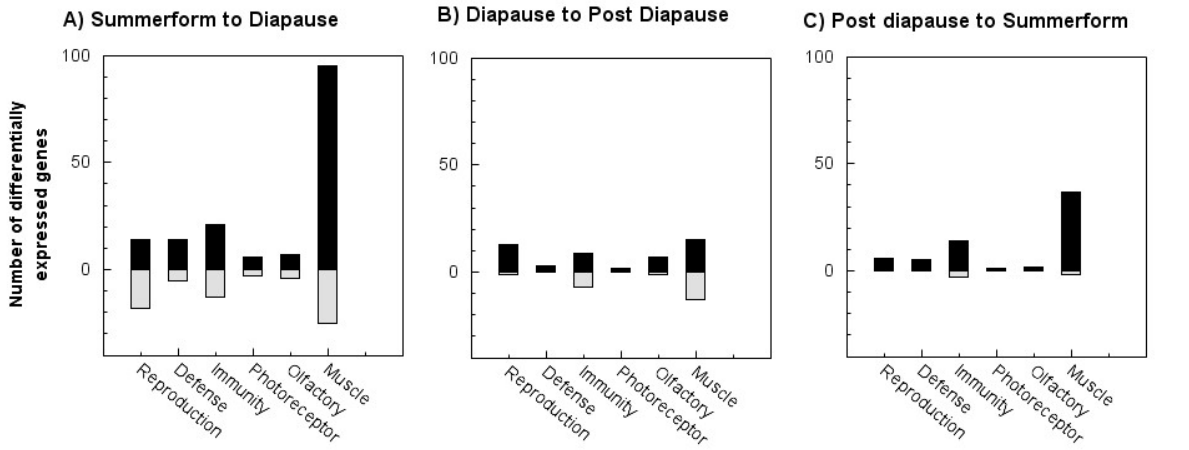

Figure 3. Number of genes that were up (black bars) or down (grey bars) regulated in diapausing versus summerform (A), post-diapausing versus diapsuing winterform (B), and summerform versus post-diapausing winterorm (C) pear psylla.

We identified 15 sensory receptor proteins putatively involved in sight, olfaction, or hearing that were upregulated in diapausing winterform psylla. Winterform psylla are attracted to color of foliage in autumn but are not attracted to pear specifically (Figure 1). Because they are attracted to the color of foliage, they often disperse from pear orchards after leaf drop and overwinter on evergreen conifers. Post-diapause winterforms are not attracted to the color of foliage and disperse from conifers to pear trees. We currently do not know what ques pear psylla use to locate pear trees, but preliminary evidence suggests that pear psylla are attracted to pear volatiles in early spring. It is currently not known whether winterform pear psylla are also attracted to volatiles released by conifers. The upregulation of sensory receptors in diapausing winterform psylla (Figure 3A) collected in December may be due to a change in which senses (olfactory versus visual) regulate pear psylla dispersal and behavior.

Anticipated benefit to the industry.

It is not surprising that gene expression differed between winterform and summerform pear psylla. But the transcriptomes produced by this project provide valuable insight into the basic biology of seasonal morphotypes that will improve design and interpretation of future studies on the biology and management of overwintering psylla. With matching funding related to this project, we are working with AgPest100 Initiative (http://i5k.github.io/ag100pest) to sequence the genome of pear psylla, which will be the first genome of any psyllid pest of temperate tree fruits. In addition, we are working with collaborators to obtain long-read transcriptome sequences that will improve annotation of the transcriptomes developed from this current project. Once we obtain and analyze the genome of long-read transcriptomes, we will prepare a peer-reviewed manuscript changes in gene expression that correspond with changes in seasonable biology and management of pear psylla.

We are making these transcriptome libraries available on AgriVectors.org, an online bioinformatics tool developed by the PIs (Saha et al. 2021). This portal provides an open access platform that allows researchers to easily compare datasets across multiple pathosystems, including citrus psyllid and potato psyllid. Contemporary research is progressing toward the ability to use highly-specific gene-based therapies to target insects and pathogens in crops (Hunter 2017, Ghosh et

al. 2018, Das and Sherif 2020, Hunter et al. 2021, Hunter and Wintermantel 2021). In fact, the precursor to AgriVectors.org (citrusgreening.org) has already helped researchers develop several RNAi biopesticides which have been patented for control of citrus psyllid (US patent 10,344,291_B2), and several patented antisense oligos (US patent 11,001,842 B2) that target and reduce pathogens. By comparing transcriptomes between citrus and potato pathosystems, we adapted an RNA-targeting therapy developed for citrus greening disease to target RNA of the zebra chip pathogen in potato (Hunter et al. 2021), and are currently adapting RNA-based insecticides developed for citrus psyllid to target potato psyllid. ***The pear psylla transcriptomes will allow us to also adapt these bioinsecticides to target pear psylla.***

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EXECUTIVE SUMMARY
Project Title: Using transcriptomics to target key behaviors of pear psylla Key words:

Key words: pear psylla, transcriptomics, RNA-based controls

Abstract:

Pear psylla occurs as two distinct seasonal morphotypes - summerform and winterform - that differ with respect to diapause, feeding behavior, plant attraction, and association with bacterial endosymbionts. Winterforms are further divided into diapausing winterforms that occur in autumn and early winter, and post-diapausing winterforms that occur in late winter and spring. Changes in behaviors associated with summerform, diapausing winterform, and post-diapause winterform psylla are well-documented, but the timing and regulatory mechanisms controlling these behavioral changes remain unknown. Comparative transcriptomics has proven to be highly effective for examining the seasonal and other life cycle shifts in behavior or physiology in other insect pests. We used complete transcriptomes to compare gene expression among summerform, diapausing winterform, and post diapause winterform pear psylla, thus providing a better understanding of the expression-level changes underlying the seasonal biology of pear psylla. We also used funds to leverage opportunities to sequence the pear psylla genome using the latest sequencing technologies. These transcriptomic and genomic libraries will enable researchers to adapt gene-based therapies that have been developed and that are currently being tested for control of citrus and potato psyllids to control winterform and summerform pear psylla.

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

Project Title: Fire Blight Product Testing for Effective Recommendations

PI: Tianna DuPont
Organization: WSU Extension
Telephone: (509) 293-8758
Email: tianna.dupont@wsu.edu
Address: Tree Fruit Research and Extension
Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee WA 98801

Total Project Request: **2020:** $14,255 **2021:** $14,686 **2022:** $0 (reduced from $15,132)

Other funding sources: **Awarded**
Amount: $30,000
Agency Name: USDA NIFA IR4

Other funding sources: **Awarded**
Amount: $88,250
Agency Name: Gift Grants from Product Companies

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

Budget 1
Organization Name: **WSU** **Contract Administrator:** Stacy Mondy
Telephone: 916-897-1960 **Email address:** anastasia.mondy@wsu.edu

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

Footnotes: [1]Salaries for a scientific assistant 2 month/ yr.
[2]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

- Relative control from alum was 30% in 2020 and 50% in 2021 less than the 2016-2019 average relative control of 75%.
- In 2021 peroxide + peracetic acid treatments provided relative control of 63-67% with 3 applications, not significantly different than the organic standard and up from 23% relative control provided with 2 applications in 2020.
- Commercial thyme oil products averaged 43% relative control in 2019 to 2021 with three to six applications.
- In 2020 and preliminary trials in 2019 bacteriophage products performed no better than the water treated check.

MATERIALS & METHODS

A two-acre research block of mature Red Delicious apples at WSU Columbia View Orchard 48 Longview Rd. East Wenatchee, WA was used for this trial. The experiment was arranged in a randomized complete block with five single tree replications. Products were applied to the area of the tree to be inoculated (bottom 8 ft) according to manufacturer recommendations using a Stihl SR420 mist blower backpack sprayer. Products were applied to wet, near dripping at 0.4 gal/tree (100 gal/A). At 100% bloom (of the king blooms) (19 Apr 2021, 18 Apr, 2020) *Erwinia amylovora* was applied at 1 x10^6 CFU ml^{-1} (verified at 40-94 x10^6 CFU ml^{-1} 2021, 24 x 10^6 CFU ml^{-1} 2020) to lightly wet each cluster. Trees were visually evaluated for flower cluster infection weekly from when symptoms became visible 10 days after treatment for 4 weeks and infection counts summed across all dates. Fruit was evaluated for fruit skin marking before fruit colored over. Statistical analysis was performed using general linear mixed models (GLIMMIX) analysis of variance ANOVA, and multiple means comparison Fisher's T test (LSD) SAS v 9.4. Environmental conditions during bloom (14-26 Apr 2021) ranged from an average maximum temperature of 72 °F and minimum of 43 °F with 36% average humidity. A precipitation event (0.04 in) occurred on 24 Apr the evening after petal fall sprays were applied. All applications were made under fast drying conditions. In 2020 during full bloom fire blight risk was moderate with warming temperatures right after full bloom. Temperatures during the bloom period (14-22 Apr) ranged from an average maximum of 56 °F to 77 °F and average minimum of 36 °F to 51 °F. Petal fall sprays went on the evening before a significant rainfall event.

RESULTS AND DISCUSSION

Alum

Alum (Potassium aluminum sulfate) has been tested for five years in Washington. This compound is experimental (non-labeled). It has had consistent positive results with an average of 75% control relative to the untreated check in 2016, 2017 and 2019 when the product was applied at an 8 to 10 lb per 100 gal rate. This control was lower than but not significantly different than the oxytetracycline check (82% control) and the streptomycin check (91% relative control). Marking from chemical russet was negligible in all WA trials (< 1 on a 0 to 15 scale). In 2020 control from alum was 30% compared

to the water treated check. In 2021 relative control was approximately 50%, but still significantly different from the untreated check and comparable to the relative control obtained using oxytetracycline check (56% relative control) and streptomycin check (58% relative control).

Table 1. 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.

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

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.

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.

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 3). In 2020 with two applications relative control for peroxide + peracetic acid treatments was 23% not significantly different than the water treated check (Table 4). At these application timings no significant fruit marking was observed (less than 1 on a 0 to 15 scale). In comparison long term averages are 85% relative control for the streptomycin standard (N=33), 71% relative control Blossom Protect + Buffer Protect (N=16), 68% relative control oxytetracycline standard and 68% relative control Previsto. Enumeration of bacterial populations in the flower suggest that the 3-day post petal fall application in 2021 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 1-2).

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 3. 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%) (J)	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%) (O)	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%) (J) *Bacillus amyloliquefaciens*	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 4. 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%) (J)	128 fl oz	Day after inoc and 3 days after inoc[v]	28	±	3.9	c	0
hydrogen peroxide (27%), peracetic acid (5%) (O)	128 fl oz	Day after inoc and 3 days after inoc	24	±	3.8	c	0.02
hydrogen peroxide (27%), peracetic acid (5%) (O)	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	31	±	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.

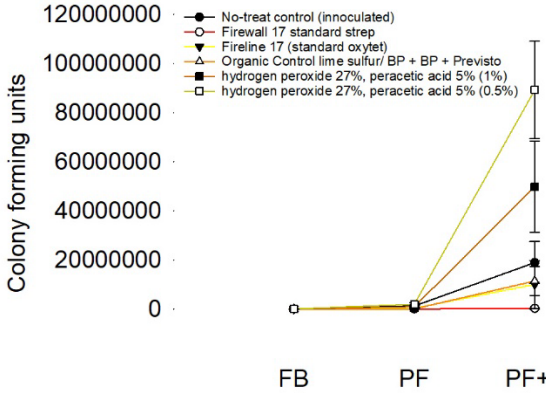

Figure 1. Effect of treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers at Full Bloom (FB), Petal Fall (PF) and Petal Fall + 1 week (PF+1) in WA in 2020.

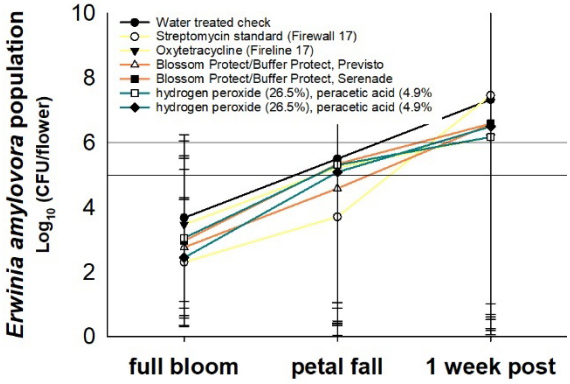

Figure 2. Effect of hydrogen peroxide and peracetic acid treatments applied to Red delicious apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers at full bloom, petal fall and 1 week post petal fall in Wenatchee, WA, in 2021.

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 Thymegard, Thymox, and Cinnerate.

Commercial thyme oil products averaged 43% relative control in 2019 to 2021 with three to six applications lower than but not significantly different than long term averages of 71% relative control Blossom Protect + Buffer Protect (N=16), 68% relative control oxytetracycline standard (N=25) and 68% relative control Previsto (N=48) (Table 5-7). In one trial the alternative organic program Blossom Protect + Buffer Protect at 50% and 100% bloom followed by Previsto at 100% bloom + 1 day and at 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.

Table 5. 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%)[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%)[v]	2 qt	100% bloom, 100% bloom + 1 day, petal fall	21.4 ± 3.9 ab	0.24
Thymol (23%)	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%) + 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%)	32 oz	100% bloom, 100% bloom + 1 day, petal fall, petal fall + 3 days	20.8 ± 3.7 ab	0.01
Thyme oil (3%)	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 6. Effect of Essential Oil/ Plant Extract Treatments on infection of *E. amylovora* in apple blossoms in Orondo, WA, in 2020 [‡]

Treatment	Rate per 100 gallon water	Application timings	Infections per 100 clusters	Fruit 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	1.24 lb			
(Blossom Protect/Buffer)	8.75 lb	50% bloom, 80% bloom,		
+ Soluble Copper (Previsto)	3 qt	100% bloom, petal fall	9.5 ± 1.3 bc	0.2
Thyme oil (23%)	2 qrt	80% bloom, 100% bloom +1, petal fall	17 ± 2.3 cd	0
Thymol (23%)	2 qrt	80% bloom, 100% bloom, petal fall	22 3.5 d	0
Cinnamon oil (60%)	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 7. 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,	6 gal	LS: 70% bloom		
Blossom Protect+ Buffer Protect,	1.24+8.75 lb	BP: 20% bloom, 80% bloom		
Previsto)	3 qt	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%)	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}.

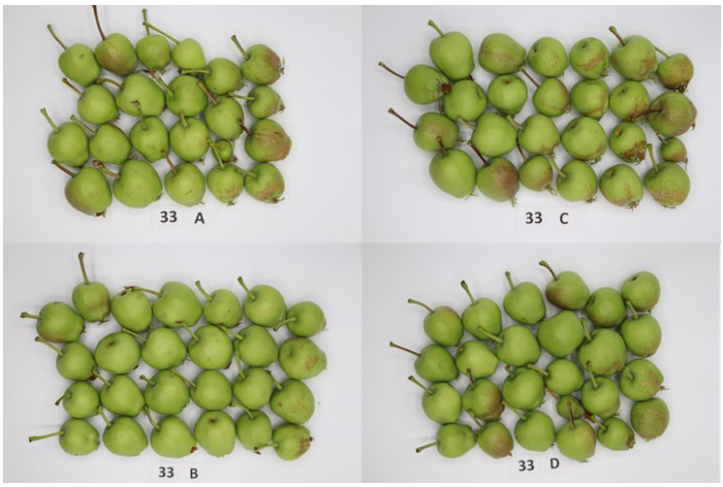

Figure 3. Russet fruit marking of Thyme oil treatment with eight applications, WA, in 2019.

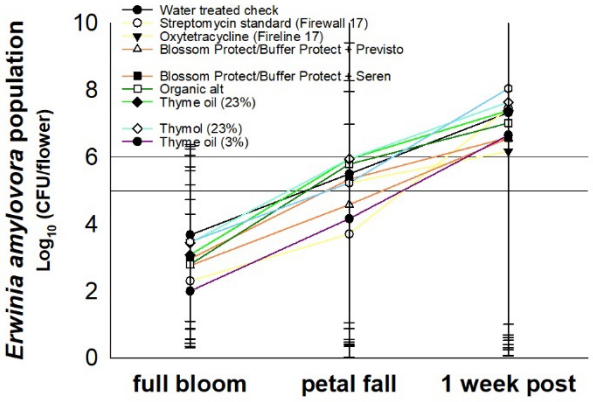

Figure 4. Effect of thyme treatments on the population size of *E. amylovora* strain 153N on flowers at full bloom, petal fall and 1 week post petal fall in Wenatchee, WA, in 2021.

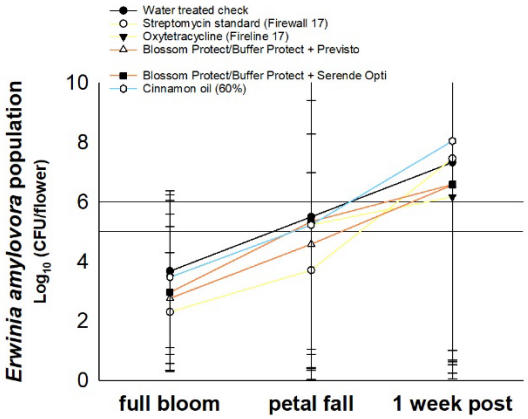

Figure 5. Effect of cinnamon oil products on the population size of *E. amylovora* strain 153N on flowers at full bloom, petal fall and 1 week post petal fall in Wenatchee, WA, in 2021.

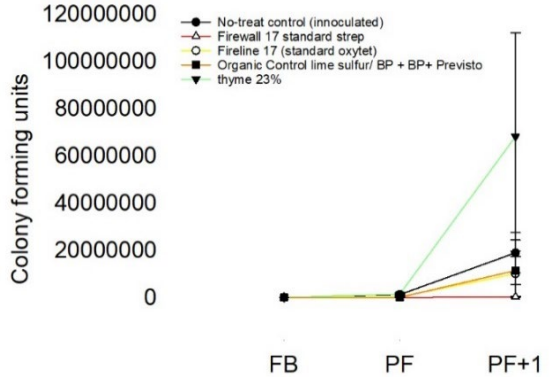

Figure 6. Effect of thyme oil treatments on on the pop size of *E. amylovora* strain 153N on flowers at full

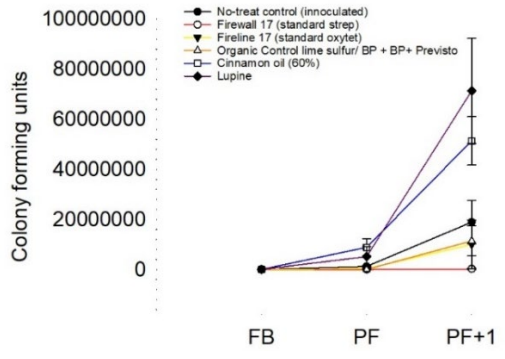

Figure 7. Effect of cinnamon oil treatments on bl the population size of *E. amylovora* strain 15

(FB), petal fall (PF) and petal fall + 1 week (PF flowers at full bloom (FB), petal fall (PF) and peta
Orondo, WA, in 2020. 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. *Bacteriophages* 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. Phage 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 (Iriarte *et al.*, 2012; Fujiwara *et al.*, Vol. 77, No. 12).

In 2020 and preliminary trials in 2019 bacteriophage products performed no better than the water treated check (Tables 8,10). Based on work by Sundin (Michigan State University) it was hypothesized that the addition of a particle film sun protectant would reduce phage die-off due to UV and enhance control potential. In 2020 addition of kaolin clay (Surround) did not improve control (Table 8).

Table 8. 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	2 qt	100% bloom 12hr before ap, +1 day, +3 days	24	±	4.8	c	0
Phage7 + Surround	2 qt + 0.1 lb	100% bloom 12hr before ap, +1 day, +3 days	31	±	3.7	c	0

Table 9. 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.

[t] Experimental biological.

[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 10. 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}.

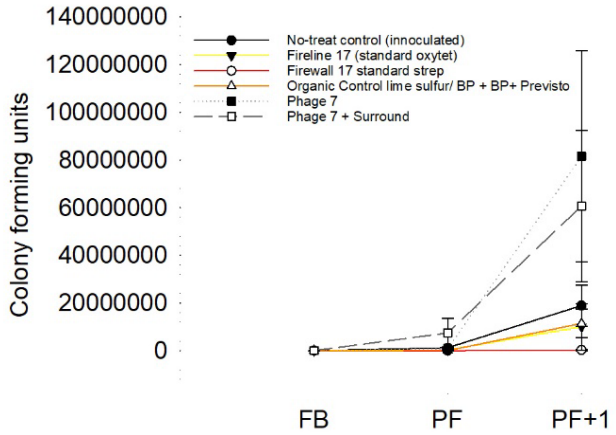

Figure 8. Effect of bacteriophage treatments on the population size of *E. amylovora* strain 153N on flowers in Wenatchee, WA, in 2020.

References

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CONTINUING PROJECT REPORT **YEAR**: 3 of 3
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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Co-PI: Achour Amiri
Organization: Washington State University
Telephone: 509-293-8752
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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:** $60,000
Amiri lab:
Agency Name: Specialty Crop Block Grant program-USDA-WSDA. **Amt. awarded:** $170,195.
Notes: "Strategies to enhance pre- and postharvest management of gray mold in pome fruit" PI: Amiri, co-PI: Tobin Peever. This grant is split 70% and 30% for apple and pear, respectively.

WTFRC Collaborative expenses: None

Budget 1: Achala KC
Organization Name: OSU Ag. Res. Foundation **Contract Administrator: Russ Karow**
Telephone: 541-737-4066 **Email address: russell.karow@oregonstate.edu**

Item	(2019-20)	(2020-21)	(2021-22)	2022
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**	**0**

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 have been observed among orchards located in different districts.
- From both OR and WA fruit samples, Botrytis were detected in pear tissues including calyx, stem-bowl, cuticle, and flesh indicating latent (dormant) infections from previous infections in the orchard
- In all locations, the size of *Botrytis* inoculum was greater in organic orchards compared to conventional orchards.
- In WA orchards, trials to detect *Botrytis* from bloom to late in storage were reconducted in 2020. More than 600 Botrytis isolates have been collected from WA and OR orchards and awaiting genetic analyses to determine the species. A delay occurred after the Postdoctoral scientist accepted another position.
- In SO trials, fungicides showed a range of effectiveness against 20 *Botrytis* isolates indicating variability in sensitivity when exposed to preharvest fungicides with different modes of action. When tested on wound inoculated fruit assays, the efficacy of Ziram, and PhD were higher than 50% for all isolates tested in this study. Whereas 25% of the isolates showed reduced sensitivity to Manzate, and Botran. Similarly, when three postharvest fungicides (ADA 72902, BioSpectra, and Scholar) were tested for their efficacy on wound inoculated fruits, their efficacy were higher than 60% for all isolates tested in this study.
- Four seasonal field spray programs to improve gray mold management were tested in 2020 and 2021 field seasons. Fruit are in cold storage and data will be available in spring 2022.

Methods

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

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

Activity 1.1. ***Infection timing:*** Amiri (Cashmere, Hood River) and KC (Medford) (Years 1 & 2):

To investigate the impact of weather conditions and fungicide sprays on pear infection timing(s) 60 pear blossoms were collected from two orchards at each district in the spring of 2019 and 2020. Afterward, 60 fruit were collected from the same trees and orchards used for flowers sampling at fruit set, mid-summer, and at commercial maturity. Blossom and fruit samples were transported in separate clean bags to the Pathology Labs at TFREC or SOREC. Thirty samples were used for molecular quantification of *Botrytis* infections and the 30 remaining samples were used for isolation of *Botrytis* on a semi-selective medium. Flowers were freeze-dried and stored at -80°C. Fruits were peeled and the peel and the flesh of the fruit were freeze-dried separately and stored at -80°C. DNA were extracted from freeze-dried samples and the presence of *Botrytis* were detected using a quantitative polymerase chain reaction (qPCR) assay (Diguta et al. 2010). Spores of *Botrytis* were enumerated from fresh (non-dried samples) on a *Botrytis* semi-selective artificial agar medium (Edwards and Seddon 2001). Data on *Botrytis* isolations in every stage were quantified and compared to weather data and fungicide applications at respective stages.

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

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

Objective 2. Enhanced approaches to manage gray mold in pear

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

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

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

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

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

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

Trials at OSU-SOREC (KC): Based on the results from Objective 1, the most susceptible stage for *Botrytis* infection will be identified and the trees will be inoculated with *Botrytis* inoculum at that stage. The treatment trees in respective research station will be sprayed with spore suspension of *B. cinerea* @ 1 X 10^5 spores/ml. The control trees will receive spore sprays but not treatment sprays. The fungicides identified from previous studies and sub-objective 2.1 with promising laboratory efficacy will be tested for their field efficacy.

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

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

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

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

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

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

We will use Pristine® (the most widely used in the PNW) for the conservative spray, Topsin®M (FRAC 1) and Pristine (FRAC 7 + 11) for the moderate spray, and add Luna® Sensation (FRAC 7 + 11) for the extensive spray. Luna is one of the most effective fungicides in conventional orchards. Penbotec (FRAC 9) it is the most systemic fungicide among the current postharvest fungicides and is thought to be the most effective against potential latent infections. Trials will be set in a randomized complete block design with four replicate trees per treatment and fungicides will be sprayed using backpack sprayers. At commercial maturity in late August-early-September of 2020 and 2021, a total of 200 fruit/treatment (50 fruit/replicate tree) will be harvested, drenched or not with the label rate of Penbotec (Table 2), and stored at 1°C in a regular atmosphere for up to 8 months. Fruits will be checked for gray mold after 4 months of storage and every two months thereafter. At harvest (pre and post

Penbotec application) and after 4 months of storage, 10 fruit (each time) will be removed from each treatment and subjected to qPCR analyses (Objective 1) to detect and quantify *Botrytis* inoculum. The type of fungicides and application time may be modified in Year 3 based on results from Year 2. An economic study will be conducted to estimate the costs and benefits of each spray regime in relation to the rates of gray mold after storage.

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

RESULTS AND DISCUSSION

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

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

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

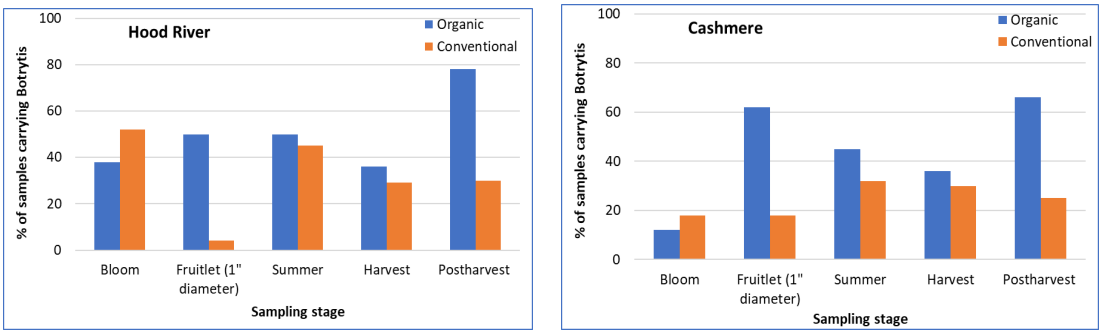

Figure 1. Evolution of *Botrytis* incidence on organic and conventional Anjou pear in Hood River and Cashmere throughout the 2019-20 preharvest growing season and after 6 months of CA storage as detected by qPCR.

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

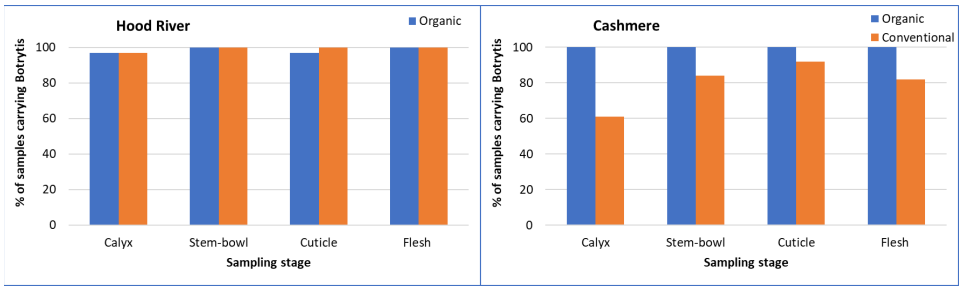

Figure 2. Incidence of *Botrytis cinerea* on different organs of the fruit at commercial maturity (harvest time) Anjou pear in organic and conventional orchards in 2019.

Trials at SO (Year 2)

Comice pears were collected in a commercial orchard in Southern Oregon starting in early April to late August of 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.

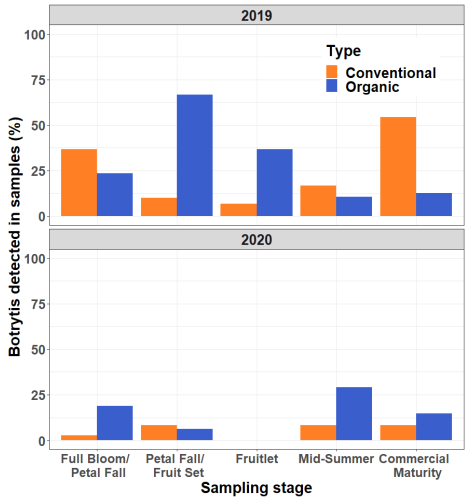

Figure 3: Percentage of *Botrytis cinerea* detected from pear samples collected in Medford organic and conventional orchards at different stages during their development in 2019 and 2020.

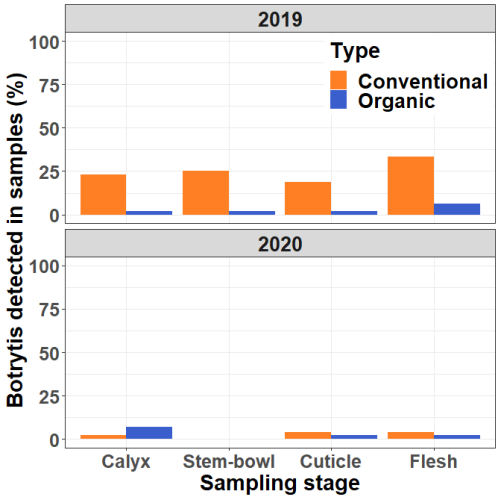

Figure 4: Percentage of *Botrytis cinerea* detected from pear tissues collected in Medford organic and conventional orchards at commercial maturity in 2019 and 2020.

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

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

Preharvest fungicides, Manzate Pro-Stick, Ziram 76DF, Ph-D, and Botran 5F were tested for their effectiveness against 20 *Botrytis* isolates. When tested on wound inoculated fruit assays, the fungicides showed a range of effectiveness against 20 *Botrytis* isolates indicating variability in sensitivity when exposed to preharvest fungicides with different modes of action. The ranges in fungicide efficacies were 32.31% to 99.22%, 21.15% to 89.53%, 61.39% to 96.15%, and 76.35% to 100% for Manzate, Botran, Ziram, and Ph-D respectively. 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.

Based on these results and previous studies, discriminatory doses of seven fungicides (two from this study and five from previous study) are identified and approximately 150 Botrytis isolates are being screened for their sensitivity against these seven fungicides.

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

Based on these lab results and the epidemiology study (objective 1), another gray mold management program have been developed (Table 3). Both programs (Table 2 and Table 3) were applied in a research block in SOREC in 2020 and 2021. Data from 2020 trials are being processed and fruits from 2021 trials were harvested on September, 2021. These fruits are in normal atmosphere cold storage that will be assessed for gray mold and overall rot development in Mid-March, 2022.

Table 3. Description of spray regimes tested in 2020 and 2021 at SOREC, OR.

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

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

Since the inception of this project, the results of this study have been presented in two local growers meetings in Medford, OR, one regional scientific society meeting, and two regional growers meetings including Orchard Pest and Disease Management and Washington State Tree Fruit Association annual meeting. A non-refereed technical report has been published and two manuscripts are under active preparation.

Future work:

2022:

Conduct the genetic analyses of *Botrytis* isolates, obtain data from cold storage facilities on samples collected in 2021, and more outreach programs via online webinars and/or workshops.

Project Title: New active ingredients for pear superficial scald control (PR-19-103)

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Cooperators: Dr. Jingi Yoo

Report Type: Final Project Report

Total Project Request for Year 1 Funding: $84,894
Total Project Request for Year 2 Funding: $86,893
Total Project Request for Year 3 Funding: $89,036

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

WTFRC Budget

Item	2019	2020	2021
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental		6695	6695
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total		6695	6695

Budget 1
Co PI 2: Carolina Torres
Organization Name: Washington State University
Contract Administrator: Anastasia Mondy
Telephone: 916-897-1960
Contract administrator email address: arcgrans@wsu.edu
Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: ckruger@wsu.edu

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

Budget 2
Primary PI: David Rudell
Organization Name: USDA-ARS
Contract Administrator: Chuck Myers and Sharon Blanchard
Telephone: 510-559-5769 (CM), 509-664-2280 (SB)
Contract administrator email address: Chuck.Myers@usda.gov, Sharon.Blanchard@usda.gov

Item	2019	2020	2021
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	1000	1000	1000
Travel			
Miscellaneous*	11,500	4805	4805
Miscellaneous			
Plot Fees			
Total	12,500	5805	5805

Footnotes: One-eighth instrument service contract

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

Goals and Activities for the next year:
Project Year 3 goals are to confirm scald control properties and impacts on fruit finish of squalane (E7 formulation) using multiple orchards and harvest maturities from different growing regions. Scald control efficacy of delayed drenches of up to 3 months is being tested on additional orchards. Control of CO_2-related disorders using squalane (E7 formulation) is being evaluated. Analysis of scald control mechanism using squalane is expected to be completed.

SIGNIFICANT FINDINGS:
1. Formulations containing squalane reduced or eliminated superficial scald of 'd'Anjou'.
2. Control using squalane emulsions was comparable with ethoxyquin drenches.
3. Squalane (E7 formulation) emulsion drenches can impact peel degreening.
4. Squalane is the active ingredient in these formulations.

METHODS

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

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

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

Year 1: Superficial scald control using the existing formulation and other formulations containing squalane needed to be demonstrated on 'd'Anjou'. In Year 1, we tested the previously established rate on 'd'Anjou' pears from an orchard in each of the Hood River, Yakima, Wenatchee, and Okanogan regions. We harvested 1296 fruit twice [2 weeks (early) and 1 week (late) before commercial harvest] from external canopies and double that from the Wenatchee location. Fruit were transported to TFRL, initial fruit quality evaluated, and 432 drenched 0.5% squalane formulation (E7), 432 drenched with 2000 ppm ethoxyquin, and 432 drenched with washed with water. Additional pears (36 fruit/treatment/storage duration) from each location were drenched with 3 concentrations of another emulsion containing 0.5%, 1%, and 2% squalane, Triton X-100, and water.
Year 2: Repeated Year 1 harvest protocol from the same Hood River and Wenatchee locations. We added a 1.0 % E7 squalane treatment to test if scald control is improved without phytotoxicity at a higher rate. Another activity, testing the scald control efficacy of E7 treatments during 0.6% O_2 CA, were performed by placing pears in CA at harvest, treating one group with 0.5% E7 immediately and after 1, 2, and 3 months of storage. All scald evaluations will be on 100 pears per treatment.
Year 3: In Year 3, our focus has been on finishing our examination of the squalane emulsion on pears from multiple locations and maturities from the Hood River and Wenatchee Valley areas. Pears were harvested around commercial maturity from 4 locations around Hood River and 3 locations in the Wenatchee Valley, with 1 location in the Wenatchee Valley harvested at 5 different maturities. Pears from one Wenatchee Valley and one Hood River location are represented in all 3 years of the project.

Pears were treated with 0.5% squalane (E7 formulation) or 2000 ppm ethoxyxquin immediately after harvest.

Storage and quality analysis. Pears from both harvest from every orchard as well as those treated with the Triton formulation (Years 1 and 2) were stored in commercial CA rooms (33°F; 1% O_2, 1.5 % CO_2) for 3, 6, or 8 months (Years 1-3), respectively. Pears from the Wenatchee location were also stored in air (33°F) for 3, 6, or 8 months (Years 1 and 2). For the delayed squalane trial (Years 2 and 3), pears were stored in TFRL CA chambers (33°F; 1.0% O_2, 0.5% CO_2) for 8 months.

Disorder and quality analysis. Scald incidence and severity as well as phytotoxicity and fruit quality are being evaluated upon removal from storage as well as after 7 and 14 at 68 °F (if intact) days of storage. Fruit quality and maturity was evaluated on all treatments at all sampling periods using fruit weight, I_{AD}, °Hue (green to yellow), firmness, soluble solids, starch index, titratable acidity, and whole fruit ethylene production. In Year 2, pears from each treatment will be peeled for subsequent metabolic analysis.

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

Year 1: Peel from pear from the Wenatchee location that were drenched with 0, 0.5, 1, and 2 % squalane emulsion formulated with Triton X-100 and stored in CA was sampled at 3, 6, and 8 months for chemical analysis to determine the mode of action of squalane.
Year 2: Test for scald control activity of other ingredients. Squalane in formula E7 was replaced with the same concentration of soybean oil (triacylglyceride) and tested alongside squalane-based E7 on pears harvested from both locations. Oleic acid, soybean oil, and squalane were formulated with Triton X-100 to confirm any scald-control properties using a dose response test. Peel will also be sampled at multiple pullouts from air and 3, 6, and 8 months CA from pears treated as in activities under objective 1. All scald control evaluations are performed on over 100 pears per treatment.
Year 3: Metabolic analysis of peel sampled from Year 2.

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

Year 1: 180 pears (from each of 2 harvests) from the Yakima location were selected for an experiment looking into how antioxidant (ethoxyquin) and squalane treatments impact peel injury caused by elevated CO_2 in storage. 36 fruit (per treatment) at each harvest were left untreated or treated with 2000 ppm ethoxyquin (drench), 2000 ppm DPA (drench), 1% squalane/oleic acid emulsion (drench), or 2% squalane/Triton X-100 emulsion (drench). Pears are stored at 33ºF, 0.5% O_2, 5% CO_2 to check for peel injury related to CO_2 sensitivity. Pear appearance will be evaluated at 6 months.
Year 3: CO_2 injury reduction by squalane (E7 formulation) is under evaluation. d'Anjou pears were harvested from Wenatchee Valley locations at around commercial maturity. Pears were treated with 0.5% squalane (E7 formulation) or 2000 ppm ethoxyquin immediately after harvest. Pears in TFRL CA chambers (33°F; 0.5% O_2, 5% CO_2) for 3, 6, or 8 months.

RESULTS AND DISCUSSION

Drench properties and immediate impacts on appearance and finish

Ripeness and other fruit quality attributes were not impacted by any of the treatments in either year. All emulsifiable concentrate formulations, applied as drenches, spread nicely on the fruit surface. The E7 formulation dried more slowly than the Triton X-100 formulation, but residue quickly

disappeared leaving no trace. None of the treatments have negatively impacted fruit quality. None of the formulations tested in Year 1 developed any detectable symptoms of phytotoxicity.

There was also no phytotoxicity in Year 2 associated with the principal squalane (E7) emulsion at any applied rate. The highest rate of squalane (4 mL L-1) formulated with Triton X-100 caused minor darkened lenticels on pears from both locations. Oleic acid formulated in Triton X-100 caused more severe staining of the peel. Both formulations were merely meant to test control mechanisms and are not proposed for use in fruit production. In Year 3, there has not been peel damage associated with any treatment as of 3 months.

Wenatchee

Treatments	Hue°					
	Storage duration (months+weeks, 33F+68F)					
	At Harvest	3M	6M	6M+2W	8M	8M+2W
T1 - Control	113.5	112.0 a	109.1 a	95.7 c	109.7 a	95.6 bc
T2 - 0.5% E7 (Squalane)	113.5	111.9 a	109.7 a	101.9 ab	111.3 a	101.2 a
T3 - 1% E7 (Squalane)	113.5	108.6 a	111.8 a	103.8 a	111.2 a	101.0 a
T4 - 0.5% E7 (Soybean oil)	113.5	112.3 a	110.3 a	97.8 bc	110.8 a	98.8 ab
T5 - Ethoxyquin (2000ppm)	113.5	112.9 a	111.6 a	94.1 c	110.9 a	93.2 c

Hood River

Treatments	Hue°					
	Storage duration (months+weeks, 33F+68F)					
	At Harvest	3M	6M	6M+2W	8M	8M+2W
T1 - Control	113.0	108.0 a	105.1 a	90.5 c	106.0 a	92.1 c
T2 - 0.5% E7 (Squalane)	113.0	108.8 a	108.4 a	97.1 b	106.9 a	102.3 a
T3 - 1% E7 (Squalane)	113.0	108.9 a	108.4 a	103.6 a	106.5 a	97.5 b
T4 - 0.5% E7 (Soybean oil)	113.0	110.9 a	108.4 a	94.9 bc	103.6 a	93.2 c
T5 - Ethoxyquin (2000ppm)	113.0	110.4 a	108.2 a	91.9 bc	104.8 a	94.1 bc

Different superscripts within the column are significantly different at $p<0.05$ by Tukey's HSD test.

Figure 1. Squalane (E7) drench reduced d'Anjou peel degreening after both 6 and 8 months CA storage in year 2. Squalane drenches when formulated with Triton X-100 had no impact on color. Squalane did not impact color in Year 1 in any formulation. A lower hue angle indicates more yellow than green peel in this evaluation.

Pear peel remained greener in pears treated with the squalane (E7) emulsion during ripening following 6 and 8 months of CA storage in Year 2. This is best indicated by the hue angle where a lower value indicates, in this case, peel that is more yellow (Figure 1). There was no color difference among any other treatments in Year 2, including squalane drenches formulated with Triton X-100. There was no difference of peel color among treatments during Year 1.

Scald control using emulsifiable concentrates containing squalane

In Year 1, out of 4 orchards, superficial scald only developed on pears from the Wenatchee and Hood River orchards following 6 months CA. Superficial scald development and etiology followed expected patterns on pears from the orchards providing a realistic testing scenario for these ingredients. Scald was not present upon removal from CA, even after 8 months, only developing and worsening over the 14 days at 68 °F (simulated retail shelf). Scald incidence was generally greater Hood River for the location, although it diminished more on pears harvested on the second harvest compared with Wenatchee. Scald did not develop on air stored fruit before it ripened *to the point of spoiling.*

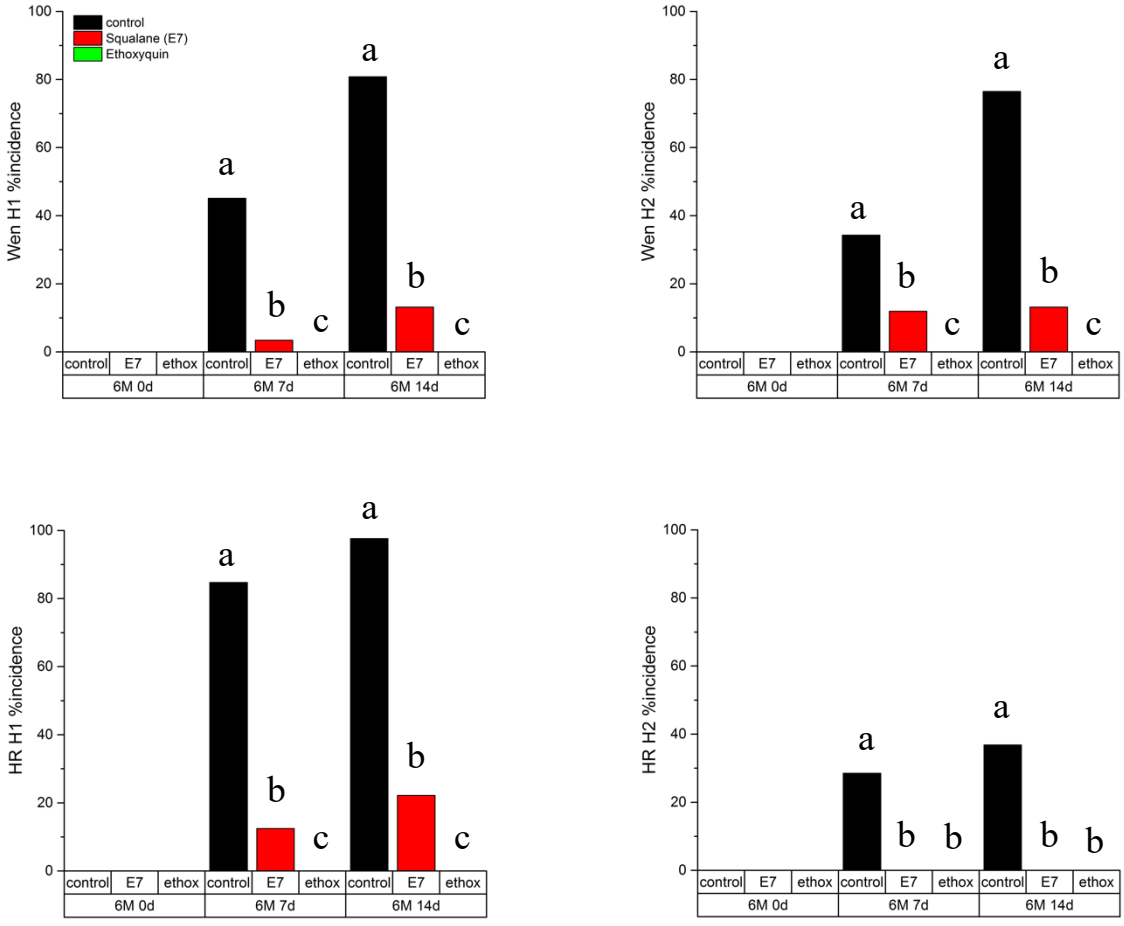

Figure 2. Squalane (red) and ethoxyquin (green) emulsions control or reduce scald compared to untreated (black) 'd'Anjou' pears. Pears harvested twice from Hood River (HR) and Wenatchee (Wen) were stored for 6 months in CA (33°F; 1% O_2, 1.5 % CO_2) and superficial scald rated at 0, 7, and 14 d at 68 °F. Significance was tested using a pooled z-test. Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

Our existing squalane emulsifiable concentrate (E7), applied at a rate of 0.5%, controlled or reduced scald to varying degrees depending upon orchard, harvest maturity, and storage duration (Figures 2 and 3). Ethoxyquin (2000 ppm) drench-controlled scald in most cases except pears harvested from Hood River at the early date, stored 8 months, and held at 68 °F + 14 d. Much less scald also

developed on ethoxyquin treated pears from Wenatchee by 8 months storage plus 7 days at 68°F on this treatment.

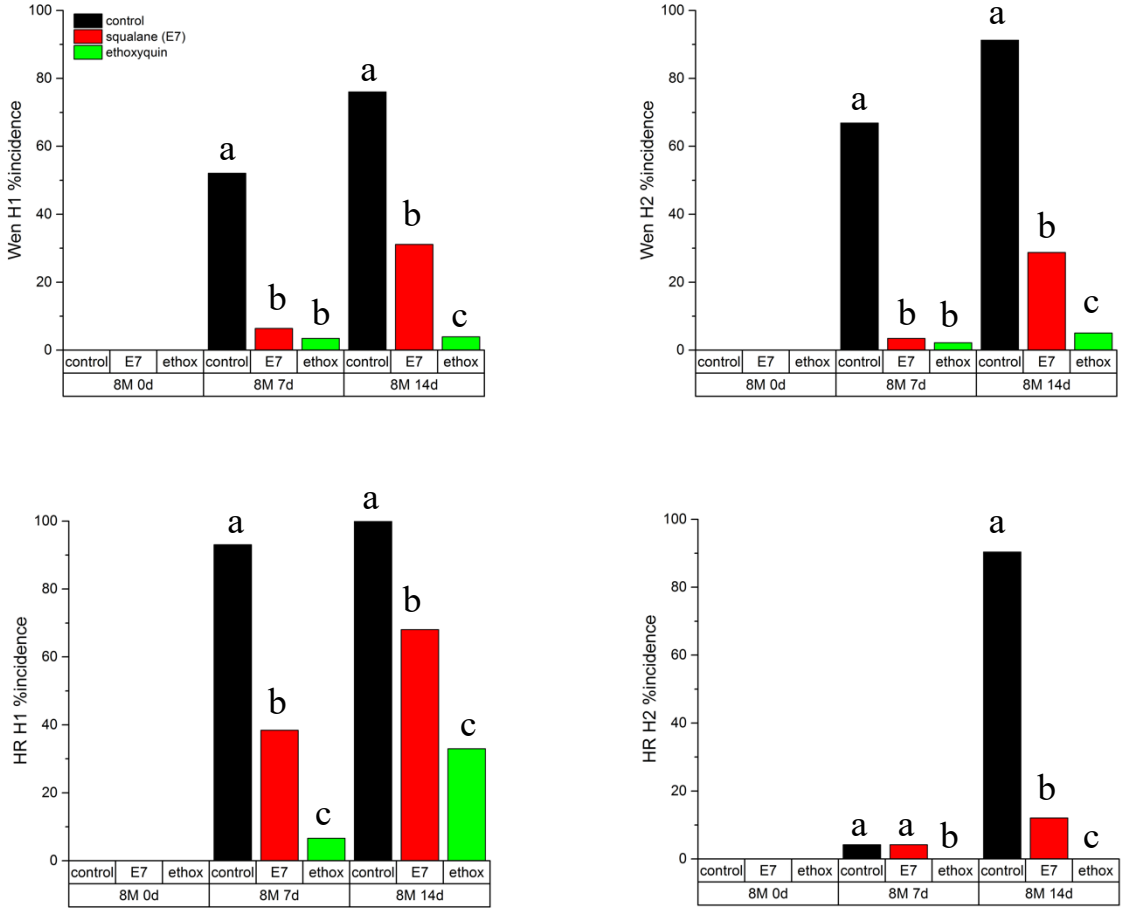

Figure 3. Squalane (red) and ethoxyquin (green) emulsions control or reduce scald incidence compared to untreated (black) 'd'Anjou' pears. Pears harvested twice from Hood River (HR) and Wenatchee (Wen) were stored for 8 months in CA (33°F; 1% O_2, 1.5 % CO_2) and superficial scald rated at 0, 7, and 14 d at 68 °F. Significance was tested using a pooled z-test. Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

While control using squalane (E7 formulation) drenches at the 0.5 % rate was not equal to that of the ethoxyquin, incidence was significantly reduced in all but the most severe cases where ethoxyquin was also inadequate (Hood River, Harvest 1, 8M CA). E7 reduced scald incidence to same levels as ethoxyquin on both harvests from the Wenatchee location following 8M CA + 7d and below 14% after 6 or 8M +7 d in every other instance except Hood River, H1, 8M + 7d. E7 controlled scald on pears stored for 6 months from Hood River, H2.

In Year 2, scald only developed on pears from the Hood River location. As in Year 1, scald began to develop during post-storage ripening after 6 months and was more severe after 8 months CA storage. Scald was reduced or eliminated by both rates of squalane (E7 formulation) drench to the same extent as the 2000 ppm ethoxyquin drench (Table 1). Soybean oil (substituted for squalane in E7) drench also controlled scald but only following 6 months CA.

Table 1. Squalane (E7 formula) drench controlled superficial scald of d'Anjou pears harvested from the Hood River area as effectively as ethoxyquin in Year 2. Pears were stored 3, 6, or 8 months in 1.5% O_2:1% CO_2 at 33°F and ripened for up to 14 d at 68°F. Scald was also controlled when squalane was substituted with soybean oil in this formulation after 6 M CA storage but not after 8 months. Pears harvested in the Wenatchee Valley in Year 2 did not develop scald.

	Superficial scald incidence (%)								
Treatment	Storage duration								
	3M	3M+7d	3M+14d	6M	6M+7d	6M+14d	8M	8M+7d	8M+14d
control	0	0	0	0	0	85 a	0	78 a	100 a
0.5% squalane (E7)	0	0	0	0	0	6 b	0	0 b	6 d
1% squalane (E7)	0	0	0	0	0	0 b	0	0 b	22 c
0.5% soybean oil (E7)	0	0	0	0	0	0 b	0	67 a	83 b
Ethoxyquin (1000 ppm)	0	0	0	0	0	2 b	0	0 b	9 cd

Significance was tested using a pooled z-test (n=54, $p<0.05$). Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

Squalane mode of action in superficial scald control

The squalane (E7) emulsion controls d'Anjou superficial scald. However, it is conceivable, given the nature of the chemicals, that inactive ingredients in the E7 formulation may have a role in scald control. Developing an emulsifiable formulation from the E7 formulation without squalane to test whether squalane is the principal active ingredient was not impossible. Our goal was to establish if squalane is the sole active ingredient in this formulation and determine a mode(s) of action.

To begin to determine if squalane was the sole ingredient actively controlling scald, in Year 1, we formulated a simple emulsion by mixing squalane at concentrations of 0, 0.5, 1.0, and 2 mL L^{-1} with Triton X-100, a surfactant. Emulsions using this formulation appeared stable and complete (no oil droplets on the surface of the drench). While control was evident, especially at the 2 mL L^{-1} rate, following both 6 and 8M storage and both 7 and 14d on pears from both locations, a dose response was not universally observed (see Year 2 report). Differences of formulation may influence efficacy of the squalane resulting in the observed differences of scald control. Also, even though instability of the emulsion was not obvious, variability of fruit or solution temperature may have impacted efficacy. However, given the many instances of dose-driven scald control in these tests, we can assume there is some relationship with squalane and scald control.

In Year 2, we extended our analysis to include, where possible, emulsifiable concentrates of the principal inactive ingredient (oleic acid) with Triton X-100. We also substituted soybean oil (triglyceride) for squalane in both the E7 formulation as well as with the Triton X-100 to approximate the "oiliness" of the squalane formulations, to test if that may be a scald control mechanism. While squalane formulations reduced or eliminated scald, soybean oil only reduced scald following 6 months CA and only the E7 formulation was effective (Table 2). Soybean oil easily formed more stable emulsions than squalane in the E7 or Triton X-100 emulsions. It is still possible that a higher concentration of soybean oil may afford more scald control but not at the comparable rate to squalane. A second dose-response study of squalane formulated with Triton X-100 using a greater concentration range (up to 4% squalane) yielded a clearer dose response effect on scald incidence than in Year 1. This was further supported by a comparison of 0.5% and 1% squalane (E7) emulsions where the 1% provided more scald control (Table 1). Evidence-to-date indicates that squalane is the

active ingredient controlling scald in these formulations, although the coating effect of higher rates of soybean oil than those tested may afford some scald reduction and should be tested.

Table 2. d'Anjou pear superficial scald reduction by squalane emulsion formulated with Triton X-100 is dose dependent. Pears were stored 6 or 8 months in 1.5% O_2:1% CO_2 at 33°F and ripened for up to 14 d at 68°F. Only timepoints where scald was present are reported. Oleic acid (an inactive ingredient in formula E7) and soybean oil did not impact scald incidence when formulated with Triton X-100.

	Superficial scald incidence (%)		
Treatment	Storage duration		
	6M+14d	8M+7d	8M+14d
Control (5 mL/L Triton)	15 a	80 ab	96 ab
Control (7.5 mL/L Triton)	0 b	61 bc	85 c
1 mL/L Squalane (5 mL/L Triton)	6 ab	78 ab	96 ab
2 mL/L Squalane (5 mL/L Triton)	0 b	46 c	81 c
4 mL/L Squalane (7.5 mL/L Triton)	0 b	28 d	72 c
1 mL/L Soybean oil (5 mL/L Triton)	0 b	61 bc	100 a
2 mL/L Soybean oil (5 mL/L Triton)	6 ab	65 bc	87 bc
4 mL/L Soybean oil (7.5 mL/L Triton)	0 b	59 c	87 bc
1 mL/L Oleic acid (5 mL/L Triton)	4 ab	61 bc	94 abc
2 mL/L Oleic acid (5 mL/L Triton)	0 b	87 a	98 ab
4 mL/L Oleic acid (7.5 mL/L Triton)	9 a	59 c	91 bc

Significance was tested using a pooled z-test (n=54, $p<0.05$). Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

Delayed treatment with squalane emulsion following storage in ULO CA controls scald

Short-term storage of pears before packing is often necessary during harvest when packing lines are fully committed. In air or conventional CA storage, scald mitigation treatments should be applied as rapidly as possible to be most effective. However, in apples this period can be extended using ultra-low oxygen (ULO; 1% or less O_2) conditions. A preliminary experiment was performed in Year 2 to determine if squalane (E7) emulsion would be effective following up to 3 months of ULO-CA. Pears were placed immediately into ULO CA (1.0% O_2: 0.5% CO_2) following harvest. Trays of fruit were drenched with 0.5% squalane (E7) emulsion or 2000 ppm ethoxyquin at 0, 1, 2, and 3 months, air dried, and immediately placed back into ULO-CA for a total of 8 months. Appearance, scald risk assessment chemicals (CTOL), and quality was evaluated at 8 months + 7 d. This preliminary study indicated that scald was reduced or controlled following delayed treatments under these conditions (Figure 4). CTOL levels (scald risk assessment) indicated lower scald risk that was later reflected by reduced scald incidence. An expanded version of this experiment is being performed in Year 3.

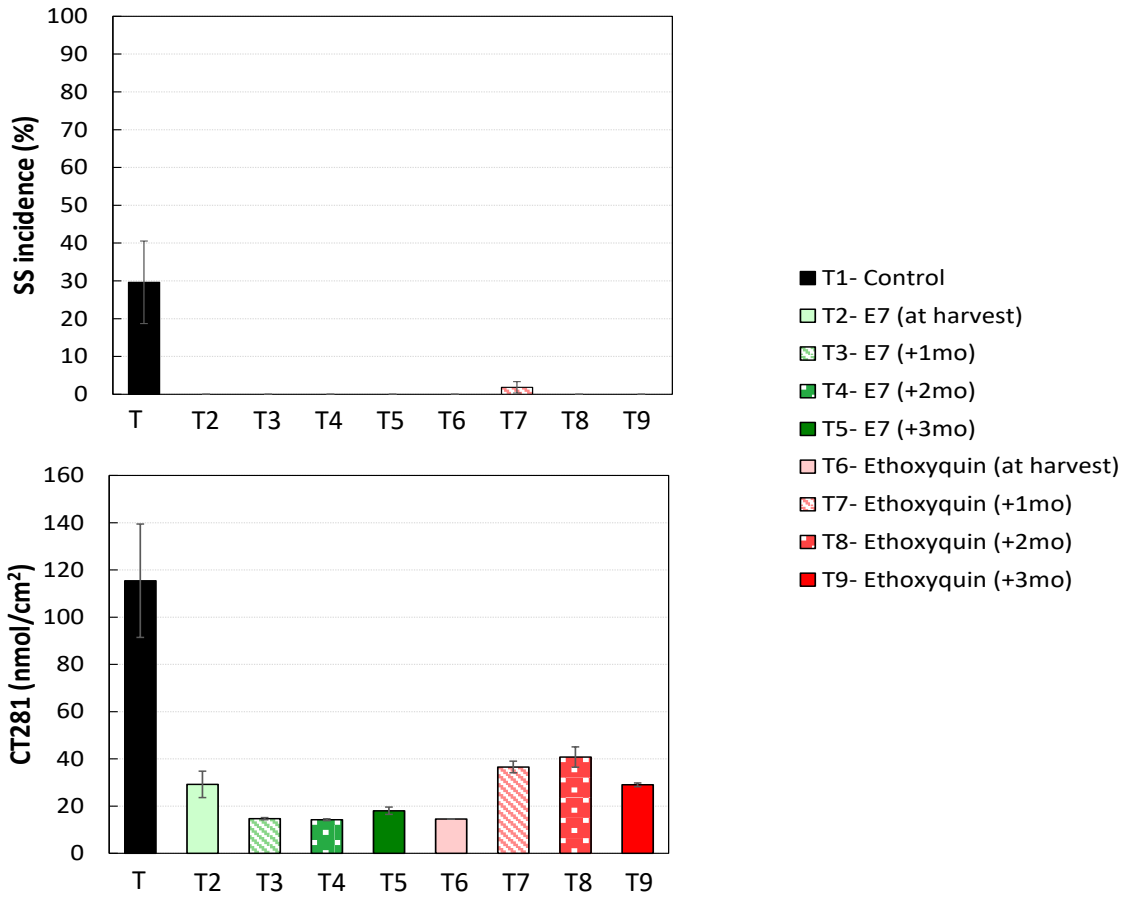

Figure 4. Scald control was effective even if squalane (E7 formula) and ethyoxyquin drenches were delayed up to 3 months for d'Anjou pears stored in ULO CA (1.0% O_2:0.5% CO_2). CTOL (CT281) (scald risk indicator) levels at 8 months were reduced by both active ingredients. Pears were stored for 8 months at 33°F and ripened 7 d at 68°F.

Conclusions

Squalane-based emulsions reduced or eliminated scald. Reduction of scald using squalane (E7 formulations) drenches was as or nearly as efficient at controlling scald as ethoxyquin during ripening following 8 months of CA storage. Delaying drenching treatment with squalane (E7) or ethoxyquin up to 3 months during ULO CA (1.0 % O_2: 0.5 % CO_2) was as effective at controlling scald during ripening following 8 months storage as immediate treatment. The primary formulation did not cause any phytotoxicity at any of the effective rates. Peel degreening was reduced by squalane treatment in one out of two years. Beyond understanding that squalane can control scald, the mechanism for control is not known. Studies of mechanism indicate that squalane is the primary active ingredient in the E7 formulation as oleic acid (inactive ingredient) did not control scald.

Project Title: New active ingredients for pear superficial scald control (PR-19-103)

Executive Summary

Keywords: pear, cold chain, fruit finish, superficial scald, squalane, scald control

Abstract: With diminishing market acceptability of ethoxyquin, new tools and strategies are required to control d'Anjou superficial scald that do not have negative impacts on eating quality. A drench containing squalane was effective for controlling superficial scald of Packham's Triumph is also as or nearly as effective at controlling scald of d'Anjou as ethoxyquin following 8 months of conventional CA storage. Furthermore, delaying drenching up to 3 months in ULO CA conditions was as effective at controlling scald as drenching immediately following harvest. No negative impacts on other appearance or quality attributes were indicated when using effective rates of the principal formulation. Our evaluation of mechanism indicates squalane is the active ingredient, although more work using this type of formulation and soybean oil or other triglycerides is warranted given incomplete results. Commercialization of the squalane emulsion for this purpose is underway.

Project outcome:

1. A drench that controls d'Anjou superficial scald with similar efficacy as ethoxyquin without negatively impacting appearance or quality.

Significant Findings:

1. Formulations containing squalene reduced or eliminated superficial scald of 'd'Anjou'.
2. Control using squalane emulsions was comparable with ethoxyquin drenches.
3. Squalane (E7 formulation) emulsion drenches can impact peel degreening.
4. Squalane is the active ingredient in these formulations.

Future Directions:

1. Test other application methods such as fogging in storage or orchard spray application.
2. Test different CA atmosphere conditions that can be used effectively with delayed squalane application to control scald.
3. Further evaluation of soybean oil and other triglycerides formulated similarly to squalane as scald control drenches.
4. Find additional pear scald control strategies that can be used in a variety of regulatory conditions.

Pear Consumer Preference Testing –
Washington Tree Fruit Research Commission
Continuing Report

Prepared: January 11, 2022

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

Objectives

The objective of this research is 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, seeking to understand what sparks consumer interest in pears in the current PNW consumers. It must also be acknowledged that while this proposal will provide information regarding consumer preferences, due to the limited budget, the consumer testing will only be performed in one testing location. Evaluating different consumers in different regions of the United States would provide a more robust picture of the variation in consumer preferences of pears.

Significant Findings

- Trained sensory panel profiling found differences among Winter and Summer pears in all sensory attributes.
- These pears were then presented to consumers for their assessments with results to follow.

METHODS

Pears

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. Pears used in this study were provided by producers of commercial U.S. grown tree fruit, researchers from WSU-Wenatchee, and

USDA ARS sites who had ample supply and agreed to participate in the study. All samples were harvested and transferred to the Stemilt Packing House in Wenatchee, WA for storage and conditioning prior to the assessments.

A large sample set of 23 pears (11 summer and 12 winter pear varieties) were obtained for descriptive analysis and instrumental measures evaluation. Pears were evaluated at two time points, October and December, depending on the seasonality of the variety. Using 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 in Portland, Oregon. Each set of six pears represented a range of seasonal pear sensory attributes on offer within the U.S. Varieties tested and sourcing are listed in Table 1.

Table 1. Pear varieties, source, month tested and inclusion into the consumer evaluations.

Pear Variety	Season	Source	Testing Date	Consumer Trials
Bartlett	Summer	Stemilt	October	√
Coldsnap ™ (HW614) - Harovin Sundown	Summer	Stemilt	October	√
Happi Pear ™ (HW624)	Summer	Stemilt exclusive	October	√
Harrow crisp	Summer	Stemilt	October	
Harrow sweet	Summer	Stemilt	October	
PiqaBoo ™ (P009)	Summer	Stemilt	October	√
Reddy Robin (PREMP109)	Summer	Brandt's Fruit	October	√
Seckel	Summer	Mt. Adams Fruit	October	√
Starkrimson	Summer	Stemilt	October	
Summer Blood Birne	Summer	USDA ARS National Clonal Germplasm Repository	October	
Sylvania	Summer	Mt. Adams Fruit	October	
Abate Fetel	Winter	OSU Mid-Columbia Agricultural Research and Extension Center	December	
Bosc	Winter	Stemilt	December	√
Comice	Winter	Mt. Adams Fruit	December	√
Concorde	Winter	Prey's Fruit Barn	December	√
Forelle	Winter	Mt. Adams Fruit	December	
Gem	Winter	Duckwall Fruit	December	√
Green Anjou	Winter	Stemilt	December	√
Packham's Triumph	Winter	OSU Mid-Columbia Agricultural Research and Extension Center	December	
Paragon	Winter	OSU Southern Oregon Research & Extension Center	December	√
Red Anjou	Winter	Stemilt	December	
OHUS-US783012-022	Winter	USDA ARS Appalachian Fruit Research Station	December	
US79453-007	Winter	USDA ARS Appalachian Fruit Research Station	December	

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.

The pears used during the training for the evaluation of the summer pears were USDA varieties collected at Corvallis, OR (e.g., Paragon, Triumph de Vienne, Dessertnaia, Richard Peters, Premices de Maria Lesueur, Doyenne blanc, Madame favre, Mela di laconi, General le clere, Vavilov and commercially available varieties such as Bartlett, Starkrimson, D'Anjou, Asian, Bosc.

Each session was structured to achieve a specific objective. In the first three sessions, vocabulary development took place. 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). This process continued until agreement among panelists was reached regarding the meaning, relevance, and the intensity of each of the attributes. The final list of attributes (Table 2**)** comprised 18 attributes, of which eight were related to aroma/ flavor, three to taste, one to mouthfeel and six to texture.

Table 2. List of attributes, definitions, and references for the sensory profiling of pears from the PNW.

Attribute	Definition	Reference	Intensities
AROMA/FLAVOR			
Pear	The aromatics /taste of Bartlett pears	Pears in heavy syrup 70g of pears + 30g of syrup in a 250ml bottle	**10**
Vanilla	Aroma associated with vanilla	1ml of pure vanilla extract (McCormick-Pure vanilla extract) in 100ml of water	**10**
Floral	Aroma associated with flowers/honey	200μl linalool in 400ml of apple juice (Tree Top, 100% apple juice-from concentrate)	**10**
Fruity	Sweet aromatic, characteristic of ripe fruit	Canned mix fruit (peaches, pears and pineapple-Del Monte) 70 g of mix fruit +30g syrup	**10**
Apple	Aroma associated with fresh apple	100g of freshly cut Fuji apple in a 250ml bottle	**10**
Fermented	Aroma associated with fermented fruit	Semi sweet hard cider (Seattle Cider Co) (100ml in a 250ml bottle)	
Grassy/green	Aroma associated with green wood stems; twiggy Aroma associated with unripe or "green" fruit that is similar to grass/leaves	100μl of Cis-2-hexen-1-ol diluted in 100ml of Bartlett pears water (Del Monte, no sugar added, sliced pears)	**11**
Stemmy/woody	Aroma associated with fruit stalks/cores	Broken stems in a 50ml bottle	**8**
TASTE			
Sweet	Basic taste stimulated by sugar and high-potency sweeteners	2 %(w/v) sucrose solution 6 %(w/v) sucrose solution	**3** **12**
Sour	Basic taste stimulated by acids	0.5g malic acid/ 1L water	**6**
Bitter	Basic taste stimulated by solutions or substances such as caffeine	0.35g caffeine/ 1L water	**5**
MOUTHFEEL			
Astringency	The sensation associated with drying of the mouth	0.5g tannic acid/500mL water	**9**

TEXTURE			
Crispy	The amount and pitch sound generated when the sample is first bitten with the **front teeth**	1 cm^3 banana 1 cm^3 celery	**0** **12**
Crunchy	The amount of noise generated when chewing with the **back teeth**	1 cm^3 banana 1 cm^3 carrot	**0** **14**
Juicy	The amount of juice released by the sample during the **1st three chews**	1 cm^3 banana Orange (one segment)	**0** **12**
Firm	Force required to bite completely through the sample during the first bite/chew	1 cm^3 banana 1 cm^3 carrot	**2** **12**
Grainy/gritty	The presence of small hard particles in the flesh	Apple sauce (20g) Cooked corn meal* (20g)	**4** **14**
Skin toughness	The amount of chewing required to **cut through and breakdown the skin with the back teeth**	Granny smith apple with skin on	**13**

The evaluation of the intensity of each attribute was measured with a 15 cm unstructured continuous line scale with 1.5cm anchors at the ends. The position of reference standards was marked on each attribute line scale. From session 5 onward, the intensities for the Barlett were also marked on each attribute line scale. During the sensory profiling of both summer and winter pears' varieties this pear became a reference as well.

The training sessions were conducted on a discussion room following the COVID-19 protocols of social distancing defined by Washington State University (WSU). The project was approved by the Institutional Review Board (IRB) # *19063-001*.

During the training process, the performance of each panelist was monitored. Replicates of some pear varieties, mostly Barlett, were evaluated to determine the panelists' performance.

A total of six attributes were evaluated: shape, russet coverage, blush coverage, skin appearance, skin color, and flesh appearance. For shape evaluation, the shape Chasset classification (Chasset, 1920) was used.

For color evaluation rate-all-that-apply (RATA) was used. To represent the color intensities of the pears paint chip colors (Rodda Paint Company) were used. The following paint chip colors were used: red (1100, 1101 and 1103), yellow (0819,0820 and 0821), green (0792,0793 and 0781) and brown (0897, 0898 and 0900).

Final evaluations

Final evaluations took place following training. Each of the pear varieties from both summer and winter season, were tested in duplicate by each of the ten panelists. The evaluation took place at the WSU Sensory Evaluation facilities, in booths under white light and positive pressure control. Water and

unsalted crackers were provided to the panelists as palate cleansers. Questionnaires were designed and data was collected with Compusense® software.

Data analysis

Data were analyzed applying three-way Analysis of Variance (ANOVA) at a 95% confidence level, with mean separation using Tukey's HSD. Principal Component Analysis (PCA) was also applied. XLSTAT 2021.5.1 (Addinsoft, 2022) software was used to run the different techniques for data analysis.

Results

In Figure 1 and 2, the PCA results for the summer and winter pears sensory profiling are presented.

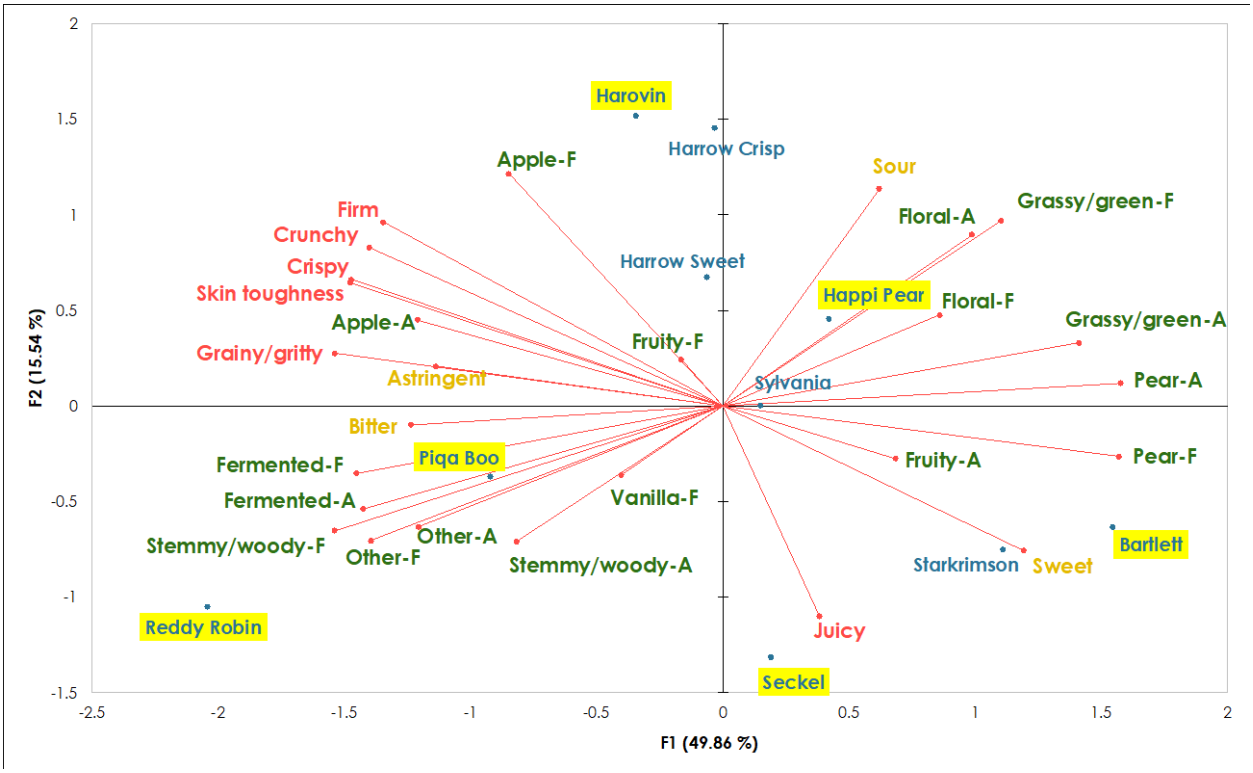

Figure 1. PCA of all significant attributes of the **summer season pears** as determined by the trained panel (n=10). The aroma and flavor-related attributes are presented in green. The aroma-related attributes are represented as -A and the flavor-related attributes are represented as -F. The basic taste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

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 Happi Pear, Sylvania had higher associations with positively loaded attributes on PC1 while varieties like Harovin and Harrow Sweet had higher association with negatively loaded attributes.

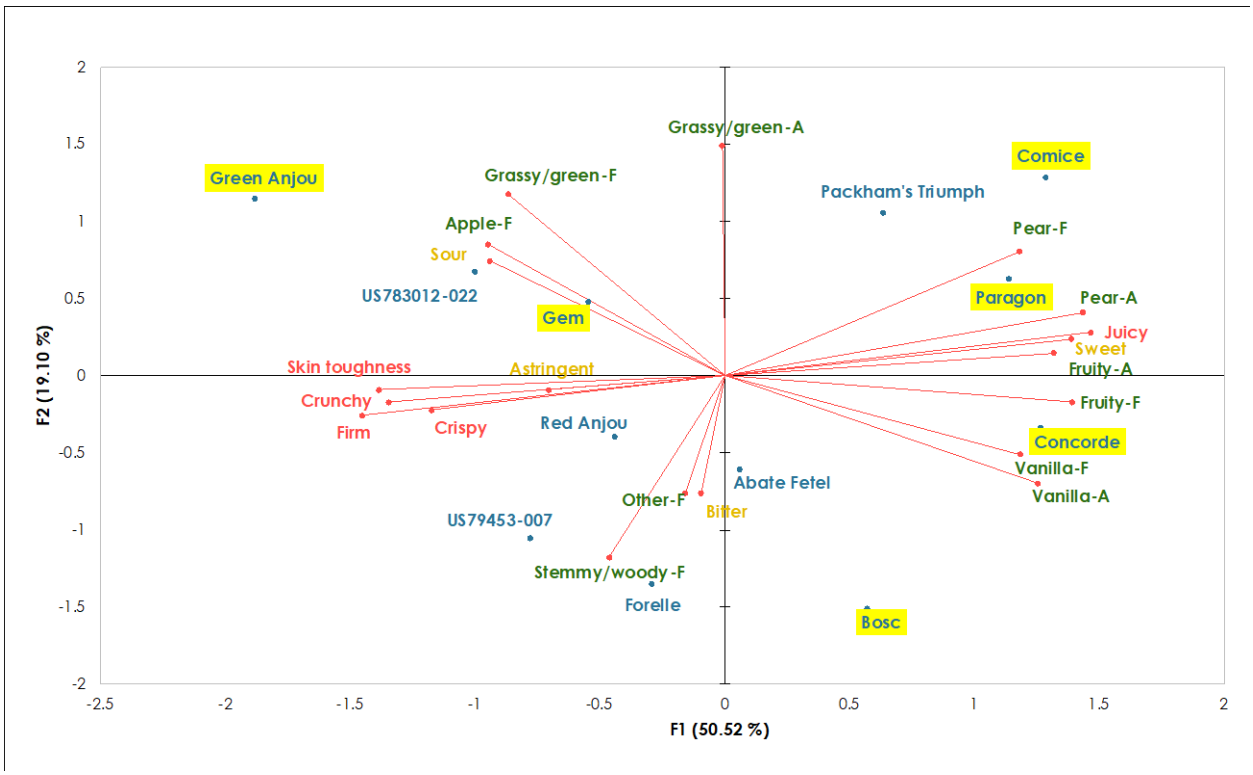

Figure 2. PCA of all significant attributes of the **winter season pears** as determined by the trained panel (n=10). The aroma and flavor-related attributes are presented in green. The aroma-related attributes are represented as -A and the flavor-related attributes are represented as -F. The basic taste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

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 had higher association with negatively loaded attributes.

Instrumental measurements of the pears evaluated by the trained panel

A series of instrumental measurements were conducted on the pear varieties evaluated by the trained panel, stem length and thickness, firmness, and soluble solids measurement.

For each of the pear varieties, between three to six fruits were selected to measure the stem length and thickness. The length of the stem was measured with a 25cm ruler, and the stem thickness was measured as an average of the top, middle and bottom section of the stem. For the stem thickness measurement, an electronic digital caliper (MAX-CAL) was used. Both length and stem thickness were reported in mm.

The firmness of three to six pears was measured with a GS-14 Fruit Texture Analyzer (GÜSS Instruments, South Africa). The following conditions were used: 8.0 mm probe set at 5.0 mm flesh penetration. The measurements were taken at 3 equidistant points around the equatorial region of each fruit following peel removal. Mean firmness value for each fruit was used for the final data analysis.

The soluble solid content was measured for the pears evaluated in both seasons by extracting approximately 0.5-1.0 mL juice from three to six pears from each variety. °Brix were determined with a handheld refractometer (Pocket Refractometer PAL-1, ATAGO, Japan).

Consumer Sensory Evaluation

Two large-scale consumer sensory evaluation tests were conducted at the Oregon State University Food Innovation Center (OSU FIC) in Portland, Oregon, USA. The OSU FIC Agriculture Experiment Station is an off-campus unit of the university, which supports mission-oriented research in the areas of consumer sensory science, product development and food safety. The sensory and consumer laboratory complex includes 10 booths and staging and reception areas, descriptive analysis/focus room and observation area, and a commercial kitchen. The booth area is equipped with odor and temperature control and special lighting to ensure controlled test environment for sensory and consumer studies.

The OSU FIC sensory services are well known and used by global corporations and highly successful startups alike, due to their extensive database of over 40,000 target market consumers from the Portland Metro Area. Portland, Oregon is well known for savvy, locally minded connoisseurs of food and beverages. The markets of the West Coast and Portland, specifically, are leading indicators of product innovation and new trends. The rich agricultural bounty of the region fosters product innovation by highly trained chefs and entrepreneurs, who create exceptionally high-quality products. Portland consumers have come to both enjoy and expect novel, locally sourced food and beverage offerings and are considered a bellwether for understanding future product category achievement.

Consumer sensory evaluations were conducted to determine consumer acceptability and market feasibility of U.S. grown pears. Two large-scale consumer sensory evaluation tests were conducted to assess the quality of 12 pear varieties (six varieties per test) to understand the effect of appearance, flavor and texture on consumer acceptability, willingness to pay and purchase intent. Twelve pears (six each of both summer and winter pear varieties) were tested from growers across the U.S.

Over 100 consumers were used for each sensory study (ie. October and December), consistent with the methodology for statistical significance in consumer acceptability. Consumers were recruited from the Portland Metro Area through the OSU FIC database. Target market participants were pre-screened using questions about pear purchase behavior, consumption habits and demographics. Consumers who participated were given a $40 incentive to participate in the one-hour sensory test.

At the OSU FIC sensory facilities, consumers were seated in individual testing booths with touch screen monitors under white lighting. Twelve one-hour testing sessions were conducted over two days, each with 10 consumers per session for a total of 120 consumers maximum per trial. Pears for the sensory evaluations were sliced just prior to each session by sensory staff with a methodology replicated throughout the research to ensure consistency in quality.

Sensory data were collected using a computerized data collection system utilizing 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 will be subjected to both analysis of variance and penalty analysis and will be statistically analyzed at the 95% confidence limit. Perceptions about the pear category in general were also probed.

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 questionnaire. The bids for different pear sample will allow us to estimate the WTP for each pear sample, and the marginal value of the salient pear quality characteristics. Also, we collected sociodemographic and some purchase pattern data that would allow us to identify a profile of the consumers who are willing to pay price premiums for each pear variety. Due to data entry issues, we will be able to apply fully the contingent valuation methodology for the data collected in December 2021.

Instrumental Measures

For the consumer testing, the instrumental measures of weights, firmness, soluble solids, and titratable acidity were taken by the Mid-Columbia Agricultural Research and Extension Center. Pears were analyzed on the same days as the consumer sensory evaluations in Portland.

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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Co-PI: Chris Hedges
Organization: Washington State University
Telephone: 509 881 9266
Email: john.hedges@wsu.edu

Cooperators: WA packinghouses (TBD)

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
Telephone: 509 335-2885 Email address: cahnrs.grants@wsu.edu
Contract Administrator 2 (TFREC): Shelli Tompkins
Telephone: 509 293-8803 Email address: shelli.tompkins@wsu.edu

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

- Approximately thirty percent of the survey and fruit sampling has been completed up to this date. Activities are ongoing.
- To date fruit overall quality including appearance and flavor has been 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 from 3 distinct growing regions in the PNW, 2 from NCW, 2 from the Mid-Columbia and 1 from Yakima are participating in the survey. Protocols for processing Anjou pears are currently being collected from each participating warehouse.

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

Activities:
Anjou pears from 5 diferent warehouse and lots were collected and fruit recorded at sampling day 1, 7 and 14 days after at 68F (Table 1). In general, fruit had good eating quality throughout this period. Table 1 shows the averages for flesh firmness, soluble solids, chlorophyll degradation (IAD 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.

Warehouse	Lot	Firmness (lb)			°Brix			IDA index			Visual Color		
		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	A1	5.36	2.05	0.88	17.42	16.77	16.67	1.49	1.54	0.84	2.5	2.8	3.4
	A2	5.14	1.59	0.48	13.92	13.54	13.2	1.59	1.41	0.65	2.6	2.4	3.7
B	B1	5.26	1.11	0.68	13.73	13.59	12.96	1.55	0.78	0.33	2.3	2.4	3.9
	B2	4.89	1.17	0.68	13.63	13.82	13.2	1.33	0.96	0.38	2.3	2.5	3.4
	B3	1.64	0.85	0.7	13.57	13.53	12.69	0.94	0.47	0.31	2.4	2.4	4
C	C1	4.57	0.56	0.51	14.69	14.97	14.46	1.51	0.7	0.34	1.6	2.9	4
	C2	5.06	0.64	0.55	14.01	14.01	14.18	1.57	0.94	0.47	1.4	2.5	3.8
	C3	4.73	0.77	0.54	13.02	13.36	13.17	1.52	0.87	0.34	1.7	3	3.8
D	D1	4.16	0.7	0.44	14.27	14.12	14.64	1.49	1.05	0.41	2.9	3.1	4
	D2	4.78	0.62	0.47	15.12	14.69	14.06	1.53	1.08	0.4	2.8	3.2	4
E	E1	3.67	1.19	0.77	13.94	15.26	14.84	0.99	0.75	0.29	2.8	3.6	4
	E2	4.29	0.87	0.56	13.08	13.4	13.37	1.17	0.66	0.28	2.6	3.5	4
	E3	3.03	0.98	0.77	14.16	13.66	13.52	0.73	0.31	0.04	3.1	3.9	4

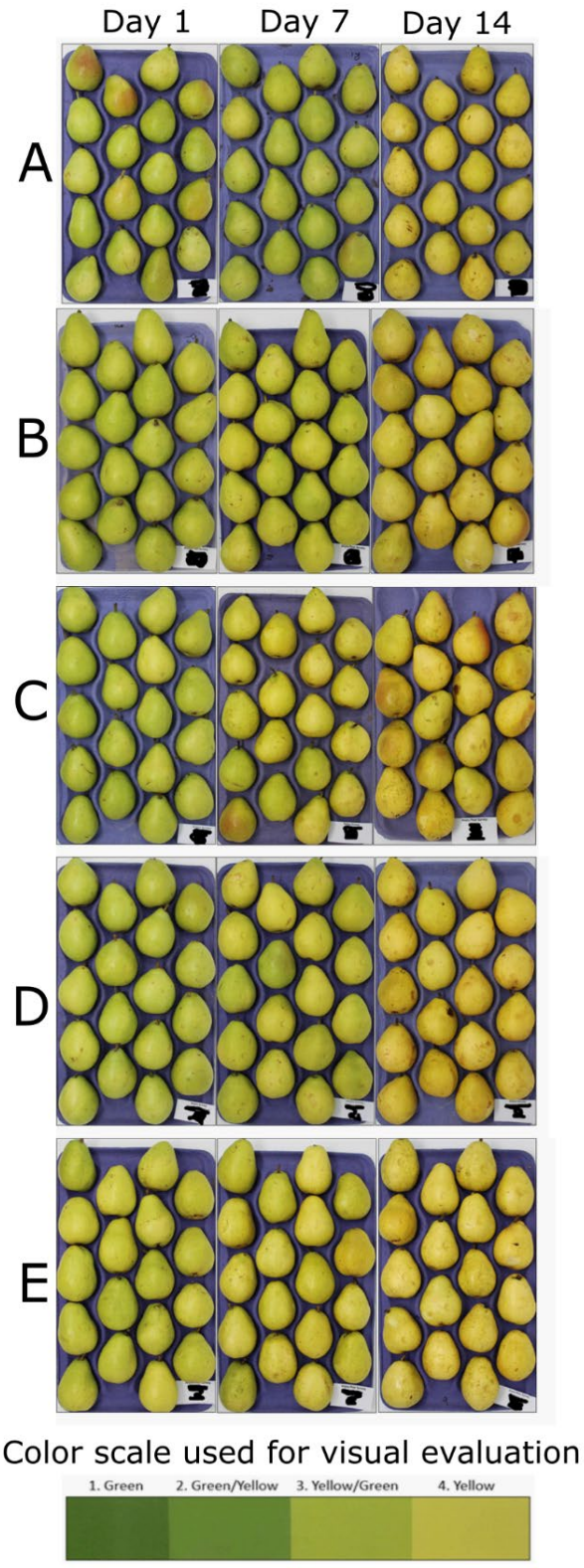

Fig 1. Anjou pear samples from five different warehouses (A, B, C, D and, E) after 1, 7 and 14 days at 68F. Color scale for visual evaluation is showed at the bottom.

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

Project Title: Pear Rootstock Breeding

PI: Kate Evans
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Co-PI (2): Amit Dhingra
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Co-PI (3): Soon Li Teh
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City/State/Zip: Wenatchee WA 98801

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

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

Other Funding Sources

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

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

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

Agency Name: Program Royalties
Amount Awarded: Ph.D. Research Assistantship (2019-2023) Zara York
Notes: "Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock breeding populations." (PI: Evans)

WTFRC Collaborative Expenses: None

Budget

Organization Name: WSU-TFREC **Contact Administrator**: Anastasia (Stacy) Mondy
Telephone: 916-897-1960 **Email**: arcgrants@wsu.edu

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

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

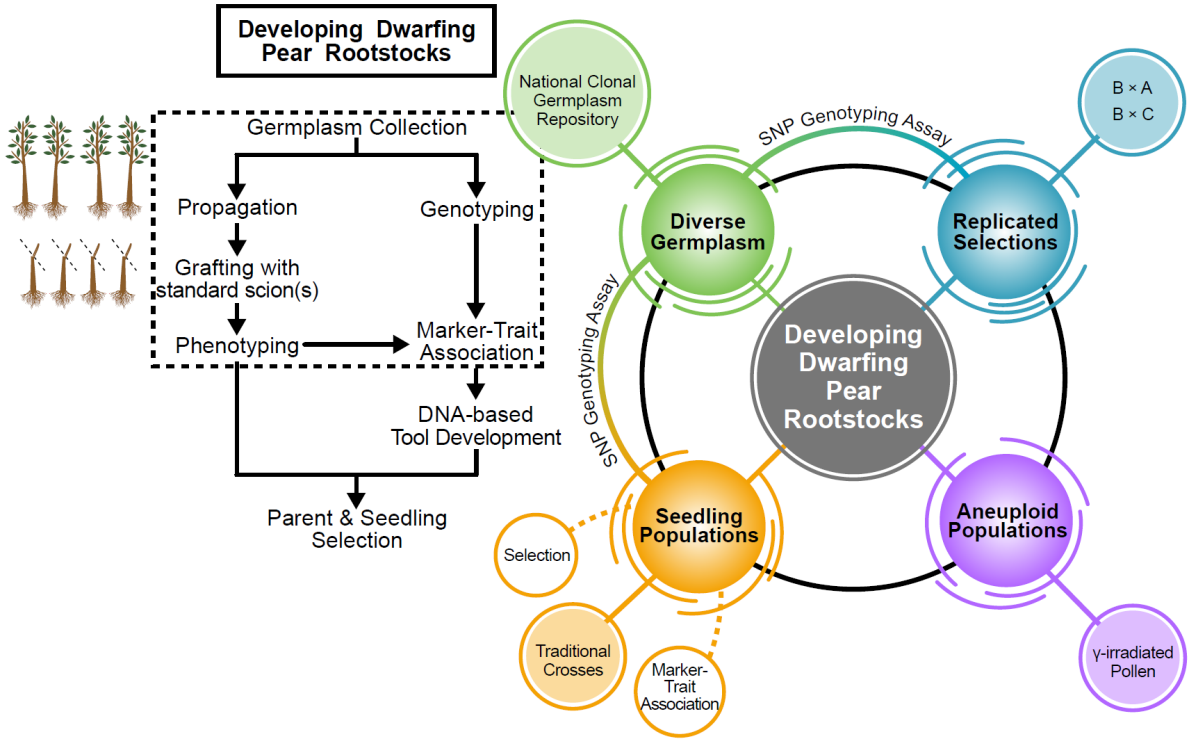
RECAP OF THE ORIGINAL OBJECTIVES

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

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

SIGNIFICANT FINDINGS

- ~2,000 seedlings (from 2016, 2017 and 2019 crosses) are being maintained at the WSU Columbia View orchard. These seedlings were budded with d'Anjou. Evaluation for dwarfing potential is ongoing. Up to 3 years of rootstock and scion trait data were collected.
- Ten preliminary precocious seedlings with medium to high early dwarfing effect were identified and micropropagated.
- Breeding parents were tested with published DNA markers reported to be linked with dwarf or dwarfing traits in apple and/or pear. None of the reported dwarf or dwarfing alleles (i.e., genetic copies) were present in the *Pyrus* rootstock parent set.
- Four high-density genetic maps for two seedling populations were constructed.
- A preliminary locus (i.e., genetic determinant) for dwarfing/vigor was mapped on chromosome 15 in one seedling population.
- ~45 replicated B × A and B × C selections were phenotyped for vigor-related traits, which were highly correlated. Trees are just starting to fruit with six accessions bearing fruit in fall 2021.



† Replicated aneuploid populations will be transferred from the Dhingra lab to the Waite USDA lab in 2022.

Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. Accomplishments highlighted within the dotted box 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.

RESULTS AND DISCUSSION

Objective 1: Develop seedling populations to produce new rootstocks

Seedling populations were generated for future selection of promising rootstocks with dwarfing potential. All seedlings were budded with d'Anjou during the fall that they were transplanted at the WSU Columbia View orchard. Vigor/dwarfing potential of rootstock seedlings and scion traits were collected annually, as shown in **Table 1**. Seedlings will be maintained for further evaluation of rootstock and scion traits, as a measure of vigor and precocity (as relevant).

Cross year	Number of seedlings	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: Data collection of various rootstock seedling and scion (d'Anjou) traits for breeding and selection.

Improvements were made to the seedling protocol in 2019 to reduce the time that seedlings spent in the greenhouse, where typically they were subjected to high disease/fungicide pressure. The 2019 seedlings were moved from the greenhouse (after germination in spring 2020) to the WSU-TFREC hoop house in summer 2020 through spring 2021 (included overwintering). They were irrigated with auto-sprinklers, protected with nets (10% shade factor), and straw-mulched for overwintering. Hoop house space, irrigation set-up and protective nets were kindly provided by Dr. Lee Kalcsits. These seedlings established significantly better than previous years seedlings when transplanted at the WSU Columbia View orchard in spring 2021.

In 2021, ten preliminary precocious seedlings were identified and micropropagated.

Seedling populations are being leveraged through ongoing collaboration with funds via Dr. Sindhuja Sankaran (WSU Department of Biological Systems Engineering) to phenotype canopy architecture using remote sensing technologies. In fall 2021, canopy structures of approximately 450 seedlings were captured, phenotyped and extracted using remote sensing tools (LiDAR and RGB). This added layer of phenotypic data will likely enable more efficient, reliable and accurate of phenotyping canopy volume and dwarfing for our future populations.

In addition, these populations were leveraged through collaboration with funds via Dr. Lee Kalcsits to understand scion-rootstock water relation, which can be indicative of vigor/dwarfing. Plant water relation is estimated through carbon isotope composition analysis. In several studies of apple rootstocks (by Kalcsits program), positive values of carbon isotope composition were correlated with lower water availability/relation, an indicator of dwarfing. In fall 2021, leaves from our pear seedling populations were collected and dried for carbon isotope composition analysis. This biochemical/physiological information may provide additional confirmation of our existing and future vigor/dwarfing data.

Objective 2: Validate published markers for parent and seedling selection
Several DNA-based markers were reported to be linked to dwarf (e.g., *PcDw*) or dwarfing (e.g., *Dw1*, *Dw2*) traits. These markers need to be tested on our pear breeding parents to determine the allelic (i.e., genetic copy) presence and polymorphism/differences (i.e., genetic copies are different among parents). If a known dwarf or dwarfing allele in present in our breeding parents, future work would assess if phenotypic differences in the seedling populations are associated with the presence/absence of the dwarf or dwarfing allele.

Fresh young leaves from the rootstock parent germplasm were collected. DNA extraction of the parent set was carried out at WSU Pullman – Dhingra lab. The DNA quality and quantity were verified to meet the threshold needed for DNA genotyping/sequencing.

DNAs of five breeding parents and one apple Bud9 reference were tested with genetic markers associated with dwarf (pear – *PcDw* locus; **two markers**), dwarfing (apple – *Dw1*, *Dw2*; **four markers**), and dwarfing (apple – *Rb1*, *Rb2*, *Rb3*; **three markers**) to determine if there are differences in the genetic copies (i.e., alleles) of these parents.

Preliminary triplicate analysis of two **dwarf markers** (pear – *PcDw* locus) showed marginal differences in the alleles between dwarf control 'Le Nain Vert' and our tested breeding parents.

Subsequently, high-resolution capillary electrophoresis was performed to quantify/validate the marginal differences. Results showed that none of the tested breeding parents contains the dwarf alleles of 'Le Nain Vert'. One hybrid breeding parent has an allele in common with 'Le Nain Vert', but the allele is not associated with dwarf.

Preliminary triplicate analysis of seven **dwarfing markers** (apple – *Dw1*, *Dw2*, *Rb1*, *Rb2*, *Rb3*) showed clear differences of alleles between dwarfing control 'Bud9' and our tested breeding parents. Subsequently, high-resolution capillary electrophoresis was performed to quantify/validate the differences. Results showed that none of the tested breeding parents contains the dwarfing alleles of 'Bud9'. One breeding parent has an allele (from *Dw1* marker) in common with 'Bud9'; however, the allele is not associated with dwarfing.

None of these published markers (dwarf and dwarfing) is useful for pre-selecting rootstocks in our current parent set.

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

Fresh young leaves from over 600 seedlings (of the four *Pyrus* seedlings populations) were collected. DNA extraction was conducted at WSU Pullman – Dhingra lab. The DNA quality and quantity were verified to meet the threshold needed for DNA genotyping/sequencing. Of the > 600 seedling DNAs, 190 were submitted in 2019 for high-resolution pear genotyping/sequencing array, which was a pear genomic tool previously developed by Dr. David Neale's group ("Development of marker-based breeding technologies"; PR-14-111). Continued close collaboration within the U.S. and international pear genomics community facilitated cost efficiencies in genotyping analysis.

Once the 190 seedlings (of two populations) were sequenced with the genotyping array, four high-density genetic maps were constructed. In combination with phenotypic data collected in *Objective 1*, these maps were used to identify genetic determinants associated with dwarfing and/or vigor-related traits – an analysis termed marker-trait association.

Based on 2020 phenotypic data, preliminary analysis revealed a dwarfing/vigor locus that was mapped on chromosome 15. This locus was identified in one population but could not yet be validated in the other. This preliminary discovery needs additional years of phenotypic data to confirm the statistical significance of this dwarfing/vigor locus.

An additional 192 individuals were genotyped in fall 2021 to further refine the genetic maps. We thank Dr. Nahla Bassil (USDA-ARS at Corvallis, OR) for DNA extraction and for coordinating the genotyping effort to improve cost efficiencies and quality control. The raw data outputs were received in December 2021, and will be processed, analyzed, and incorporated to improve the current genetic maps.

Objective 4: Expand the pear rootstock parent germplasm

In 2019, the existing rootstock breeding program was supplemented with several diverse *Pyrus* seedlings collected from USDA-ARS, Corvallis to replace a few *Pyrus* parents that died due to fire blight. In subsequent years, no additional rootstock parents were added. Newly propagated precocious selections will be added to the parent set in spring 2022.

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

The 14 unique selections ('Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice') grown in triplicate (total of ~45) are being maintained at WSU Columbia View orchard. Trees were pruned (except central

leaders) and trained to induce fruit production. We thank Dr. Stefano Musacchi for his advice on training these trees.
Trees are just starting to fruit with six accessions bearing fruit in fall 2021. Fruit count (total: 27) and weight were recorded. Ten of the 14 selections 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, fruit size, texture and skin finish will be evaluated, as relevant.

In winter 2019-2021, the trees were phenotyped for various vigor-related traits. Consistent segregations for vigor-related traits were observed among triplicated selections; however, it is still too early to draw meaningful conclusions. High correlation coefficients were reported between scion trunk cross-sectional area and scion tree height of multiple years. These scion traits were negatively or non-correlated with rootstock seedling traits (collected in 2012), suggesting that compact rootstock seedling stature is not indicative of dwarfing potential.

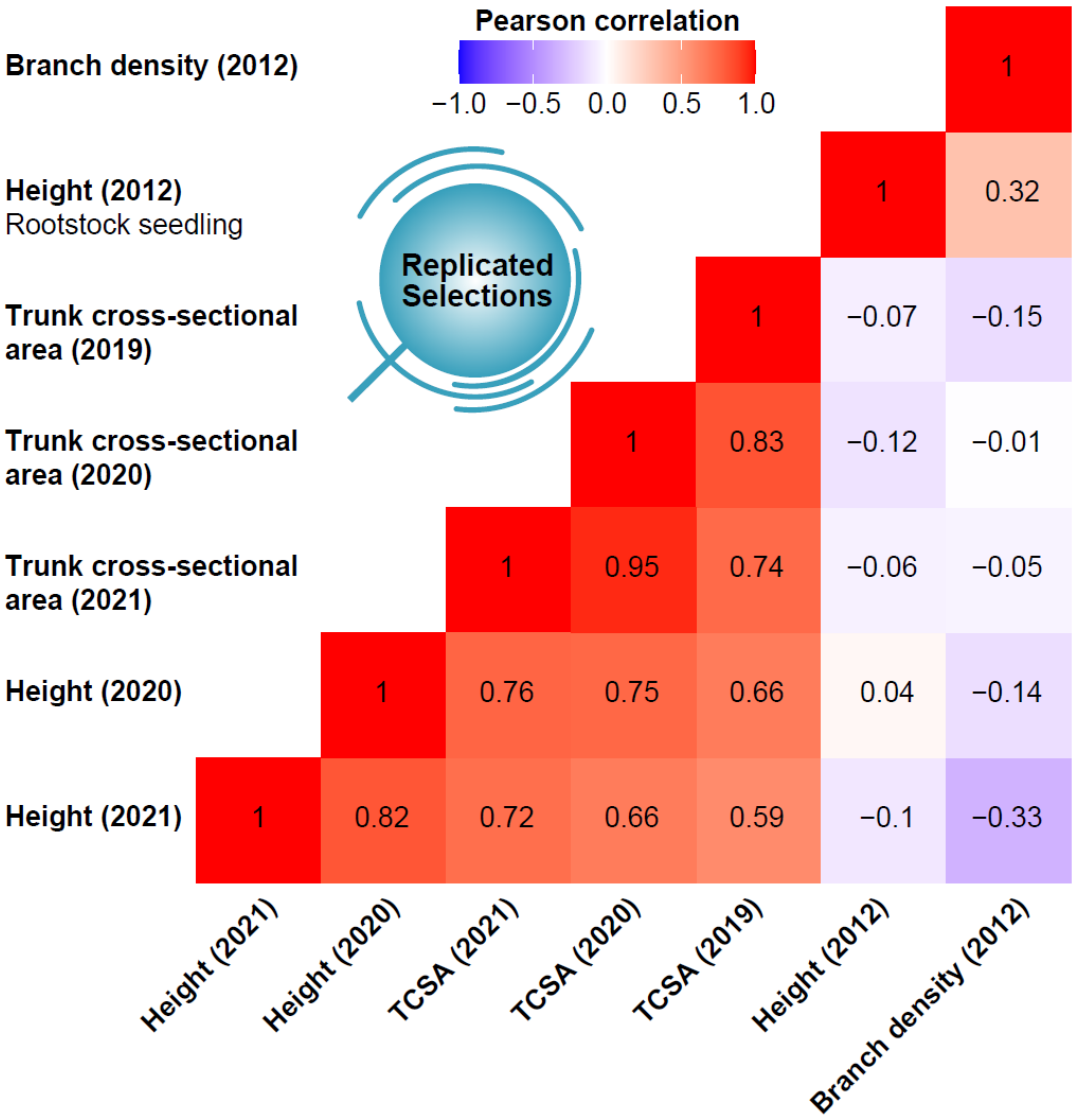


Figure 2: Correlation analysis of replicated selections of 'Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice'. Scion traits (height and trunk cross-sectional area) of 2019-2021 are highly correlated, but are negatively or non-correlated with rootstock seedling traits collected in 2012.

OUTREACH

- Soon Li Teh presented "Pear rootstock breeding program" at the WSU Sunrise Research Farm Extension Field Day at Rock Island, WA on August 7, 2019.
- Soon Li Teh presented "Initiating pear rootstock breeding at Washington State University" at the 2019 Annual Meeting for National Association of Plant Breeders (NAPB) at Pine Mountain, GA on August 25 – 29, 2019.
- The WSU pear rootstock breeding program was featured as a Good Fruit Grower article, "Rooting out Solutions for Pear Growers" on September 2019 Issue (https://www.goodfruit.com/rooting-out-solutions-for-pear-growers/).
- Soon Li Teh and graduate student, Zara York presented an overview of pear rootstock breeding at the WSU Tree Fruit Breeding 101 – Extension Field event at Orondo, WA on October 24, 2019.
- Zara York presented "Advancing genetic resources for pear rootstock breeding" Research News Flash talk at the Washington Horticultural Association Show, Wenatchee, WA in December 2019.
- Soon Li Teh presented "Initiating pear rootstock breeding at Washington State University" at the 10th Rosaceae Genomics Conference (virtual/online) on December 9 – 11, 16 – 18, 2020.
- Zara York presented "Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock populations" at WSU Department of Horticulture – Research Proposal Expo via Zoom on April 21, 2020.
- Zara York, Soon Li Teh and Kate Evans presented "Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock populations" at the 2020 Annual Meeting for National Association of Plant Breeders via Zoom on August 18, 2020.
- Soon Li Teh led a pear discussion group during a "U.S. Nationwide Pear Researcher Meeting" (virtual format) coordinated by Dr. Jessica Waite on March 9-10, 2021.
- Soon Li Teh gave a field tour on "Overview of WSU apple scion and pear rootstock breeding programs" to students of the Cascade Christian Academy High School at Orondo, WA on May 4, 2021.
- Kate Evans hosted the WSU cohort of 'FACT: Research Experience for Undergraduates on Phenomics Big Data Management' at WSU Columbia View orchard, describing the rationale and process of pear rootstock breeding on July 9, 2021.
- Soon Li Teh delivered a guest lecture on "Pear rootstock breeding" at WSU Department of Horticulture (*HORT 503* – virtual format) on November 15, 2021.
- Soon Li Teh and Tory Schmidt (WTFRC) facilitated a panel discussion on "Evaluating new rootstocks for pears" at NCW Pear Day on January 20, 2022.

EXECUTIVE SUMMARY

Project Title: Pear Rootstock Breeding

Key words: breeding, dwarfing, precocious, *Pyrus*, rootstock

Background: The pear industry lacks dwarfing rootstocks that can transform orchard structures to enable application of new technologies to improve efficiencies. This project aimed to build on previous breeding progress to develop a long-term, dedicated pear rootstock breeding program at the WSU-TFREC, Wenatchee. Evaluation of rootstock populations began in this project, which also included the first steps toward establishing necessary genotyping resources to inform breeding for dwarfing. In summary this project encompassed: (1) developing seedling populations to produce new rootstocks; (2) validating published dwarf and dwarfing markers for potential use in selection; (3) conducting marker-trait association for dwarfing traits; (4) expanding pear rootstock parent germplasm; as well as (5) evaluating B × A and B × C selections.

Outcomes and significant findings: Approximately 2,000 seedlings that were budded with d'Anjou are being maintained at the WSU Columbia View orchard. Evaluation of their dwarfing potential is ongoing, with up to 3 years of rootstock and scion trait data collected. Ten preliminary precocious seedlings with medium to high early dwarfing effect were identified and micropropagated. Breeding parents were tested with published dwarf and dwarfing DNA markers. None of the reported dwarf or dwarfing alleles (i.e., genetic copies) were present in the rootstock parent set. Genetic maps of two seedling populations were constructed. A preliminary dwarfing locus (i.e., genetic determinant) was mapped on chromosome 15 in one seedling populations. 45 replicated selections (Bartlett × d'Anjou and Bartlett × Comice) were phenotyped for vigor-related traits, which were highly correlated. Trees are just starting to fruit with six accessions bearing fruit in fall 2021.

Future directions: Current seedling populations will continue to be evaluated to produce more robust phenotypic data that can be integrated with existing genotypic information to facilitate future selection of desirable rootstocks. Additional years of phenotypic data will be used to: (1) validate the preliminary dwarfing locus on chromosome 15; and (2) identify other dwarfing and/or precocious locus/loci. The ten precocious seedling candidates will be replicated and planted at the WSU-Sunrise orchard for use as future crossing parents. More fruit are expected from the B × A and B × C selections, which will be evaluated for fruit quality and skin finish.

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

Primary PI: **Jessica Waite**
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Cooperators: Sean Cutler, UC Riverside; Kate Evans, WSU; Amit Dhingra, WSU; Chris Dardick, USDA-ARS Kearneysville

Report Type: Continuing Project Report

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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Station Manager/Supervisor: Jim Mattheis
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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

- The inducible Rapid-Cycle Breeding (RCB) construct was successfully modified to generate two new versions: one carrying the CiFT gene (Flowering Locus T gene from citrus), and another carrying the BpMADS4 (the MADS4 flowering gene from birch).
- OHxF 87, OHxF 97, and Bartlett budwood was obtained, cleaned, and micropropagated for this work.
- Sufficient plant material was generated to transform both OHxF 87 and Bartlett leaves with the CiFT inducible RCB construct.
- More OHxF 97 material is currently being generated to transform with the CiFT construct. Further, more OHxF 87 and Bartlett material is being generated to transform with the BpMADS4 inducible construct.
- Currently, we are in process of hiring a technician to support this work.

Methods

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

1a. Selection of germplasm to be transformed

We previously proposed to start with compatible germplasm containing traits of interest, namely OHxF 87 and OHxF 97 (recently confirmed to actually be Old Home x Bartlett crosses by (Montanari *et al.*, 2020)) for their graft compatibility and resistance to fire blight (Brooks, 1984). We additionally included Bartlett to use as a control for micropropagation protocols, as these are well established for the cultivar. We obtained OHxF 87 and 97 budwood from the germplasm repository (USDA NCGR) in Corvallis and followed cleaning and micropropagation protocols described by Reed et al. 2013. We obtained Bartlett cultures from our cooperator (Dr. Amit Dhingra) and maintained them using the same micropropagation protocols (Reed *et al.*, 2013). In addition to these protocols, input was provided by the Dardick group (Cheryl Vann). We were able to generate substantial tissue from OHxF 87 and Bartlett, however following these protocols, OHxF 97 was slower to respond. Consequently, we have worked to optimize nutrient and hormone inputs for this line and are currently generating more material. This is a current limitation of all pear tissue culture, as each cultivar has different specific input needs, which are currently determined empirically and rarely published.

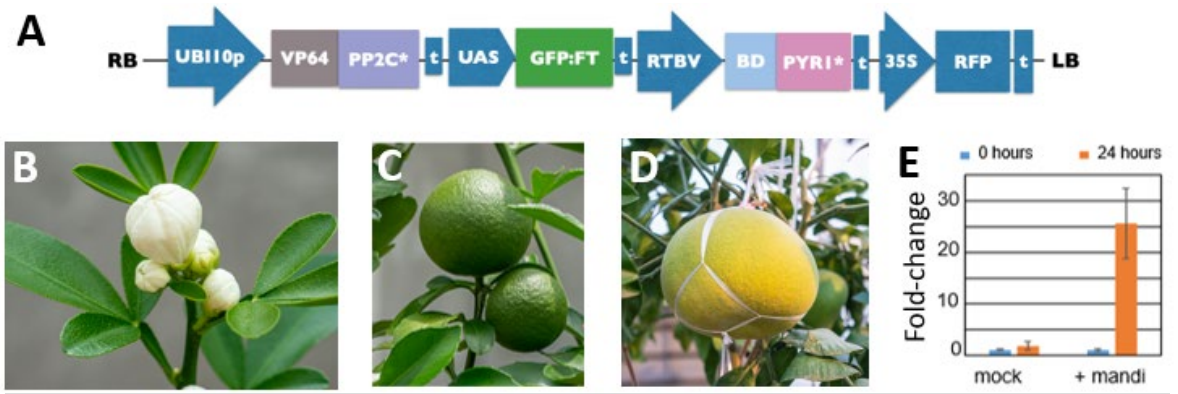

Figure 1. (A) The iFT construct to be used for this study. **(B)** Inducible flowering in juvenile citrus. ~8-month old iFT Carrizo, ~2-weeks post-inducer treatment (50 µM mandipropamid, foliar application, applied three times over six days). **(C)** Fruit formation in an iFT citrus strain. An ~12-month old juvenile iFT transgenic with fruit. **(D)** Successful use of iFT juvenile pollen in crosses. **(E)** High-level gene induction after mandipropamid treatment. iFT Carrizo leaves were either treated with a mock or 50 µM inducer and transgene FT mRNA-levels measured by qPCR at T = 0 hours (pre-treatment) or 24-hours post-mandi application.

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

The initial transgene construct that was applied successfully to citrus plants is visually described in Fig 1A. Briefly, the construct contains (a) two proteins (PYR1 and PP2C) that have been engineered to only interact with one another, and respond to the presence of a low-cost agrochemical called mandipropamid by specifically activating transcription of (b) the flowering gene FLOWERING LOCUS T (FT) from Arabidopsis, downstream of a promoter that is activated by PYR1/PP2C proteins (Fig 1A). To ensure efficacy in pears, we developed two additional constructs containing different flowering genes that have been previously shown to promote early flowering in apple and pear. One modified construct was modified to express a version of FT from citrus (CiFT), as this has been shown to induce flowering in European pear (Matsuda *et al.*, 2009) (Fig 2B). A second modified construct expresses BpMADS4 as a target flowering gene, as this has worked well in apple systems (Flachowsky *et al.*, 2007) (Fig 2C). To do this, we had both the CiFT and BpMADS4 genes

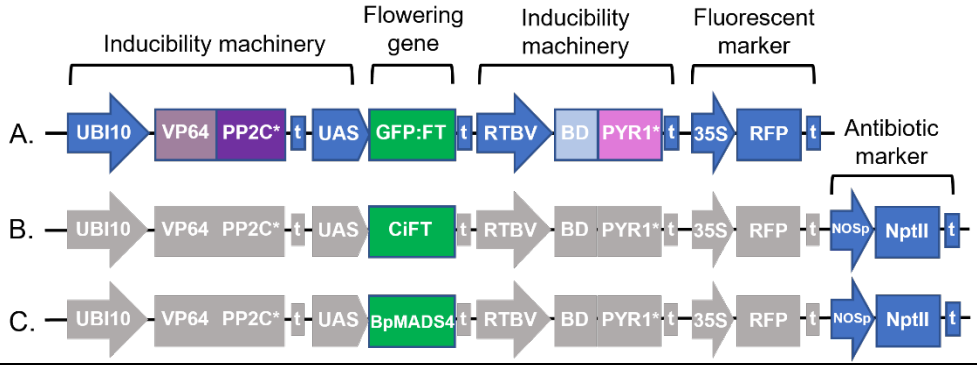

Figure 2. (A) The original iFT construct obtained from the Cutler lab, containing genes involved in the inducibility machinery, a red fluorescent marker (RFP) and the Arabidopsis FT flowering gene. **(B)** Modified version of the original iFT construct. All inducibility machinery and fluorescent marker were maintained (light grey boxes), the CiFT flowering gene replaced Arabidopsis FT, and an antibiotic marker was added. **(C)** Modified version of construct, as above, but the flowering gene is replaced with BpMADS4.

synthesized, and inserted them in place of Arabidopsis FT (Fig 2). The initial construct contains a fluorescent marker to be able to select plants that contain the transgene. In addition to this, we added an antibiotic resistance marker, so that transformed plantlets will be selectable both by antibiotic selection and fluorescence (Fig 2B and C). We did this because antibiotic selection works well for European pear transformation and allows us to select and maintain transformants by adding antibiotics to the media, as well as quickly screen for the transgene using a fluorescent microscope available in the lab. Once the new constructs were generated, we then checked them for quality control, both by digestion and sequencing, and subsequently transformed them into the EHA105 agrobacterium strain commonly used for pome fruit transformation.

1c. Transform germplasm

When enough leaf tissue was generated for one of the OHxF cultivars (87), we moved forward with transforming our first construct. We chose the version containing the CiFT gene to begin with, as it has been shown to promote flowering in pears when overexpressed. We also transformed Bartlett, as we had sufficient leaf tissue and we know more about its tissue culture requirements.

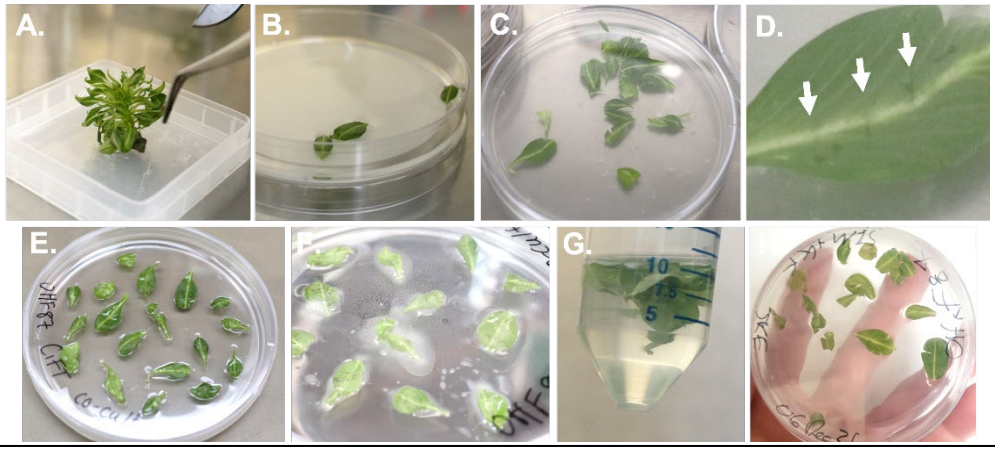

Figure 3. (A) Images showing several steps of the transformation process. First, plantlets were removed from their media for ease of removing leaves, placed on a sterile surface, and leaves were removed. **(B)** Once removed, leaves were placed in sterile dishes containing water to maintain humidity. **(C)** Leaves were infiltrated, incisions were made along the midrib, and here the leaves were allowed to soak in the inoculum of Agrobacterium containing the CiFT construct. **(D)** An up-close image of a leaf with incisions across the midrib, indicated by arrows. **(E)** Leaves initially placed on co-cultivation media. **(F)** Leaves after three days of growth on co-cultivating media. Agrobacterium growth can be seen here. **(G)** Leaves are being washed in a wash media to remove excess Agrobacterium after initial co-cultivation. **(H)** Leaves after first 3 weeks of growth in the dark, no having been moved to unlit shelves.

The protocol we followed was a modified version of the one described by (Mourgues *et al.*, 1996), obtained from the Dardick lab. In addition, we added a vacuum infiltration step, recently described by (Chevreau *et al.*, 2019). Briefly, leaves were removed and kept in petri dishes containing water to maintain humidity until inoculation (Fig 3A and B). They were then placed in dishes filled with agrobacterium (strain EHA105) containing the CiFT construct, and vacuum infiltrated for 1 minute. We then made 4-7 incisions across the midrib of the leaves using a scalpel blade and allowed the leaves to soak in the inoculum for 20 minutes (Fig 3C and D). Leaves were then blotted on filter paper and placed on co-cultivation medium to promote culturing (Fig 3E). After 3 days growing in the dark at 25C (Fig 3F), leaves were washed to removed excess agrobacterium (Fig 3G), dried and

cleaned of any browned material. They were then placed on shoot induction medium containing selective antibiotics. Plates were grown for 3 weeks in the dark at 25C, then moved to unlit shelves (Fig 3H). After 4 weeks, leaves were transferred to fresh shoot induction medium with antibiotics. All leaves have begun to form some callus, and when regenerants begin to form, plates will be moved to lit shelves. When regenerants are 1-2cm in diameter, they will be transferred to their own boxes containing shoot growth medium with antibiotics. Leaves and new regenerants will be transferred to fresh media every 3-4 weeks.

Transformations were carried out on 50-60 leaves from each of OHxF 87 and Bartlett. We are currently growing more of each cultivar to obtain enough leaves to transform additional constructs (containing BpMADS4 or Arabidopsis FT), as well as optimizing growth protocols for OHxF 97. This year we will also request additional OHxF 97 budwood to have more material for culturing. We will aim to generate 20 lines for each construct to ensure we obtain lines with high flowering responsiveness to chemical induction. At the time of report submission (20 January 2022), we expect to begin seeing regenerated plantlets in the next month, and growth to 1-2cm in the subsequent 2-3 months.

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

2a. Rescue transformants, confirm presence of construct
In the coming year (February 2022-January 2023) we will rescue transformed plants growing on antibiotic selection, indicating that they contain the RCB construct. Additionally, we will be able to confirm that transformants have the red fluorescent marker, as well as 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. A potential difficulty we may encounter this year is getting transformed plants to root. Pear cultivars are particularly difficult to root, however we have had success with several Bartlett cultures in the lab, we are aware of multiple rooting treatments to try, and we are hopeful that we will be successful in rooting transformants.

2b. Test flowering-induction in response to chemical induction and select clones to move forward
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 towards the end of year two into the beginning of year 3.

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 (Fig 1E), as well as flowering occurring in the axillary bud associated with leaves sprayed after about 2-3 weeks (Fig 1B). 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.

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 (Fig 1C and D).

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 (Montanari *et al.*, 2016; Peil *et al.*, 2009; Zurn *et al.*, 2020), 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

This year we were successful in obtaining and modifying the rapid-cycle breeding constructs to be used for pear. Budwood was obtained from rootstock varieties OHxF 87 and 97, cleaned and micropropagated through tissue culture to obtain leaf material for transformation. Both OHxF 87 and Bartlett leaves were transformed with the construct containing the CiFT gene, and OHxF 97 is continuing in culture until enough leaves are grown. Further, we have recently completed a hiring search for a new technician whose primary focus will be on this project, and they will be starting in the coming months.

Rapid cycle breeding (RCB) systems developed in other crops, for example apple and plum, have been successful in shortening the time from seed germination to flowering to ~1 year, and greatly reduced breeding times to produce elite germplasm (Elo *et al.*, 2007; Flachowsky *et al.*, 2007; Srinivasan *et al.*, 2012). In apples, a flowering activator from Birch (BpMADS4) is overexpressed, and this system has been successfully used to generate advanced fire-blight resistance selections, reaching the fifth generation within 7 years after initial crosses (Schlatholter *et al.*, 2018). A recent publication this year also demonstrated that using whole genome sequencing, researchers were able to determine the specific site of RCB transgene insertion in the apple genome and that there were no unexpected insertions, which should make selecting against the transgene in the final breeding step simpler (Patocchi *et al.*, 2021). This work informs the techniques we will use in the future to select and confirm RCB lines.

Work in citrus using the original inducible RCB construct (the inducible FT, or iFT, system, containing the Arabidopsis FT gene) is ongoing at UC Riverside, where they recently successfully tested crossing capability in transgenic plants. They now have progeny from these crosses that are

currently being tested. Additionally, transformation of the iFT system has been successful in multiple citrus varieties recently.

Developing this system for pear now will ensure that as germplasm containing important traits is being identified and bred, for example dwarfing and precocious individuals from the Evans lab rootstock breeding program or existing germplasm with disease resistances, we will have a tool ready to be able to stack these traits with fire-blight resistance from the OHxF lines. An additional important step that will make breeding and stacking traits faster is the development of genetic markers for these traits. Efforts to identify and confirm genetic regions associated with dwarfing and precocity are ongoing and proposed by the Evans group (PI: Evans; "Pear Rootstock Breeding"; PR-19-108, and a new proposal this year), and efforts to develop markers for fire-blight resistance in pear are ongoing at the USDA National Crop Germplasm Repository in Corvallis, OR in the lab of Dr. Nahla Bassil.

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FINAL PROJECT REPORT **YEAR**: 3 of 3
WTFRC Project Number: PR-19-101

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

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

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

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

Other funding sources: **Awarded**
Amount: $73,459 (2017 – 2019)
Agency Name: Fresh & Processed Pear Committee Research
Notes: "Greenhouse screening of 49 dwarf rootstock candidates" (PI: Dhingra; Co-PI: Evans)
Synergistic project to evaluate the dwarfing potential of aneuploid pear rootstock seedlings.

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

WTFRC Collaborative Expenses: None

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

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

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

RECAP OF THE ORIGINAL OBJECTIVES

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

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

This is a synergistic project aimed towards developing dwarfing, precocious and disease resistant pear rootstocks. It involved collection of 65 diverse accessions of pear germplasm selected for the desirable traits. These accessions were collected with support from a previous project (PI: Evans “Pear rootstock breeding”; PR-15-105). The goal of this project was to establish all the remaining accessions in tissue culture as well as establish ten clones from each accession so that they could be used for grafting and subsequent phenotyping for dwarfing.

SIGNIFICANT FINDINGS

- Of the 65 accessions 61 were successfully cloned either in the micropropagation system or in soil. The endophytic contaminants proved fatal for four of them despite multiple attempts during the course of the project.
- Due to the need for repeated establishment and management in tissue culture and greenhouse, the entire focus was centered around objective 1.
- Given the wide range of diversity of the accessions, the growth rates, caliper and architecture of the clones in soil are highly variable at the time of this report.
- The accessions were collected multiple times in 2019 and 2021, therefore the clones in soil derived from different accessions are not of the same age. Samples were not collected in 2020 due to COVID.
- The asynchronous establishment of the accessions in tissue culture over the years and their subsequent establishment in soil has resulted in a population that will need additional time to reach the requisite caliper for grafting.
- For most accessions, there are more than ten clones each in soil and for others back up material is available in tissue culture.
- Both the cultures and the replicated clones will be provided to Dr. Jessica Waite at USDA to continue the maintenance of the genetic material.

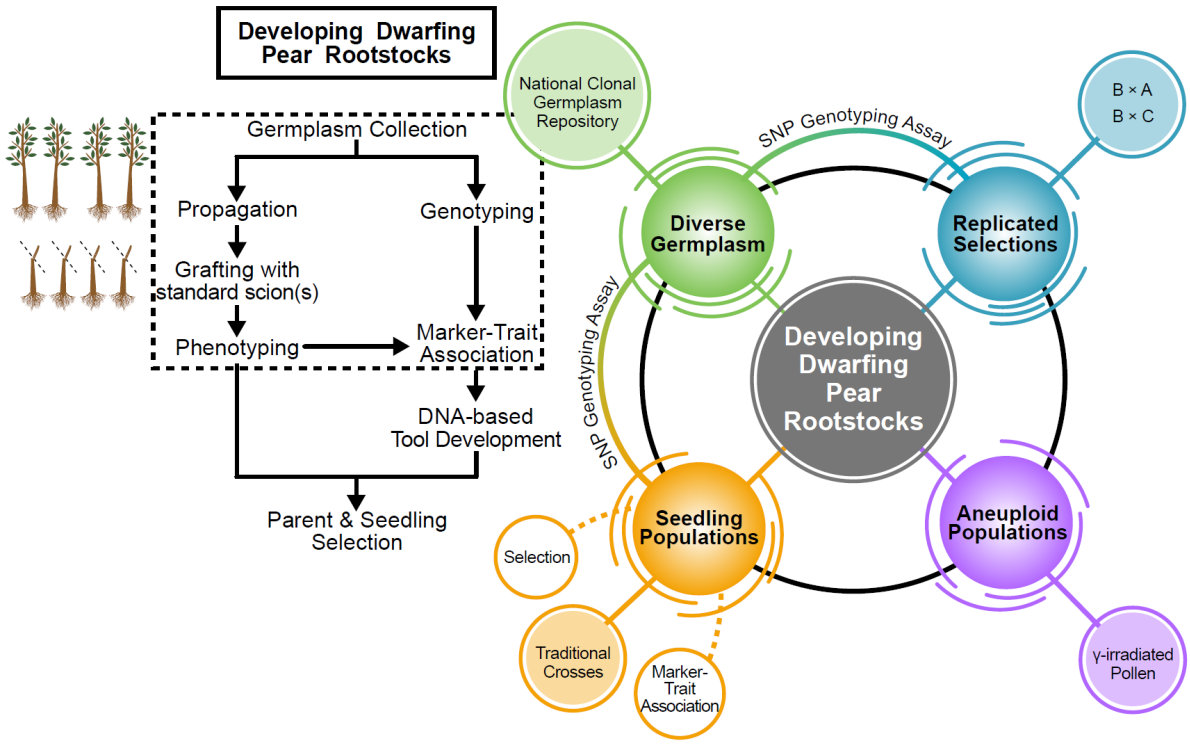

Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. This illustration outlines the four synergistic projects. This project focuses on the diverse germplasm accessions. Replicated populations of the diverse germplasm both in soil and tissue culture will be transferred from the Dhingra program to the Waite USDA lab in 2022.

RESULTS AND DISCUSSION

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

Of the 65 accessions, 61 were successfully established either in tissue culture or in soil as a source of clean and genetically true-to-type plant material. These diverse accessions were previously genotyped using the Pear SNParray produced as part of a collaborative project with UC Davis - PI: Neale, Co-PI Dhingra, “Development of marker-based breeding technologies”; PR-14-111.

Throughout the project, heavy bacterial and fungal infestation in plant material derived from the germplasm repository necessitated repeated initiation of the accessions in tissue culture. Due to COVID, no new initiations were done in 2020.

The plant material that has gone through the process of initiation is maintained either in tissue culture or in the greenhouse. The tissue culture material is further divided into dormant (39 deg F) or active growing (75 deg F). This allows for management of the material and avoiding any cross contamination. We observed that the endophytic microbes in clean material manifested after 6-8 months and destroyed some of the cultures.

Due to the repeated initiations, the plant material in the greenhouse is also divided into two categories. There is a set of clones that is older than 1 year and another that is younger. These plant materials are growing well but their growth rate, caliper and architecture are highly variable.

The replicated clones and plant material in tissue culture will be transferred to the Waite USDA lab in Wenatchee in 2022 to continue this project.

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

Table 1: Status and number of clones available for all the accessions representing a diverse set of *Pyrus spp*. Four rows highlighted in gray represent the accessions that failed to be established despite multiple attempts.

Sample#	Row USDA Corvallis	position	# of boxes in TC @4C	Total # of shoots in TC @4C	# of boxes in TC @ 24C	Total # of shoots in TC @ 24C	# of rooted saplings 1 year+	# of small rooted plantlets < 1 year old	Total number of rooted clones in soil
1	**NF 23**	**1**	2	**18**	**0**				**0**
2	**NF 23**	**15**	0	**0**	**0**		20		**20**
3	**NF 23**	**14**	0	**0**	**2**	**10+**	9		**9**
4	**NF 24**	**11**	0	**0**	**2**	**10+**	12	11	**23**
5	**NF 25**	**8**	0	**0**	**2**	**10+**	17	5	**22**
6	**NF 28**	**9**	0	**0**	**3**	**15+**	15		**15**
7	**NF 30**	**4**	0	**0**	**0**			8	**8**
8	**NF 31**	**16**	1	**5**	**0**				**0**
9	**NF 32**	**13**	2	**18**	**0**				**0**
10	**NF 33**	**4**	0		**0**		13		**13**
11	**NF 34**	**2**	0	**0**	**0**				**0**
12	**NF 34**	**7**	0	**0**	**0**				**0**
13	**NF 52**	**1**	0	**0**	**0**	**10+**	17	9	**26**
14	**1**	**17**	0	**0**	**2**	**10+**	10		**10**
15	**1**	**21**	0	**0**	**2**	**10+**	30		**30**
16	**2**	**3**	2	**12**	**1**	**6**	7	6	**13**
17	**2**	**23**	0	**0**	**3**	**15+**	18	6	**24**
18	**2**	**27**	0	**0**	**1**	**6+**	15	3	**18**
19	**3**	**15**	0	**0**	**0**		8	2	**10**
20	**3**	**25**	2	**16**	**0**				**0**
21	**4**	**19**	0	**0**	**0**				**0**
22	**4**	**21**	0	**0**	**2**	**10+**	10	9	**19**
23	**4**	**45**	1	**7**	**1**	**5**		1	**1**
25	**5**	**11**	0	**0**	**2**	**8**	19	4	**23**
26	**5**	**21**	2	**6**	**0**				**0**
27	**6**	**45**	0		**0**		11		**11**

28	**8**	**23**	0	**0**	**2**	**10+**	4		**4**
29	**8**	**25**	0	**0**	**3**	**15+**	8	5	**13**
30	**10**	**13**	0		**0**		12	2	**14**
31	**12**	**25**	0	**0**	**2**	**10+**	1		**1**
32	**12**	**41**	1	**1**	**0**				**0**
33	**14**	**3**	2	**16**	**0**		2		**2**
34	**14**	**43**	0		**0**		2	21	**23**
35	**15**	**19**	0	**0**	**1**	**6+**	15		**15**
36	**16**	**29**	0	**0**	**1**	**4+**	12	3	**15**
37	**16**	**37**	0	**0**	**2**	**5+**	20		**20**
38	**6**	**43**	2	**10**	**0**				**0**
39	**17**	**35**	0		**0**		14	8	**22**
41	**19**	**11**	0	**0**	**2**	**8+**	7	3	**10**
42	**19**	**17**	0	**0**	**2**	**6**	17		**17**
43	**21**	**9**	0	**0**	**0**		4		**4**
44	**21**	**23**	2	**12**	**0**				**0**
45	**21**	**43**	2	**18**	**0**		3		**3**
46	**22**	**7**	0	**0**	**0**		15	3	**18**
47	**22**	**41**	0	**0**	**0**				**0**
48	**23**	**31**	0	**0**	**0**	**10+**	13		**13**
49	**23**	**47**	1	**5**	**1**	**10**	10		**10**
50	**25**	**29**	0	**0**	**2**	**10+**	14	6	**20**
51	**25**	**59**	1	**9**	**3**	**12**	15		**15**
52	**26**	**25**	0	**0**	**2**	**10**	20		**20**
53	**27**	**1**	1	**5**	**1**	**5+**	9	3	**12**
54	**29**	**53**	1	**7**	**0**				**0**
55	**30**	**5**	1	**4**	**0**				**0**
56	**30**	**35**	2	**8**	**0**				
57	**31**	**19**	0	**0**	**2**	**12+**	15	8	**23**
58	**47**	**5**	2	**12**	**0**		17		**17**
59	**65**	**17**	1	**3**	**0**				**0**
60	**67**	**1**	2	**10**	**0**		20		**20**
61	**67**	**47**	2	**12**	**0**				**0**
62	**67**	**9**	1	**4**	**0**				**0**
63	**67**	**17**	0	**0**	**3**	**15+**	2	11	**13**
64	**68**	**7**	0	**0**	**1**	**5**			**0**
65	**40**	**1**	2	**8**	**0**				**0**
OHxF87							32		

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

Due to variable growth patterns and the need to repeatedly initiate plant material, the goals of this objective were not achieved. The OH×F 87 clones did achieve the desired caliper for budding. The non-domesticated germplasm presented the unexpected challenge of much slower growth rate and a highly variable architecture. It is expected that the Waite USDA labs will utilize this material to continue the budding work. The Waite USDA lab also plans to phenotype this population for rooting potential as part of another complimentary project.

OUTREACH

- Soon Li Teh presented "Pear rootstock breeding program" at the WSU Sunrise Research Farm Extension Field Day at Rock Island, WA on August 7, 2019.
- Soon Li Teh presented "Initiating pear rootstock breeding at Washington State University" at the 2019 Annual Meeting for National Association of Plant Breeders (NAPB) at Pine Mountain, GA on August 25 – 29, 2019.
- The WSU pear rootstock breeding program was featured as a Good Fruit Grower article, "Rooting out Solutions for Pear Growers" on September 2019 Issue (https://www.goodfruit.com/rooting-out-solutions-for-pear-growers/).
- Soon Li Teh and graduate student, Zara York presented an overview of pear rootstock breeding at the WSU Tree Fruit Breeding 101 – Extension Field event at Orondo, WA on October 24, 2019.
- Soon Li Teh presented "Initiating pear rootstock breeding at Washington State University" at the 10th Rosaceae Genomics Conference (virtual/online) on December 9 – 11, 16 – 18, 2020.
- Soon Li Teh led a pear discussion group during a "U.S. Nationwide Pear Researcher Meeting" (virtual format) coordinated by Dr. Jessica Waite on March 9-10, 2021.
- Soon Li Teh delivered a guest lecture on "Pear rootstock breeding" at WSU Department of Horticulture (*HORT 503* – virtual format) on November 15, 2021.
- Amit Dhingra visited Fowler Nurseries, Sierra Gold Nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in November 2019.
- Amit Dhingra presented a seminar at Pairwise Inc. in North Carolina regarding pear genomics and rootstock breeding in September 2019.
- Amit Dhingra hosted farmers from Yakima in February 2020 and shared details about the pear rootstock breeding project
- Amit Dhingra visited nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in February 2020
- Amit Dhingra shared the approaches of pear rootstock breeding using greenhouse based generation cycling as part of a AG2PI workshop in July 2021
- Amit Dhingra shared the pear rootstock research at the annual Fruit Conference in New Braunfels, TX in October 2021
- Amit Dhingra provided an update on pear rootstock research at the Texas Nursery and Landscape Association Lone Star Horticulture Forum January 2022

EXECUTIVE SUMMARY

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

Key words: *Pyrus*, genetic diversity, germplasm, dwarfing, precocious

Background: There is a need for dwarfing rootstocks for Pyrus to enable high density production to enhance orchard profitability. However, very little is known about how a dwarfing rootstock might control vigor, which makes selecting new improved rootstocks challenging. In order to enhance the chances of obtaining the genetic donors of dwarfing, 65 germplasm accessions were identified. These accessions also harbored other traits such as disease resistance and stress tolerance.

This project focused on completing the establishment of all 65 accessions in vitro as well as obtain a minimum of 10 clones each for grafting 'Bartlett' and 'D'Anjou' scions.

Outcomes and significant findings: Of the 65 accessions, 61 genotypes have been successfully established either in vitro or in the greenhouse. Each accession has a unique growth rate and architecture as observed by different caliper obtained over the same period of growth. The endophytes in the budwood material collected from the repository necessitated iterative plant material collection.

Future directions: The clones that are established in soil as well as in vitro material will be provided to the Waite USDA lab. The plan is to plant them and grow these accessions out for grafting in subsequent seasons. Both the Dhingra program and Waite USDA program plan to continue leveraging this useful germplasm for additional genetics and genomics studies. The phenotyping results from grafting experiments will contribute to the larger project of developing a genetic understanding of dwarfing in *Pyrus*.

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

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

PI: Todd Einhorn
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Address 2: 3005 Experiment Station Drive
City/State/Zip: Hood River/OR/97031

Cooperators: Sara Serra, Steve Castagnoli, USDA-NCGR curator (tbd), Adam McCarthy, Stemilt

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.

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.

Objective 2: • Determine the propagation potential of previously identified cold-hardy quince clones.

Significant Findings:

- **Seven of the 14 cold hardy quince clones not yet previously tissue cultured were successfully micropropagated from shoot tips. These represent diverse germplasm of cold hardy and likely dwarfing pear rootstocks and include the three hardiest quince taxa of the entire collection. Modified media will be used to micropropagate the remaining seven clones. In addition, two quince rootstock standards (BA29C and MA) were successfully micropropagated as well as accessions under evaluation in 2017 field trials. Multiplication of accessions with limited or no shoots will continue, but rooting of accessions that initiated shoots in 2021 will be delayed until 2022, after attempts to culture unsuccessful accessions are executed. The objective is to produce trees of the same age for future field performance trials.**
- **Based on growth habit, vigor, canopy balance, precocity and production during the first two cropping years (2021 comprising the first significant crop), three to four rootstock accessions remain promising candidates for pear scions.**
- **High-performing 'D'Anjou' trees on size controlling quince rootstocks had ~20 to 36 lbs of fruit per tree in WA and OR, respectively. The higher yields in OR are associated with the larger tree size (i.e., greater canopy volume given the highly fertile soil and cooler growing season). Fruit size for these combinations were excellent at both sites (i.e., >225 g/ 80 and 90 box size) equating to ~40-70 fruit per tree and ~20 to 40 bins per acre at the tree density of the planting (1210 trees/acre).**
- **High performing Bartlett trees on size controlling quince rootstocks had 20 to 35 lbs of fruit per tree in OR and WA, respectively. Reasons for the lower yields in OR are attributed to the lack of pruning in 2020 due to COVID. This resulted in large canopies with fruiting wood that extended into alleys and neighboring trees requiring significant pruning in spring 2021. Fruit size for high-performing combinations was excellent in WA (i.e., >225 g) but smaller in OR (~190 g). Smaller fruit size, despite lower crop load, was due to retention of weak fruiting wood in the trees following pruning to ensure some vegetative vigor control by fruit. WA and OR 'Bartlett' produced ~ 39 and 22 bins per acre at the tree density of the planting (1210 trees/acre).**
- **Tree survival of the high-performing combinations was excellent in 2021. Mortality was significantly observed only for combinations that are failing and/or struggling to grow.**
- **The relative ranking of vegetative growth (vigor) of all combinations, assessed by pruning weights and trunk cross sectional area, showed general agreement between sites. Cropping performance in WA and OR of specific combinations, however, did not always agree. Reasons for this are unclear and will be assessed in 2022.**
- **Nearly all quince rootstocks conferred precocity. In WA, Bartlett generally had more flower clusters than 'd'Anjou' (150 and 100 per tree, respectively). The opposite was observed in OR, again, due to heavier 2021 pruning applied to 'Bartlett'. The establishment of strong fruiting wood in both cultivars and sites will facilitate high cropping potential in future years. Consistent annual yield performance and its influence on tree health and fruiting require multiple years of evaluation.**

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.

Mortality

By the end of 2021 after ~4 years, a significant difference in survival rate emerged for both cultivars. Figure 1 reports the average percent survival for each combination in WA (this includes alive and struggling trees). Among 9 combinations with interstem, Anjou/Comice/68.002 showed the highest mortality rate in WA since the orchard planting (57%) followed by Anjou/Comice/99.002 (44%), Anjou/Comice/118.001 (31%), and Anjou/Comice/70.001 (23%). Bartlett/Comice/68.002, 99.002, 118.001, and 70.001 had 56%, 28%, 23% and 12% mortality in WA, respectively (Figure 1B). 68.002 had the highest proportion of dead trees with both scions after approximately 4 years from planting. For high-performing combinations, significant changes in mortality between 2020 and 2021 were not observed. 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). Mortality data in OR will be collected at the time of pruning in early spring 2022 but high-performing combinations did not show increased mortality rates during the 2021 season.

Pruning and bloom

For both WA and OR, pruning weights were calculated as the total weight of wood removed per plot (i.e., replicate), then divided by the number of trees per plot to produce an estimate of kg wood pruned per tree (Table 1; Figure 2). Pruning weights ranged from ~ 0.5 kg per tree for struggling combinations, to 1.25 kg per tree for those with good vigor in WA (Table 1). These weights were similar for Bartlett and 'd'Anjou'. OR 2021 pruning weights were ~ double those in WA (Fig. 2). There was general agreement between the most vigorous and least vigorous combinations for each cultivar between sites. Cumulative pruning weights (between 2018 and 2021) revealed significant differences between combinations with Comice interstems (Table 1). The most vigorous combination of Anjou/Comice was 65.001, while 68.002 resulted in the least vigorous trees (Table 1). Bartlett/Comice/65.001 and Bartlett/Comice/57.001 had the most cumulative pruning weights, while Bartlett/Comice/68.002 and Bartlett/Comice/118.001 had the least (Table 1).

Flower clusters were counted on individual trees of both varieties at each site (data is only provided for OR site due to space limitations). The range of flower clusters per tree in WA was 63 to 152 for 'd'Anjou' and 108 to 163 for Bartlett'. 'D'Anjou' flowering in OR was markedly higher (100-300 clusters per tree; Fig. 3) due to the significantly larger canopies in OR as compared to WA (see pruning weight or TCSA data). For Bartlett, the reverse was observed (40-100 clusters per tree; Fig. 3), due to the severe 2021 pruning imposed on OR trees. 'Bartlett' require short-pruning to maintain productive wood, especially when trees are established in tight spacings, as demonstrated by CO-PI Musacchi (e.g., click pruning). The tip bearing habit and propensity for 'Bartlett' to develop unbranched limbs with considerable blind wood, lends itself to this pruning technique. Because COVID restrictions precluded our pruning of the plot in 2020, trees in OR developed a high percentage of blind wood at distances from the tree row that required severe restructuring of limbs in 2021. We maintained as much 'weak' fruiting wood as possible in order to produce fruit to aid in the control of vigor in 2021. Blocks

were not thinned in 2021, chemically nor manually. Fruit set (%) data were collected at both sites and was based on the number of fruit divided by the total number of flowers. In general, Bartlett and Anjou presented a similar fruit set % regardless of the combinations (13% and 15% respectively). Secondary bloom was monitored in WA only. In May 2021, there were no significant differences among combinations, but some combinations tended to show a higher secondary bloom (for instance, Bartlett/Comice/22.001, 118.001 and Anjou/Comice/67.001; data not shown).

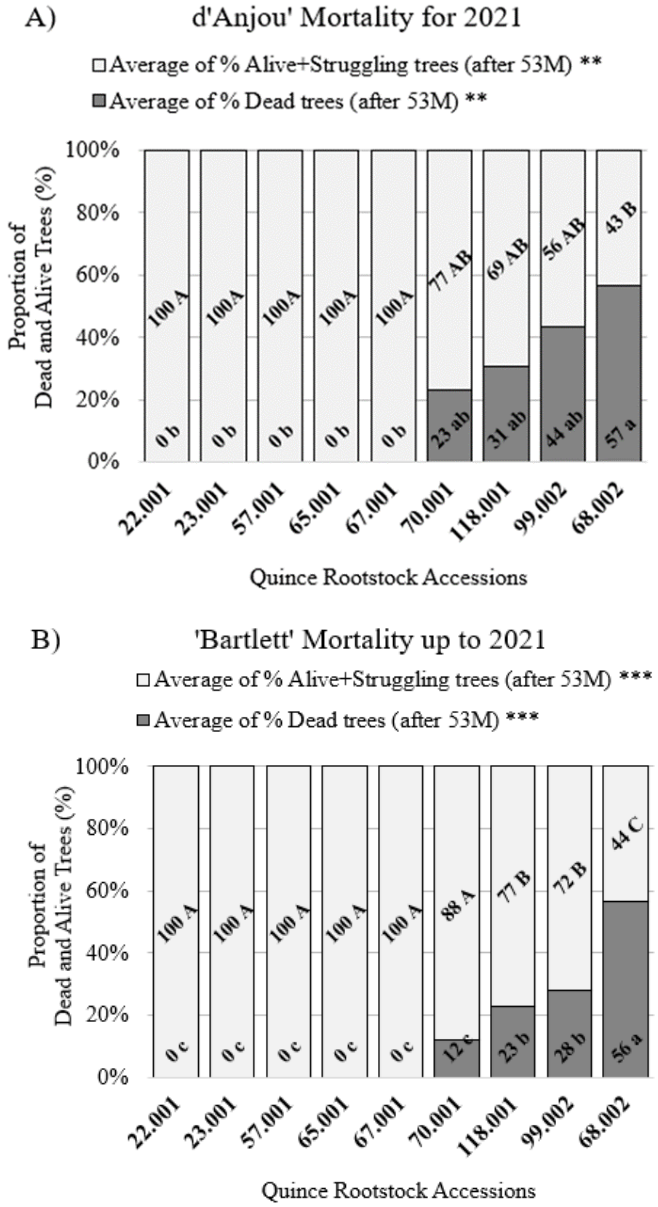
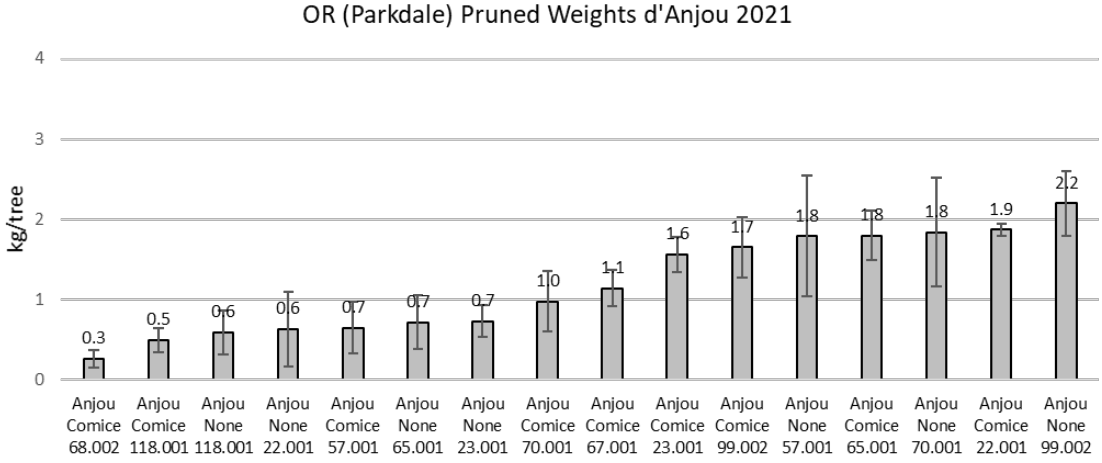


Figure 1. *Status of trees alive & struggling and dead at the end of 2021 expressed as the average of % of alive and dead trees across 3 blocks in November 2021 in Entiat (WA) for Anjou (A) and Bartlett (B) grafted on 9 different quince accessions with Comice interstem. N=3 where each rep is a block, percentage of dead trees (dark grey) and alive trees (light grey) is shown. Significance: **=p<0.01 and ***=p<0.001. Letters discriminate the means based on SNK (p=0.05).*

Table 1. *2021 dormant winter pruning (kg/tree) and cumulative pruning weights from 2018-2021 in Entiat (WA) for Anjou and Bartlett with Comice interstem and grafted on 9 different quince accessions (table sorted by cv and CYD acc. #). Combination without interstem were excluded from statistical analysis.*

Cultivar	Rootstock	Interstem	Count of reps 2021	Pruned Weight (kg/tree) 2021	Pruned weight in 4 years 2018-2021 (kg/tree)	
d'Anjou	22.001	Comice	3	0.99	1.90	AB
	23.001	Comice	3	1.19	2.23	AB
	57.001	Comice	4	1.16	2.18	AB
	65.001	Comice	3	1.29	2.44	A
	67.001	Comice	3	0.95	1.75	AB
	68.002	Comice	3	0.36	0.72	B
	70.001	Comice	7	0.74	1.28	AB
	99.002	Comice	4	0.80	1.46	AB
	118.001	Comice	3	0.45	0.97	AB
Significance				NS (p=0.0730)	* (p=0.0200)	
Bartlett	22.001	Comice	8	1.06	2.40	AB
	23.001	Comice	3	0.86	2.23	AB
	57.001	Comice	3	1.32	2.76	A
	65.001	Comice	3	1.42	3.09	A
	67.001	Comice	4	0.70	1.53	AB
	68.002	Comice	3	0.45	0.82	B
	70.001	Comice	4	1.10	2.01	AB
	99.002	Comice	10	1.06	2.09	AB
	118.001	Comice	6	0.27	0.73	B
Significance				NS (p=0.0593)	** (P=0.0049)	

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).



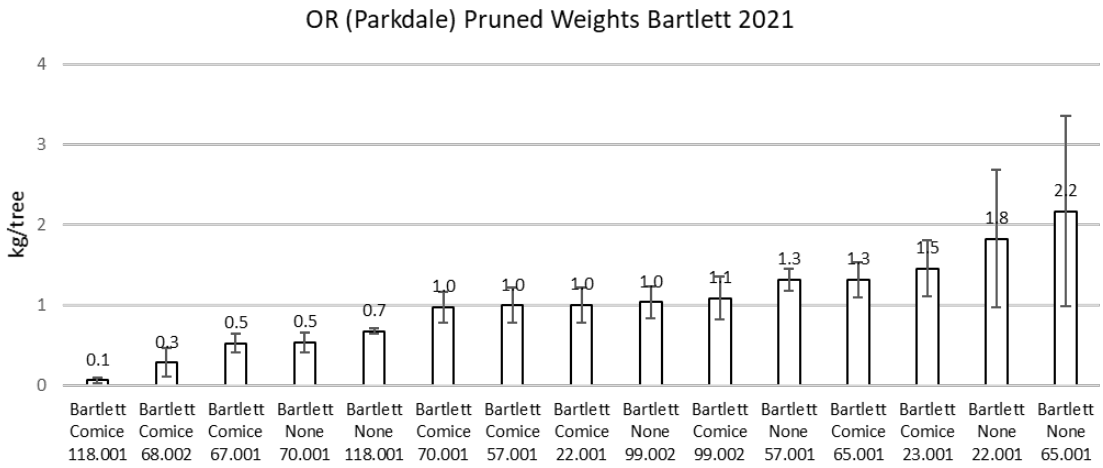


Figure 2. *2021 dormant winter pruning (kg/tree) in Parkdale (OR) for combinations of quince accessions and 'd'Anjou' (above) or 'Bartlett' (below) with and without Comice interstem. Data are sorted according to vigor, from lowest to highest and are means of 3 reps (±SE).*

Rootsuckers and TCSA

Suckering was observed in nearly all combinations with Comice interstem in WA. At the end of 2021, Anjou/Comice/67.001 reported the highest number of suckers (approximately 15/tree and 5/tree respectively), significantly higher than all the other combinations (data not shown). The lowest values were found in the combinations Anjou/Comice/23.001, and 57.001. Similarly, for Bartlett, combinations with Comice on 67.001 and 68.002 reported the highest number of suckers per tree, approximately 16 and 20 suckers per tree, respectively, (data not shown). OR root sucker data will be collected in spring 2022 when pruning is conducted, however, suckering data from previous seasons agreed with WA data.

Trunk cross sectional areas (TCSA) of scions measured in November 2021 at 10 cm above the graft union (always on the scion) are reported in Figure 4 for WA only. The largest TCSA in November 2021 was observed for Anjou/Comice/99.002, and the lowest TCSA belonged to the combinations Anjou/Comice/68.002 and 118.001 (Figure 4A). Anjou/Comice/68.002 confirmed its low vigor as reported from the pruning weights (Table 1). Similarly, Bartlett's highest TCSA mean at the end of season 2021 was reported for Bartlett/Comice/65.001 and the lowest in Bartlett/Comice/118.001 (Figure 4B). OR trunks will be measures in spring 2022 when pruning is conducted, however, previous years data showed that trees in OR were roughly double the size of those in WA and there was general agreement for weak and vigorous combinations.

Productivity

Harvest 2021 represented the second, and most significant, crop for both cultivars at both sites. Three representative trees per replicate were selected for each combination and harvested individually. The average fruit weight was calculated by dividing the total kg per tree by the number of harvested pears per tree and expressed in grams. In general, Anjou produced less on a tree basis than Bartlett in WA (respectively 8.4 kg/tree and 11.8 kg/tree; Fig. 5). The reverse was observed in OR (Fig. 6). In either of the two cultivars, statistically significant differences did not emerge in 2021 despite an approximate two-fold difference between the highest and lowest yielding combinations (Figs. 5 and 6). 'Bartlett' yields were higher in WA than OR (Fig. 6) due to the severe pruning in 2021 at the OR site. Irrespective,

high yielding ‘Bartlett’ combinations still produced ~10 kg per tree in OR (i.e., ~50 fruit per tree);fruit weight in OR, however, was smaller than WA (Figs. 5 and 6). Fruit size was excellent in WA (box size 90s). The smaller fruit in OR are associated with weaker wood and further illustrate the benefits of short pruning as discussed above. ‘d’Anjou’ yields were higher in OR than WA due to markedly larger trees (greater canopy volume) in OR. ‘d’Anjou’ fruit size was excellent at both sites (box size 80s and 90s). The highest yielding combinations produced ~40 bins per acre. Differences in the relative ranking of combinations for 2021 yield did not agree between sites. We plan to assess these differences in 2022.

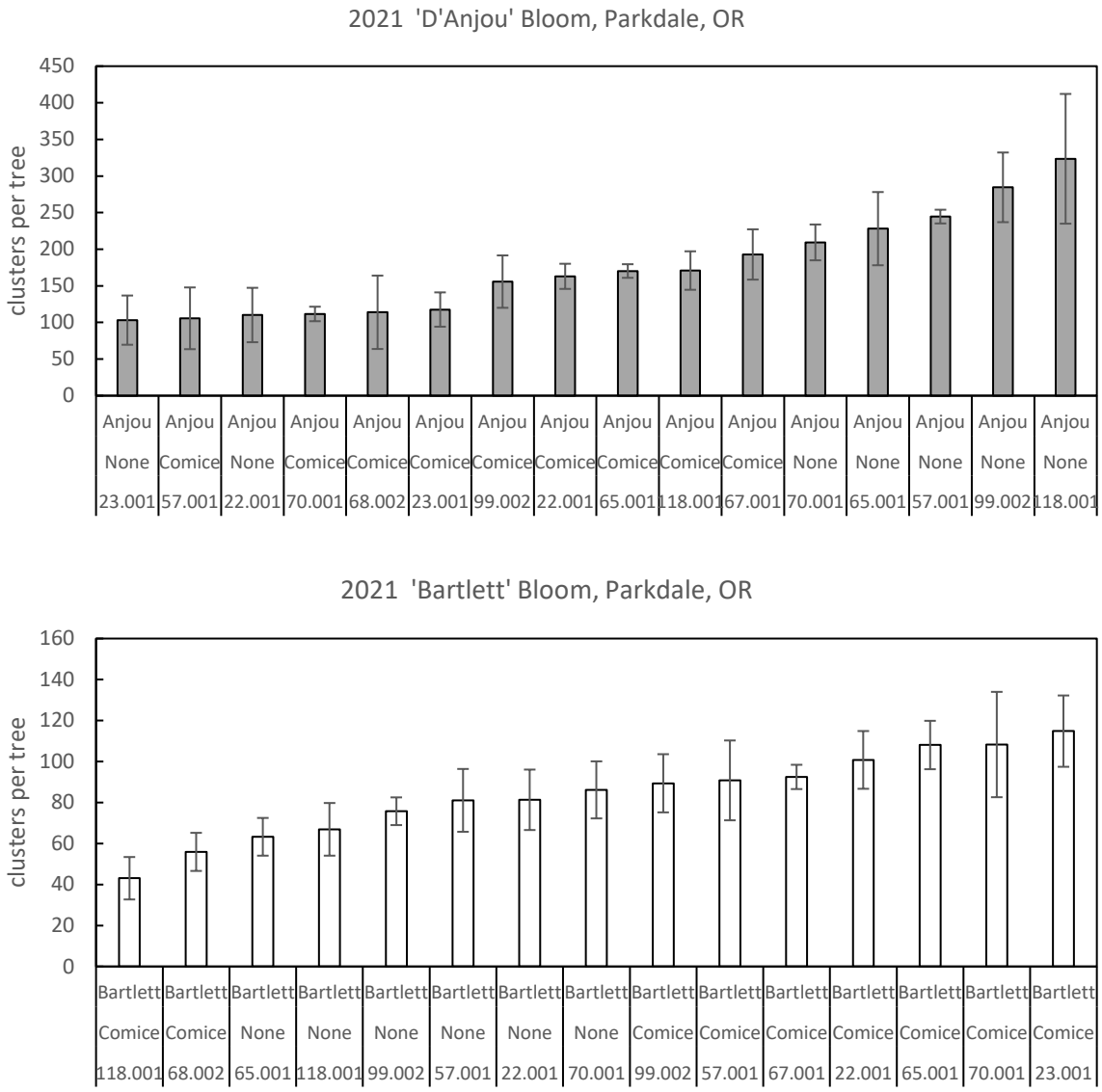


Figure 3. *2021 bloom (number of clusters/tree) in Parkdale (OR) for combinations of quince accessions and d’Anjou’ (above) or ‘Bartlett’ (below) with and without Comice interstem. Data are sorted from lowest to highest and are means of 3 reps (±SE).*

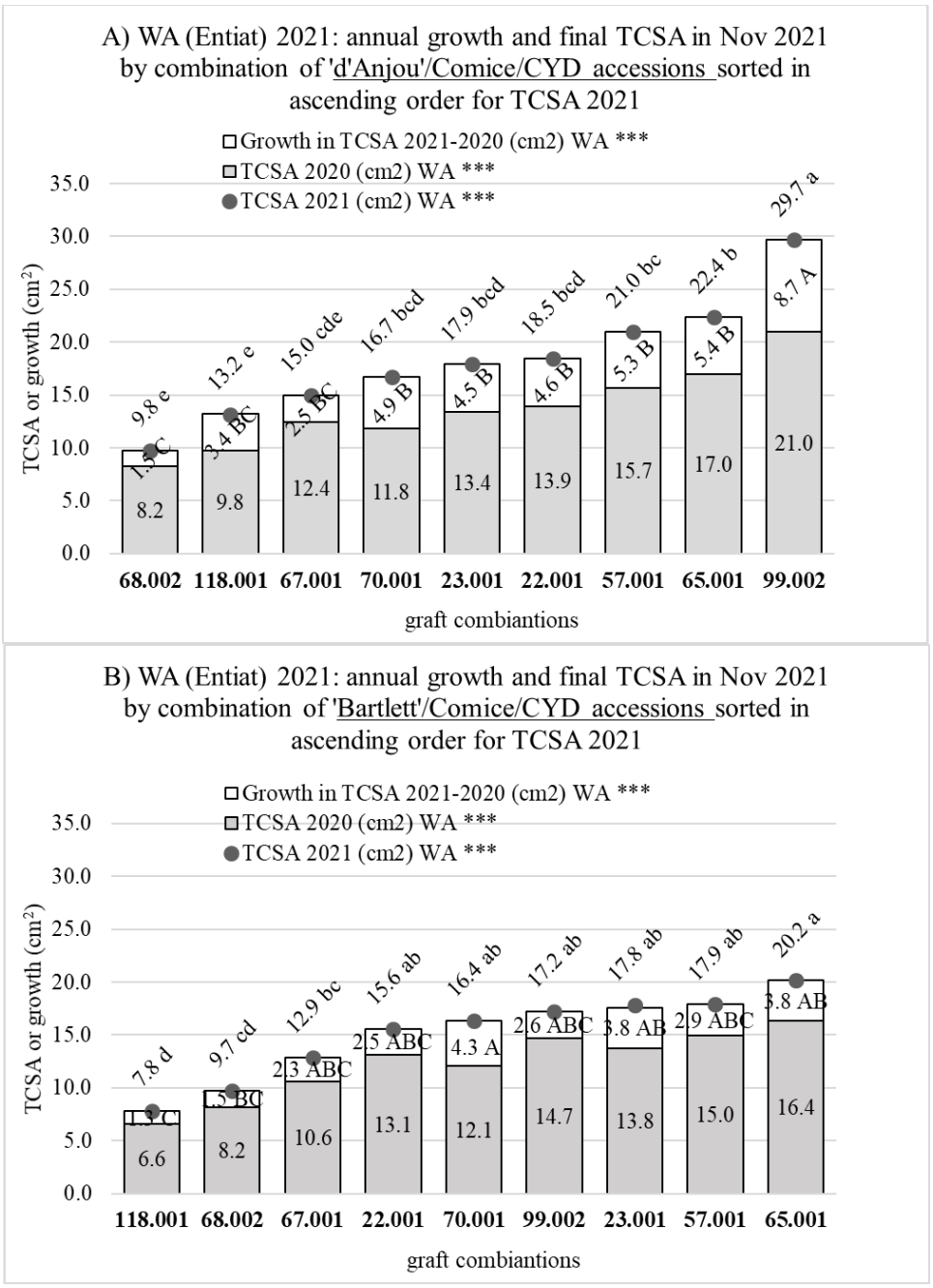


Figure 4. *Trunk cross-sectional area (TCSA) in 2020 and 2021 expressed as cm^2, measured 10 cm above the graft union between the interstem and scion, for Anjou (A) and Bartlett (B) grafted on 9 different quince accessions in Entiat (WA). The chart is sorted by ascending tree size for each variety. Combinations without interstem (direct graft) were excluded from statistical analysis and not displayed here. Means associated with different letters are significantly different at $P<0.05$.*

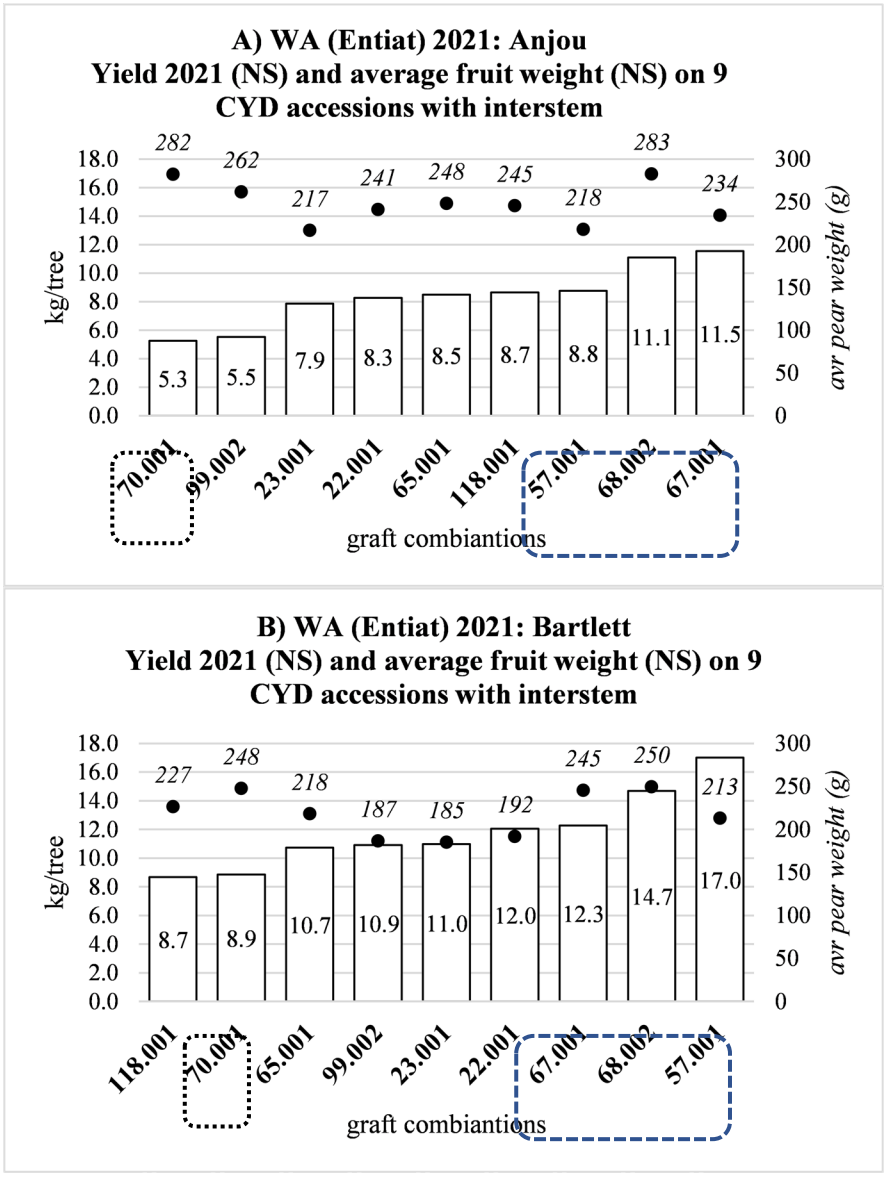


Figure 5. *Yield data for 2021, expressed as kg fruit/tree and average pear weight (g), are presented for Anjou (A) and Bartlett (B) grafted on 9 different quince accessions in Entiat (WA). The chart is sorted by ascending yield/tree for each variety. Combinations without interstem (direct graft) were excluded from statistical analysis and not displayed here. NS= not significant differences emerged between the combinations for the indicated parameters (see title). Dashed boxes at the two extremes of the production range for each cv in 2021 show consistency in cultivars' performances using those CYD accessions as rootstocks.*

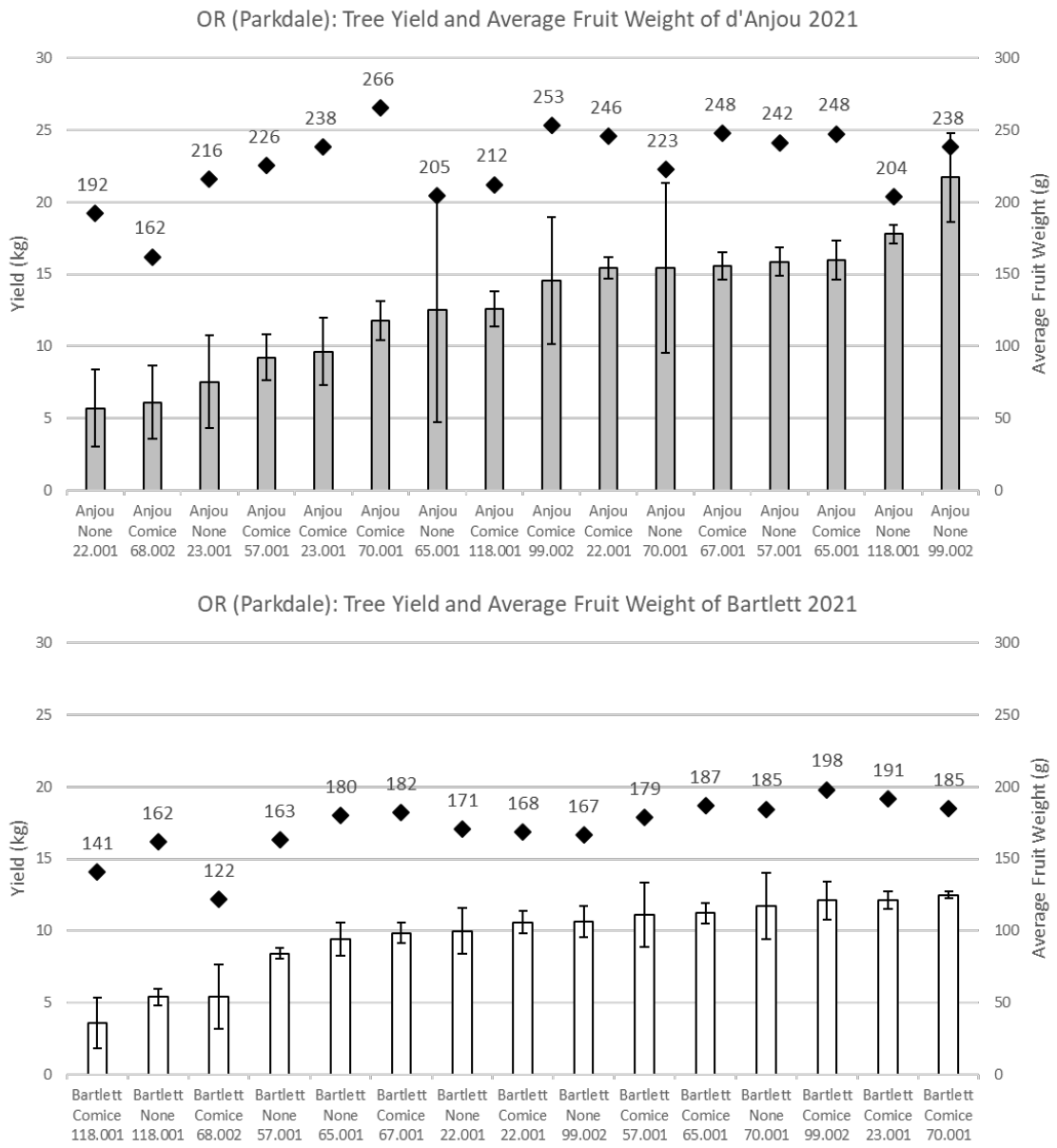


Figure 6*. Yield data for 2021, expressed as kg fruit/tree and average pear weight (g), are presented for Anjou (top) and Bartlett (bottom) grafted on 9 different quince accessions in Parkdale (OR). The chart is sorted by ascending yield/tree for each variety. Data are means of 3 reps (±SE).*

Fruit quality

Fruit quality data were collected at both sites but space limitations do not allow discussion or presentation of those data. We will highlight key findings during our presentation at the 2022 NW Pear Research Review

Objective 2: Determine the propagation potential of previously identified cold-hardy quince clones not included in the field trial described above.

Several attempts were made to establish cultures in 2021, despite beginning in late spring. We successfully cultured 15 of the cold hardy clones from USDA-NCGR including half of the new

accessions not previously cultured or evaluated in field performance trials (Table 3). These include the top three cold hardy accessions in the collection. We will continue to modify our medium (i.e., recipes) and customize our approach in 2022 for clones that either have low multiplication rates (shown as number of jars; Table 3) or failed to initiate shoots (lower portion of Table 3). There were only six genotypes that could not be cultured in 2021. Root induction will be delayed until 2022 after our attempts to initiate enough shoots on the complete collection. This is consistent with our proposal in order to develop trees of the same age for future field trials. Further, dormant bud wood will be collected from the USDA/NCGR in-situ collection in February 2021 and grafted to commercial quince to establish a mother block to improve supplies, increase efficiency, and to assess any phenotypical changes that may occur throughout micropropagation (such as expression of juvenility characteristics following tissue culture).

Table 3. *January 2022 status of micropropagation efforts of cold hardy quince accessions collected from the NCGR.*

Yes or No indicates if accessions were included in 2017 field trials (data in parentheses are cold hardiness rankings)	**Accession: NCGR identifiers**		**Number of jars in tissue culture**
Standard	Standard	BA 29C	25
No (10)	CYD 32.002	Tashkent AR-232 Seedling 4 (A)	4
No (22)	CYD 34.001 (IGC 34)	Sorbopyrus 'Smokvarka'	1
Standard	CYD 64.001	Quince A	4
No (2)	CYD 104.001	Aiva from Gebeseud	15
No (3)	CYD 67.004	Akhtubinskaya O.P. Seedling (B)	16
No (1)	CYD 120.001	C. oblonga- Arakseni, Amernia	5
No (8)	CYD 126.001	C. oblonga- Megri, Amernia	22
No (12)	CYD 128.001	C. oblonga-Babaneuri, Georgia	12
Yes (18)	CYD 99.002	Kashnko no.8	25
Yes (9)	CYD 118.001	C. oblonga-Seghani, Amenia	3
Yes (13)	CYD 68.002	Krukouskaya O.P.Seedling	24
Yes (5)	CYD 70.001	Sokorospelka O.P. Seedling	6
Yes (14)	CYD 22.001	W-4	21
Yes (16)	CYD 23.001	WF-17	6
No (4)	CYD 32.004	Tashkent AR-232 Seedling 4 (B)	0
No (7)	CYD 29.001	Quince W	0
No (15)	CYD 123.001	Trentholm	0
No (17)	CYD 75.001	Bereczki	0
No (20)	CYD 13.001	Pyronia veitchii	0
No (24)	CYD 71.001	Teplovskaya O.P. Seedling	0
Yes (6)	CYD 57.001	Quince S	0
Yes (19)	CYD 65.001	Quince C7/1	0
No (11)	n/a	Van Deman	0