2025 NW Pear Research Review

WTFRC intern Raesibe Kgaphola is pictured above inoculating pears with bacteria and preparing them for storage in packed boxes as part of a research initiative.

Photo Source: Michael Meyer

February 20, 2025

Hybrid Format
Hood River, OR

Project Title: What factors impact mite outbreaks in pear?

Report Type: Final Project Report

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Project Duration: 2 Year

Total Project Request for Year 1 Funding: $33,054
Total Project Request for Year 2 Funding: $33,857

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Other related/associated funding sources:
Requested

Funding Duration:	June 2024 – May 2027
Amount:	$109,581
Agency Name:	Washington Tree Fruit Research Commission (ACP) & Fresh and Processed Pear Committee Research
Notes:	New funding request to pursue research on whirligig mite releases and conservation. In addition to unrelated work in potatoes, this proposal was brought about by determining that whirligigs were highly abundant in some pear orchards surveyed in this project. This project will determine if whirligig releases have potential; we will seek additional funding from Specialty Crop Block to expand the work if results are promising.

Budget 1
Primary PI: Rebecca Schmidt-Jeffris
Organization Name: USDA-ARS
Contract Administrator: Mara Guttman
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Supervisor: Rodney Cooper
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Item	2022	2023
Salaries[1]	$9,297	$9,529
Benefits[1]	$744	$762
Wages	$0	$0
Benefits	$0	$0
Equipment	$0	$0
Supplies[2]	$9,000	$9,000
Travel[3]	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$19,041**	**$19,291**

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

Budget 2

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

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

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

Budget 3
Primary PI: Chris Adams
Organization Name: OSU
Contract Administrator: Charlene Wilkinson
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Station Manager/Supervisor: Steve Castagnoli
Email Address: steve.castagnoli@oregonstate.edu

Item	2022	2023
Salaries[1]	$2,187	$2,252
Benefits[2]	$875	$901
Wages[3]	$3,900	$4,017
Benefits[3]	$390	$402
Equipment	$0	$0
Supplies	$0	$0
Travel	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$7,352**	**$7,572**

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

OBJECTIVES

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

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

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

SIGNIFICANT FINDINGS

- Wenatchee Valley had substantially higher twospotted spider mite populations than Yakima Valley or Hood River. Hood River locations in 2022 had very few spider mites, but were more similar to Yakima numbers in 2023. In 2023, Hood River sites had higher pear psylla populations than Wenatchee, contrary to expectations. Yakima Valley had much higher rust mite populations than the other two regions in both years of the survey.

- While phytoseiids ("typhs") were found in the survey, they were much less common than in apple orchards. This suggests that in pear orchards where pest mites do not flare, other natural enemies may be responsible for biological control. Yakima orchards were dominated by *Typhlodromus caudiglans*, whereas Wenatchee orchards were dominated by *Galendromus occidentalis*. This likely reflects the prey preferences of these two phytoseiids (rust mites and twospotted spider mites, respectively) and the abundance of particular prey items. Relatively few phytoseiids were found in Hood River orchards, even in orchards where spider mites were relatively abundant.

- Weed wash samples effectively detected phytoseiids and spider mites in the ground cover. Orchards with very high TSM populations in the weeds also had outbreaks in the trees, but there was no consistent correlation between ground cover and canopy populations at moderate or low densities. There was also no clear trend on where TSM appeared first (weeds versus canopy). The phytoseiid community in the weeds was more diverse than the canopy.

- The composition of the orchard natural enemy community varied dramatically by region. Very few natural enemies were captured on beat trays in Hood River in 2022, so we focused on 2023 to compare regions. Wenatchee sites had more *Deraocoris*, *Camplyomma*, and *Chrysopa* lacewings; Yakima sites had more *Stethorus,* other ladybeetles, whirligig mites (*Anystis*), phytoseiids, and spiders (anyphaenids, oxyopids, philodromids, salticids); Hood River sites had the fewest natural enemies but did have more *Chrysoperla* lacewings and linyphiid and theridiid spiders than the other two regions. Other predatory hemipterans (besides *Deraeocoris* and *Camplyomma*) were relatively rare, but more abundant in Yakima and Hood River.

- We tested 1,375 natural enemies captured on beat trays for the presence of twospotted spider mite and pear psylla DNA. *Deraeocoris* was the most abundant predator and tested positive at the highest rates for pear psylla and TSM. *Chrysopa* lacewings, *Anystis*, and several spider families also frequently tested positive for pear psylla. *Chrysoperla* lacewings, *Anystis*, and several spider families had high positive rates for TSM. Accounting for predator abundance, the majority of TSM positives were *Deraeocoris*, spiders, *Campylomma*, and *Stethorus*, and the majority of pear psylla positives were *Deraeocoris*, spiders, *Campylomma*, and *Chrysopa*.

METHODS

This two-year (2022-2023) study was conducted in commercial pear orchards in each of three pear-growing regions: Wenatchee, Yakima, and Hood River. Orchards represented a variety of management types (e.g., conventional, organic, soft IPM) and mite outbreak frequency and intensity. Each orchard was sampled every 1-3 weeks, with sampling frequency increasing during late July to mid-August when mite outbreaks are most likely to occur.

At each sampling date, a 50-leaf sample was collected from throughout the orchard block. Leaves were brushed with a mite brushing machine and the resulting sample will be counted using a microscope. We counted eggs and motiles of spider mites (twospotted spider mite, European red mite, brown mite), pear rust mites, pear psylla eggs and nymphs, and predatory mites. Any predatory mites found were removed from the sample and stored in 70% ethanol, then slide-mounted for identification. Five sticky cards were also placed throughout the orchard block. From these, we counted ladybeetles, lacewings, *Deraeocoris*, anthocorids (to genus), *Stethorus*, *Campylomma*, *Geocoris*, and *Nabis*. We conducted beat samples on 5 trees spaced roughly evenly throughout the orchard block. Any small predatory insects (of the appropriate size to eat mites) were directly placed in molecular grade ethanol for later counting and gut content analysis by PCR.

We also assessed herbicide strip weediness. We measured the distance from the edge of the herbicide strip to the trunk for the five sample trees to determine the herbicide strip size. For the same set of trees, we also estimated percent composition of bare ground, grass, and broadleaf weeds in the space adjacent to the tree (0.5×0.5 m quadrat). The presence/absence of dominant weed species was also recorded. Weeds were also collected from within the quadrat, brought to the lab, and then rinsed with ethanol to remove any arthropods. The ethanol “rinsate” was then poured through a vacuum filter with filter paper. Spider mites and phytoseiids captured on the filter paper were counted.

We determined that typical molecular gut content analysis using universal COI primers is not suitable for this study. Neither our pest mites nor pear psylla amplify well with this primer. Instead, we used species-specific primers for each predator collected in the study to determine whether it had recently fed on a pest of interest. An existing twospotted spider mite specific primer was determined to be suitable for our use. A colleague (B. Ohler) recently designed a highly specific pear psylla primer, which will also screen our predators with. We have successfully sequenced pear rust mites and are in the process of designing and optimizing a primer to detect their DNA. All other molecular work has been completed.

We have conducted some preliminary modelling to determine which factors are likely associated with mite outbreaks at the surveyed sites. However, we will continue to refine our analysis to better handle the large quantity of data collected.

RESULTS AND DISCUSSION

In 2022, we monitored a total of 20 locations: nine in Yakima Valley, six in Wenatchee Valley, and five in Hood River. In 2023, we monitored 18 locations: seven in Yakima Valley, six in Wenatchee Valley, and five in Hood River. In 2022, pest mites were nearly absent at all locations in Hood River. We also noted that almost no natural enemies were captured on beat trays in Hood River; therefore, new sites were selected in 2023. Yakima Valley dropped three sites and added one in 2023, whereas all the Wenatchee Valley sites remained the same between both years.

To compare pest and natural enemy populations between the regions, we calculated seasonal averages only using dates with strong overlap in pear psylla degree days (PPDD). There were fewer dates of overlap between regions in 2022 than 2023. The PPDD ranges used were 1150-3050 in 2022 and 2120-4400 in 2023. Wenatchee Valley sites had much higher twospotted spider mite populations than the other two regions, with Yakima Valley intermediate (Fig. 1). Phytoseiids were generally

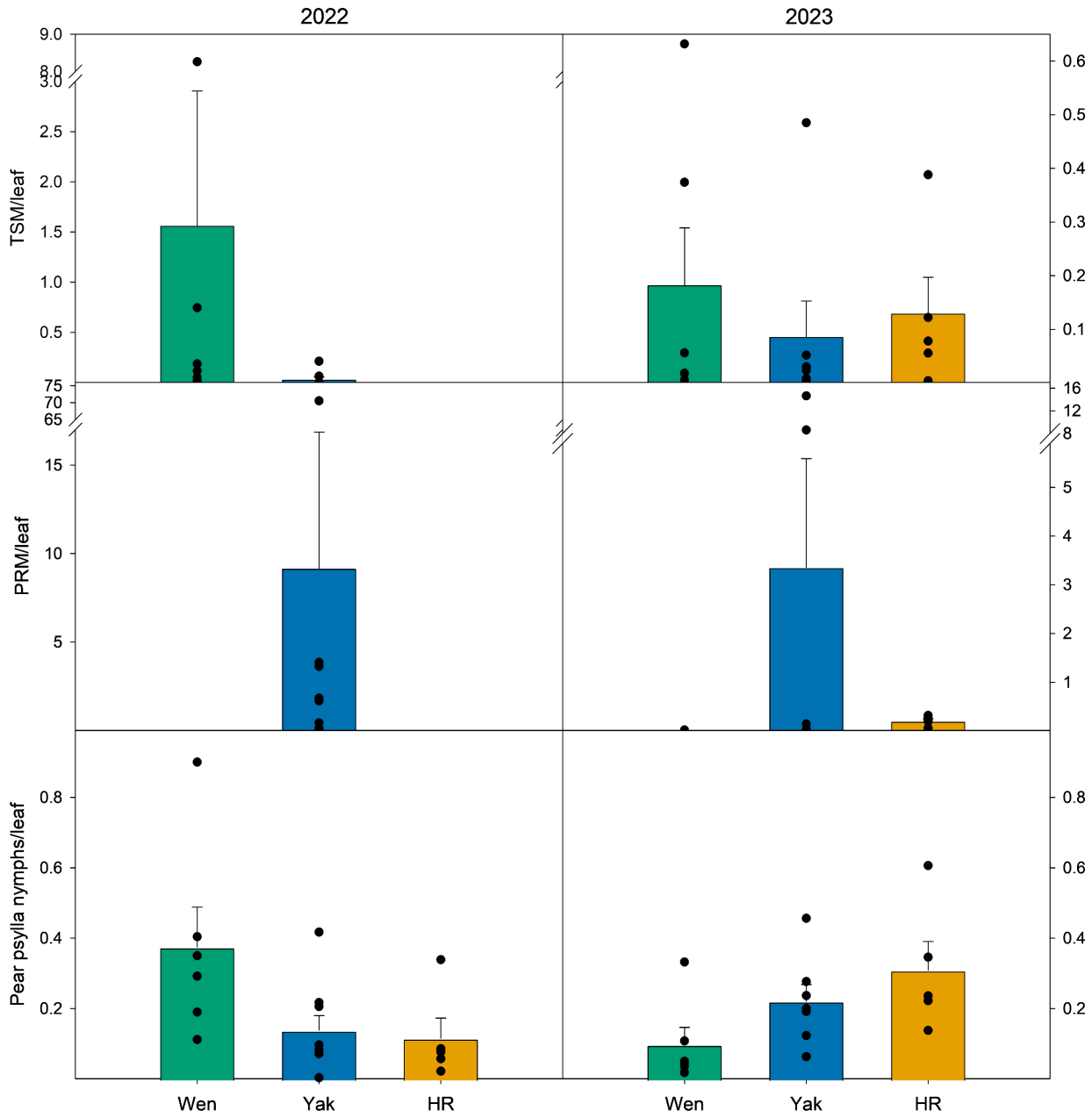

Fig. 1. Mean (±SE) pest counts per leaf in the three growing regions in 2022 and 2023, during the sampling period with the most PPDD overlap between the sites monitored. The black circles are the means for the individual orchards surveyed. Y-axis breaks are used to incorporate outlier data points for mite counts.

uncommon, especially compared to prior work in apple orchards (Schmidt-Jeffris et al. 2015). They were rarest in Hood River (3 total specimens found across all orchards throughout the season). In Yakima, one orchard reached 1.10 phytoseiids/leaf in 2023, but this was extraordinarily high compared to the other orchards. In general, orchards rarely exceeded 0.1 phytoseiids per leaf. In Yakima, *Typhlodromus caudiglans* is the dominant predatory mite, likely due to high populations of its preferred rust mite prey (Fig. 1). In Wenatchee, *G. occidentalis* is the dominant predatory mite, again due abundant preferred prey (spider mites). Only one organic orchard in Yakima had a different dominant canopy species (and in fairly low abundance), which we have yet to identify. Our results indicate that orchards with high populations of rust mites can expect *T. caudiglans* to be their most common phytoseiids, whereas those with twospotted spider mites can expect *G. occidentalis*.

Alcohol weed washes were effective at detecting spider mites and phytoseiids in the ground cover and were a much more efficient method of collecting mites than examining plant material. There was a correlation between TSM abundance in weed washes and in the canopy, although this relationship was not consistent across all orchards (Fig. 2). In general, orchards where TSM were absent from the weeds did not exceed per leaf thresholds, but moderate levels of TSM in the weeds were less indicative of levels in the canopy. Phytoseiid diversity was higher in the weeds than the canopy and *G. occidentalis* in particular was less abundant. The presence of *G. occidentalis* in the weeds was almost always associated with an orchard where TSM exceeded thresholds and were therefore also abundant in the weeds. Corresponding with leaf counts, Wenatchee had the highest number of TSM in weed wash samples both years (Table 1). In 2022, Wenatchee had more phytoseiids in the weed wash samples than the other regions and ~50% were *G. occidentalis*. In 2023, Yakima had over 10× as many phytoseiids in weed washes as the other regions (Table 1); this was driven primarily by one orchard where over 70 *T. caudiglans* females were found during the course of sampling; on 9 Sep 2023 alone, 54 phytoseiids were found in the weed wash sample for this location. This orchard

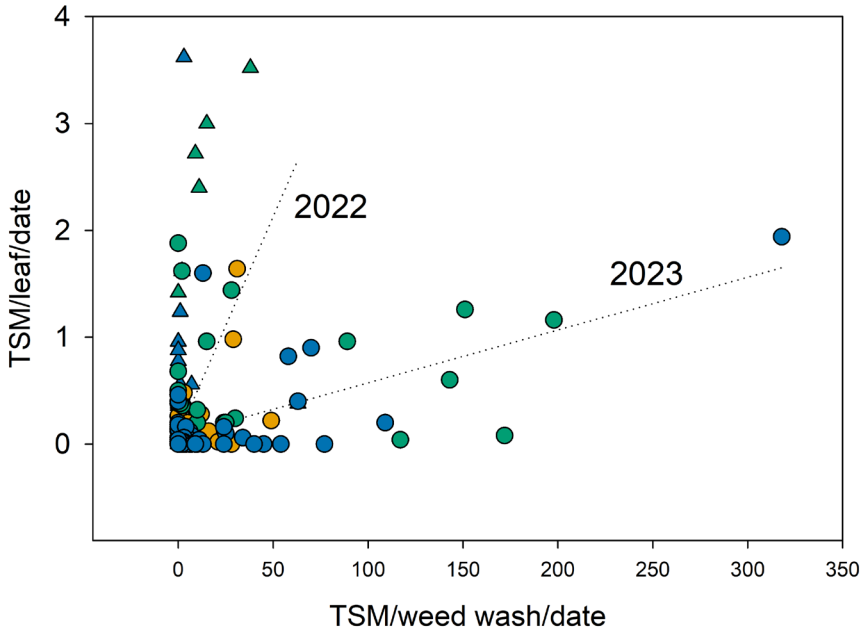

Fig. 2. TSM in weed wash samples versus leaf samples on each date studied in each orchard.

Table 1. Mean (±SE) mites per weed wash sample per date in each region.

	Region	*n*	TSM	Phytoseiids
2022	Wen	6	6.69 ± 3.11	0.31 ± 0.21
	Yak	9	0.34 ± 0.11	0.05 ± 0.03
	HR	5	0.03 ± 0.03	0 ± 0
2023	Wen	6	14.58 ± 13.39	0.21 ± 0.07
	Yak	7	10.21 ± 5.90	2.35 ± 0.80
	HR	5	5.27 ± 1.86	0.10 ± 0.07

Table 2. Mean (±SE) natural enemies per sample (5 beat trays) for each region in 2023. Phytoseiid counts shown are per leaf. In each row, the darkest box corresponds to the region where a natural enemy was most abundant.

	Region	*n*	TSM	Phytoseiids
2022	Wen	6	6.69 ± 3.11	0.31 ± 0.21
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	HR	5	5.27 ± 1.86	0.10 ± 0.07

year	area	_FREQ_	antho	anthocorisSE	anyphenid	anyphaenidSE	brown	brownSE	camp	campySE	chryso
2020	H						0.033	0.033			

was dominated by *T. caudiglans* (79%), which was likely feeding on the unusually high rust mite population (>15/leaf).

The most common natural enemies in beat tray samples were *Deraeocoris*, spiders, *Stethorus*, and *Campylomma* (Table 2). Poor beat tray capture in Hood River in 2022 made it difficult to compare natural enemy populations between the regions, so only data from 2023 samples are shown. Wenatchee sites had more *Deraocoris*, *Camplyomma*, and *Chrysopa* lacewings; Yakima sites had more *Stethorus,* other ladybeetles, whirligig mites (*Anystis*), phytoseiids, and spiders (anyphaenids, oxyopids, philodromids, salticids); Hood River sites had the fewest natural enemies but did have more *Chrysoperla* lacewings and linyphiid and theridiid spiders than the other two regions. Other predatory hemipterans (besides *Deraeocoris* and *Camplyomma*) were relatively rare, but more abundant in Yakima and Hood River. The lower abundance of natural enemies in Hood River in 2023 may have contributed to the higher pear psylla populations.

Across both years, Yakima orchards had wider herbicide strips (2.3 ± 0.3 m) than orchards in Hood River (1.2 ± 0.1 m) or Wenatchee (1.1 ± 0.1 m). All orchards in Yakima had clover, dandelion, mallow, and chickweed present and black medic, pigweed, and field bindweed were also common (Table 3-4). In Hood River, clover, dandelion, and mallow were also the three most common weeds (Table 3-4). Wenatchee differed from the other two areas – field bindweed was the most common, followed by dandelion, mallow, and lambs quarter. Some weeds were found in a majority of orchards, but still relatively uncommon within samples (e.g., lambs quarter in Wenatchee and Yakima). Yakima orchards tended to be grassier than Wenatchee and Hood River orchards (Fig. 3), potentially indicating that the herbicide strip was managed less heavily.

We compared orchards where spider mites exceeded the threshold (0.5/leaf) at least once to those that did not. Of the more common weed groups, field bindweed and lambs quarter incidence differed the most between the “exceeded” and “did not exceed” groups; orchards that exceeded thresholds had more than twice as many samples with bindweed present as those that did not (lambs quarter 76% higher in “exceeded” orchards). This indicates that these weeds in particular should be monitored for spider mites and may need to be carefully controlled to reduce outbreak risk. However, bindweed was also more common in Wenatchee (where spider mite outbreaks more commonly occur); more

complex modelling procedures may better determine which of these variables is truly associated with TSM outbreaks versus regional differences. There were similar difficulties determining which natural enemies were associated with lower pest populations using more basic modelling procedures.

The gut content analysis data used two species-specific primers (TSM, pear psylla) and provides insight regarding which natural enemies are consuming key pear pests in the field. However, our analysis only indicates that a predator recently ate one of these pests and not how many or when. Most differences in results between regions were due to the abundance of particular groups of predators and the availability of the two pests, therefore we are showing the combined results of both years for all three regions. A high percentage of *Deraeocoris* tested positive for TSM (40%) and pear psylla (59%) (Table 5), resulting in this species making up the largest total number of positives. Of the groups where >20 individuals were collected, *Deraeocoris*, *Anystis*, and *Chrysoperla* most frequently test positive for TSM and *Deraeocoris*, *Anystis*, and *Chrysopa* most frequently test positive for pear psylla (Table 5). However, when the abundance of the natural enemy groups was considered, *Dereaocoris*, spiders, and *Campylomma* accounted for the majority of positives for both pests (Fig. 4). In 2023, we identified the spiders to family and are able to further breakdown which groups are consuming the two pests. Anyphaenids, oxyopids, philodromids, and salticids were the most abundant. A relatively high proportion of all these groups tested positive for both pests (Table 6). Oxyopids and anyphaeids contributed to a relatively high proportion of both the TSM and pear psylla positives, and salticids to the pear psylla positives (out of all positive natural enemies combined). Earwigs are undersampled on beat trays, leading our gut content work to likely underestimate their importance in pear orchards.

Table 3. Percent of orchards where a given weed was present, 2022-2023.

	Yakima	Wenatchee	Hood River
Clover	100	50	90
Dandelion	100	67	80
Mallow	100	67	70
Chickweed	100	17	10
Black Medic	90	0	0
Pigweed	80	0	30
Field Bindweed	80	100	10
Lambs Quarter	70	67	0
Shiny Geranium	70	0	0
Broad Leaf Plantain	60	0	30
Prostrate Knotweed	40	0	0
Narrow Leaf Plantain	20	0	0
Purslane	10	0	0
Horsetail	0	50	0
Ribes	0	33	0

Table 4. Percent of samples (quadrats) where a given weed was present, 2022-2023.

	Yakima	Wenatchee	Hood River
Dandelion	47.0	6.7	16.8
Clover	25.5	1.5	14.3
Mallow	21.4	6.9	11.4
Field Bindweed	18.9	33.8	0.2
Chickweed	18.2	0.1	0.1
Prostrate Knotweed	9.0	0.0	0.0
Lambs Quarter	5.2	3.2	0.0
Pigweed	4.8	0.0	5.2
Broad Leaf Plantain	4.6	0.0	1.8
Black Medic	3.9	0.0	0.0
Shiny Geranium	1.6	0.0	0.0
Purslane	1.2	0.0	0.0
Narrow Leaf Plantain	0.8	0.0	0.0
Ribes	0.0	0.0	0.0
Horsetail	0.0	5.4	0.0

Spiders and whirligig mite (*Anystis*) are likely underappreciated as predators in orchards, although their role may be more important in the Yakima Valley where they appear to be more abundant. Surprisingly, only 22% of *Stethorus* tested positive for TSM; they were abundant in some orchards where TSM populations were low, indicating they may be eating another food source. Additionally, we did not observe *Stethorus* larvae in any of our orchards, indicating that they may be reproducing

exclusively in extra-orchard habitat. The two green lacewing genera collected (*Chrysopa* and *Chrysoperla*) appear to substantially differ in prey preferences; few *Chrysoperla* tested positive for pear psylla whereas 52% of *Chrysopa* were positive. If *Chrysoperla* minimally contribute to pear psylla predation, then scouts may need to distinguish between green lacewing genera to estimate the expected level of biological control. Finally, the gut content results provide additional evidence that *Deraeocoris* is a key predator of both TSM and pear psylla in pear psylla orchards. Its role in controlling TSM should be further investigated. We will continue to work with this data set to develop models that highlight factors that contribute to spider mite outbreaks.

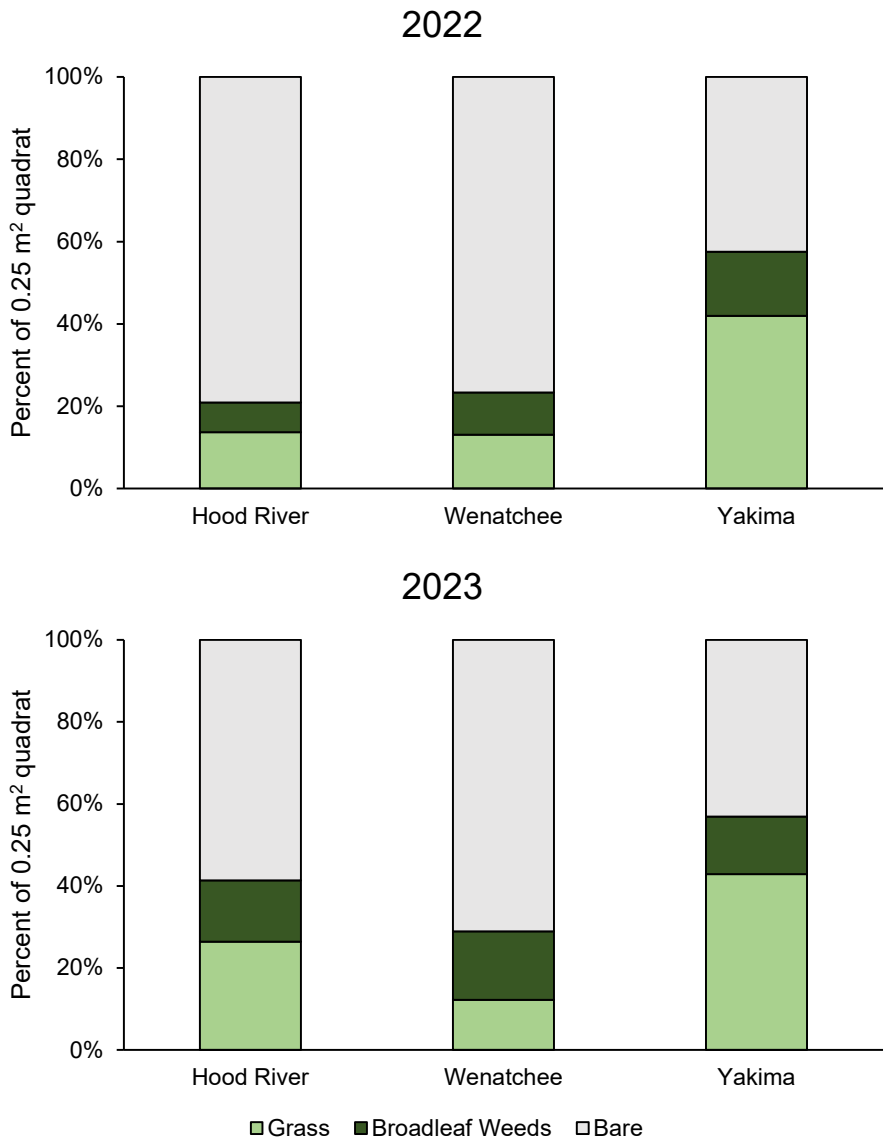

Fig. 3. Ground cover composition 0.5 m into the row from the base of the tree.

Table 5. Percent of samples from each natural enemy group testing positive for TSM and pear psylla (PP) DNA using species-specific primers.

	n	% TSM+	% PP+
Earwigs	34	3	29
Campylomma	188	23	24
Deraeocoris	505	40	59
Anthocoris	7	14	57
Orius	13	23	15
Geocoris	3	67	33
Nabis	15	33	27
Berytidae	3	0	33
Hemerobiidae	8	0	38
Chrysopa	42	21	52
Chrysoperla	36	31	14
Stethorus	161	22	1
Other ladybeetle	38	16	24
Spider	292	24	30
Opiliones	8	13	0
Anystis	22	32	45

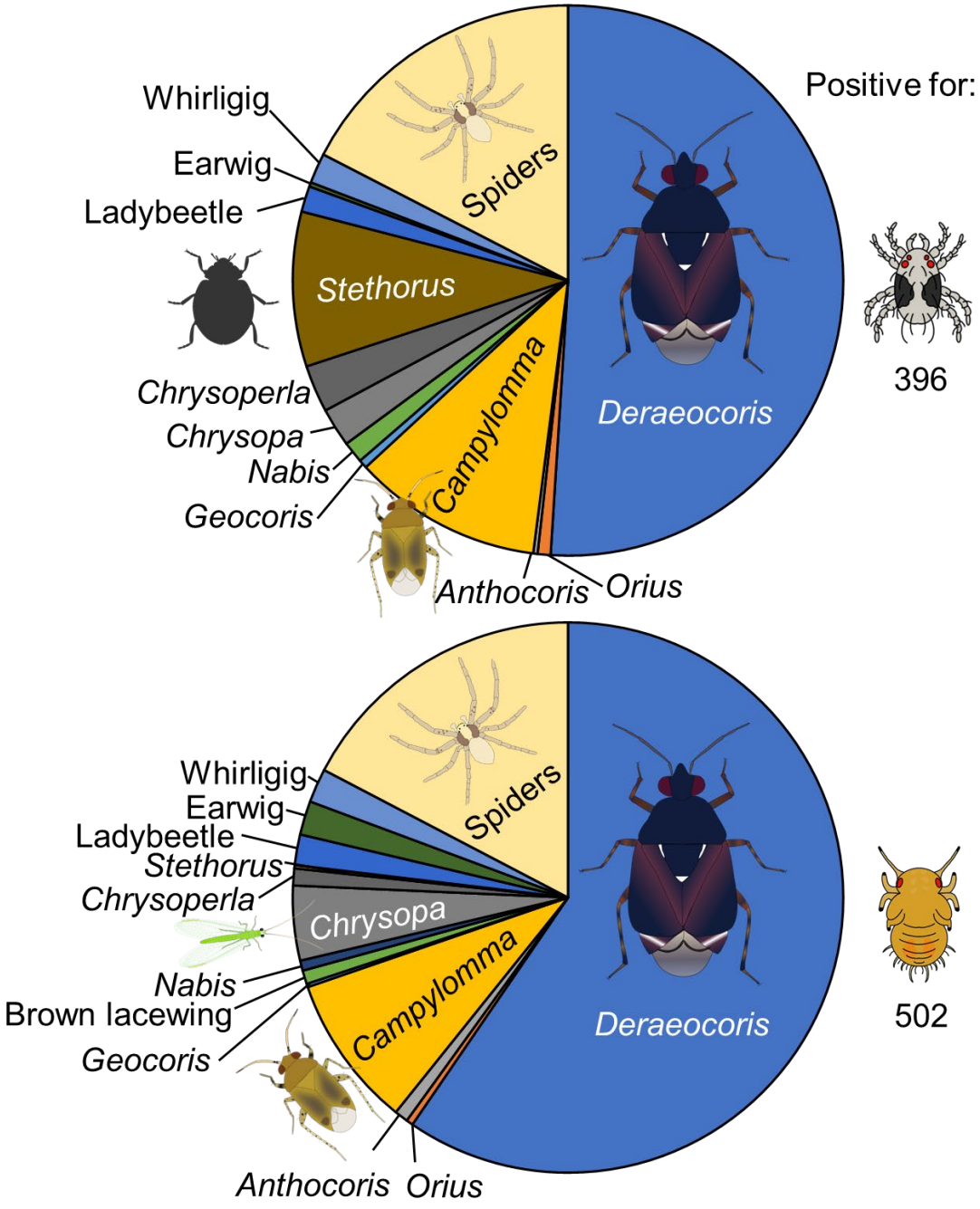

Fig. 4. The relative proportion of each natural enemy group making up samples that tested positive for TSM or pear psylla DNA.

Table 6. Percent of samples from each natural enemy group testing positive for TSM and pear psylla (PP) DNA using species-specific primers in 2023. In 2023, spiders were identified to family.

	n	**% TSM+**	**% PP+**
Earwigs	18	6	28
Campylomma	161	26	25
Deraeocoris	377	45	60
Anthocoris	5	20	80
Orius	4	50	0
Geocoris	1	100	0
Nabis	11	36	27
Berytidae	1	0	0
Hemerobiidae	1	0	0
Chrysopa	24	25	50
Chrysoperla	29	38	7
Stethorus	115	30	1
Other ladybeetle	14	14	7
Opiliones	7	14	0
Anystis	18	39	50
Anyphaenidae	32	38	47
Dyctinidae	1	0	0
Linyphiidae	16	44	31
Oxyopidae	42	38	29
Philodromidae	33	30	6
Salticidae	30	23	53
Theridiidae	10	20	40
Thomisidae	6	17	17

EXECUTIVE SUMMARY

Project title: What factors impact mite outbreaks in pear?

Key words: spider mite, rust mite, *Tetranychus urticae*, *Epitrimerus pyri*, natural enemies

Abstract:

After pear psylla, twospotted spider mite (TSM) and pear rust mite are the key pests of pears in the Pacific Northwest. TSM outbreaks can be sporadic and difficult to predict. We sought to determine which factors, including spray programs, mowing, weed community, orchard dustiness, weed management, and the natural enemy community, best predicted flare ups of either mite pest. We also used molecular gut content analysis as a method for determining which predators likely played the largest role in biological control. We collected data from orchard managers and sampled the weed community and ground cover composition, as well as sampling for arthropods using leaf samples, weed washes in ethanol (for spider mites and phytoseiids), beat trays, and sticky cards. Sampling was conducted in 2022-2023 in orchards in three pear growing regions: Wenatchee, Yakima, and Hood River. Preliminary data analysis suggests that the presence of field bindweed may contribute to TSM outbreaks, but more sophisticated analysis is needed to account for correlation between the variables measured. Field bindweed was present in most orchards in Yakima and Wenatchee, but absent in Hood River; it was particularly abundant in Wenatchee. In general, Yakima orchards had larger pear rust mite populations and Wenatchee orchards had larger TSM populations. This corresponded to these areas having phytoseiid communities dominated by *Typhlodromus caudiglans* and *Galendromus occidentalis*, respectively; this is likely due to the prey preferences of each predator for the most common pest of each region. Both TSM and phytoseiids could be found in weed wash samples; very high abundance of TSM in the orchard canopy corresponded with high abundance in weed wash samples, but moderate levels did not strongly correlate with leaf counts. The relative abundance of natural enemy groups varied by region. Hood River had fewer natural enemies than the other two locations. In Wenatchee, *Deraeocoris* and *Campylomma* were the most abundant and highly dominant. In Yakima, *Deraeocoris* and *Campylomma* were also the most abundant, but spiders and *Stethorus* also made up a large portion of the natural enemy community. We determined that COI-barcoded primers were not suited for our target pests and instead used species-specific primers for TSM and pear psylla; we are working on developing a primer to target pear rust mite and have identified a promising sequence. In addition to being abundant, *Deraeocoris* tested positive for both pest species at high rates, indicating that it may be the most critical predator in pear orchards. Spiders and whirligig mite (*Anystis*) also tested positive at high rates and are likely underappreciated as predators in orchards, although their role may be more important in the Yakima Valley where they appear to be more abundant.

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

Report Type: Final Project Report

Primary PI: Christopher Adams
Organization: OSU
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Cooperators: GS Long, Wilbur-Ellis, W. Ag. Improvement, Chamberlin

Project Duration: 3 Year

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

Other related/associated funding sources: Applied for WSARE
Funding Duration: 2025 - 2028
Amount: $342,000
Agency Name: WSARE
Notes: We applied for this grant in 2023 and were highly rated but not funded. We re-submitting the grant this spring (2024) with more of an emphasis on on-farm outreach and extension.

WTFRC Collaborative Costs:

Item	2021	2022	2023
Salaries [1]	$13,000.00	$13,000.00	$13,000.00
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies [2]	$6,000.00	$6,000.00	$6,000.00
Travel [3]			
Plot Fees			
Miscellaneous			
Total	$19,000.00	$19,000.00	$19,000.00

Footnotes:

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

[2]Research consumables

Budget 1
Primary PI: Christopher Adams
Organization Name: OSU
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228
Contract administrator email address: Charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Brian Pierson
Station manager/supervisor email address: brian.pierson@oregonstate.edu

Item	2021	2022	2023
Salaries [1]	$13,000.00	$13,000.00	$13,000.00
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel [2]			
Plot Fees			
Miscellaneous			
Total	$13,000.00	$13,000.00	$13,000.00

Footnotes:

[1]GS-4 technician for 4 months per year, 100% FTE at 8% benefits, Year 2 includes 2.5% COLA increase. Technician would conduct sampling in the Yakima area, process/count samples, and slide mount mites for identification (Schmidt-Jeffris will identify). This technician will also conduct surface sterilization and PCR for gut content analysis for all samples (Yakima, Wenatchee, and Hood River).

[2]Molecular supplies for gut content analysis, sticky cards for field sampling – to be purchased for entire project team.

[3]Fuel to field sites will be provided by USDA base funds and is not requested.

Budget 2

Co PI 2: Rebecca Schmidt-Jeffris
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Station Manager/Supervisor: Rodney Cooper
Station manager/supervisor email address: Rodney.cooper@usda.gov

Item	2021	2022	2023
Salaries [1]	$13,000.00	$13,000.00	$13,000.00
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel [2]			
Plot Fees			
Miscellaneous			
Total	$13,000.00	$13,000.00	$13,000.00

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

Budget 3
Co PI 2: Rob Orpet
Organization Name: WSU
Contract Administrator: Shelli Tompkins
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Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@wsu.edu

Objectives

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

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

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

Significant Findings

- We have shown that lure-baited monitoring traps can be used to attract and collect natural enemies in managed pear orchards. These traps are superior to beat trays because they collect data continually over the period of a week. Plant volatile baited traps collect unbiased data that is not influenced by differences in human collection technique.

- We have measured the abundance and timing of 12 natural enemies of pear psylla across the entire Hood River valley, and in several Wenatchee valley orchards, over three years.

- We provided weekly communication about natural enemy abundance and timing to stake holders through weekly extension emails, who said they used these numbers to make management decisions.

Methods

Natural enemy lures containing 4 compounds acetic acid, methyl salicylate, phenylacetaldehyde, and 2-phenylethanol, a combination that has been shown to attract key indicator groups of natural enemies, were made at the OSU MCAREC lab. These lures were hung on yellow sticky traps and placed at 20 pear orchards that were recommended by collaborative crop consultants. Traps were checked and replaced weekly from April to September. Captured insects were identified to family level, species complex (e.g. Lacewings), or to species when possible.

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

To evaluate the usefulness of natural enemies traps we will need to show that trapping can be as good or better at measuring the building natural enemy population, as scouting. Scouting for natural enemies only provides a snapshot in time of the pest and predator populations and may be negatively influenced by weather or sampling technique, which makes it difficult to know if you have an accurate picture of the insect community. Traps have the advantage of collecting data continually over the period between trap checking. Lure baited traps left in the field for a week provide a more consistent measure of the local arthropod community and is more consistent than a person tapping limbs. Catch data was shared with consultants in real time during the study and reviewed retrospectively to see how recommendations and predictions of pest and natural enemy populations matched with catch data. Cooperating crop consultants have been asked to keep detailed notes of psylla and natural enemies 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 compared

crop consultant's management decisions and scouting counts with trap capture for that same period of time.

Weekly psylla counts were sampled by randomly collecting 10 pear shoots from each site and counting the number of eggs, young nymphs, and old nymphs from 5 leaves from each shoot. This method is regularly used by crop consultants to help guide management decisions. The addition of this data will give a clearer image of how psylla populations grew or decreased each week at each site.

We believe that lure baited monitoring will be the new standard for monitoring pear orchards for natural enemies. We have approached private industry (AlphaScents) to develop a commercial lure that can be used by crop consultants.

Results and Discussion

A total of 837 four-part plant volatile lures were manufactured in Hood River for the three trapping seasons. The traps placed at 20 pear orchards in Hood River Co (Fig 1.A.) yielded a total of 5,037 natural enemies in 2021. Of these the most common insects found were green lacewings (1,680), Dereaocoris (1,836), Yellow Jackets (809), and earwigs (232). In 2022 traps placed in the same 20 orchards yielded a total of 5,037 natural enemies. Of these the most common insects found were green lacewings (1,091), Dereaocoris (1,303), Yellow Jackets (1,040), Syrphidae (615), Trechnites (696), and earwigs (274) (Fig. 3 A and B). In 2023 traps placed in the same 20 orchards yielded a total of 4,522 natural enemies. Of these the most common insects found were green lacewings (1,861), Trechnites (1,038), Yellow Jackets (564), Deraeocoris (464), Campylomma (136), and earwigs (107)

In Chelan County, WA 9 traps placed along US route 2 near Cahsmere (Fig 1.B.) that yielded a total of 3,773 natural enemies. Of these the most common insects found were green lacewings (1,112), Trechnites (1,743), and Dereaocoris (462), in 2022 In 2023 these same sites had a total of 3,773 natural enemies. Of these the most common insects found were green lacewings (1,112), Trechnites (1,743), and Dereaocoris (462) (Fig. 3 D and E).

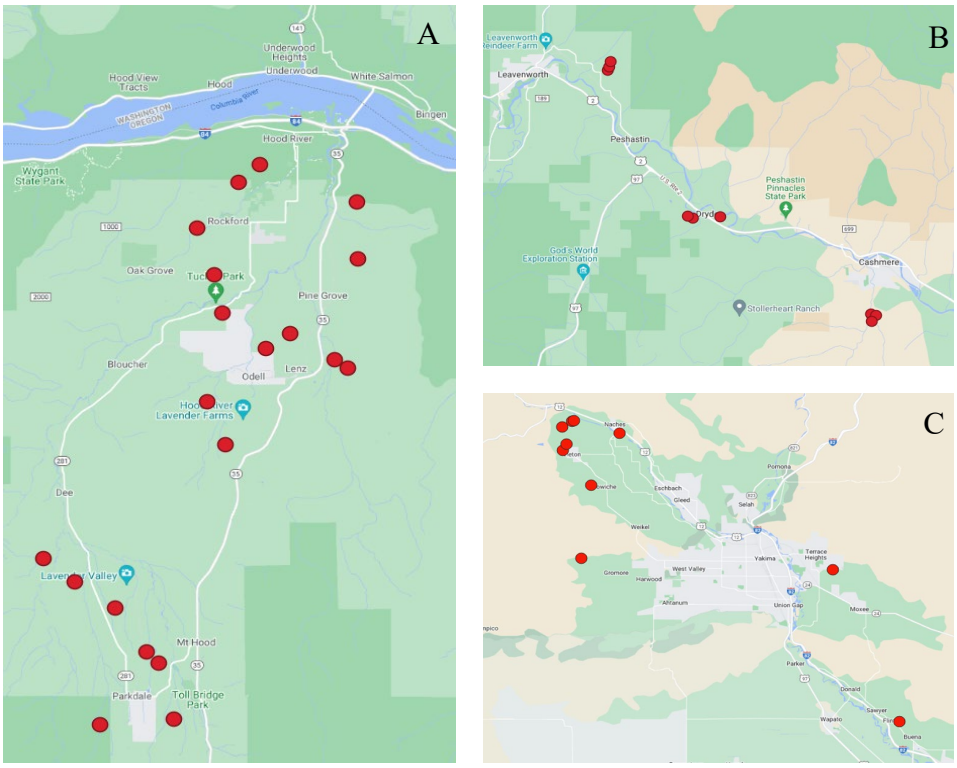
In Yakima County, WA 10 traps placed in pear orchards (Fig 1.C.) yielded a total of 1,602 natural enemies. Of these the most common insects found were green lacewings (994), Dereaocoris (409), Coccinellidae (322), and Yellow Jackets (320) in 2022. In 2023 these same sites had a total of 1,602 natural enemies. Of these the most common insects found were green lacewings (653), Dereaocoris (342), and Trechnites (142)

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

Earlier researchers have suggested that natural enemies need to be present in large numbers early in the season to be effective at rendering biological control against pear psylla. In Orchards identified by crop consultants as "easy" to control with natural enemies, we find large populations

of natural enemies early in the season and at ratios of up to 100:1 (natural enemies to pear psylla). Where populations of natural enemies are not present early in the season or when ratios of natural enemies to pear psylla is not sufficient, we see lack of control. Tracking natural enemies with lure baited sticky cards also indicates where psylla sprays are impacting natural enemies and, in some cases, we can see where insecticide sprays were applied when no psylla were present. However, pesticide applications did not always correlate with reduced natural enemies or psylla control, suggesting that the system is more dynamic with both psylla and natural enemies moving between blocks at a landscape level. The lure-baited trap allowed us to see these trends with less labor and time and with more consistency than the standard limb tapping. Crop consultants reported that this tool improved their management decisions and helped them improve sprays timing.

Researchers have been working on this objective for fifty years. This same question was Larry Gut's Master's degree in 1985, his dissertation sits on my shelf. The last three seasons have been some of the most unusual in memory with snow during bloom, a heat dome in the summer, followed by an unusually wet spring. None of these past years can be considered average so finding significant trends has been challenging. However, we did see is that multi-year drop in Deraeocoris, bald-faced hornets, yellow jackets, and syrphid flies over this three-year period that corresponded with an increase in pear psylla over the same time period. While we still have great variability between sites within each year, this multi-year population trend in several key natural enemies follows the classic predator prey relationship. Despite this variability crop consultants can, for the first time, compare individual sites to area-wide averages to help make decisions. While the number of any one natural enemy has not correlated with control, we are encouraged by the high level of enthusiasm from our crop consultant collaborators, who feel that this new tool saves them considerable time and improves the quality of the data they use to inform management decisions.



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

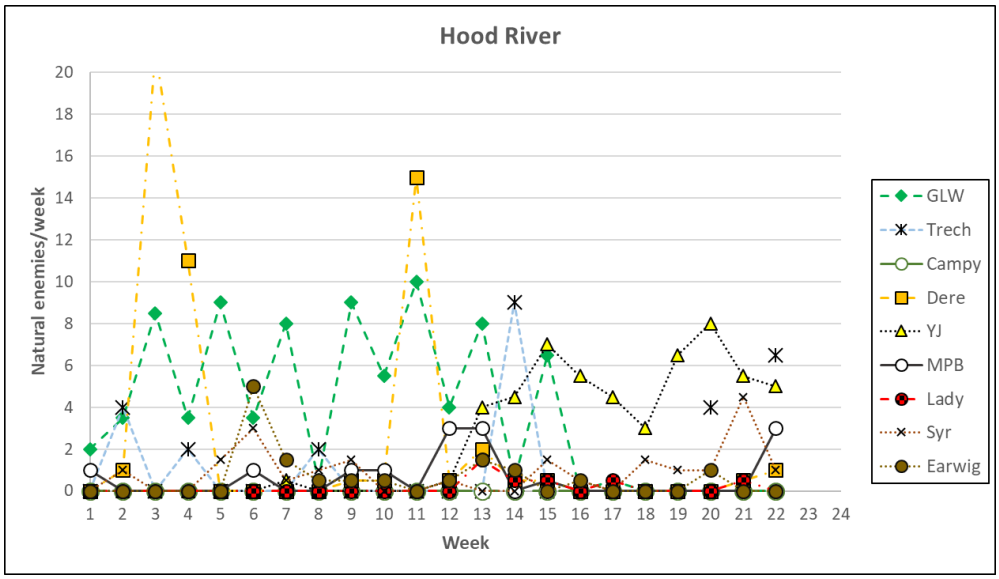


Figure 2. An example of the average natural enemy counts found in the Hood River region, sent out weekly to growers and crop consultants in 2021 - 2023. These area-wide averages were used by crop consultants, in conjunction with local trapping, to make decisions. Although crop consultants could not agree on a magic number of any one insect.

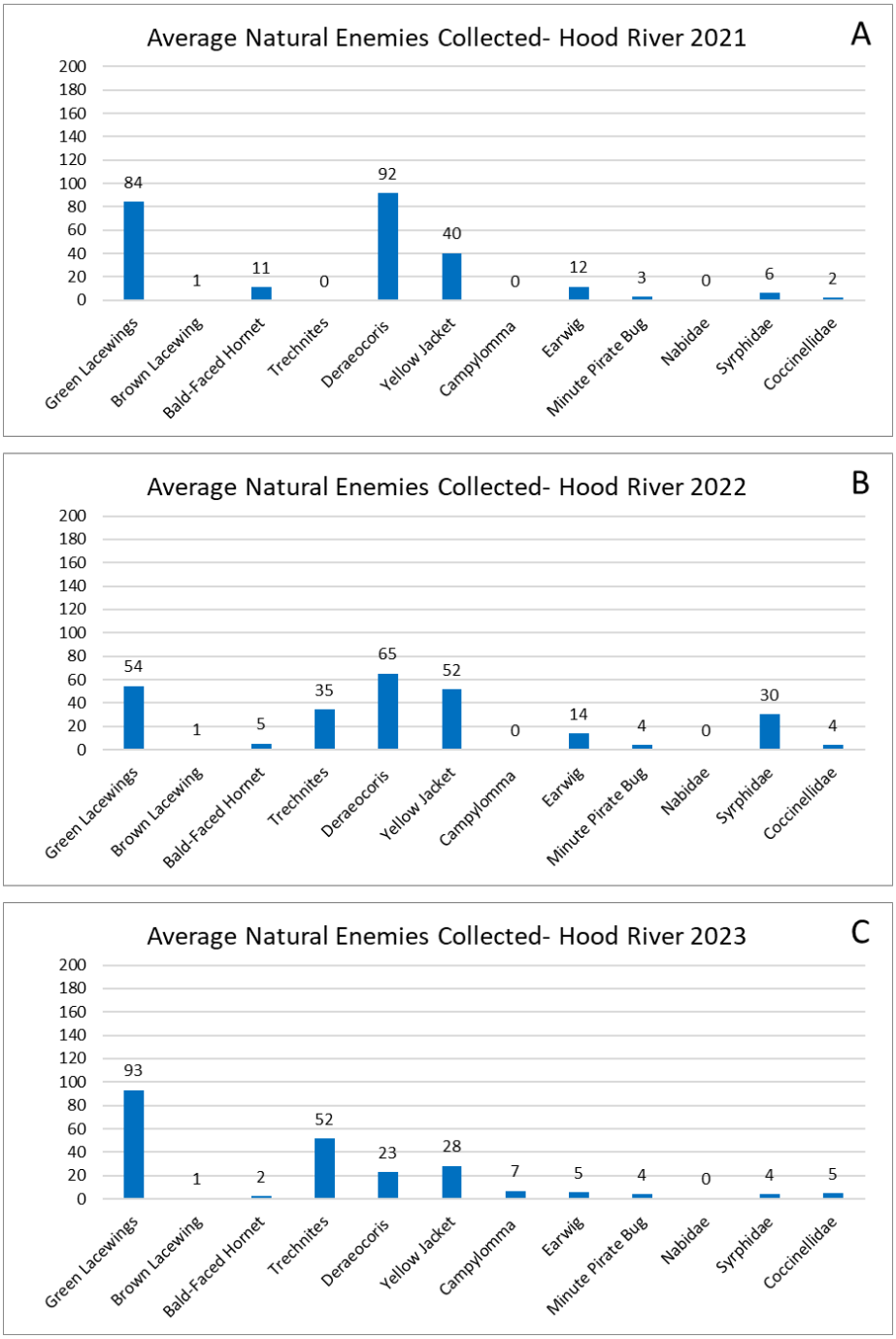


Figure 3 (A-C). Average natural enemy capture in Hood River by year shows a multi-year decreasing trend in deraeocoris that correlates with last high year's pear psylla counts. No other insect has shown a clear correlation.

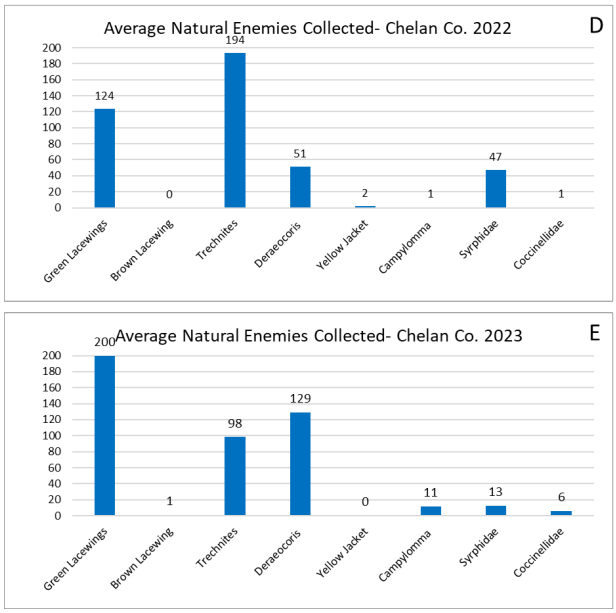


Figure 4 (D &E) Average natural enemies Chelan CO in 2022 (D) and 2023 (E).

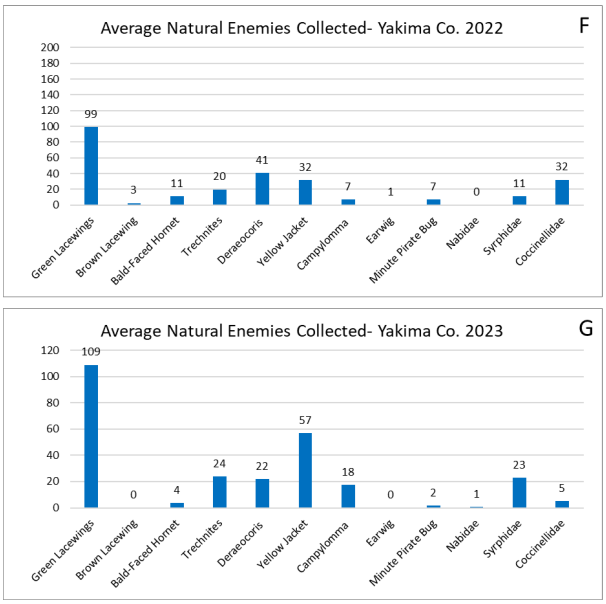


Figure 5 (F & G) Average number of natural enemies collected Yakima Co. in 2022 (F), 2023 (G).

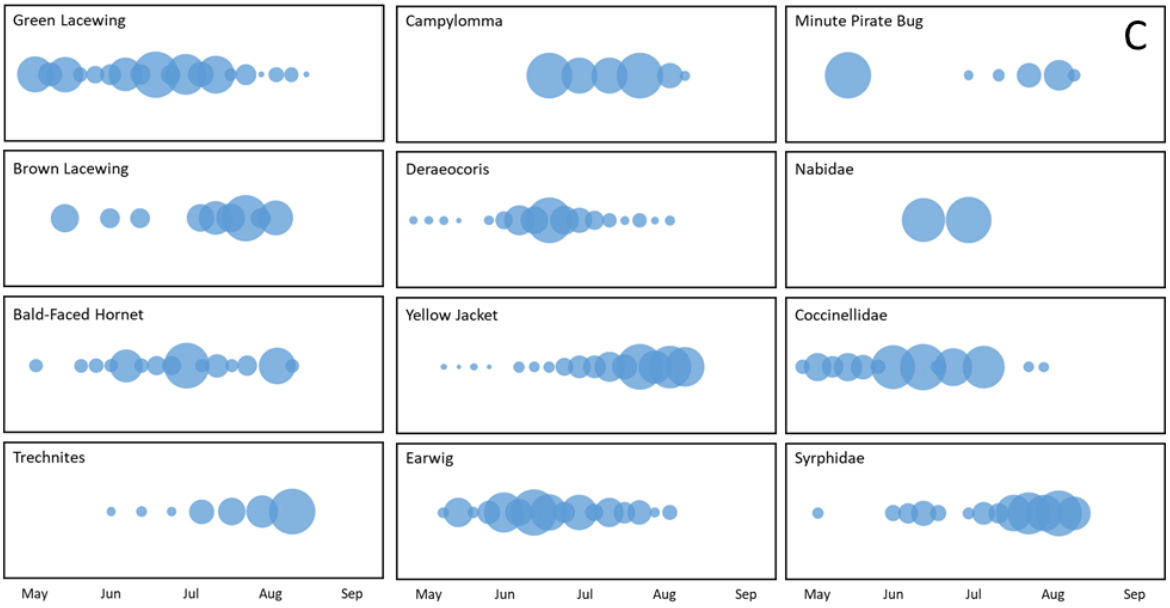


Figure 6. The relative abundance of natural enemies throughout the season in Hood River illustrates the timing of natural enemy occurrence.

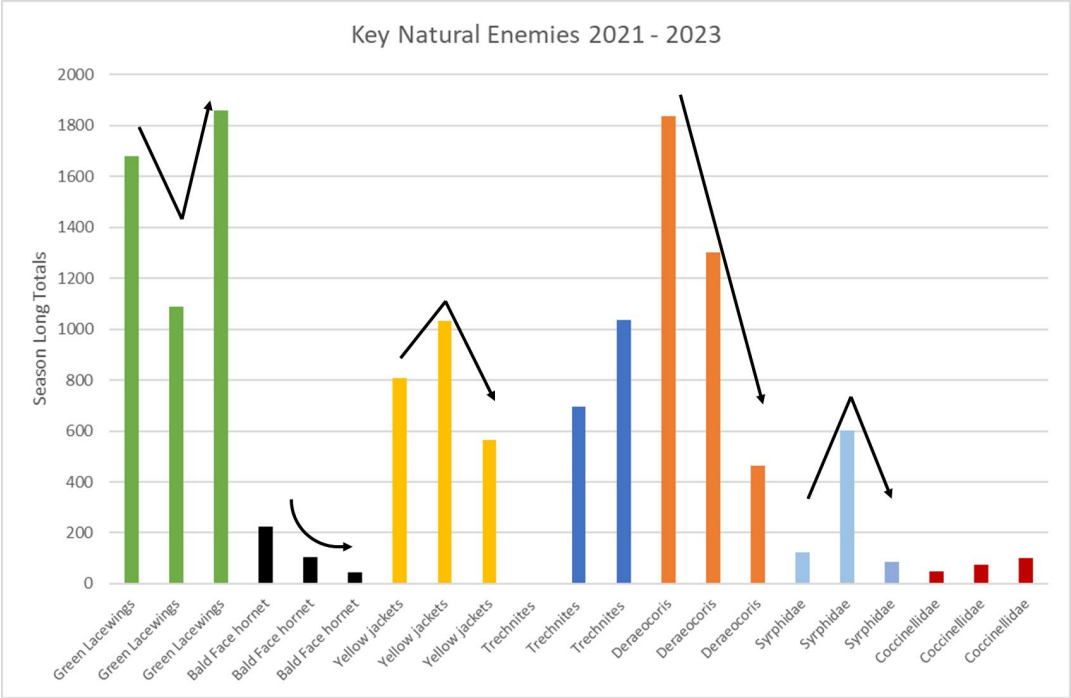


Figure 7. Natural enemies season-long totals over three years. Over the three-year period of this research pear psylla numbers (not shown) for the region increased every year.

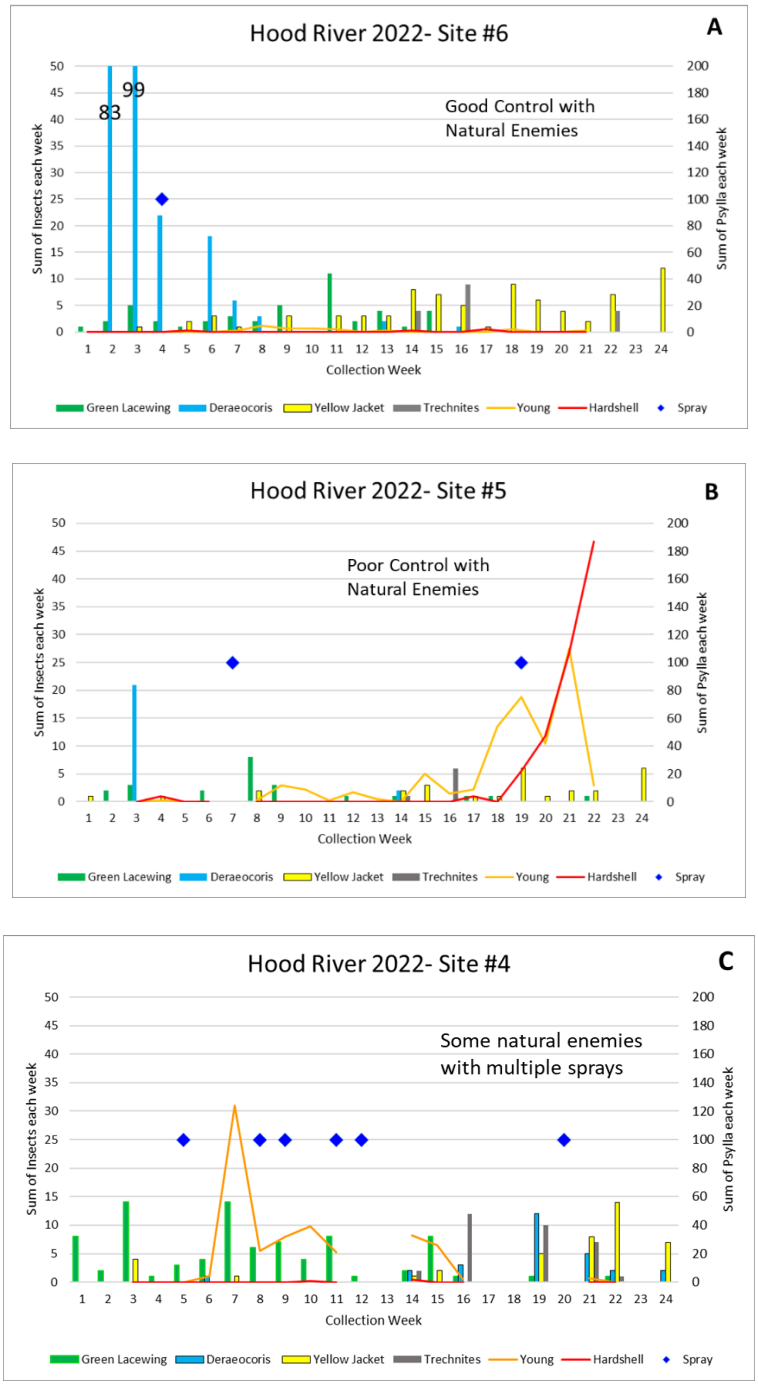


Figure 8. Representative orchards showing season long catch. Counts of natural enemies, young pear psylla nymphs (young), and mature psylla nymphs (hard-shell) at select sites in Hood River Co. Figure A shows ideal natural enemy control and low psylla and minimal pesticide sprays. Figure B shows minimal pesticide sprays but a lack of natural enemy control, and end of season increase in psylla population. And Figure C shows insufficient natural enemy control, multiple pesticide applications, and low (overall) psylla populations.

References

Booth, S.R. (1992). The Potential of endemic natural enemies to suppress pear psylla, Cacopsylla pyricola Forster, in the Hood River Valley, Oregon. Corallis, OR: Oreong State University Dissertation.

Brunner, J. F. (1975) Economic injury level of the pear psylla, Psylla pyricola Forster, and a discrete time model of a pear psylla-predator interaction. Wenatchee, WA: Washington State University.

Burts, E.c. (1983) Effectiveness of a soft-pesticide program on pear pests. Journal of Economic Entomology, 76, 936-941

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

DuPont S. T., C. Strohm, L. Nottingham, Dalila Rendon. (2021). Evaluation of an integrated pest management program for central Washington pear orchards. Biological Control, 152; 1049-9644.

Horton, D. R. (1994) Relationship among Sampling Methods in Density Estimates of Pear Psylla (Homoptera: Psyllidae): Implications of Sex, Reproductive Maturity, and Sampling Location. Annal of the Entomological Society of America, 87, 5;1, 583-591.

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. Amerasekare. (2016). Evaluating plant volatiles for monitoring natural enemies in apple, pear and walnut orchards. Biological Control, 102, 53-56.

Tougeron, K., C. Illtis, F. Renoz, L. Albittar, T. Hance, S. Demeter, G. J. LeGoff. (2021). Ecology and biology of the parasitoid Trechnites insidiosus and its potential for biological control of pear psyllids. Pest Management Science, DOI 10.1002/ps.6517.

Mills, N. J., E. H. Beers, P.W. Shearer, T. R. Unruh, K.G. Amarasekare. (2016) Comparative analysis of pesticide effects on natural enemies in western orchards: A synthesis of laboratory bioassay data. Biological Control, 102, 17-25. http://doi.org/10.1016/j.biocontrol.2015.05.006.

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.

Unruh, T.R., P. Shearer, R. Hilton, J.C. Chiu. (2015). Pesticide resistance in pear psylla and identification of resistance related genes. Continuing report on project PR-14-103. Washington Tree Fruit Research Commission.

Van de Baan, H. & B. Croft. (1990). Factors influencing insecticide resistance in Psylla pyricola (Hymenoptera: Psyllidae) and susceptibility in the predator Deraeocoris brevis (Heteroptera: Miridae). Environmental Entomology, 19, 1223-1228. https://doi.org/10.1093/ee/19.5.1223.

Van de Baan, H.E., B.A. Croft, E.C. Burts. (1990). Resistance to the pyrethroid fenvalerate in pear psylla, Psylla pyricola Foerster (Homoptera: Psyllidae), in the northwestern USA. Crop Protection, 9, 185-189. https://doi.org/10.1016/0261-2194(90) 90161-Y.

Executive Summary

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

Key words: Pear psylla management, natural enemies, new lure-baited trap

Abstract: Pear psylla is the most important pest to control for the fresh-market pear producers. Heavy infestations can create large amounts of honeydew leading to sooty mold and russeting, and in some cases tree decline. Pear psylla develops pesticide resistance quickly, making it challenging for growers to completely suppress populations and thus psylla remains a constant threat. Natural enemies of pear psylla have been shown to provide effective free biocontrol in some growing regions, and carful integrated pest management has been shown to increase the numbers of natural enemies available and reduce psylla populations. Assessing the level of available biocontrol remains a challenge for crop consultants. Sampling currently requires extensive scouting, tapping limbs of two dozen trees across 20 acres. The objective of this research was to build on earlier research by Jones et al. (2016) to develop lure-baited monitoring traps for natural enemies of pear psylla to improve data collection and provide crop consultants with a new tool for measuring biocontrol. To do this we selected four plant volatiles that were shown to be attractive to several key natural enemies and produced lures in our lab. The four-component lure included acidic acid, methylsalicylate, 2-phenylethanol, & phenylacetaldehyde. Lures were paired with a yellow sticky card and placed in 20 orchards in Hood River and Yakima over three seasons, insect data was collected weekly and shared with stakeholders through weekly emails. Lure-baited traps reliably collected data on twelve key natural enemies of pear psylla. Lure-baited traps collected more insect data than limb-tapping and caught several species of adult (flying) insects that are not measured from limb tapping. Time required to collect data was significantly less with lure-baited traps vs. limb tapping, which is key for crop consultants. We saw that blocks with high numbers of natural enemies did see lower psylla pressure on average. Biocontrol was not always available in every block, even when pesticides were withheld. Action thresholds for individual insects are still needed but the most abundant natural enemies were green lacewings and *Deraeocoris brevis*. With the exception of green lacewings and the parasitic wasp *Trechnites insidious,* all natural enemies were at a three-year low while psylla was at a three-year high in 2023. This pattern looks similar to the classic predator-prey relationship where the predators crash the prey population, and then their numbers in turn decline from a lack of resources. If this pattern holds, we should be able to predict “good years” and “bad years” for psylla pressure. The most important natural enemy appears to be *Deraeocoris brevis.* Green lacewings can be found in similarly high numbers, but do not seem to follow the predator-prey relationship. Yellow jackets and hornets are important generalist predators but likely eat as many beneficial insects as psylla, so it is difficult to quantify their net affect. Another confounding factor is that some of these insects are moving across the landscape between blocks and are not restricted to a single orchard. Action thresholds will require more research to measure factors such as landscape level movement, total egg capacity, and number of psylla consumed per insect per day. In conjunction with this research, we added pitfall traps for spiders (because I hired someone passionate about spiders, and wanted to give him a project). In that study we found 7 families of spiders, including one new record for the state. Bi-catch from these pitfall traps included dozens of ground beetles, known to be excellent indicators of overall bio-control. These data suggest that the system is more complex and we are only measuring a small part of the system. While action thresholds have not been established at this time, crop consultants feel they have a better understanding of the amount of biocontrol available in these orchards, and have used this data to make their own management decisions. Additional funding has been applied for with WSARE so that we can continue to work on developing action thresholds.

Project Title: Assessing and supporting effective areawide pear pest management

Report Type: Continuing Project Report

Primary PI: Dr. Robert Orpet
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City/State/Zip: Wenatchee/WA/98801

Cooperators: Louis Nottingham, Molly Sayles (WSU)

Project Duration: 3 Years
Total Project Request for Year 1 Funding: $79,989
Total Project Request for Year 2 Funding: $79,770
Total Project Request for Year 3 Funding: $77,304

Other related/associated funding sources: *Awarded*
Funding Duration: 2022–2024
Amount: $246,524
Agency Name: Washington State Department of Agriculture
Notes: Funded project "Scouts and Thresholds: Implementing Biological Base Pear IPM" helps support Objs. 2 and 3 of this proposal. PI: DuPont

Other related/associated funding sources: *Awarded*
Funding Duration: 2022–2023
Amount: $40,000
Agency Name: WSU BIOAg Program
Notes: Funded project "Growers' perceptions of IPM in pear across regions in the Pacific Northwest complements Obj. 1 of this proposal. PIs: Nottingham, Orpet, Sayles

Other related/associated funding sources: *Awarded*
Funding Duration: 2024
Amount: $22,314
Agency Name: Washington Commission on Integrated Pest Management
Notes: Funds complement Obj. 2 of this proposal. PI: Orpet

Other related/associated funding sources: *Awarded*
Funding Duration: 2024
Amount: $15,000
Agency Name: Western IPM Center – Planning Document Grant
Notes: Funds requested to support Obj. 1 of this proposal. PI: Orpet

Other related/associated funding sources: *Awarded*
Funding Duration: 2024–2026
Amount: $200,000
Agency Name: USDA Crop Protection & Pest Management
Notes: Funds will be requested to support Obj. 2 of this proposal. PIs: DuPont, Orpet, Adams, Schmidt-Jeffris

Budget 1
Primary PI: Robert Orpet
Organization Name: Washington State University
Contract Administrator: Office of Research Support and Administration
Telephone: 509-335-9661
Contract administrator email address: ORSO@wsu.edu
Station Manager/Supervisor: Kimi Lucas (interim)
Station manager/supervisor email address: kimi.lucas@wsu.edu

Item	2023	2024	2025
1 Salaries	$38,250.00	$39,780.00	$41,371.00
Benefits	$11,284.00	$11,735.00	$12,204.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
2 Supplies	$12,100.00		$4,000.00
3 Travel			
4 Plot Fees	$9,055.00	$9,507.00	$9,959.00
Miscellaneous			
Total	$70,689.00	$61,022.00	$67,534.00

Footnotes:
[1]Orpet salary: $7,083 x 12 mo x 45% (x 1.04 for each additional year), benefits at 29.5%. Funds originally budgeted for Orpet in 2025, will instead be applied to salary of co-PI Curtiss at $7,083 x 12 mo x 10%, benefits of 32.2% + a Research Intern at $4,371.44 x 12 mo x 52.75%, benefits of 45.8%
[2]Supplies: Mailing for 2000 stakeholders = $10,000 (yr1); materials for extension workshop meetings ($2,100 yr1, $4,000 yr2) includes room rental, food, color printing)
[4]Plot fees for WSU Sunrise Research Orchard ($2,663 per acre X 3.4 acres in year 1, 5% increase for each additional year

Budget 2
Primary PI: Rebecca Schmidt-Jeffris
Organization Name: USDA-ARS
Contract Administrator: Chuck Myers
Telephone: 510-559-5769
Contract administrator email address: Chuck.Myers@usda.gov
Station Manager/Supervisor: Rodney Cooper
Station Manager/Supervisor email Address: rodney.cooper@usda.gov

Item	2023	2024	2025
Salaries	$3,523.00	$7,222.00	$3,701.00
Benefits	$1,127.00	$2,311.00	$1,184.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$4,650.00	$9,533.00	$4,885.00

Footnotes:
[1]GS-6 Biological Science Technician; $40,262 annual salary, 7 months of work annually at 15% FTE in 2023 and 2025 and 30% FTE in 2024, with 32% fringe rate and COLA for Year 2 and 3 at 2.5%.

Budget 3
Primary PI: Chris Adams
Organization Name: OSU
Contract Administrator: Charlene Wilkinson
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Item	2023	2024	2025
Salaries	$3,523.00	$6,981.00	$3,701.00
Benefits	$1,127.00	$2,234.00	$1,184.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$4,650.00	$9,215.00	$4,885.00

Footnotes:
[1]Technician; $40,262 annual salary, 7 months of work annually at 15% FTE in 2023 and 2025 and 29% FTE in 2024, with 32% fringe rate and COLA for Year 2 and 3 at 2.5%

Objectives

1. Conduct an industry-wide (WA, OR) pear grower and consultant survey of management practices, then compare regions with reference to surveys from 2011, 2000, and 1990.

Deviations: Grower contacts were obtained from California via agricultural commissioners, permitting expansion to California. The survey is scheduled to go out in January, 2025.

2. (a) Expand current evaluation of phenology-based pear psylla IPM that will otherwise end in 2023. This involves researcher-led scouting, extension support, and industry-led implementation; **(b)** Quantify IPM outcomes (spray costs, pest populations, packout reports, profit) relative to conventional orchards in Wenatchee, Yakima, and Hood River regions.

Deviations: none.

3. Quantify correlations between IPM outcomes (from Obj. 3) and landscape (pear monoculture, natural enemy habitat, climate) between and within pear growing regions.

Deviations: none.

Significant Findings

- Pear IPM worked well relative to standard management in Wenatchee, Yakima, and Hood River.
    - Wenatchee Anjou downgrades from pear psylla were 12% in IPM and 10% in conventional (N = 6 pairs of commercial orchards, two-year average for 2022–2023).
    - Yakima: 12% Anjou downgrades in IPM and 25% in conventional (N = 2 pairs of commercial orchards, 2024).
    - Hood River, 2024: 0.5% Anjou downgrades in IPM and 0 in conventional (N = 2 pairs of commercial orchards, 2024).
- Pest management chemical costs (insecticides, miticides, and mating disruption) were less with IPM than standard management.
    - Average materials costs were $998/acre for IPM sites vs. $1,390/acre for conventional sites in Wenatchee, 2023. Spray records are still being obtained for all regions in 2024.
- The conventional Wenatchee and Hood River orchards studied experienced much higher high pear psylla abundance during fall than paired IPM orchards.
- Yakima pear orchards studied all had very low fall abundance.
- Geographic information system analysis indicates Wenatchee and Hood River have similar intensities of pear density, whereas Yakima pear acreage is smaller and spread over a wider area. Work is in progress to correlate local landscape factors within regions to pear pest outcomes.

Methods

Objective 1. A pear pest management survey has been drafted and will be distributed January 2025. Previous surveys on pear pest management were collated and reviewed by Orpet and Goldberger. Then, a new survey was drafted. Similar to the older surveys, the major portion of the new survey is in reporting a sample spray program for a representative pear block and reporting on other IPM practices. Feedback was solicited from the co-PIs and other community members. The new survey was reviewed by Washington State University Institutional Review Board and determined exempt from federal regulations on human subjects research. This is a required step for any research involving data collection from human subjects.

Orpet networked to obtain a list of 1,500 mailing or e-mail addresses of pear growers and consultants in British Columbia, Washington, Oregon, and California. Contact information came from Pear Bureaeu Northwest, other institutions, and county agricultural commissioners. Pear Bureau Northwest

is providing an endorsement letter. The sample size is in line with the older surveys from 1990 (1,086 mailed and 331 returned), 2000 (863 mailed, 129 returned), and 2011 (1001 mailed, 360 returned).

Objective 2. For part 2a, PI Orpet continued to work with nine pear growers and four consultants in Wenatchee Valley to evaluate pear IPM guidelines. The same plots were used as in 2023, except two new conventional plots were added to replace ones that growers switched to IPM during 2024. This resulted in six pairs of commercially managed plots for conventional–IPM comparison in Wenatchee plus two first-year IPM plots (the old conventional plots from 2023), three organic plots, and a pair of plots managed by WSU at Rock Island. The co-PIs Schmidt-Jeffris in Yakima and Adams in Hood River led sampling at four additional orchards each. They attempted to each select two conventional orchards that would use broad-spectrum sprays paired with two orchards following current phenology-based IPM guidelines. Spray records are still being obtained, but the PIs feel that the conventional orchards outside of the Wenatchee region likely use fewer broad-spectrum sprays, and the IPM orchards may follow current guidelines less strictly than in Wenatchee. Results were shared weekly in an online newsletter *Pear Entomology Weekly*. The data was displayed alongside phenologically appropriate pear IPM management guidelines – e.g., selective spray options for dominant pear psylla life stages, tips on tree washing, and reminders on codling mating disruption.

For part 2b, downgrade evaluations were conducted within a week of harvest for Bartlett, Anjou, or both cultivars at each study orchard by inspecting and categorizing 100 fruit at each location. Spray records are still being obtained, so materials costs for insecticides, miticides, and codling moth mating disruption will be calculated for 2024 later using an updated cost list.

Objective 3. Insect monitoring datasets are now collated for analysis, and landscape variables to correlate with are being obtained using a geographic information systems (GIS) approach.

Insect data for landscape analysis is available from two sources: (1) monitoring described in Objective 2 from Wenatchee orchards (2022–2024), and (2) data from 50–81 orchards contributed by co-PI DuPont and colleague Lima via the ‘Scouting Network’ funded through WSDA (2023–2024). Data include weekly counts of pear psylla adults, natural enemies, and nymphs. Orchards from DuPont used a mix of management. Spray records are still being obtained for definition.

Landscape factors are being calculated to correlate with insect data. The hypothesis is that IPM should work better when an orchard is surrounded by more of any of these: natural habitat, IPM pears, or organic pears. We thought that IPM and organic orchards would have greater abundance of pear psylla natural enemies if non-orchard habitat is serving as a refuge and source population of beneficials. Conventional orchards, which largely eliminate pear psylla natural enemies with sprays, were hypothesized to have no effect from non-orchard habitat. To quantify pear orchard landscapes for analysis, maps are being built for GIS analysis. Locations of all organically certified pears were obtained from Washington State Department of Agriculture. Locations of IPM orchards near project sites are being obtained by interviewing growers and consultants on the project. Natural habitat is being designated using layers from USDA Cropscape satellite information.

Results and Discussion

Objective 1. The pear pest management survey has not yet been distributed, so no new results have been collected yet. A review of older surveys was included in last year’s report on this project.

Objective 2. Scouting of orchards undergoing IPM trials showed variation but generally success across 2023–2024 in Wenatchee, Yakima, and Hood River.

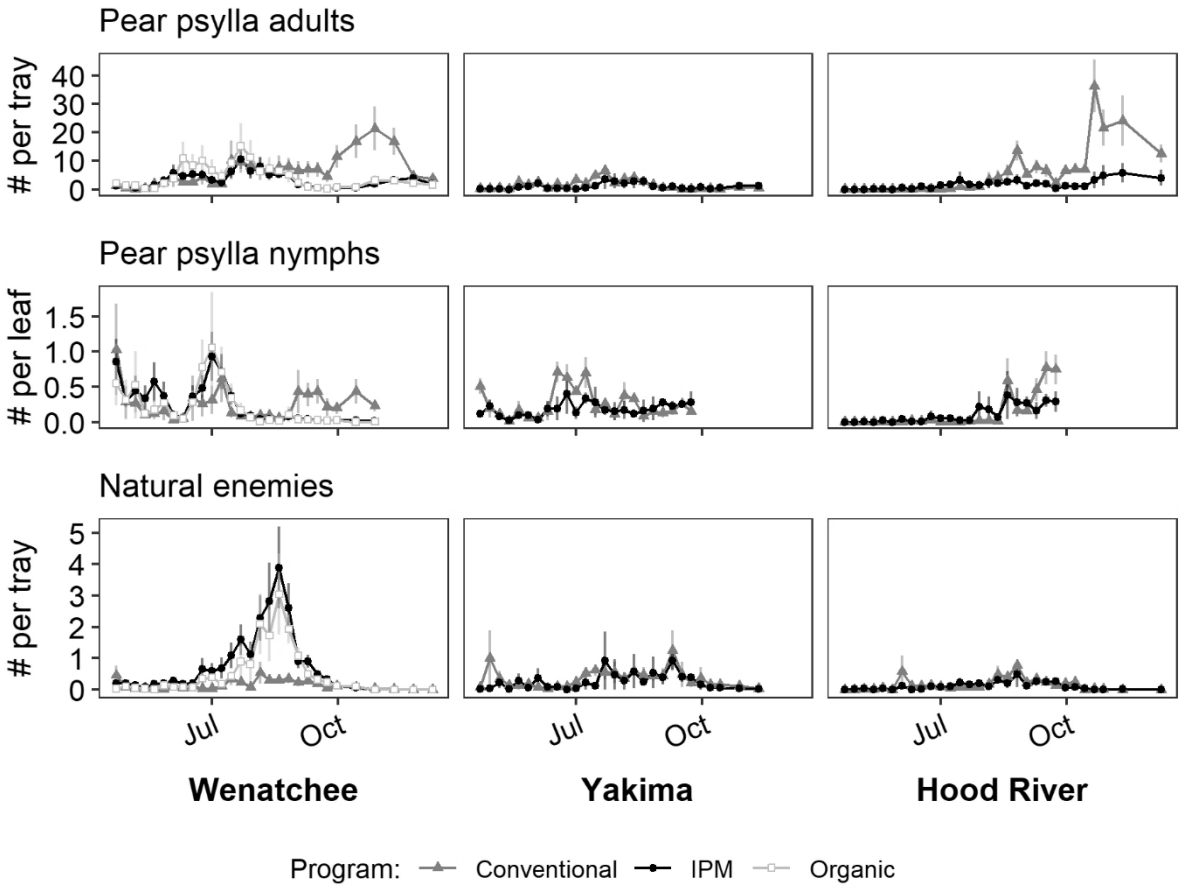

Figure 1. Mean (with standard error) pear psylla adults (per tray), pear psylla eggs (per leaf), pear psylla nymphs (per leaf), and natural enemies (Deraeocoris, Campylomma, Trechnites, *lacewing larvae, lacewing adults, ladybug adults, and ladybug larvae). Wenatchee: N = 7 conventional and 9 IPM orchards. Yakima: N = 2 conventional and 2 IPM. Hood River: N = 2 conventional and 2 IPM.*

Population dynamics of pear psylla life stages and pear psylla natural enemies for Wenatchee, Yakima, and Hood River during 2024 are in Figure 1. Pear psylla nymphs were more abundant in IPM than in conventional orchards before July, but pear psylla became more abundant in conventional orchards for the rest of the season. Biocontrol agents responded to pear psylla in Wenatchee and Yakima, but biocontrol populations were usually low in Hood River. Hood River had very low summer pear psylla populations, so there was nothing for natural enemies to respond to for most of the season. There was a spike in natural enemy abundance in Yakima during September from small black ladybugs (*Stethorus*) that occurred regardless of management program. These were excluded from Figure 1 to avoid obscuring patterns of pear psylla natural enemies since the *Stethorus* greatly expand the y axis scale. These ladybugs eat mites and may have flown in from nearby hops fields. Like in 2023, Wenatchee conventional orchards in 2024 experienced an uptick in pear psylla adults in fall, while IPM and organic orchards had a smaller and delayed uptick at the end of the season. Hood River followed the same pattern in 2024, and Yakima pear psylla adults remained low.

Comparing conventional and IPM pear psylla damage in 2024, IPM orchards were slightly worse Wenatchee (Table 1), variable in Yakima (Table 2), and Hood River had hardly any detectable pear psylla marking (Table 3). When all Wenatchee data are analyzed together, the difference between conventional and IPM pear psylla damage (as in Table 1) is statistically similar (difference of means = 3.1 less % US1 in IPM , two-tailed T = 0.30, df = 14, P = 0.77). If Rock Island and the 1st-year IPM sites are excluded to balance the dataset and give the most Wenatchee-representative comparison, the

difference is marginally significant (difference of means = 6.8 less % US1 in IPM, two-tailed paired T = 2.3, df = 5, P = 0.069). This means that the variation within a management program across locations in pear psylla damage is generally greater than the variation that can be explained by differences in the pest management program. Both management programs had examples of relatively clean and relatively marked up pears. Relatedly, extensive frost damage in 2024 made it difficult to accurately score pear psylla marking, so damage was likely overestimated for both programs in 2024.

Table 1. Wenatchee 2024 %US1-rated pears in orchards of different management from in-field inspection and interpretation of grading within a week of harvest. Preharvest assessments were 100 pears per cultivar per site.

			Pear psylla damage (%)				
Site	**Mgmt.**	**Date**	**US1**	**Fancy**	**3rd**	**Cull**	**Codling moth**
Rock Island	Conv.	10-Sep	97	3	0	0	0
	IPM	10-Sep	94	6	0	0	0
Monitor	Conv.	17-Sep	90	5	5	0	0
	IPM	17-Sep	74	18	8	0	0
Cashmere	Conv.	17-Sep	87	13	0	0	0
	IPM	17-Sep	76	15	9	0	0
	Org.	17-Sep	69	21	9	1	0
Dryden	Conv.	18-Sep	93	7	0	0	0
	IPM	18-Sep	90	7	0	3	0
	Org.	18-Sep	89	6	0	5	0
Peshastin	Conv.	30-Sep	100	0	0	0	0
	IPM (yr1)	26-Sep	96	4	0	0	0
	IPM (yr3)	17-Sep	98	2	0	0	0
	Org.	26-Sep	97	3	0	0	0
HWY 97	Conv.	25-Sep	92	8	0	0	0
	IPM (yr1)	18-Sep	92	7	0	1	0
	IPM (yr3)	18-Sep	95	4	0	1	0
Leavenworth	Conv.	06-Sep	43	29	19	9	0
	IPM	06-Sep	31	38	18	12	0
		CONV	86	9.3	3.4	1.3	0
AVG PEAR PSYLLA US 1:		IPM	82	9	3.9	1.9	0
		ORG	85	10	3.0	2.0	0

Table 2. Yakima 2024 %US1-rated pears in orchards of different management from in-field inspection and interpretation of grading within a week of harvest. Preharvest assessments were 100 pears per cultivar per site.

		Pear psylla damage				
Site	**Date**	**US1**	**Fancy**	**3rd**	**Cull**	**Codling moth**
1 (IPM)	27-Aug	80	14	3	1	0
2 (Conv.)	27-Aug	86	13	1	0	0
3 (IPM)	27-Aug	96	4	0	0	0
4 (Conv.)	27-Aug	64	20	10	6	0

Table 3. Hood River 2024 %US1-rated pears in orchards of different management from in-field inspection and interpretation of grading within a week of harvest. Preharvest assessments were 100 pears per cultivar per site.

	Pear psylla damage			
Site	**US1**	**Fancy**	**Cull**	**Codling moth**
1 (IPM)	99	1	0	0
2 (Conv.)	100	0	0	4
3 (IPM)	100	0	0	0
4 (Conv.)	100	0	0	0

Comparison of Wenatchee results to last year also highlights the variability within management programs. In 2023, there was minimal damage in IPM and organic orchards (99% of Anjous in Wenatchee sites US1) and there was damage in some conventional orchards (95% of Anjous US1).

We know that some growers had codling moth and boxelder bugs problems on edge rows, but except for some codling moth in one Hood River orchard (Table 3) no damage from these pests was detected with our sampling protocol, which does not focus on edge rows.

Once spray records are collected for all locations, management costs for 2024 can be compared.

Extension activities integrated with this research were successful. Of the six growers on the project trialing IPM, two have adopted IPM for all their pear acreage and four increased acreage during 2024. DuPont and Sayles organized monthly Pear Pest Management Discussion group meetings April 11 (26 participants), May 9 (23 participants), June 13 (21 participants). Sayles organized a field day for Pear IPM (July 24) and DuPont organized an August field day which included IPM Aug 6 (approx.100 participants). The *Pear Entomology Weekly* newsletter currently has 230 subscribers.

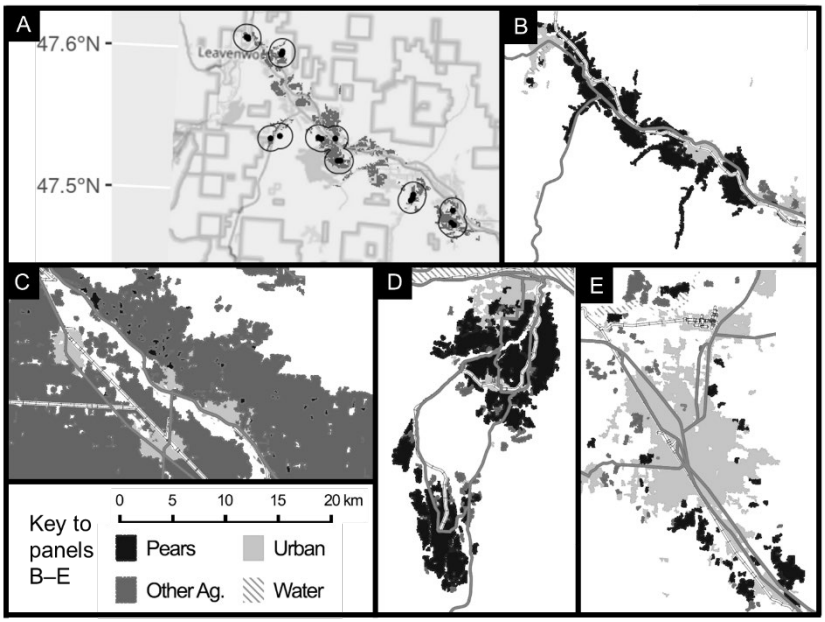

Figure 2. Map of Wenatchee area study locations (back points), partially annotated with nearby pear acreage (in grey) within 1-km radii (circles around points) (A); pear acreage, other agriculture, and urban land from USDA-CropsScape satellite data in Wenatchee (B), Yakima (C), Hood River (D), and Medford (E). Thank you Izzy McDonald for map creation.

Objective 3. Figure 2A shows locations of Wenatchee sites from Table 1. Some of the orchards were in canyons and relatively isolated from other pear acreage in the valley, and others were closer to the main concentration of pears around Highway 2. Work is ongoing to annotate the map and quantify habitats types within radii around each site. Pest data from orchards on co-I DuPont's 'Scouting Network' will further diversify the landscape variation for study.

Maps were also created to compare landscapes of Wenatchee (Figure 2B), Yakima (Figure 2C), Hood River (Figure 2D), and Medford (Figure 2E). The maps indicate that Wenatchee and Hood River have dense pear monocultures. Although there is more diversity of crops in the Hood River region, Wenatchee's pears are more thinly spread across the valley and side canyons (Figure 2B), whereas as Hood River has wider continuous areas of crops (Figure 2D). Yakima and Medford have less pear acreage and it occurs in smaller pear islands separated by other agricultural production or urban development. This may partially explain the patterns seen in Figure 1. Areawide winterform pear psylla movement in fall from areas of high populations seems less likely to influence local pear psylla populations in Yakima, where less pear acreage surrounds a given pear orchard.

Proposal Title: OPTIMIZATION OF HONEYDEW WASHING SYSTEMS IN PEAR ORCHARDS

Report Type: Final Report

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Project Duration: 2-Year
Total Project Request for Year 1 Funding: $ 54,000
Total Project Request for Year 2 Funding: $ 56,000

Other related/associated funding sources: None
WTFRC Collaborative Costs: None

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Item	2023	2024	
Salaries	$19,267.00	$20,038.00	
Benefits	$5,836.00	$6,069.00	
Wages	$24,273.00	$25,244.00	
Benefits	$2,477.00	$2,576.00	
RCA Room Rental			
Shipping			
Supplies	$1,110.00	$1,005.00	
Travel	$250.00	$250.00	
Plot Fees	$787.00	$818.00	
Miscellaneous			
Total	$54,000.00	$56,000.00	$0.00

Footnotes: Salaries: RT Curtiss (@ 0.1 FTE), RJ Orpet (@ 0.1 FTE), L Nottingham (@ 0.02 FTE). Benefits: RT Curtiss (32.9%), RJ Orpet (32.9%), L Nottingham (28.6%). Wages (Time-slip @ $20/hr, 40hr/wk, 30 wk/year). Supplies: Misc. field and lab supplies ($1110 in year 1, $1005 in year 2). Plot Fees: $787 in year 1, $818 in year 2. Travel: Fuel and vehicle costs to reach field sites in WA $250/yr.

ORIGINAL PROJECT OBJECTIVES

1) Monitor seasonal honeydew deposition to understand when washing should be applied
2) Compare honeydew washing efficacy with overhead, air blast, and handgun sprayers, and at seasonal (phenology-based) wash timings
3) Evaluate the impact of surfactants and/or soaps on honeydew removal
4) Provide Extension

SIGNIFICANT FINDINGS

Objective 1 – 2022-2023 key findings

- Honeydew levels and psylla populations were highest in conventional orchards by the end of the season
- Most fruit damage occurs in conventional orchards within 2-3 weeks of harvest
- Natural enemies were highest in IPM and Organic orchards

2023-2034 key findings

- As in the first year, honeydew and psylla populations were higher in conventional orchards at the end of the season
- 2024 was a poor fruit set year, so fruit evaluations were difficult in some locations. However, the same fruit damage patterns were observed in both years of this project.
- Natural enemies also followed similar patterns in year two

Objective 2 – 2022-2023 key findings

- Honeydew levels were highest in plots not receiving washing treatments (controls)
- Plots washed every two weeks had lower honeydew levels than plots treated based on psylla phenology or other treatment timings
- It was difficult to apply enough water to wash trees with the air blast sprayer
- Psylla adults, nymphs, and eggs were not impacted by washing treatments

2023-2034 key findings

- Although 2024 was an extremely poor fruit-set year in our washing plots, and we were not able to evaluate honeydew and damage on fruit, we were still able to measure honeydew on leaves.
- Plots receiving washing treatments had lower honeydew levels
- In 2024 we increased overhead wash times from 8 hours to 24 hours, and more effectively removed honeydew.
- Other than removal of honeydew, pear psylla were not impacted by washing

Objective 3 – 2022-2023 key findings

- The surfactant tested did not improve washing efficacy

2023-2034 key findings

- After two tests with surfactant, washing efficiency was not improved over water alone. This treatment is not currently legal and would be an off-label use of these products. We do not recommend adding surfactant to wash water.

Objective 4 – 2022-2023 key findings

- Information generated from these studies was shared with farmers at 6 events in 2023

2023-2034 key findings

- Information from this study was presented at 4 events in 2024 and will be presented in at least two in 2025 after project conclusion.

METHODS

Objective 1: Monitor seasonal honeydew deposition to understand when washing should be applied

Weekly through both years, at least nine commercial study sites located in the Wenatchee River Valley had pear psylla and natural enemy populations, and honeydew levels monitored. Study sites had one of three management systems: organic-, conventional- and IPM-based pest management. Plots were monitored for natural enemies from March to October using beat trays, rolled cardboard traps, and yellow sticky cards with volatile lures. Pear psylla populations were monitored by beat tray and leaf sampling. Honeydew was monitored on leaves with a method to measure BRIX, and on fruit with visual inspection. Natural enemies that were monitored included adult *Trechnites insidiosus*, adult and immature stages of Aranae (spiders), Anthocoridae (minute pirate bugs), *Campylomma verbasci* (common mullein bugs), Chrysopidae (green lacewings), Coccinellidae (ladybird beetles), *Deraeocoris brevis*, *Forficula auricularia* (Dermaptera, European earwigs), Geocoridae (big-eyed bugs), Hemerobiidae (brown lacewings), and Nabidae (damsel bugs). Pear psylla counted from leaves samples included eggs, young psylla nymphs (instars 1-3), old psylla nymphs (instars 4-5), and mummified (parasitized) psylla nymphs. Mealybugs, European red mites, spider mites, and rust mites were also be counted on glass plates from leaf samples.

Pear psylla honeydew on leaves in commercial sites was measured weekly to understand the correlation with infestation and injury levels. Ten leaves were collected from each of 10 randomly selected trees distributed throughout each plot. Additionally, we monitored individual fruit at the unsprayed WSU-TFREC orchard through the entire season to understand the pattern of damage caused by honeydew. Fruit were evaluated in commercial orchard sites at mid- and end-of-season one week prior to harvest. Fruits were categorized based on USDA pear packing grades for pear psylla marking (USDA, 2007) by the U.S. #1, Washington Fancy, or Cull designation.

Objective 2: Compare honeydew washing efficacy

Honeydew washing methods were compared in small plots at the unsprayed WSU-TFREC and Sunrise pear orchards. In a randomized block designed experiment we compared efficacy of overhead washing systems, tractor with airblast sprayer, tractor with handgun, and unwashed control at managing honeydew. We used honeydew presence on fruit and leaves as a trigger for treatments other than the calendar treatment.

Table 1. Example experimental layout used in washing study.

	WSU-TFREC				**WSU-Sunrise**			
	Block 1		***Block 2***		***Block 3***		***Block 4***	
	Anjou	***Bartlett***	***Anjou***	***Bartlett***	***Anjou***	***Bartlett***	***Anjou***	***Bartlett***
Treatment in plot	Overtree wash	Airblast sprayer	Handgun sprayer	Control	Airblast sprayer	Control	Handgun sprayer	Overtree wash
	Airblast sprayer	Control	Overtree wash	Handgun sprayer	Control	Handgun sprayer	Overtree wash	Airblast sprayer
	Handgun sprayer	Overtree wash	Control	Airblast sprayer	Overtree wash	Airblast sprayer	Control	Handgun sprayer
	Control	Handgun sprayer	Airblast sprayer	Overtree wash	Handgun sprayer	Overtree wash	Airblast sprayer	Control

In 2024, fruit set in the TFREC orchards was particularly poor, and very few fruit were present in the orchard. Due to this unforeseen circumstance, we were unable to evaluate fruit damage as a measure of honeydew washing.

Objective 3: Evaluate surfactants' and/or soaps' impact on honeydew removal

We compared water alone with soaps' ability to remove honeydew from pear trees.

Objective 4: Provide Extension

Project findings will be submitted for peer reviewed publication after project end. In addition, we provided information directly to the industry. Our overall goal was to help farmers produce clean fruit through sustainable pest management programs with reduced inputs that conserve natural enemies.

RESULTS AND DISCUSSION

1) Monitor seasonal honeydew deposition to understand when washing should be applied

In 2023 and 2024 we monitored honeydew deposition in the 17 and 19 commercial pear orchards respectively, that were under focus in Dr. Orpet's project "Assessing and supporting effective areawide pear pest management."

In 2023, seven orchards were under conventional management programs, seven were under integrated management programs, and three were under organic management programs. Generally, across orchards, honeydew levels were higher in IPM orchards than conventional orchards early in the season, but by mid-season, conventional orchards' honeydew load typically increased and exceeded the levels measured in IPM and organic orchards (Fig. 1).

In 2023, fruit damage assessments were conducted at all sites, and we found that pre-harvest damage was lowest in IPM- and organic-managed orchards but was generally low across all sites. We found a correlation between higher leaf BRIX measurements and fruit downgrading (Fig. 3) in commercial orchards. Generally, the correlation between honeydew levels and fruit damage was clearest in conventionally managed orchards, where most damage occurred within two weeks of harvest due to lack of tools and natural enemies.

Natural enemy monitoring efforts followed typical trends observed in other years. Few natural enemies were found in conventionally managed orchards, while natural enemy populations increased through the season in IPM- and organic-managed orchards.

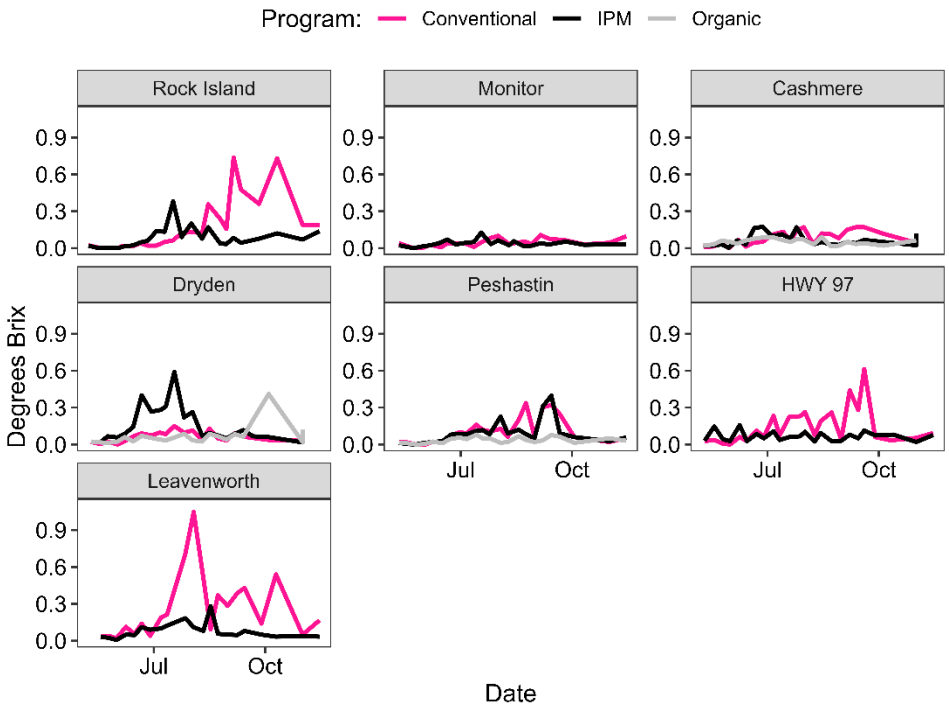

Figure 1. BRIX measurements in Washington commercial pear orchards (n=17) by region in 2023.

Figure 2. Example photograph of an individually tracked fruit at mid-season.

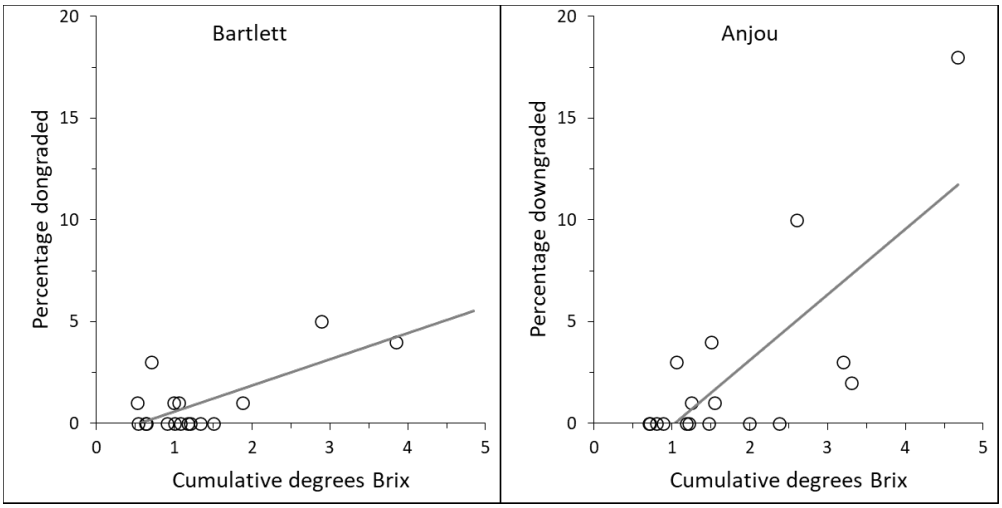

Figure 3. Relationship between the brix measurements and percentage fruit downgraded on Washington commercial pear orchards (n=17) in 2023.

In 2024, seven orchards were under conventional management programs, nine were under integrated management programs, and three were under organic management programs. Honeydew levels were higher in IPM orchards than conventional orchards early in the season, but by mid-season, conventional orchards' honeydew load increased and exceeded the levels measured in IPM and organic orchards (Fig. 4).

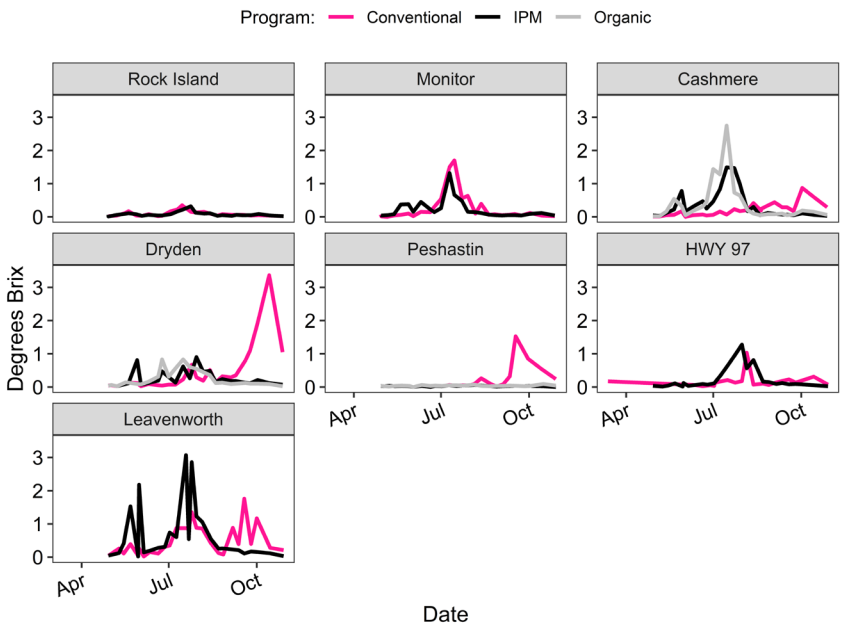

Figure 4. *Honeydew (as degrees Brix) on pear leaves measured weekly at seven locations in at 19 total orchards of variable management in 2024.*

Fruit damage assessments were conducted at all sites in 2024 on Anjou fruits within a week of their commercial harvest. There was a correlation between cumulative BRIX measurements and fruit downgrading (Fig. 5) in commercial orchards.

In addition to honeydew monitoring at the commercial orchards, individual fruits were visually and photographically monitored at the WSU-TFREC and -Sunrise orchards weekly through the season (e.g., Fig 2). Analysis of weekly fruit photographs from 2023 and 2024 is ongoing. However, at the otherwise unmanaged WSU-TFREC orchard, damage was high early in the season when psylla

pressure was high, but due to high rates of predation by yellowjackets, damage reduced through the season both years as pears grew, and damage was diluted across the increased fruit surface area.

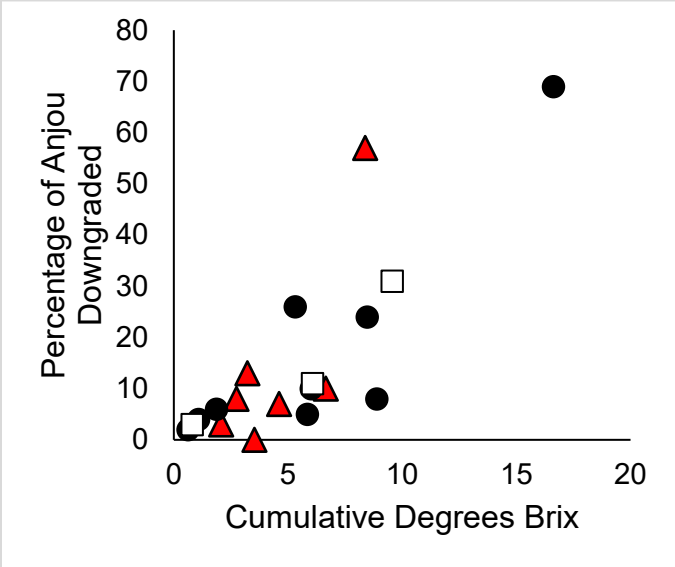

Figure 5. *Cumulative Degrees Brix from the beginning leaves until harvest time and percentage of Anjou fruits rated as downgraded in the field at harvest time at seven conventional (triangles), 9 IPM (circles), and three organic (squares) orchards in 2024.*

2) Compare honeydew washing efficacy with overhead, air blast, and handgun sprayers, and at seasonal wash timings.

Figures 6-8 show that washing had an impact on honeydew levels, but not psylla eggs, nymphs, or adults. However, the key observation from 2023 was that more water was needed to effectively remove honeydew. In 2023, we found it extremely difficult to apply enough water using the air blast sprayer. To spray water to the top of the trees, smaller droplets were required, however, the consequence was faster drive speeds. We attempted to ride the brake and drive slower than 1 mph and make 4 passes per plot, however, we still were not satisfied with the level of washing achieved with the air blast sprayer. These observations are reflected in Fig. 6 that shows poor results using the air blast sprayer compared to other methods. Calendar sprays, every two weeks achieved the lowest overall honeydew levels in among the plots, however, we believed better results could be achieved in 2024 with longer wash times.

In 2024, like much of the Wenatchee Valley, our test orchards had poor fruit set. It was difficult to find any fruit in some plots. Because of this, fruit evaluation was impossible to use as a measure of washing effectiveness. Without being able to measure honeydew accumulation on fruit and use it as a trigger for treatments, and unsatisfactory results in previous years with the airblast sprayer, we eliminated tractor-based treatments in 2024. In a slight modification from 2023, we evaluated overhead washing systems using 24-hour sets in 2024 instead of the 8-hour sets used in 2023. We expected to obtain significantly lower leaf honeydew compared to control plots and 2023 findings. In both years, with 8- and 24-hour sets, we did achieve better honeydew removal in washed plots than unwashed control plots. However, 2024 appeared to have a higher honeydew load on plots and we did not lower honeydew below 2023 levels (Fig. 9). Pear psylla populations were once again unaffected by the washing treatments in 2024 (Fig. 10). Immature pear psylla and eggs on leaves were also unaffected by the washing treatments in 2024 (Fig. 11).

3) Evaluate the impact of surfactants and/or soaps on honeydew removal

Figures 6-8 show that washing with surfactant may have a minor an impact on honeydew levels, but not psylla eggs, nymphs, or adults. However, the surfactant we tested did not achieve better results than water alone. In 2024, due to poor fruit set, lack of significant effect in previous years, and legal concerns with using soaps and surfactants for this purpose, we eliminated the

surfactant treatment. Previous studies reached similar conclusions, and we recommend against using this tactic to wash honeydew from fruit. Water alone, in high enough volume is just as effective, and is legal.

4) Provide Extension

PI Curtiss provided information to pear farmers at one formal extension event in 2023. The event detailed mid-season observations and extensively covered the need for high volumes of water for successful washing. Also in 2023, Co-PI Orpet co-organized (with ST DuPont and MW Sayles) one grower panel and four discussion meetings where stakeholders exchanged knowledge on washing strategies.

In 2024, we shared information on honeydew washing with growers at four extension events. Talks detailed year one findings, and discussions centered around soaps and surfactants ensued. Our recommendation against using these products for this purpose were reiterated.

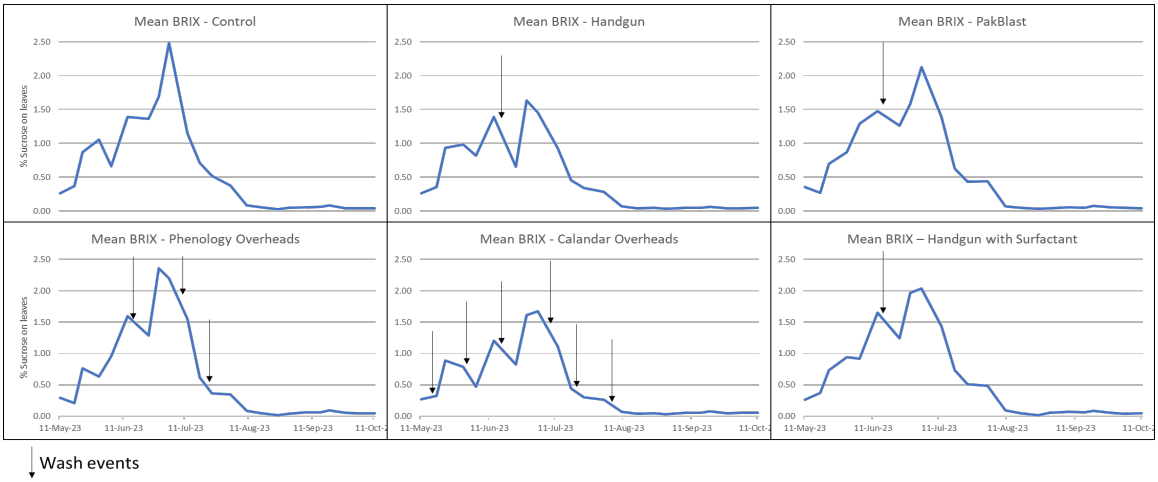

Figure 6. *Season-long BRIX measures (% soluble solids) in plots receiving six washing treatments in 2023. Arrows indicate timings of washings.*

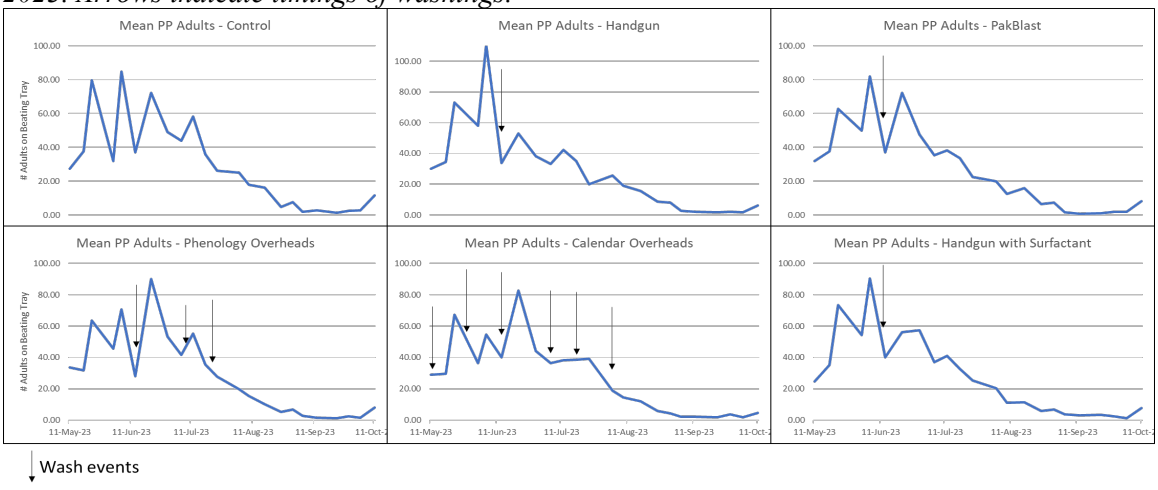

Figure 7. *Beating tray samples (number per tray) for adult pear psylla in plots receiving six washing treatments in 2023.*

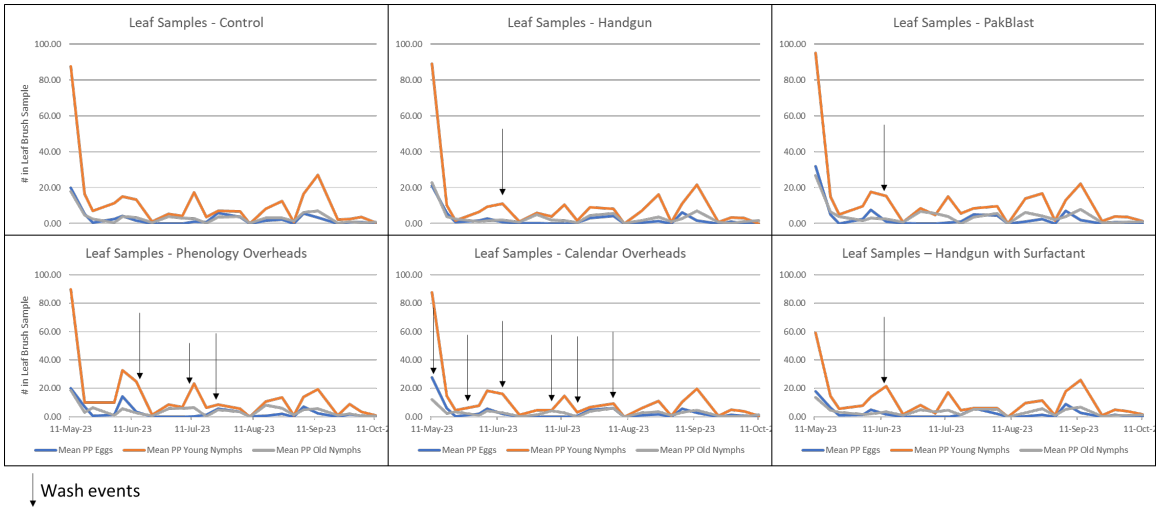

Figure 8. *Leaf brush samples for pear psylla eggs and nymphs (number per sample) in plots receiving six washing treatments in 2023.*

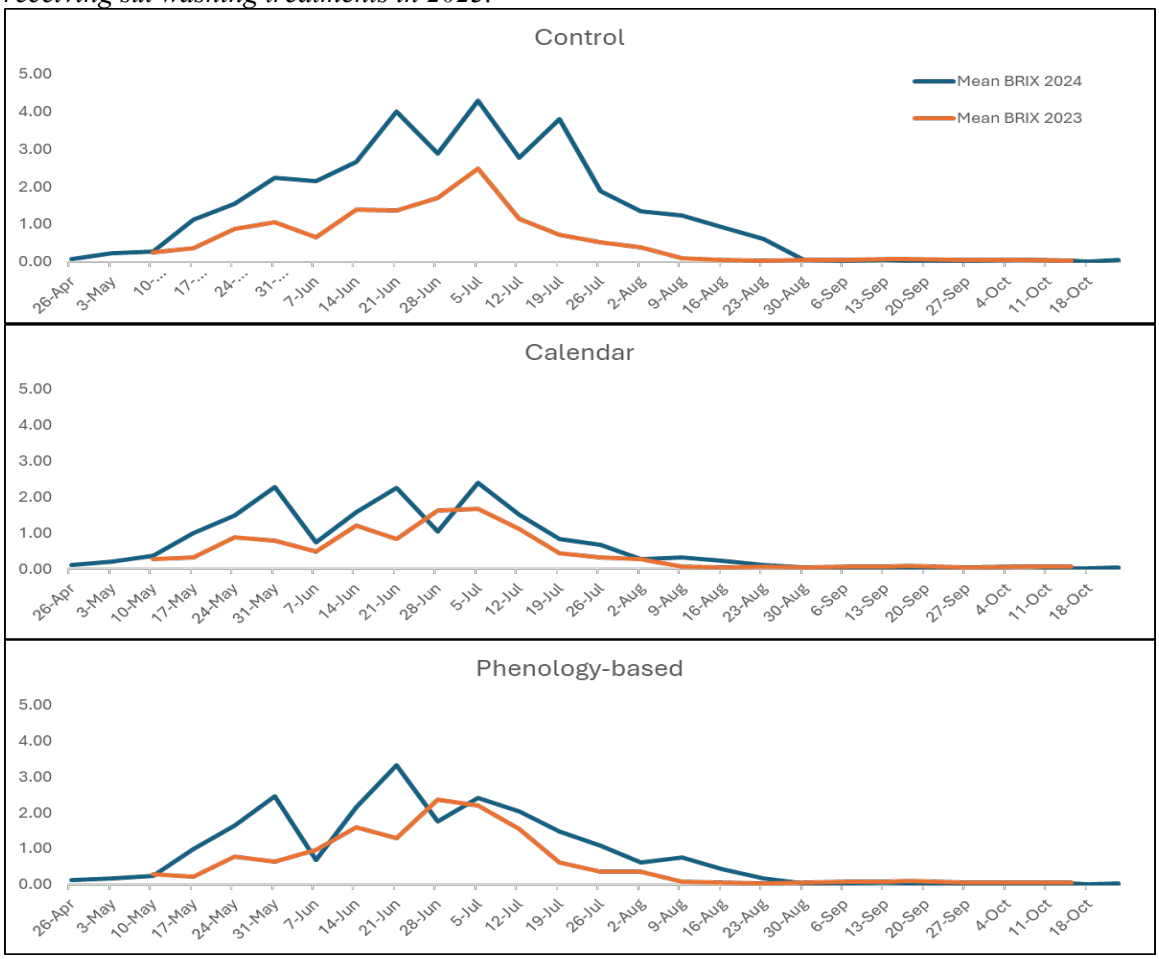

Figure 9. *Season-long leaf BRIX measures (% soluble solids) in plots receiving overhead washing treatments in 2023 and 2024.*

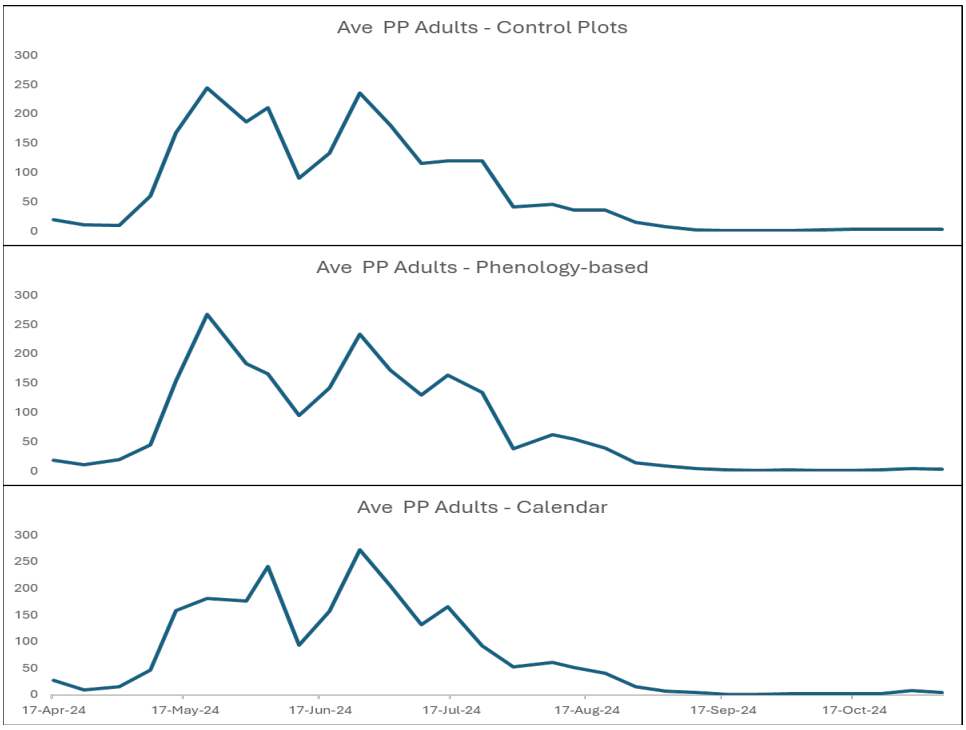

Figure 10. *Beating tray samples (number per tray) for adult pear psylla in plots receiving overhead washing treatments in 2024.*

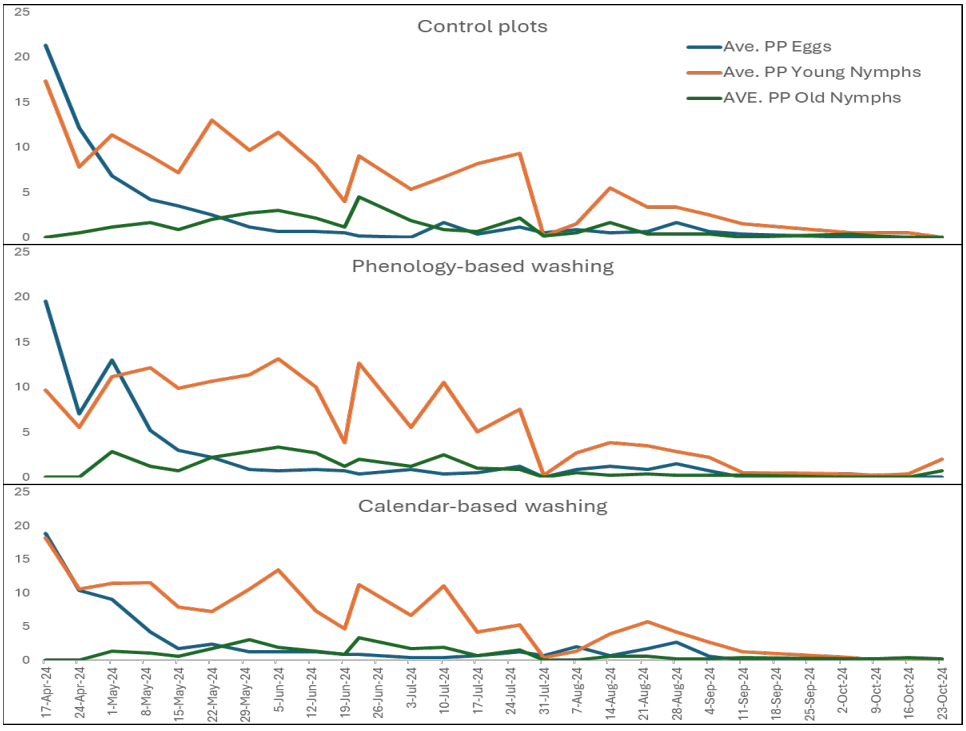

Figure 11. *Leaf brush samples for pear psylla eggs and nymphs (number per sample) in plots receiving six washing treatments in 2024.*

Executive Summary

Project title: Optimization of honeydew washing systems in pear orchards

Key Words: Overhead washing, Pear psylla, *Cacopsylla pyricola*, IPM, natural enemies

Abstract: Pear psylla honeydew is the primary cause of fruit downgrading. Fruit is marked by honeydew and growth of black sooty mold in the highly concentrated sugar water solution. However, honeydew is soluble and may be washed off with water. Water may be applied to trees in the field by several methods, including overhead systems, tractor-driven air blast sprayers, or hose-based systems. This project was designed to understand honeydew deposition patterns in commercial and experimental orchards, to test honeydew washing tactics, and provide the industry with scientifically-based guidance on optimizing washing. From this study we now understand that conventionally-managed orchards typically experience honeydew accumulation later in the season than IPM-and organic-managed orchards. In addition, IPM and organic orchards have lower honeydew pressure before harvest but may be damaged by honeydew earlier in the season. Of the honeydew washing tactics we tested, overhead washing systems used every two weeks resulted in the lowest overall honeydew load on leaves and fruit. Although it is possible to run washing systems timed to pear psylla phenology and remove significant amounts of honeydew, our studies found this method to be inferior to regular washes. We tested washing with a tractor-driven air blast sprayer but found this tactic to be unsatisfactory. We were not able to drive the tractor slowly enough, nor spray enough water into the trees to effectively wash off honeydew. The addition of soaps or surfactants to the tank of an air blast sprayer, however illegal, did not improve washing; we do not recommend this tactic. Although pear psylla themselves were not impacted by washing, neither were the natural enemies, leaving them free to contribute to pear psylla management. Based on our findings, it appears that regular washing using overhead systems is the most effective tactic in high honeydew pressure situations.

Proposal Title: On-farm evaluation of pear psylla and natural enemy thresholds
Report Type: Continuing Report

Primary PI: **Tianna DuPont**
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Cooperators: Cooperators in the 2023 Scouting Network (WSDA SCBG leveraged project) include Scott Cummings, Neil Johnson, Chamberlin Ag.; Kevin Kenoyer, Randy Nelson, Wilbur-Ellis; Chuck Weaver, Jake Carson, GS Long; Chris Strohm, NWW; Dave Burnett, Rollin Smith, Glad Brosi, Evelyn Arnold, Erica Bland, Jon Torrence, Keith and Kathy Archibald, Wayne Reiman, Kevin Carney, Loren Baird, Mel Weythman, Jorge Zavola, Blaine Smith, Shawn Cox. Cooperators in the 2024 Scouting Network (SCBG leveraged project) include Keith Archibald, Randy Arnold, Erica Bland, Loren Baird, Glade Brosi, Dave Burnett, Kevin Carney, Kramer Christensen, Sean Cox, Fisher, Josh Hill, Darrin Kenoyer, Matt McDevitt, Todd McDevitt, Dillon Miller, Michelle Nicholson, Sam Parker, Dave Piper, Wayne Reiman, Rollin Smith, David Thacker, Jorge Zavola, Mike Taylor, Chris Strohm, Northwest Wholesale; Scott Cummings, Neil Johnson, Bruce Kiyokawa, Chamberlin Ag; Randy Nelson, Kevin Kenoyer, Wilbur Ellis; Jake Carson, Chuck Weaver, GS Long

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $19,050
Total Project Request for Year 2 Funding: $19,813
Total Project Request for Year 3 Funding: $20,605

Budget 1
Primary PI: Tianna DuPont
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Item	2024	2025	2026
Salaries	$14,093.00	$14,657.00	$15,243.00
Benefits	$4,957.00	$5,156.00	$5,362.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$19,050.00	$19,813.00	$20,605.00

Footnotes:

Other related/associated funding sources: Awarded
Funding Duration: 2021 – 2024
Amount: $246,524
Agency Name: Washington State Specialty Crop Block Grant
Notes: Scouts and Thresholds: Implementing Biologically Based Pear IPM – Funding developed a scouting network.

Other related/associated funding sources: Awarded
Funding Duration: 2024
Amount: $18,925
Agency Name: Washington State Commission on Integrated Pest Management
Notes: On-farm Evaluation of Pear Psylla and Natural Enemy Thresholds

Other related/associated funding sources: Awarded
Funding Duration: 2025-2026
Amount: $323,134 ($231,982 to this PI)
Agency Name: USDA NIFA Crop Protection and Pest Management
Notes: Implementing New Tools for Pear Integrated Pest Management

Objectives

1) Test the accuracy of natural enemy inaction thresholds: if natural enemy inaction thresholds are met do pear psylla populations remain under control.
2) Test the accuracy of pear psylla economic thresholds: If psylla populations are ≤ ET do pest populations remain below economic injury levels (EIL)?
3) Test the accuracy of pear psylla population models during second and third generation.

Significant Findings

- Natural enemy inaction thresholds (*D. brevis* immatures per greater than 0.2, European earwigs per trap are greater than 2, *C. verbasci* immature per beat tray are greater than 0.1 or European earwigs per beat tray are greater than 0.05) predicted no significant change in third generation young psylla nymph populations.
- One hundred percent of sites where young psylla nymphs were below the economic injury level in both the second and third generation had higher than 95% US1.
- Psylla population prediction models which predict psylla nymph and adult numbers 200 PPDD in the future based off of current numbers, psylla phenology and natural enemy numbers performed better for nymphs and with less accuracy for adults using 2023 data than the using the data used to create the model (2017-2021).

Methods

Data analyzed for the following evaluation of thresholds was collected by leveraging scouting information collected through the 'Scouting Network' funded through state WSDA funds. Data collected included weekly counts of psylla adults and natural enemy adults and immatures per beat tray as well as psylla nymphs per leaf and end of season fruit quality. Orchards included 87 registered scouting blocks encompassing 914 acres where 50 to 81 were scouted each week. Orchards were scouted by 6 scouts, 2 grant funded WSU scouts, 2 from chemical distributor and fruit warehouses and 2 grower scouts.

Scouting: Scouting methods were per (DuPont *et al.*, 2023). Plots were scouted once per week from April to October using beat trays, leaf samples, and earwig traps. Within each plot, thirty samples of canopy dwelling arthropods was collected using the beat tray method. Each beat tray sample (one 'tray') involves holding a 45 by 45 cm white sheet 30 to 45 cm underneath a horizontal branch and striking it three times with a stiff rubber stick to dislodge insects onto the tray, which were then counted. Branches selected for sampling were 1 to 2 m above ground and 1.5 to 4 cm in diameter. The number of pear psylla adults and natural enemies per beat tray were counted. Major natural enemies included in analysis were adult *T. insidiosus*, Aranae (spiders), Anthocoridae (minute pirate bugs), *C. verbasci* (common mullein bugs), Chrysopidae (green lacewings), Coccinellidae (ladybird beetles), *D. brevis, Forficula auricularia* (European earwig), Geocoridae (big-eyed bugs), Hemerobiidae (brown lacewings), and Nabidae (damsel bugs). Additionally, leaf samples were taken to determine densities of pear psylla eggs and nymphs. During the first generation 25 fruit spurs, 1 from each of 25 randomly selected trees was collected for determination of psylla eggs and nymphs (Burts and Retan, 1973; Deronzier and Atger, 1980; Beers *et al.*, 1993; California, 1999; Horton, 1999a; DuPont *et al.*, 2023). During the second generation one hundred leaves were collected from ten randomly selected trees distributed throughout each plot (Burts, 1988; DuPont and Strohm, 2020; DuPont *et al.*, 2021; DuPont *et al.*, 2023). Lower canopy leaves were selected with two leaves from the center (representing hard to spray), 1 leaf from the middle of each of 2 leaders, and one from outer section of the scaffold 1.2 to 1.8 m from the ground. Upper canopy leaves were collected using a telescopic pruner from 2 suckers/shoots/spurs 1 on each side of the tree in areas that are difficult to spray (upper canopy/ back of limbs) (Horton, 1994; DuPont *et al.*, 2021). Collected leaves were kept cool and returned to the lab to be processed using a leaf brusher (Leedom Enterprises). Leaves were run through two motorized brushes which dislodge arthropods onto a revolving glass plate,

creating a composite sample of arthropods which were counted under a stereoscopic microscope (Burts, 1988; Horton, 1999b). Arthropods collected from the leaves included pear psylla eggs, young pear psylla nymphs (instars 1-3), old pear psylla nymphs (instars 4-5), mummified pear psylla nymphs, mealybugs, European red mites, *Panonychus ulmi;* spider mites *T. urticae, T. mcdanieli*; and pear rust mites, *Epitrimerus pyri* (Nalepa).

Fruit: Using funding from this proposal and leveraged WCIPM funds, fruit data was collected. One week prior to harvest, pear fruits were inspected on 20 randomly selected trees at each site and categorized as either U.S. #1 (best), Washington (WA) Fancy (downgraded, but marketable), or Cull (unmarketable) based on USDA pear packing grades for pear psylla marking (USDA 2007). Fruit was evaluated for bartlett (34 orchard plots) and dAnjou (43 orchards).

Statistics: Objective 1: To address the hypothesis that when natural enemies are above the threshold proposed by DuPont et al. 2023, third-generation psylla young nymphs did not significantly increase, we tested the relationship between pear psylla densities and natural enemy densities (for 2024). To determine the impact of natural enemy populations thresholds, a linear regression of the population of young psylla nymphs between the beginning (PPDD = 2,575) and maximum of the third-generation before harvest (PPDD = 4,100) was conducted for 2 categories where natural enemies are above or below identified thresholds. The category "above" is designated where the average *D. brevis* immatures per beat tray for a two week period are greater than 0.2, European earwigs per trap are greater than 2, *C. verbasci* immature per beat tray are greater than 0.1 or European earwigs per beat tray are greater than 0.05. If natural enemies are not above identified thresholds, the category will be designated as "below." The model was tested using a linear mixed model.

Objective 2: To test the association between pear psylla young nymphs ET at both 1300 and 2600 PPDD with population levels that exceed the EIL, we analyzed the data using a McNemar test. Similar to contingency tests McNemar's test can be used to analyze categorical data but when variables are related. McNemar's test was used to show the number of observations that fall into a combination of categories: if psylla levels ≤ ET at 1300 and 2600 PPDD and psylla max for the generation did not exceed the EIL or if psylla levels ≥ ET at 1300 and 2600 PPDD and psylla max for the generation did exceed the EIL. EIL designated for young nymphs was designated as 0.9 young nymphs per leaf second generation corresponding to the low yield low price scenario of 40 bins per acre and $23.30 per box US1 and ET of 0.15 nymphs per leaf at 1300 PPDD for IPM at the associated yield and price. EIL for the third generation young nymphs was designated as 1 young nymphs per leaf second generation corresponding to the low yield low price scenario of 40 bins per acre and $23.30 per box US1 and ET of 0.77 nymphs per leaf at 2600 PPDD for IPM at the associated yield and price. The relationship between EIL and fruit quality was assessed using a McNemar test for when psylla were below the EIL for both second and third generations and the fruit grade for insect damage at less than one week before harvest. A Phi Coefficient was used to assess the strength of associations among the variables. A Fisher exact test was used to test frequency estimations and determine whether there is an association between the economic injury levels and end of season fruit quality.

Objective 3: To test the accuracy of pear psylla population model projections the mean average error was calculated for predicted versus actual populations using 2023 data for second and third generation nymphs and adults where mean average error (MAE) equals the sum of the absolute errors divided by the sample size $MAE = \frac{\sum|predicted - actual|}{number\ of\ data\ points}$. Young nymph generations were defined as second-generation 1015 to 2690 PPDD, and third-generation 2240 to 3600 PPDD. Adults first-generation were 50 to 790 PPDD, second-generation 895 to 2315 PPDD, third-generation 2305 to 3600 PPDD. PPDD per generation were designated from where 10 to 90% cumulative proportion of the generation was predicted to occur (Jones

et al. 2020). Confidence intervals (CI) were determined based on the MAE where $CI\ (90\%) = MAE + \frac{\sigma}{\sqrt{n}}\ x\ 1.64$.

Results and Discussion

Objective 1. Natural enemy inaction threshold validation. The relationship between natural key natural enemies at the beginning of the third generation and the maximum number of psylla nymphs of the third generation was analyzed using linear regression. Where the average number of natural enemies for two weeks early third generation was above the threshold (*D. brevis* immatures per greater than 0.2, European earwigs per trap are greater than 2, *C. verbasci* immature per beat tray are greater than 0.1 or European earwigs per beat tray are greater than 0.05) the number of young psylla nymphs per leaf did not increase (no significant change; p=0.0948) but where the number of natural enemies was below the threshold psylla numbers had a significant increase (0.0253). This analysis supports the published model where when natural enemies are above thresholds psylla populations tend not to increase.

Objective 2. Pear psylla ET and EIL validation. A McNemar's test was used to determine the number of observations that fall into a combination of categories: if psylla levels are ≤ ET at 1300 and 2600 PPDD and did not exceed the EIL or if psylla levels ≥ ET at 1300 and 2600 PPDD and exceed the EIL. A Fisher exact test was used to test frequency estimations and determine whether there is an association between the economic injury levels and end of season fruit quality.

Analysis of 2024 data showed that 14% of the time when psylla young nymphs were below the economic threshold at the beginning of the second generation, maximum numbers of young psylla nymphs in the second generation exceeded the injury level (Table 1). For the third generation, when young psylla nymphs were below the economic threshold at the beginning of the generation, maximum populations exceeded the economic injury level only 7% of the time (Table 2). This relationship was significant for the second and third generation (p<0.0001). As the ET at the beginning of the generation did not predict with 100% accuracy that psylla numbers will not exceed economic injury levels it is recommended that users sequentially assess populations on a weekly basis where managers can consider that if their pest pressure is below thresholds this week a spray can be delayed and reconsidered the following week.

Table 1. McNemar contingency table showing proportion of plots which were above or below the economic threshold (ET) at 1300 PPDD to proportion of plots above or below economic injury level (EIL) during second generation in 2024. N=71. p<0.0001. EIL defined for 40 bins per acre and US1 at $23.3 per box for IPM system.

	ABOVE EIL >0.9 psylla young nypmhs max second generation	**BELOW EIL** < 0.9 psylla young nypmhs max second generation
ABOVE ET >0.15 psylla young nymphs per leaf at 1300 PPDD	**39%**	**8%**
BELOW ET <0.15 psylla young nymphs per leaf at 1300 PPDD	**14%**	**26%**

Table 2. Contingency table showing proportion of plots which were above or below the economic threshold (ET) at 2600 PPDD to proportion of plots above or below EIL at maximum during third generation in 2024. N=71. p<0.0001 Fisher Exact test. EIL defined for 40 bins per acre and US1 at $23.3 per box for IPM system.

	ABOVE EIL >1 psylla young nypmhs max second generation	**BELOW EIL** < 1 psylla young nypmhs max second generation
ABOVE ET >0.77 psylla young nymphs per leaf at 2600 PPDD	**27%**	7%
BELOW ET <0.77 psylla young nymphs per leaf at 2600 PPDD	7%	**60%**

Of 43 orchards evaluated for end of season fruit marking from insect damage on ‘dAnjou fruit, 23 of those sites did not have honeydew washing and could be used to look at the relationship between psylla numbers and final fruit grade (regarding insect damage). One hundred percent of sites where young psylla nymphs were below the EIL in both the second and third generation had higher than 95% US1 (Table 3). In 30% of cases the EIL was exceeded in either the second or third generation and fruit grade still exceeded 95% US1 (Table 3). This may be due to a drenching rain on August 23, 2024 (0.61 in) nicely timed to wash honeydew from fruit and prevent fruit marking. Overall this relationship between combined EIL and insect related fruit downgrades was significant (p<0.001) with a strong relationship (Phi Coefficient 0.84).

Table 3. Number of sites in 2024 with maximum psylla nymphs above or below the EIL for both the second and third generation compared to the percentage of US1. Rating performed on 200 fruit per block in the field using USDA grading standards for insect damage. Sites without honeydew washing included. N=23. Fisher test p<0.0001.

US1	**EILcombined**	
	aboveEIL	**belowEIL**
57	1	0
62	1	0
67	1	0
67	1	0
75	1	0
77	1	0
80	1	0
83	1	0
87	1	0
88	1	0
94.5	1	0
95.0	1	0
96	0	1
97	0	1
97	0	1
98	0	1

98	1	**1**
99	4	**1**
Total	**17**	**6**

Objective 3. The psylla population models are used to create predictions of where psylla numbers may be next week based on current scouting data for both psylla and natural enemies. These numbers can then be compared to economic thresholds to help growers make decisions. For example if current and projected psylla numbers are below the threshold and natural enemies are above the threshold a grower may decide to delay a spray, waiting to re-evaluate the following week.

By comparing psylla numbers projected by the model to the actual number that was recorded the following week we can calculate the mean absolute error (MAE) and compare those values to the MAE of the published model. Using 2023 data the MAE for second generation nymphs was 0.2 and for third generation was 0.22 (Table 4). The MAE for second generation adults was 1.01 and for third generation adults was 1.53. In comparison in the published model the comparison of the modeled population compared to the predicted population one week in the future (time between pest population measured and intervention likely to occur) had a mean absolute error of 0.33 (second-generation psylla young nymphs), 0.98 (third-generation psylla young nymphs), 0.10 (second-generation psylla adults), and 0.15 (third-generation psylla adults).

As such the model for predictions performed better on 2023 data than in the original model for nymphs but not as well for adults. The large MAE for adults in 2023 stem from plots that had high numbers of psylla adults and so the sensitivity may be better for the lower numbers.

Confidence intervals calculated from the MAE are helpful to illustrate how we can use this information. For example if an orchardist receives a report like that in figure 2 where the predicted young psylla nymph number for the upcoming week (200 PPDD in the future) is 0.1 at a 90% confidence interval of 0.27 the individual could be 90% confident that the number would not exceed 0.37 young psylla nymphs per leaf the following week.

Table 4. Mean average error (MAE) for psylla population predictions.

	MAE published model	MAE 2023	90% CI	95% CI
young nymphs gen 2	0.33	0.2	0.25	0.26
young nymphs gen 3	0.98	0.22	0.27	0.28
adults gen 2	0.10	1.0	1.28	1.33
adults gen 3	0.15	1.5	1.89	1.97

Significance to the industry.

Thresholds and scouting add additional tools to the integrated pest management toolbox designed to improve pest management, reduce costs and improve fruit quality. Integrated pest management programs generally include particle films, oils and insect growth regulators early in the season followed by selective insecticides targeted to young nymphs (Figure 1). During the latter part of the second and third generation orchards can use scouting data to determine if psylla numbers are below inaction thresholds (Figure 2) and natural enemy numbers are above thresholds (Figure 3) and then with greater confidence determine if they can delay sprays or reduce the number of materials in an upcoming spray. Testing of both psylla and natural enemy thresholds is critical to determine at what level of confidence these thresholds can be used as a guide.

The use of thresholds and scouting can have significant impacts reducing costs and improving fruit packouts. For example, in the second year of the scouting network pilot funded by WSDA, 2024, there were 87 registered blocks with a total of 914 acres with 50 to 81 blocks scouted each week. Six scouts were involved in the project including 2 WSU grant funded scouts and 1 chemical distributor sponsored, 1 packing house, and 2 grower paid scouts. 45 orchardists and consultants participated in the project. Participants were surveyed through interviews and online surveys. 15 orchardists managing 2095 acres of pears and 5 consultants providing recommendations for 2424 acres of pears participated in the survey. 85% of orchardists and consultant participants surveyed reported making changes to their pest management programs as a result of the project. Growers reported using scouting network information to inform their spray decisions (87%, 859 acres), adjust spray timings based on scouting data (27%, 382 acres), reduce 1 to 3 spray applications (67%, 525 acres), use psylla thresholds (53%, 635 acres), use natural enemy thresholds (53%, 823 acres), reduce the number of broad spectrum insecticides used (60%, 1143 acres), and implement selective programs to maintain biocontrol (soft, IPM) 73%, 969 acres). As a result of these changes orchardists reported the scouting network facilitated the adoption of integrated pest management (IPM) practices (67%), increased natural enemies on

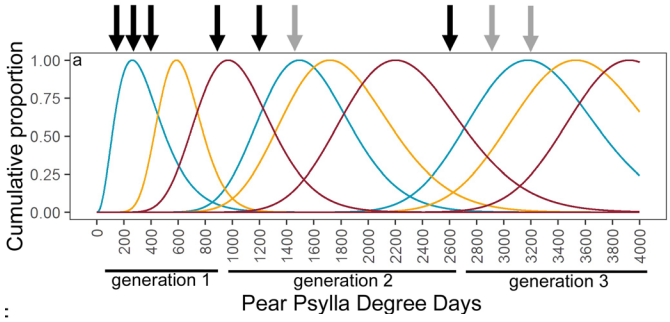

Figure 1. Pear psylla phenology model. Black arrows indicate recommended timings for spray applications. Grey arrows indicate optional timings where sprays should be applied if psylla numbers are high. Figure generated by R. Orpet.

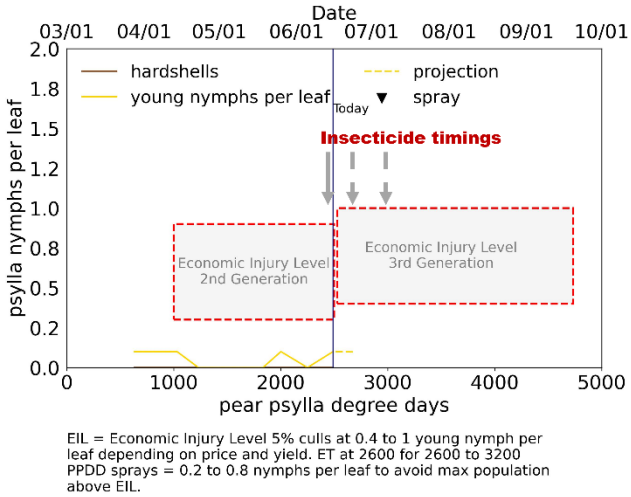

Figure 2. Example of scouting data received and how it can be used to compare to inaction thresholds. The panel shows data for young psylla nymphs per leaf (yellow line) and projected young psylla nymphs per leaf (yellow dashed line) compared to the range of the economic injury level.

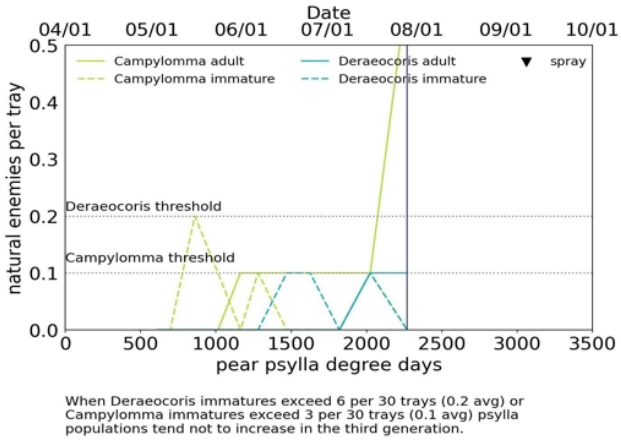

Figure 3. Example scouting data showing *Campylomma* (green line) and *Deraeocoris* adult and immature data (blue lines) compared to thresholds at which psylla populations tend not to increase in the third generation.

the farm (47%), and provided moderate or significant reductions in pesticide expenses (53%). In 2023, after the first year of the Scouting Network 12 growers managing 1,644 acres of pears and 8 consultants providing recommendations for 4,847 acres of pears participated in the survey. 80% of growers and consultants reported making changes to their pest management programs as a result of the project. Growers reported using scouting network information to inform their spray decisions (75%, 654 acres), adjusted spray timing based on scouting data (42%, 271 acres), reduced spray applications and spray costs (33%, 230 acres), increased natural enemy populations and associated biocontrol (50%, 366 acres). As a result of these changes, improved timing and effectiveness of insect pest control applications (58%, 398 acres), fruit quality improved (50%, 350 acres) and IPM programs were utilized (58%, 340 acres). Additionally, consultants reported using scouting network information to inform their spray recommendations (88%, 1486 acres), adjusted spray timing based on scouting data (75%, 1432 acres), reduced late season spray recommendations by 2-3 sprays (50%, 1358 acres), used psylla thresholds for decision making (75%, 1418 acres), and used natural enemy thresholds for decision making (38%, 558 acres).

Extension

Pear Pest Management Discussion group meetings were co-hosted with Molly Sayles and Robert Orpet: April 11 (26 participants), May 9 (23 participants), June 13 (21 participants) 2024. Weekly scout trainings were conducted April-Aug for 6 scouts.

References

Beers, E., Brunner, J., Willett, M., Warner, G., 1993. Orchard pest management. Good Fruit Grower, Yakima, WA.
Burts, E.C., 1988. Damage threshold for pear psylla nymphs (Homoptera: Psyllidae). Journal of economic entomology 81, 599-601.
Burts, E.C., Retan, A.H., 1973. Detection of Pear Psylla. In: Service, C.E. (Ed.). Washington State University, Pullman, WA.
California, U.o., 1999. Integrated Pest Management for Apples and Pears. University of California, Statewide Integrated Pest Management Project, Oakland California.
Deronzier, S., Atger, P., 1980. Dynamics of pear psylla, psylla pyri in the low Rhone valley - winter and spring time. Acta Oecologica-Oecologia Applicata 1, 247-258.
DuPont, S.T., Strohm, C., Kogan, C., Hilton, R., Nottingham, L., Orpet, R., 2023. Pear psylla and natural enemy thresholds for successful integrated pest management in pears. Journal of Economic Entomology.
DuPont, S.T., Strohm, C., Nottingham, L., Rendon, D., 2021. Evaluation of an integrated pest management program for central Washington pear orchards. Biological Control 152.
DuPont, S.T., Strohm, C.J., 2020. Integrated pest management programmes increase natural enemies of pear psylla in Central Washington pear orchards. J. Appl. Entomol. 144, 109-122.
Horton, D.R., 1994. Relationship among sampling methods in density estimates of pear psylla (Homoptera, Psyllidae) - Implications of sex, reproductive maturity and sampling location. Annals of the Entomological Society of America 87, 583-591.
Horton, D.R., 1999a. Monitoring of pear psylla for pest management decisions and research. Integrated Pest Management Reviews 4.
Horton, D.R., 1999b. Monitoring of pear psylla for pest management decisions and research. Integrated Pest Management Reviews 4, 1-20.

Project Title: Program for Control of Shoot Blight and Fire Blight Cankers on Pear

Report Type: Final Project Report

Primary PI: Srdjan G. Acimovic
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Project Duration: 2-Year

Total Project Request for Year 1 Funding: $20,513
Total Project Request for Year 2 Funding: $21,257

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2024
Amount: $286,650
Agency Name: USDA Crop Protection and Pest Management Program
Notes: Title “Creating Next-Gen controls for fire blight cankers, blossom and shoot blight with copper, PGR-s, plant activators and anti-biofilm enzymes”

Other related/associated funding sources: Awarded
Funding Duration: 2023 - 2027
Amount: $5.7 million
Agency Name: USDA Specialty Crop Research Initiative (SCRI) Program
Notes: Title “An all-stage fire blight control: remote sensing, DNA, enzyme and plant activator technologies for cankers, blossom blight and shoot blight”

WTFRC Collaborative Costs: N/A

Budget 1
Primary PI: Srdan G. Acimovic
Organization Name: Virginia Tech
Contract Administrator: Jessi King
Telephone: 540-231-7521
Blacksburg, VA 24061, Campus Mail Code: 0170
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Station Manager/Supervisor: Lesley Mitchell
Station manager/supervisor email address: lesleyg@vt.edu

Item	02/01/2023	02/01/2024
Salaries (Graduate student /GRA/, 29% effort)	$9,165	$9,603
Benefits	$848	$884
Wages	-	-
Benefits	-	-
Equipment	-	-
Supplies	-*	-*
Travel	-	-
Miscellaneous	-	-
Plot Fees	-	-
Total	$10,013	$10,487

Footnotes:
Salaries: Salaries are requested for a Graduate Student (GRA Step 15) @ $2,554/month for 29% effort and benefits rate.
*Laboratory and field supplies will be covered from the above-mentioned USDA CPPM project.

Budget 2
Co PI 2: Achala N. KC
Organization Name: Oregon State University
Contract Administrator: Russ Karow
Telephone: 541-737-4066
Contract administrator email address: russell.karow@oregonstate.edu
Station Manager/Supervisor: Richard Roseberg
Station manager/supervisor email address: richard.roseberg@oregonstate.edu

Item	02/01/2023	02/01/2024
Salaries		
(FRA 1 month)	$3,750	$3,863
Benefits (FRA @ 79.99%)	$3,000	$3,090
Equipment	-	-
Supplies	$2,250	$2,318
Travel	-	-
Miscellaneous	-	-
Plot Fees	$1,500	$1,500
Total	$10,500	$10,770

Footnotes:
Salaries: Salaries are requested for a Graduate Student (FRA) @ $45,000/year for 1 month, and 80% benefit rate.
Supplies: Funding is requested for materials to collect and process samples, plates, and media to culture *E. amylovora*, labels and field supplies.
Plot Fees: Funding is requested for SOREC research plot fees for trials @ $3,000 per acre. We estimated that approximately ½ acre worth of trees and fruits will be used for this trial.
Funding request for year 2 includes additional 3% inflation.
[5] Funding request for year 2 includes 3% inflation

1. VIRGINIA

Objectives

With the goal to reduce or prevent shoot blight severity and prevent canker development on pear wood, our objectives are to:

(1) Determine Regalia efficacy on mature pear trees against shoot blight and canker development (OR) and compare it to the same effect on young trees (VA, OR),

(2) Determine if 153.6 fl oz/acre of Regalia applied in less numbers of treatments (one, two) can achieve the same effect on fire blight (VA, OR),

(3) Compare fruit russeting at harvest after Regalia, Actigard and Apogee and antibiotic spray programs (OR).

(4) Using the price lists from local pesticide distributors in WA, OR and VA, compare the cost of Regalia programs, select, and recommend the most effective and cost-beneficial program for pears that do not cause fruit russeting.

Deviations from the original objectives: fruit did not develop in sufficient numbers due to 3-hour frost during pear bloom in Winchester, VA (4/9/2023), so the russeting incidence was not rated. In Winchester, not enough pear fruit developed in 2024 in per tree basis to be able to fulfill statistical requirements, so the fruit russeting incidence was not rated in 2024.

Significant Findings

In 2023:

A) In Winchester, when compared to the untreated control with 47% shoot blight severity:

- The spray programs #2 (three spray applications of Regalia 32 fl oz/A) and #4 (one spray application of Regalia 153.6 fl oz/A) provided 100% and 78.3% shoot blight severity control, respectively.
- The spray program #7 (antibiotics with surfactant) and #8 (Apogee 6 oz/100 Gal + Cueva 120 fl oz) provided 100% and 89.4% shoot blight severity control, respectively.
- To our surprise spray program #1 (five spray applications of Regalia 30.72 fl oz/A) which is inconsistent with our previous 2-year results.

B) In Winchester, when compared to the untreated control with 44% canker incidence:

- The spray programs #2 (three spray applications of Regalia 32 fl oz/A) and #4 (one spray application of Regalia 153.6 fl oz/A) provided 100% and 76.8% canker incidence control, respectively.
- The spray program #7 (antibiotics with surfactant) and #8 (two spray applications of Apogee 6 oz/100 Gal + Cueva 120 fl) oz provided 100% and 88.6% canker incidence control, respectively.
- Five spray applications of Regalia 30.72 fl oz/A failed to control canker incidence.

In 2024:

A) In Winchester, when compared to untreated control with 47.1% shoot blight severity:

- The spray program #7 (two applications of FireWall+FireLine+Regulaid) at BL, then ~24 h after shoot inoculation, provided 100% control of shoot blight, canker incidence and canker length. This is consistent with the 2023 results of the same spray program.
- In comparison to 2023 results, the only other spray program that was numerically consistent in comparison to 2024 results was #2 (three spray applications of Regalia 32 fl oz/A) which gave 67.1% control of shoot blight severity, albeit not significantly different from #9 Untreated Control (Fig. 6). This consistency was also seen in canker incidence and length reduction (Fig. 7, 8)
- Surprisingly but unfortunately, the spray programs #4 (one spray application of Regalia 153.6 fl oz/A) and #8 (two spray applications of Apogee 6 oz/100 Gal + Cueva 120 fl), which were effective in 2023, failed to provide even numerical fire blight disease reduction in 2024.
- Finally, spray program #1 (five spray applications of Regalia 30.72 fl oz/A) was the closest to the success in fire blight control of the spray program #7 (two applications of FireWall + FireLine + Regulaid) in 2024, which is glaringly inconsistent with the 2023 results.

Methods

Cultivar. 7-yr-old (2023) and 8-year-old (2024) Bartlett trees, planted at 10 ft between trees and 16 ft between rows. Trees were assigned in a completely randomized design.

Spray equipment in 2023 and 2024. Spray programs were spray-applied to 4 trees for each spray program (4 replicates per treatment). Spray was applied dilute at 100 gal/A to drip using a tractor-carried sprayer using a brass 'Friend' handgun connected to Pak-Blast 100-gal sprayer, with diaphragm pump pressure at 250 PSI (Rear's Manufacturing, Coburg, OR) at 11.7 gal/min output to secure good spray coverage.

Spray dates. Due to the uneven onset of growth stages in young trees of the pear block in Winchester, VA, the spray application dates for each spray program in 2023 were:

#	Number of spray applications, materials, and amount	Applied at pear growth stage (spray timing)	Dates of applications
1	5 X Regalia 30.72 fl oz/A	BB, GC, WB, PF, FS	3/26, 4/2, 4/5, 4/13, 4/19
2	3 X Regalia 32 fl oz/A (lower label rate)	WB, PF, FS	3/26, 4/9, 4/13
3	2 X Regalia 76.8 fl oz/A	PF, FS	4/13, 4/19
4	1 X Regalia 153.6 fl oz/A	FS	4/19
5	2 X Regalia 76.8 fl oz/A + Apogee 10 oz/100 gals	PF, FS	4/13, 4/19
6	**2 X** Apogee 10 oz/100 gal*	PF, FS	4/13, 4/19
7	**2 X** Agri-Mycin 16 oz/A + FireLine 16 oz/A + Regulaid 32 fl oz/100 gals	BL, 24 h before shoot inoculation	4/13, 4/23
8	**2 X** Apogee 6 oz/100 Gal* + Cueva 120 fl oz (2 oz metallic copper/A)	1 to 3-inch new shoot growth, 14 days after	4/13, 4/23
9	Untreated control	-	-

Spray dates. For each spray program in 2024 were:

#	Number of spray applications, materials, and amount	Applied at pear growth stage (spray timing)	Dates of applications
1	5 X Regalia 30.72 fl oz/A	BB, GC, WB, PF, FS	3/7, 3/14, 3/19, 4/5, 4/16
2	3 X Regalia 32 fl oz/A (lower label rate)	WB, PF, FS	3/29, 4/16, 4/22
3	2 X Regalia 76.8 fl oz/A	PF, FS	4/16, 4/22
4	1 X Regalia 153.6 fl oz/A	FS	4/22
5	2 X Regalia 76.8 fl oz/A + Apogee 10 oz/100 gals	PF, FS	4/16, 4/22
6	**2 X** Apogee 10 oz/100 gal*	PF, FS	4/16, 4/22
7	**2 X** Agri-Mycin 16 oz/A + FireLine 16 oz/A + Regulaid 32 fl oz/100 gals	BL, 24 h before shoot inoculation	4/10, 4/29
8	**2 X** Apogee 6 oz/100 Gal* + Cueva 120 fl oz (2 oz metallic copper/A)	1 to 3-inch new shoot growth, 14 days after	4/22, 5/6
9	Untreated control	-	-

Maintenance sprays. 2023: Thiram 4/19/2023 and 5/5/2023 as deer repellent. **2024:** none.

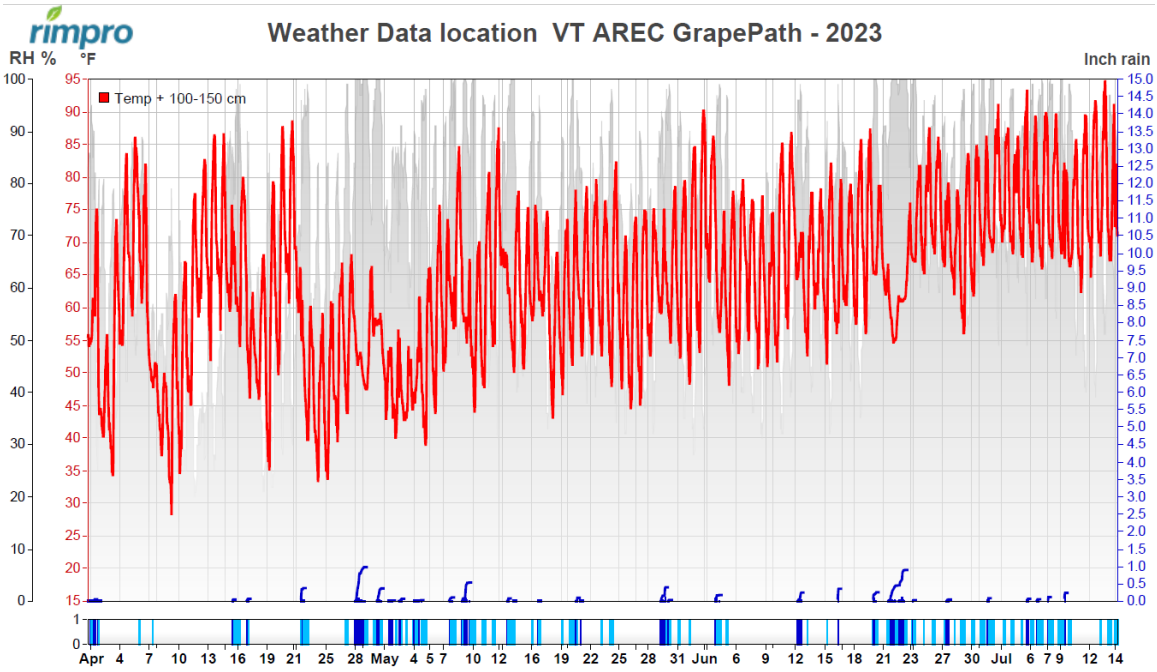


Figure 1. Weather conditions in 2023 during the fire blight experiment at Winchester, VA. Source: RIMpro B.V., Netherlands via NEWA, Cornell University, NY, U.S.A.

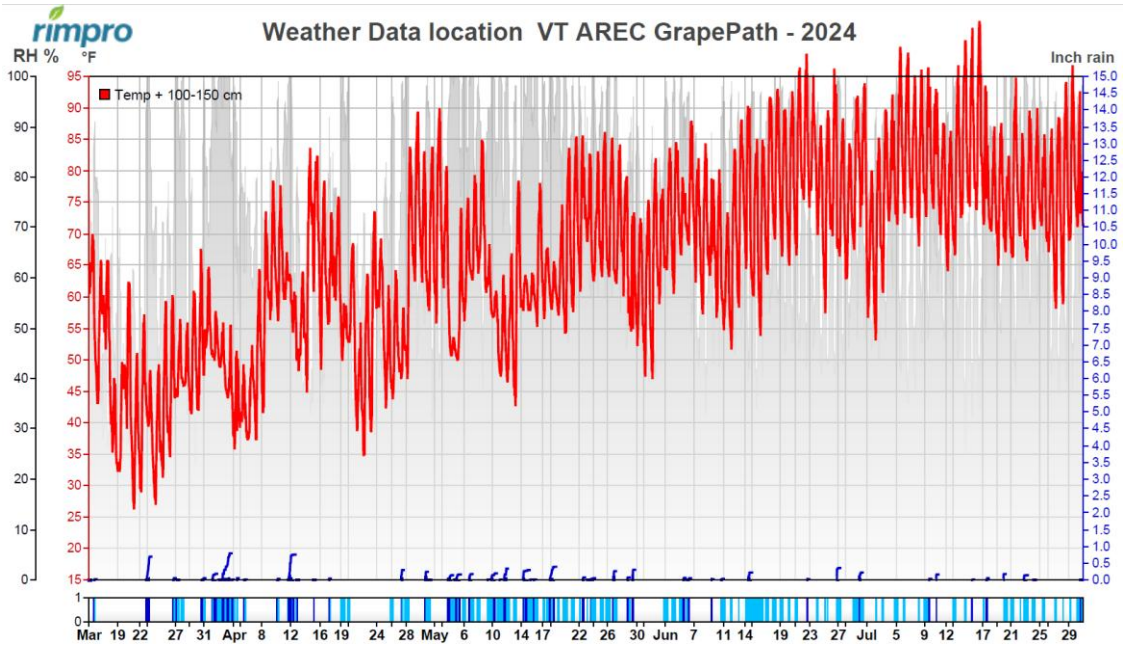


Figure 2. Weather conditions in 2024 during the fire blight experiment at Winchester, VA. Source: RIMpro B.V., Netherlands via NEWA, Cornell University, NY, U.S.A.

2023 Inoculation and disease rating. A 10 'Bartlett' shoots per tree were inoculated on **4/25/2023**. Bloom was lasting from 4/4/2023 to 4/11/2023. We used *E. amylovora* suspension of strain 110 (2 x 10^8 CFU/ml). We inoculated shoots by making a slanted sleeve incision 1 to 2 inches below the shoot tip by cutting into the soft stem tissue with a sterile scalpel. We delivered 40 microliters of *E. amylovora* cell suspension by a micropipette into the sleeve incision. For each inoculated shoot, we calculated shoot blight severity percent by multiplying the ratio of necrotic shoot length i.e. fire blight lesion length (cm) to the total shoot length (cm) by 100. We repeatedly measured the shoot blight severity, canker incidence, and canker length on the same shoots on 23 May, 23 June, and 23 July 2023 and conducted repeated measures statistical analysis accounting for time as the factor (Figures 3 – 5) Mean shoot blight severity percent, mean percent of initiated cankers on perennial pear wood (canker incidence), and mean canker length on wood per each replicate tree was calculated from 10 shoot replicates. Mean shoot blight severity, mean canker incidence, and mean canker length on perennial apple wood for each program was calculated from the four replicate tree means (Figures 3 – 5).

2024 Inoculation and disease rating. A 10 'Bartlett' shoots per tree were inoculated on **4/30/2024**. We used a stem sleeve cut inoculation method on 30 April 2024 by delivering 40 µL of *E. amylovora* (Ea110) distilled water suspension per shoot adjusted to 2 x 10^8 CFU using a micropipette. A total of 10 selected and labeled shoots per apple tree in each treatment were inoculated with E. amylovora. For each inoculated shoot, we calculated shoot blight severity percent by multiplying the ratio of necrotic shoot length i.e. fire blight lesion length (cm) to the total shoot length (cm) by 100. We repeatedly measured the shoot blight severity, canker incidence, and canker length on the same shoots on 24 May, 24 June, and 24 July 2024 (Figs 6, 7, 8). Shoot blight severity is shown in Fig. 6. Canker incidence is shown in Fig. 7 and canker length on perennial wood originating from blight lesion expansion from the inoculated shoots in Fig. 8. Mean shoot blight severity percent and mean percent of initiated cankers on perennial apple wood for each replicate tree (canker incidence) was calculated from 10 shoot replicates. Mean shoot blight severity and the mean percent of initiated cankers on perennial apple wood for each program was calculated from the four tree replicate means.

Problems or limitations that were encountered in 2023 (Virginia). Fruit did not develop in sufficient numbers due to 3-hour frost during bloom (4/9/2023) in Winchester, VA, so the russeting incidence was not rated. **Types and timing of anticipated results.** We plan to repeat the same trial in Winchester in 2024.

Problems or limitations that were encountered in 2024 (Virginia). Limited fruit which developed in 2024 on young pear trees was not sufficient in number in per tree basis to be able to fulfill statistical requirements, so the fruit russeting incidence was not rated.

Results and Discussion

VIRGINIA 2023:

Summary graphics.

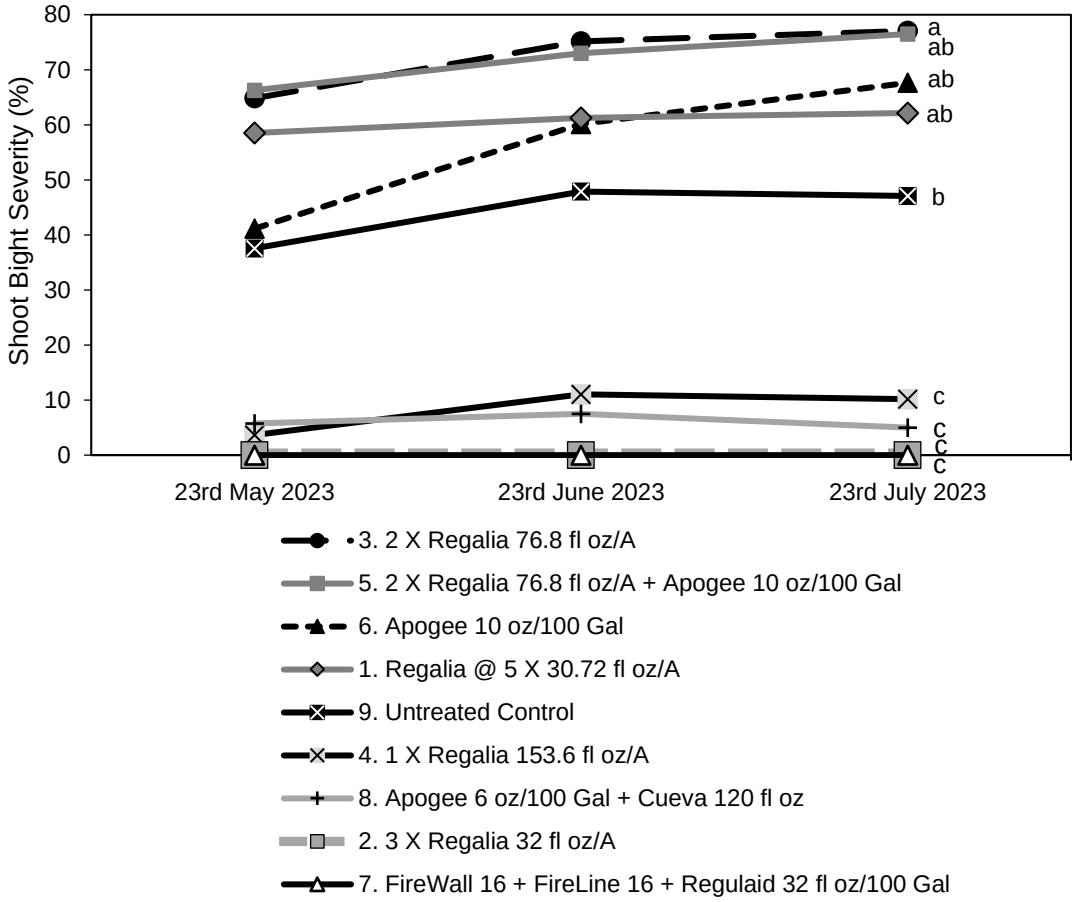


Figure 3. Shoot blight severity on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Shoots were inoculated on 25 April at 2.5- to 5-cm shoot size with *Erwinia amylovora* (2 × 10^8 CFU/ml). Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

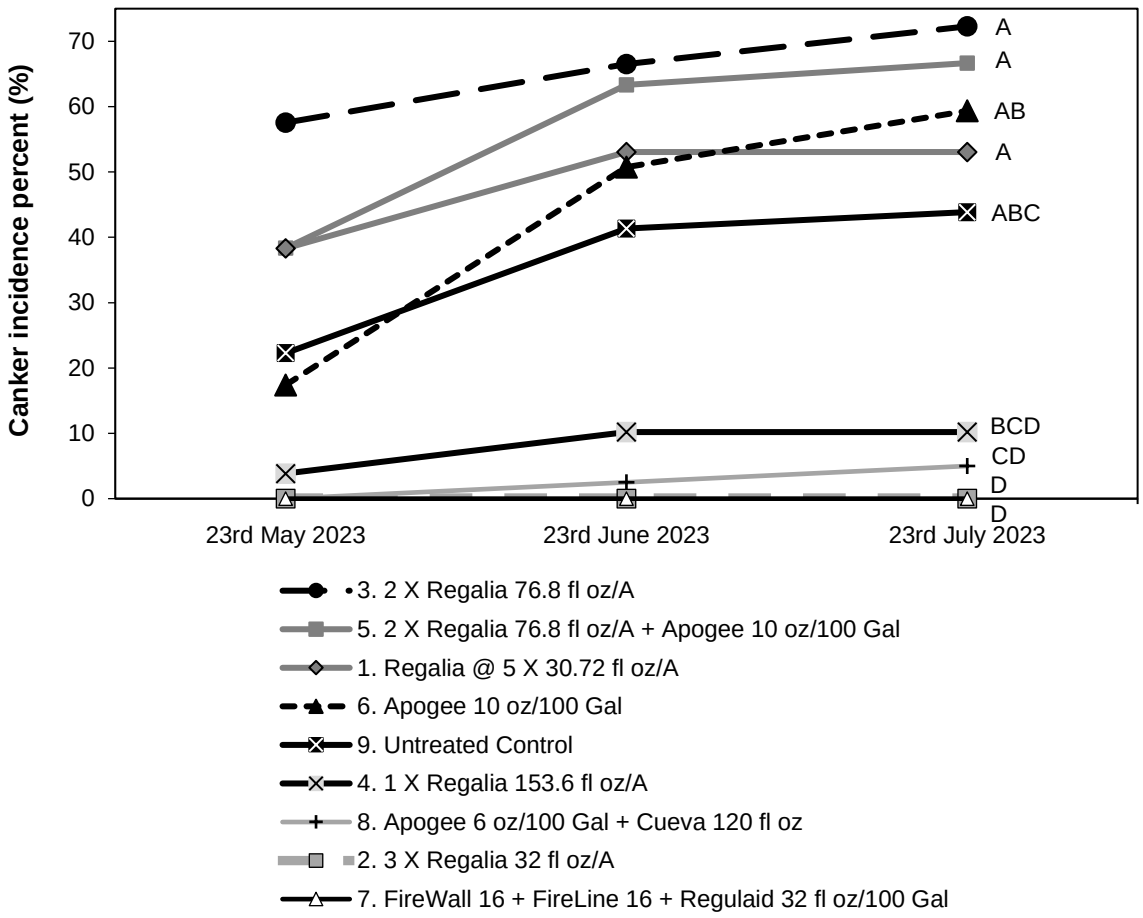


Figure 4. Canker incidence on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

Based on the 2023 data from Virginia and when compared to Oregon data from 2023 (see below), it seems that Regalia is more effective on younger pear trees (Virginia) in comparison to mature trees (Oregon). In Virginia, the most effective treatments were #2, #4, #7, and #8 (Figures 2-4). Furthermore, five spray applications of Regalia 30.72 fl oz/A seems to be inconsistent with the previous results we reported in Borba et al. (2023). We have met in person with ProFarm Group (formerly known as Marrone Bio Innovations) to record the container batch number of the Regalia (5%) used in our trial and inform us if any issues were associated with formulating this product potentially leading to poor results. At the time of creation of this report we have not heard back from ProFarm Group.

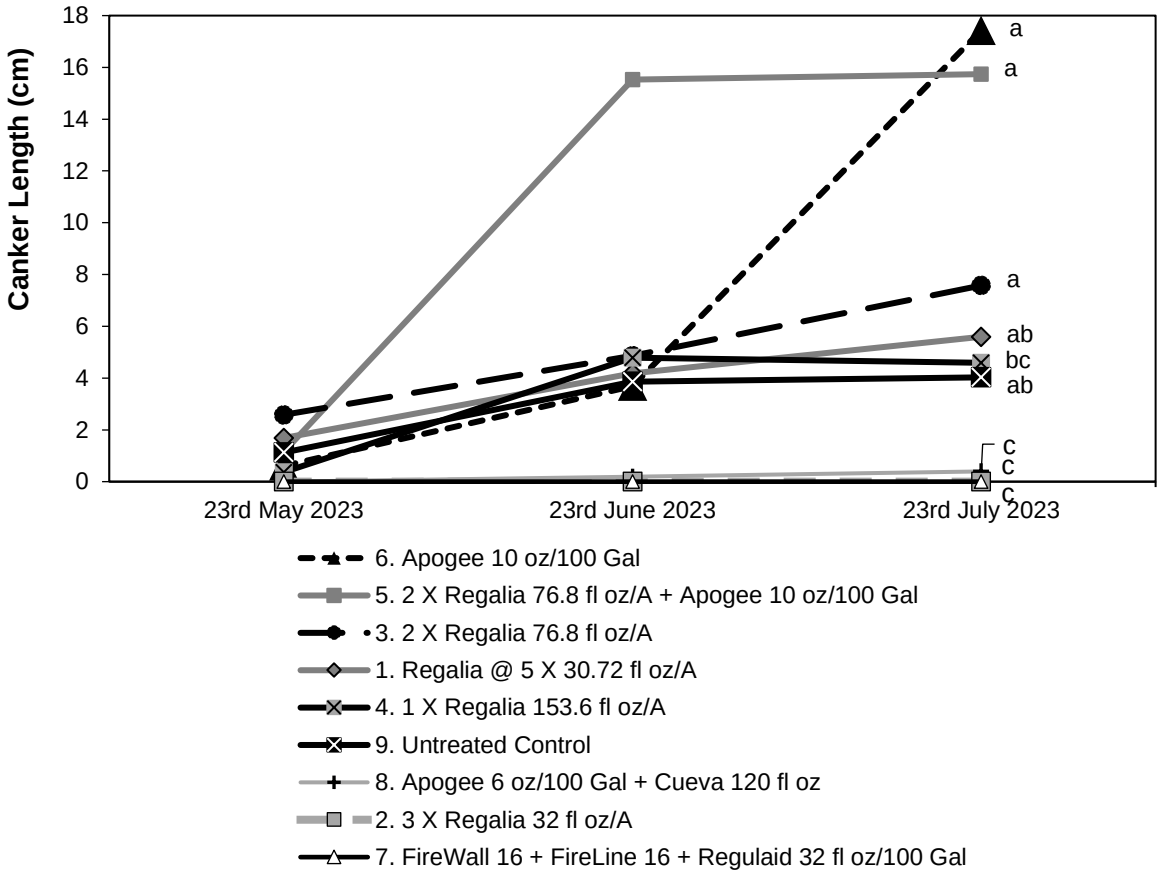


Figure 5. Length of fire blight cankers on perennial wood of pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, with each tree mean consisting of 10 shoots per tree.

***VIRGINIA 2024*:**

Summary graphics.

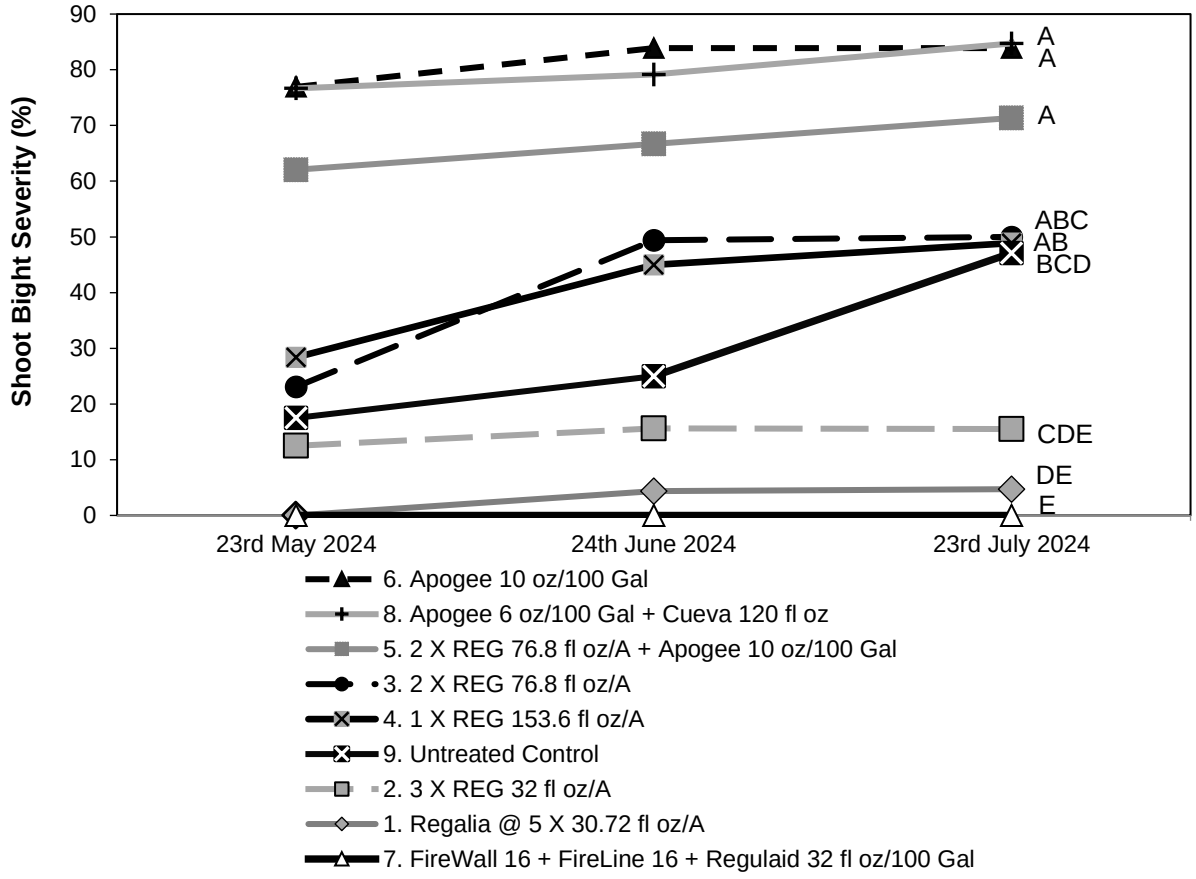


Figure 6. Shoot blight severity on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2024. Shoots were inoculated on 30 April at 2.5- to 5-cm shoot size with *Erwinia amylovora* (2 × 10^8 CFU/ml). Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

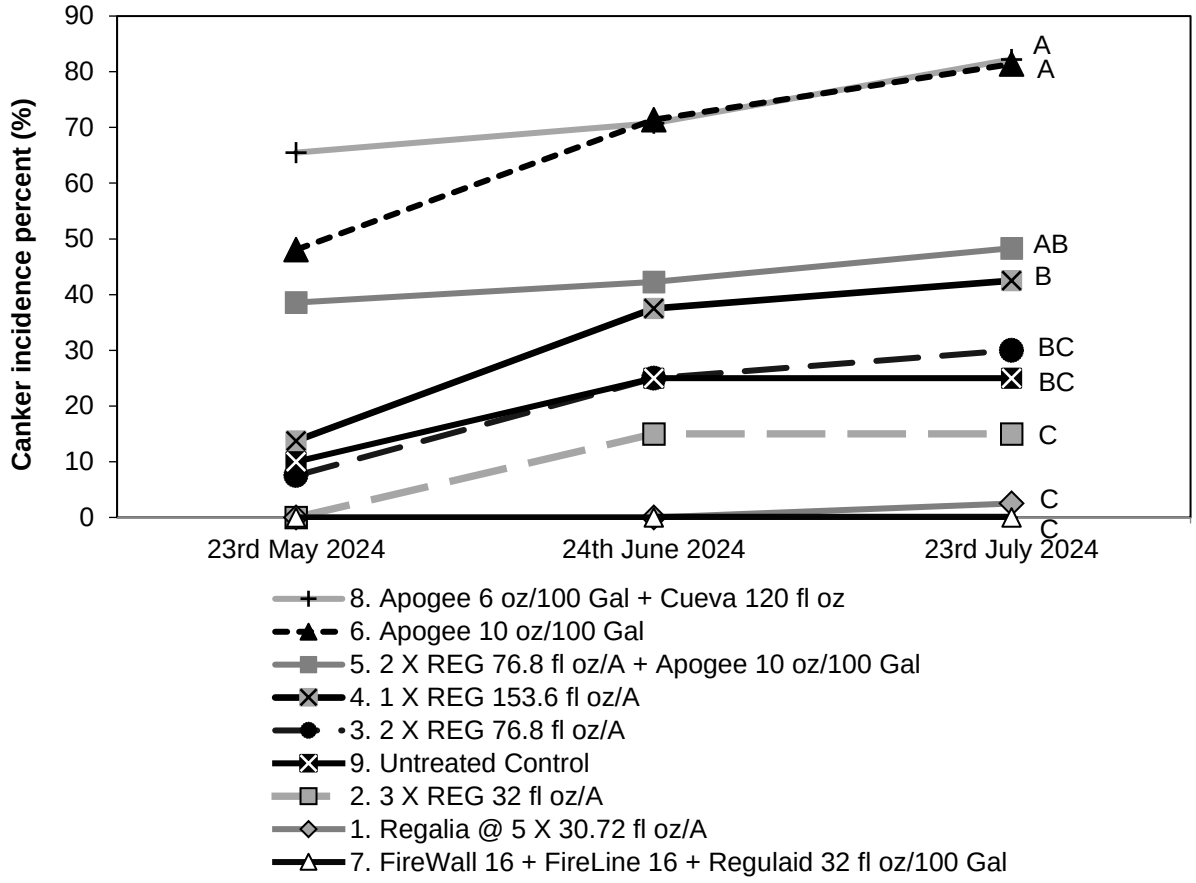


Figure 7. Canker incidence on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2024. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

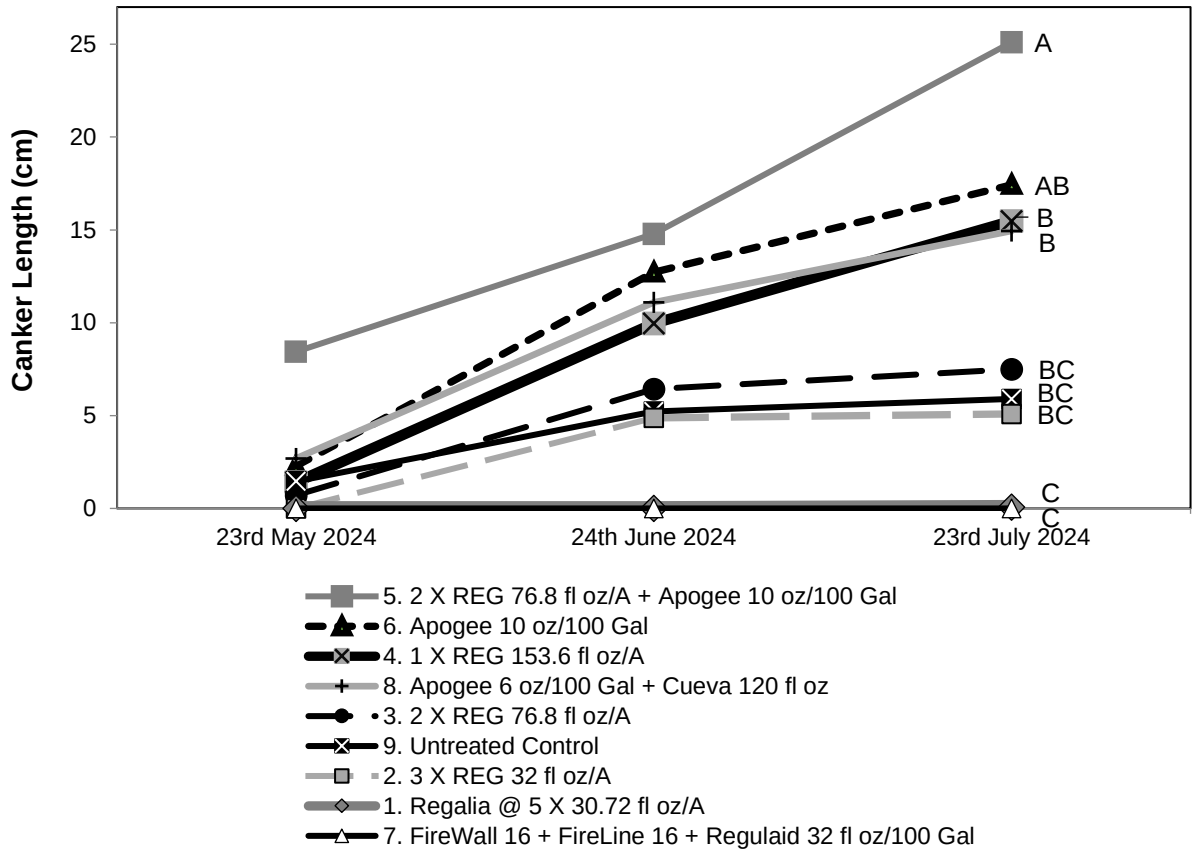


Figure 8. Length of fire blight cankers on perennial wood of pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2024. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, with each tree mean consisting of 10 shoots per tree.

Based on younger pear trees we used in Virginia the most effective treatment for controlling shoot blight severity for #7 FireWall + FireLine (Figure 4). However, this significant effect of treatment #7 has not stayed stable and did not control canker incidence and canker size (no significant differences in comparison to #9 Untreated Control. We are not sure why the fire blight did not advance more in the #9 Untreated Control, which would be expected. We are unaware of any reasons for this #9 Untreated Control failure.

Since no significant differences were visible in 2024 data set between Regalia and #9 Untreated Control, we cannot make a sound conclusion on Regalia's effectiveness on pear shoots and cankers, except that we find Regalia to be more and more inconsistent across many years of our and others research. We believe that Regalia is likely highly dependent from unknown optimal environmental, spray water, or formulation conditions to confer its desired effect. We have not tested the pH of water we used and we have not investigated the effect of environmental factors on the Regalia efficacy. However, we did hear that Regalia's formulation has undergone a change to reduce side effect of apple/pear flower phytotoxicity. This information was conveyed to use unofficially. The inconsistency in the efficacy from year to year is also in comparison to our previous results we reported in Borba et al. (2023) in Virginia, and in comparison to the results from Yan et al. (2023) in Michigan on apple, and from Boeckman et al. (2024) in Virginia on apple. Our discussions with ProFarm Group (formerly known as Marrone Bio Innovations) who produce Regalia have not

continued after we provided them with the container batch number of the Regalia (5%) used in our trials. They committed to informing us if there were any issues associated with the formulation of this product potentially leading to our poor results in 2024 when compared to earlier years, but we doubt they will contact us. At the time of creation of this report we have not heard back from ProFarm Group.

2. OREGON 2023 & 2024

Significant Findings

Other than the commercial standard of fire blight management on pears using antibiotics, no significant differences among treatments were observed for shoot blight and canker management.

Methods

The 'Bartlett' trees at Southern Oregon Research and Extension Center, in Central Point, OR, are planted at 14.8 ft. between rows and 9 ft between trees within rows. Each spray program (treatment) listed in Table 1 were applied to three replicate trees in Oregon. Trees were assigned in a completely randomized design. Inoculation of 15 shoots per tree were done after application of spray programs with *E. amylovora* suspension of 2 x 10^8 CFU/ml on May 19, 2023. Similarly, 10 shoots per tree were inoculated on May 7, 2024 for second year trial.

Treatment #	Number of spray applications, materials, and amount	Applied at pear growth stage (spray timing)	Dates of treatment application (2023)	Dates of treatment application (2024)
1	5 X Regalia 30.72 fl oz/A	BB, GC, WB, PF, FS	04/05; 04/18; 04/24; 05/09; 05/18	03/18; 03/21; 03/25; 04/10; 05/06
2	3 X Regalia 32 fl oz/A (lower label rate)	WB, PF, FS	04/24; 05/09; 05/18	03/25; 04/10; 05/06
3	2 X Regalia 76.8 fl oz/A	PF, FS	05/09; 05/18	04/10; 05/06
4	1 X Regalia 153.6 fl oz/A	FS	05/18	05/06
5	2 X Regalia 76.8 fl oz/A + Apogee 10 oz/100 gals	PF, FS	05/09; 05/18	04/10; 05/06
6	2 X d'Anjou pear rate for Vegetative Growth Control and Reduced Latent Bloom: Apogee 10 oz/100 gal*	PF, FS	05/09; 05/18	04/10; 05/06
7	2 X Pear Grower Standard in PNW: Agri-Mycin 16 oz/A + FireLine 16 oz/A + Regulaid 32 fl oz/100 gals	BL, 24 h before shoot inoculation	05/01; 05/18	04/06; 05/06
8	2 X d'Anjou pear Grower Standard for Shoot Blight: Apogee 6 oz/100 Gal* + Cueva 120 fl oz (2 oz metallic copper/A)	1 to 3-inch new shoot growth, 14 days after	05/09; 05/23	04/22; 05/10
9	Untreated control	-	-	-

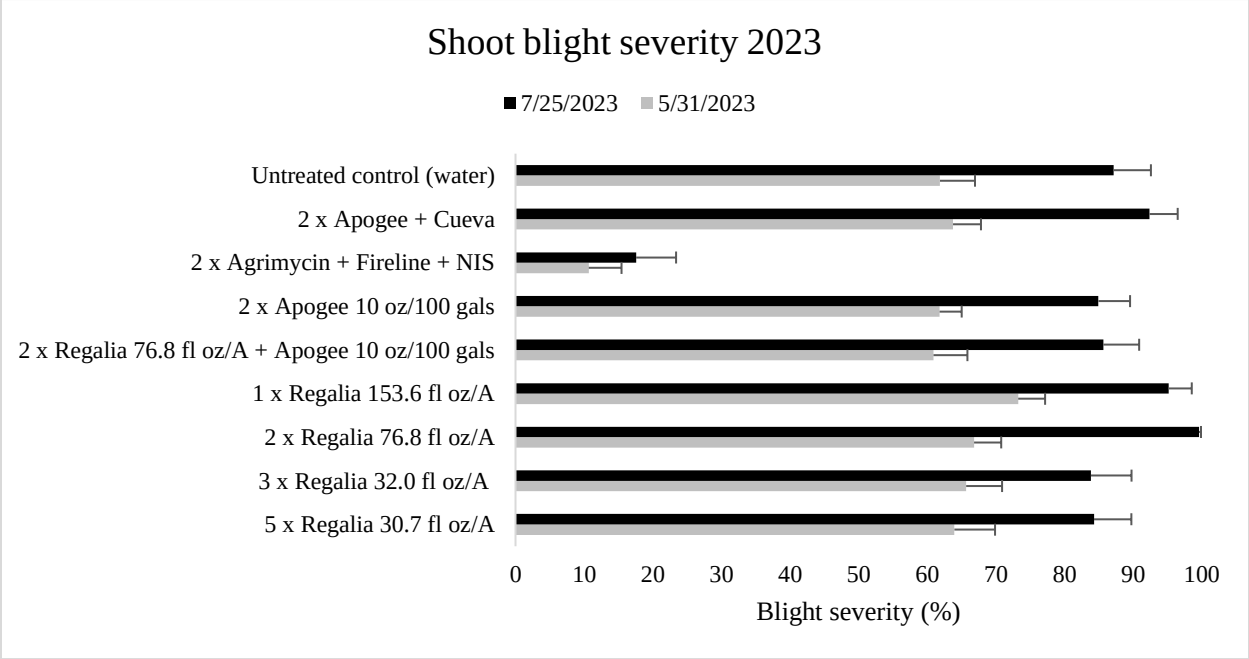
Data on total shoot length and lesion length were measured on May 31, and July 25, 2023. Similarly, data on total shoot length and lesion length were measured on May 17, and June 6, and July 17, 2024. Cankers were noticed on secondary and tertiary branches during July 25 and 17 data collection in 2023 and 2024 respectively. The total branch length and canker length were measured.

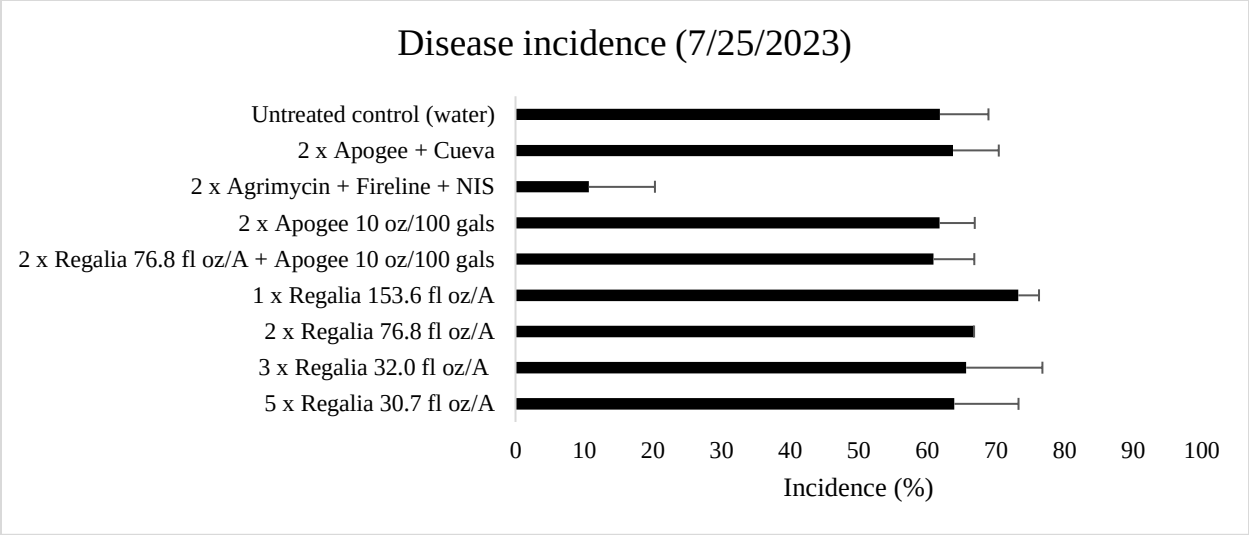
For shoot blight analysis, severity was calculated as ratio of lesion length and total shoot length expressed as percentage. Similarly, for canker severity analysis, ratio of canker length and total branch length was expressed as percentage. The number of blighted shoots were counted and disease incidence was calculated as the ratio of blighted shoots to the total number of inoculated shoots. Significance of treatment application on shoot blight and canker severity was analyzed using ANOVA and the treatment means were compared using Fisher's protected LSD ($P<0.05$).

Data on fruit russet was collected during Bartlett harvest in Southern Oregon on August 3, 2023 and August 6, 2024. Forty fruit per tree was harvested and russet on individual fruit surface was rated using modified Horsfall-Barratt rating scale and converted to the midpoint category to obtain percentage of severity. The percent severity data was analyzed using ANOVA and the treatment means were compared using Fisher's protected LSD ($P<0.05$).

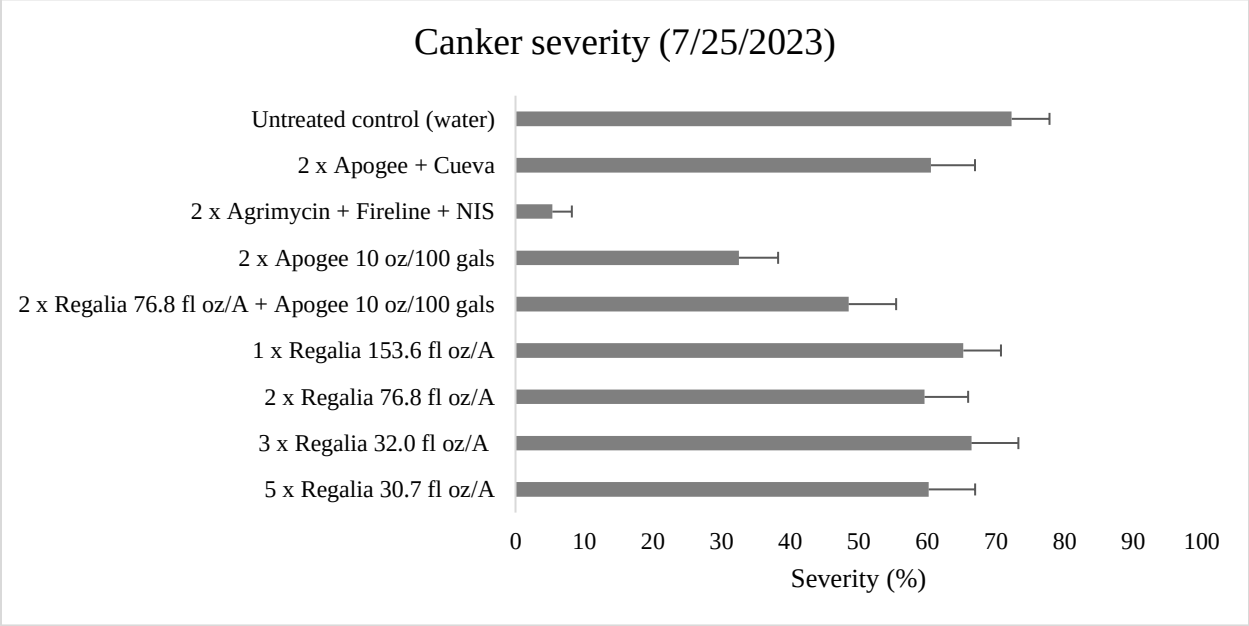
Results and Discussion

Year 1:

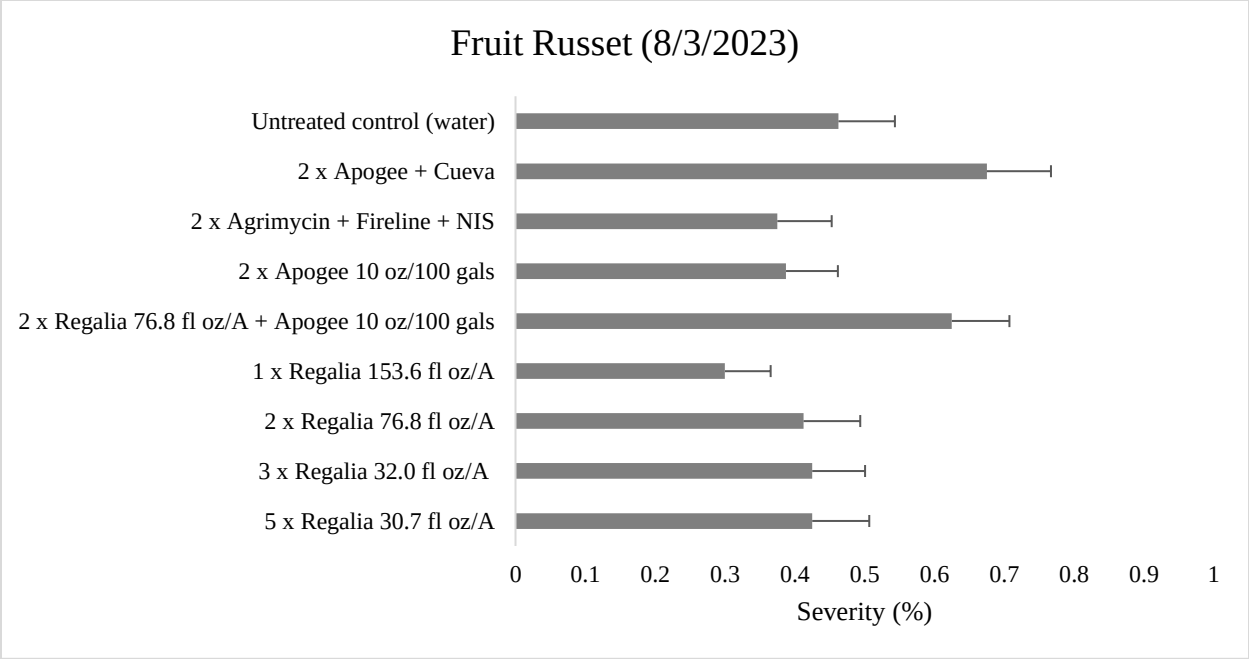




Only the commercial standard, where antibiotics were used during full bloom and one day before shoot inoculation significantly reduced the shoot blight severity and disease incidence. The severity in rest of the treatments were not significantly different from the non-treated controls.

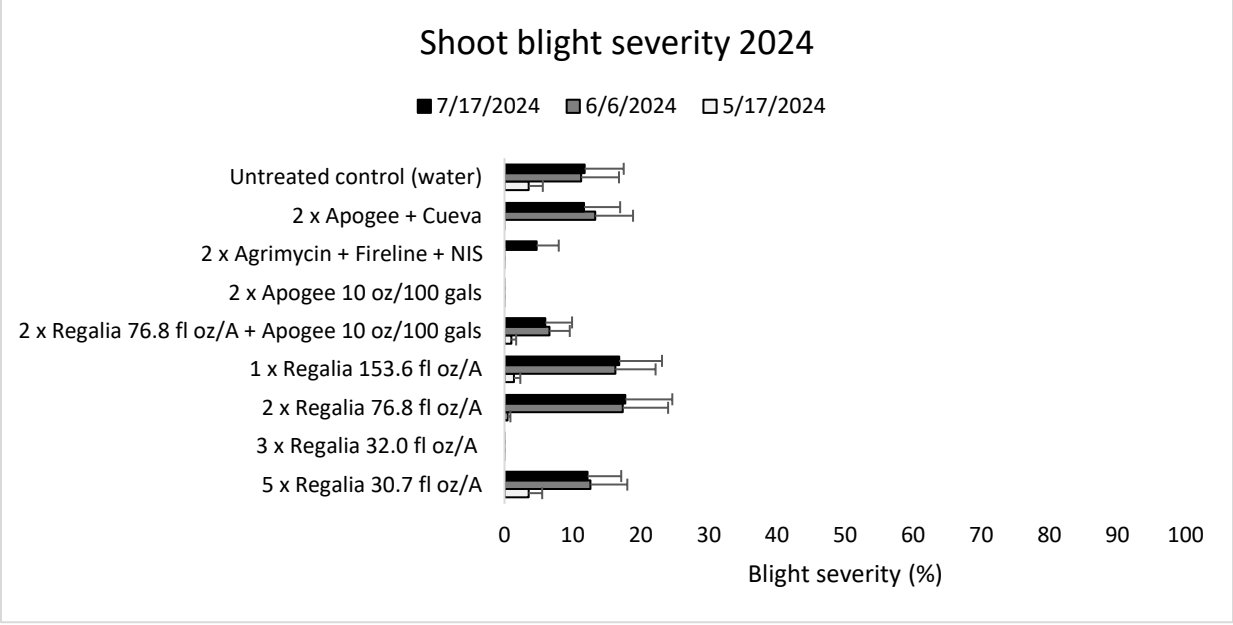


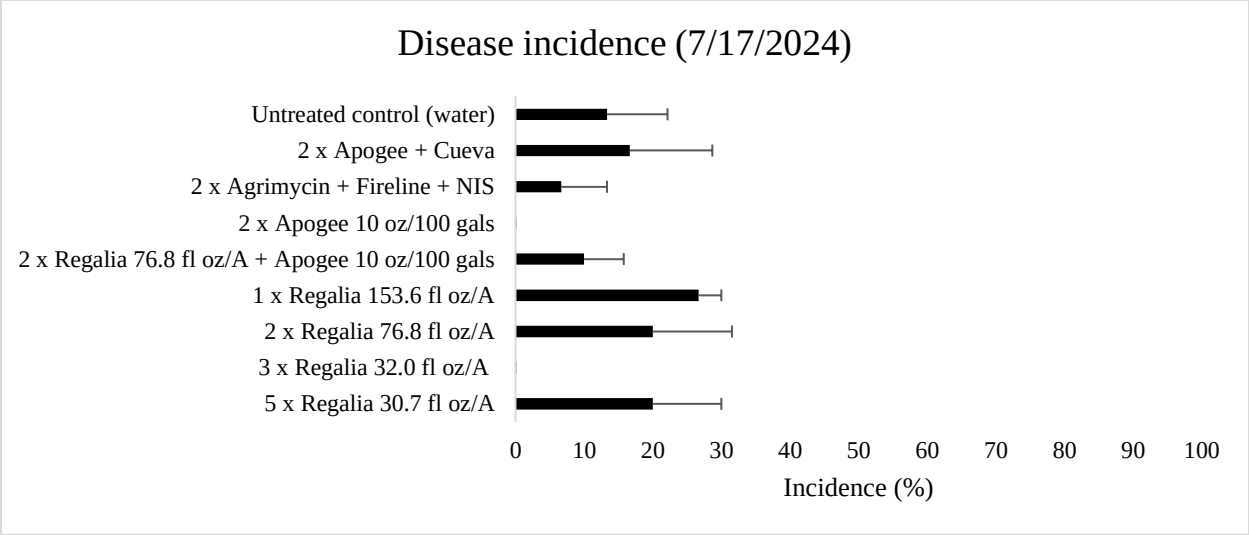
Similar to shoot blight severity, only the commercial standard with antibiotics significantly reduced the canker severity. The severity in rest of the treatments were not significantly different from the non-treated controls.



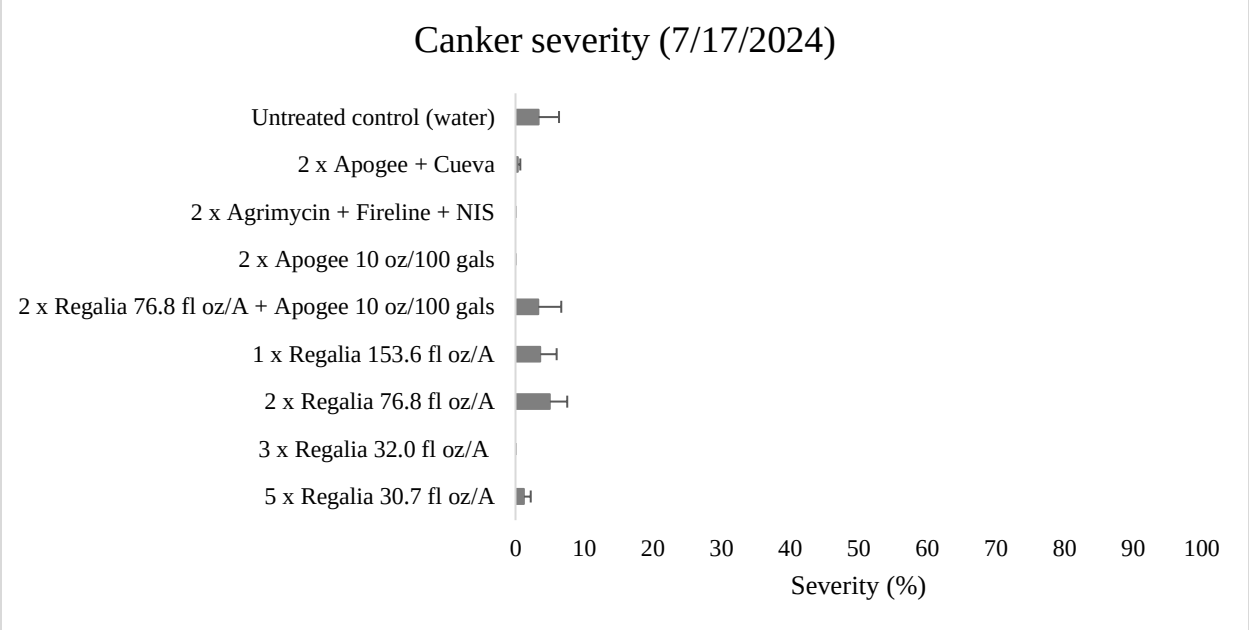
Different fruit russet response were observed on the treated fruits, however these differences were not statistically significant ($P<0.05$). The average russet were less than 1% with range of 0 to 3% russet on these fruits.

Year 2:



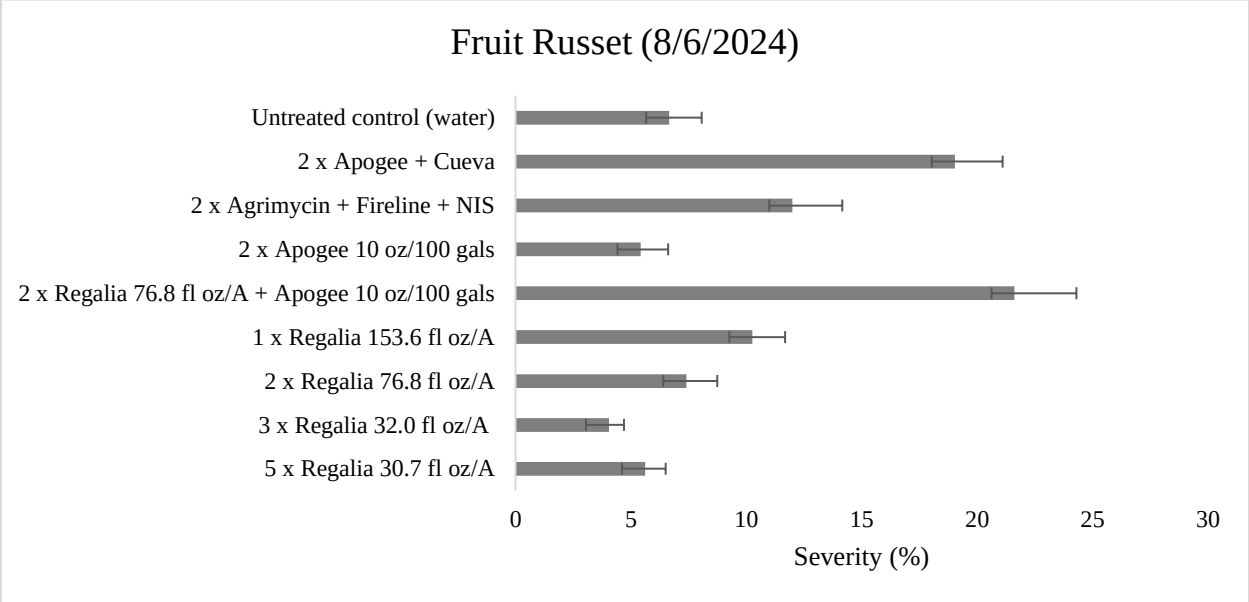


In the second year of the study, the overall fire blight incidence in inoculated shoots were very low. Even in non-treated control trees, only 13% of the inoculated shoots developed shoot blight. There was no blight in the shoots where Regalia was applied three times during WB, PF, and FS @ 32.0 fl oz/A (treatment #2); and Apogee applied two times during petal fall and fruit set (treatment #6). The highest incidence was observed on one time Regalia treated trees during fruit set @ 153.6 fl oz/A (treatment #4). Due to low disease incidence and higher variability between the treatment means, no statistically significant differences were observed among the treatments (*p= 0.204*)



Similar to shoot blight incidence, the canker incidence in the second year was low. Even though 7% of the inoculated shoots developed blight in antibiotics treated shoots (treatment #7), they did not develop any canker. Similarly, two times Apogee and Cueva applied during 1 to 3-inch new shoot growth, and 14 days after the shoot growth (treatment # 8) resulted in less than 1% canker even

though 17% of the inoculated shoots developed shoot blight. However, none of these differences are statistically significant due to low disease incidence and higher variability between the treatment means (*p= 0.449*)



Interestingly, fruit russet was the highest in second year of this trial and the treatment means were significantly different ((*p<0.001*). The highest russet was observed when Apogee was mixed with Cueva (treatment #8) and Regalia (treatment #5) and these results were similar to previous year's russet evaluation. The russet was observed in all other treatments; however these differences were not statistically different from non-treated water sprayed control.

Literature

Borba M. C., Meredith C. L., Dhar B. C., Aćimović S. G. (2023): Proof of concept for management of shoot blight and fire blight cankers on pear with preventive spray applications of giant knotweed extract. ***Frontiers in Horticulture, 1:1082284. pg. 1-14.***

Boeckman N. J., Borba M. C., Aćimović S. G. (2024): Evaluation of Giant Knotweed Extract, Regalia, and Antibiotics in Control of Shoot Blight and Fire Blight Canker Phases on Apple. ***Agronomy 2024, Special Issue: Detection and Control of Diseases and Pests in Fruits, 14(10), 2216: 1-14.***

Yuan, X.; Gdanetz, K.; Outwater, C.A.; Slack, S.M.; Sundin, G.W. (2023): Evaluation of Plant Defense Inducers and Plant Growth Regulators for Fire Blight Management Using Transcriptome Studies and Field Assessments. ***Phytopathology, 113, 2152–2164.***

Proposal Title: Screening kasugamycin resistance in *Erwinia amylovora* on pears

Report Type: Final report

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Cooperators: Garrett Bishop (G. S. Long)

Project Duration: 1 Year

Total Project Request for Year 1 Funding: $25,886

Other related/associated funding sources: Awarded.
Funding duration: 2022-2023
Amount: $158,123
Agency: WTFRC-Apple Crop Protection

WTFRC Collaborative Costs: None

Budget 1
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Item	2024		
Salaries[1]	$14,400.00		
Benefits[1]	$5,778.00		
Salaries[2]	$1,250.00		
Benefits[2]	$513.00		
RCA Room Rental			
Shipping[3]	$292.00		
Supplies	$2,805.00		
Travel[4]	$848.00		
Plot Fees			
Miscellaneous			
Total	$25,886.00	$0.00	$0.00

Footnotes: [1]Postdoc salary (Zhao lab) for 3 months at $4,800/month and postdoc benefit rate at 40.1%. [2]Technician salary (Tianna lab) for 0.25 month at $5,000/month and benefit rate at 41%. [3]shipping cost and materials (Tianna). [4]Milage for collecting samples (Tianna $348; Zhao $500).

Objectives:

1. To collect and screen kasugamycin (will also include streptomycin and oxytetracycline) resistance in pear orchards throughout the state and possibly determine the resistant/tolerant nature;
2. To deliver results to growers and provide guidance on kasugamycin use in orchards.

Significant Findings:

- No *Erwinia amylovora* isolates exhibited resistant to streptomycin and oxytetracycline in 2024;
- 7 *Erwinia amylovora* isolates exhibited resistance/tolerance to kasugamycin in 2024;
- The resistant/tolerant isolates were isolated from orchards in Sunnyside and Dryden.
- Mutation in the kasugamycin target *ksgA* gene was found in five of the resistant/tolerant *E. amylovora* isolates.
- This is the first report of kasugamycin resistant/tolerant *E. amylovora* isolates in Washington or elsewhere.
- These results suggest that growers should take immediate actions in terms of how to and what antibiotic to use for controlling fire blight disease.
- Based on our findings, we recommended that growers should mix kasugamycin with oxytetracycline or be in rotation with streptomycin for fire blight control.

Significance to the industry and potential economic benefits.
Since the identification of streptomycin-resistant strains of *E. amylovora* by Loper et al. in 1991, there has been limited data in evaluating the status of antibiotic resistance throughout the central Washington regions. The significance of this research to the industry lies in two aspects. First, this is the first report of kasugamycin resistant/tolerant *E. amylovora* isolates in Washington or elsewhere and the isolates were from orchards in two different locations, Sunnyside, and Dryden. Growers should take immediate actions in terms of how to and what antibiotic to use for controlling fire blight disease. Based on our findings, growers should rotate kasugamycin with other antibiotics such as

streptomycin/products to treat fire blight or should mix kasugamycin with oxytetracycline. In summary, the findings of the current project directly benefit the growers of Washington state by providing instant feedback to growers in antibiotics resistance situation in orchards and growers should take immediate actions to avoid control failure.

Methods and Procedures:

In 2024, we either collected symptomatic samples in central Washington by our own field trips to local area growers or samples were sent to us via mail by growers or consultants or extension specialists. We also collected asymptomatic blossom samples. Samples were placed in plastic bags and held on ice or in a refrigerator until they were processed. Samples were processed by cutting into small pieces with a sterile knife, washed briefly with sterile water, soaked in 900 µl 10 mM PBS, vortexed, and streaked for isolation onto five types of media: LB, CCT, LB + Sm 100 µg/mL, LB + Kg 100 µg/mL, LB + Tc 20 µg/mL and incubated at 82.5 Fº (28 °C) for 48 - 72 h. Colonies that appeared white in color on CCT media, slightly raised and nonfluorescent were suspected to be *E. amylovora*. Screening for resistance was performed by observing the presence of individual colonies on antibiotic media. Isolates of known resistant *E. amylovora* strains were obtained from culture collections for use as positive controls. Isolates were then confirmed by PCR using *E. amylovora* specific primers G1-F and G2-R.

Spot dilution test was performed for selected resistant/tolerant strains (**Figure 1**). Bacteria were grown on LB plates and a single colony was inoculated in LB broth and grown for 24 hr with shaking at 250 rpm. Bacterial suspensions were adjusted to an absorbance of OD_{600} = 1 in PBS and 10-fold serial dilution was made in PBS. For each dilution, 5 µL was spotted onto plates: LB and LB + Kg 50, 75, 100 125, and 150 µg/mL and incubated at 82.5 Fº (28°C) for 48 - 72 h. Bacterial growth was visually observed on plates with or without antibiotics. Growth on plates without antibiotics was used as a control to compare to the plates with antibiotics.

In addition, the minimum inhibitory concentration (MIC) was determined. Bacteria were grown on LB plates and a single colony was inoculated in LB broth with shaking at 250 rpm. Overnight bacterial suspensions were adjusted to an initial concentration of OD600 = 0.1 and 2-fold serial dilutions were performed, starting with LB + Kg1000 µg/mL and ending with LB + Kg 0.976 µg/mL. IC_{50} was defined as the concentration of antibiotics at which growth of the bacterium was 50 % less of that of the control without antibiotics. IC_{95} was defined as the concentration of antibiotics at which growth of the bacterium was 95 % less of that of the control without antibiotics.

Selected resistant/tolerant *E. amylovora* isolates were used to amplify the kasugamycin target *ksgA* gene by primers KsgA-F and KsgA-R. PCR products were then sequenced by Eton Biosciences Inc, San Diego, CA and compared to those of known sensitive strains.

Results and Discussion:

Samples were collected from three pear varieties in central Washington, i.e. Bosc, Anjou, and Bartlett. *E. amylovora* isolates were confirmed by PCR. Seven isolates collected in 2024 from Bartlett pears were shown to be resistant or tolerant to kasugamycin at 100/150 ppm, respectively. However, no isolates were found to be resistant to streptomycin or oxytetracycline. Among the resistant/tolerant isolates, colony size was significantly smaller as compared to growth of the same isolate on LB medium and spot dilution assay showed similar growth for resistant/tolerant isolates at LB with antibiotics and without antibiotics (**Figures 1 and 2**). These resistant/tolerant isolates were able to grow on plates with kasugamycin at 50, 75, 100, 125 and 150 ppm. These findings indicated that these isolates from 2024 were shown to be resistant/tolerant to kasugamycin.

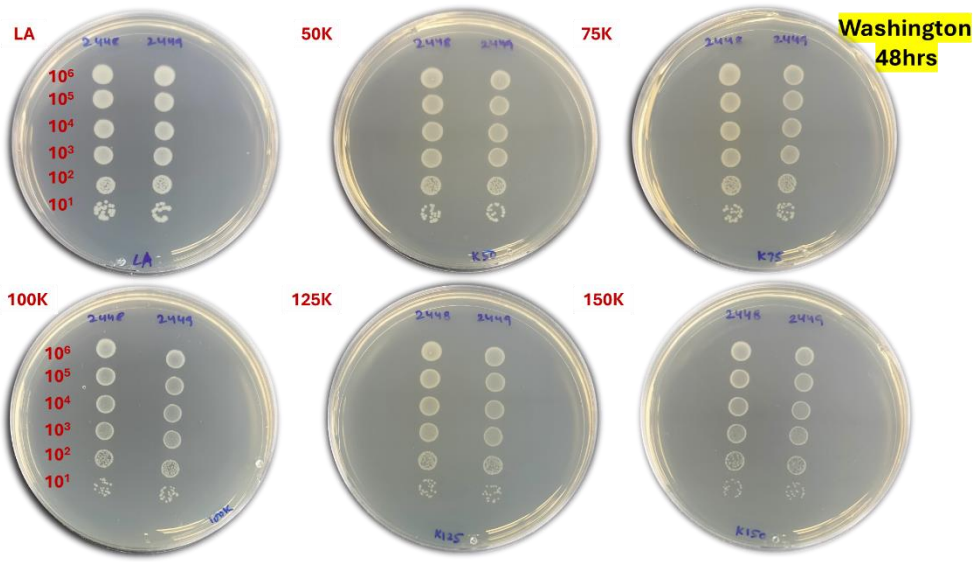

Figure 1. Spot dilution assay for two representative resistant isolates. Serial 10-fold dilutions were made in PBS. For each dilution, 5µL was spotted on LB plates containing no antibiotics or kasugamycin at 0, 50, 75, 100, 125, and 150 µg/ml. Pictures were taken 48 hours post inoculation. Both 2448 and 2449 are resistant to kasugamycin.

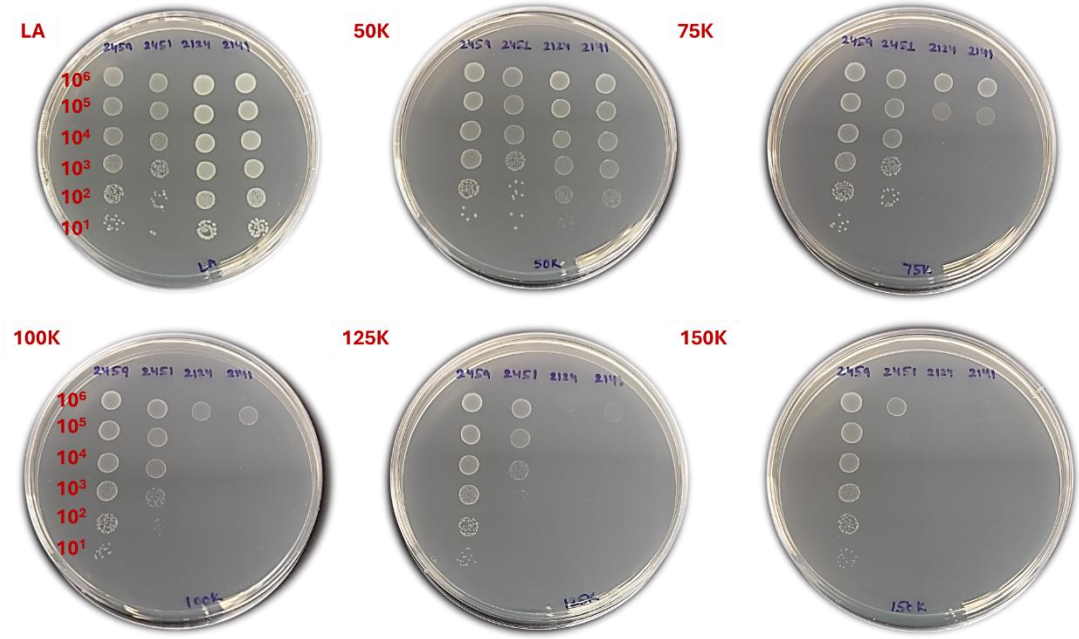

Figure 2. Spot dilution assay for one representative resistant isolate as compared to three tolerant/sensitive isolates. Serial 10-fold dilutions were made in PBS. For each dilution, 5µL was spotted on LB plates containing no antibiotics or kasugamycin at 0, 50, 75, 100, 125, and 150 µg/ml. Pictures were taken 48 hours post inoculation. Isolate 2459 is resistant; whereas isolate 2451 is tolerant and 2124 and 2141 are sensitive.

Next, we determined MIC_{50} and MIC_{95} for 7 isolates from WA. The five resistant strains isolated from 2024 had the highest MIC_{50} of more than 150 µg/ml and the MIC_{50} for two tolerant isolates was above 60 µg/ml. Similarly, the five resistant strains isolated from 2024 had the highest MIC_{95} of about 400 µg/ml and the MIC_{95} for two tolerant isolates was above 200 µg/ml. Sequence comparison of the *ksgA* gene showed mutations in five resistant isolates as compared to known type strains (data not shown). Based on previous studies, resistance to kasugamycin arises from mutations of its target gene *ksgA*, encoding an adenine demethylase. Our results indicate that resistance to kasugamycin of the five *E. amylovora* isolates is due to mutations in the *ksgA* gene. This is the first time we found mutations in the *ksgA* gene in *Erwinia amylovora*.

Executive Summary

Project Title: Comprehensive monitoring and mapping antibiotics resistance in orchards

Key words: Fire blight, antibiotics resistance, streptomycin, tetracycline, kasugamycin

Abstract: Antibiotics remain one of the best tools for managing blossom blight of apple and streptomycin remains the better choice in terms of cost and efficacy in killing pathogens as compared to tetracycline and kasugamycin. The occurrence of streptomycin resistance of the fire blight pathogen in WA pear orchards in 1980s results in increased use of tetracycline and kasugamycin. However, there has been limited data evaluating the existence and extent of antibiotic resistance of *Erwinia amylovora* in central WA since then. The purpose of the current study was to comprehensively monitor and map antibiotics resistance in orchards in WA. In 2024 growing seasons, diseased samples were collected from pear orchards and *E. amylovora* isolates were examined for their resistance to streptomycin, oxytetracycline and kasugamycin. Although no *E. amylovora* isolates exhibited resistance to streptomycin and oxytetracycline, 7 isolates exhibited resistance or tolerance to kasugamycin in 2024. WA isolates from 2024 had the highest MIC_{50} as compared to previous years. Mutation was found in the kasugamycin target *ksgA* gene in five of the resistant/tolerant *E. amylovora* isolates. This is the first report of kasugamycin resistant/tolerant *E. amylovora* isolates in Washington. These results suggest that growers should take immediate actions in terms of how to and what antibiotic to use for controlling fire blight disease. Based on our findings, we recommended that growers should mix kasugamycin with oxytetracycline or be in rotation with streptomycin.

Proposal Title: New controlled atmosphere strategies to extend 'Bartlett' pear storage

Report Type: Continuing (year 1)

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Cooperators: Pear packinghouses in the Columbia River Gorge vicinity, Dr. Yu Dong, Janet Turner, Shawn McMurtrey, Emmi Klarer, Christopher Imler

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ 99,276
Total Project Request for Year 2 Funding: $ 104,163

Other related/associated funding sources: Awarded
Funding Duration: 2024 - 2025

Amount: $95,000
Agency Name: USDA-ARS TFRL
Notes: 0.5 FTE, Biological Science Technician, GS-9 step 2,3, salary and benefits; supplies and equipment

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Rachel Leisso*
*This budget also includes funds for David Rudell, Loren Honaas, and James Mattheis as these PIs belong to the same administrative unit; see footnotes for details.
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Station Manager/Supervisor: James Mattheis
Station manager/supervisor email address: James.Mattheis@usda.gov

Item	2023	2024
Salaries	$48,505.00	$50,131.00
Benefits	$19,655.00	$20,716.00
Wages		
Benefits		
RCA Room Rental	$5,570.00	$5,570.00
Shipping		
Supplies	$5,000.00	$5,000.00
Travel	$500.00	$2,500.00
Plot Fees	$1,250.00	$1,250.00
Miscellaneous	$10,000.00	$10,000.00
Total	$90,480.00	$95,167.00

Footnotes:
Salaries: 1.0 FTE Biological Science Technician (GS-7), plus ~15 hours of overtime, annually.
Benefits: For Biological Science Technician (GS-7)
RCA room rental: per OSU-MCAREC fee book (cost per sq ft x time) (2 rooms, one with experimental CA chambers)
Supplies: harvest and storage supplies, reagents, and consumables for aroma profiling (Rudell and Leisso)
Travel: fruit transport locally and between Hood River and Wenatchee for fruit storage
Plot fees: 0.25-acre rental, OSU-MCAREC
Miscellaneous: sequencing (Honaas)

Budget 2
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Item	2023	2024
Salaries	$4,167.00	$4,292.00
Benefits	$2,629.00	$2,704.00
Wages		
Benefits		
RCA Room Rental		
Shipping	$500.00	$500.00
Supplies	$500.00	$500.00
Travel	$1,000.00	$1,000.00
Plot Fees		
Miscellaneous		
Total	$8,796.00	$8,996.00

Footnotes: 1: Salaries for a Faculty Research Assistant @ $50,000/year for 1 month, and 63.1% benefit rate. The FRA is expected to assist with inoculum preparation and isolation for rot related data, data collection, and analysis. 2: Shipping will consist of inoculum or fruit shipping during the study period between southern Oregon and Hood River. 3: Supplies for pathogen isolation and culture, as well as the harvesting supplies. 4: Travel between southern Oregon and Hood River for rot evaluation/ rot related data collection.

Objectives

1. **Evaluate ultra-low oxygen in comparison to other controlled atmosphere (CA) regimes for long-term 'Bartlett' storage.**

   This objective will determine if ultra-low oxygen CA (0.5% O_2 + <0.5% CO_2) offers significant storage extension without loss of quality relative to other CA programs.

2. **Determine optimum maturity for long-term CA storage for Bartlett.**

   Research activities in this objective will systematically evaluate the optimum firmness at harvest for long-term low-oxygen CA (~1.5% O_2 + <0.5% CO_2) for 'Bartlett' pear. Fruit size distribution for early (22.5 lb firmness), on-time (19.5 lbf), and late (17 lbf) harvests will be determined by harvesting whole trees, and the effects of maturity on storability will be evaluated by storing fruit at 1.5% O_2 + <0.2% CO_2.

3. **Evaluate the influence of modified atmosphere packaging (MAP) (LifeSpan, Amcor, Australia) on fruit quality post long-term CA storage.**

   This objective will determine storage longevity and fruit quality of 'Bartlett' fruit held at 30 and 45 °F in boxes with MA liners following long-term CA storage. Although research indicates utility for certain types of MAP for 'Bartlett' when used immediately after harvest, whether MA continues to provide significant benefit and retains aroma and quality in late-term storage post-CA has not been examined.

Significant findings 2024. Since the start of this project was postponed due to a delay in a new CA system installation, the project years are 2024 and 2025, with the second year a no cost extension (NCE). CA installation was completed in the USDA-ARS Hood River Worksite August 2024.

- Evaluating earlier harvest timing for extending CA shifted the relative size distribution of fruit; postharvest evaluation will take place in January-February 2025:
  1. Harvest 1 (21.2 lbf), 56% of the fruit were in the size categories 80, 90, or 100 (44 lb box)
  2. Harvest 2 (19.5 lbf), 65% of the fruit were size categories 70, 80, or 90 (44 lb box)
  3. Harvest 3 (18.5 lbf), 74% of the fruit were size categories 60, 70, or 80 (44 lb box)
- Fruit from the Harvest 3 (18.5 lbf) were estimated to be 38% larger than Harvest 1; fruit from Harvest 2 were 17% larger than the Harvest 1.
- Post-storage (5.5 months postharvest) MAP extended quality pre- and post- ripening relative to fruit packed in perforated polyethylene bags among fruit stored longer than 2 weeks post-6 months storage.
- Fruit stored in MAP also retained superior quality in a holding temperature of 45 °F through 4 wk, indicating utility in circumstances where cold chain breaks may occur.
- No negative effects of post-storage MAP (5.5 months postharvest) relative to perforated polyethylene bags at either 30 °F or 45 °F were observed.
- MAP also decreased average Mucor lesion size by 22% relative to control fruit.
- At the time of writing, fruit in comparative 'Bartlett' CA regimes (0.5, 0.8. 1.5, 2.5% O_2; CO_2 <0.5%) are still in storage and will be evaluated mid-February 2025.

Methods

Experimentation. Experimental approaches are briefly recapped for each objective in Results and Discussion.

Fruit. In 2024, all fruit were obtained from the Columbia River Gorge and Hood River Valley. In 2025, fruit for experimentation will be obtained from additional pear production regions.

CA. Controlled atmosphere conditions are maintained in a custom constructed cabinets served by an automated control panel (Empire Control Systems, Chelan, WA) equipped with a gas sample analyzer (Bridge Analyzers Inc., Bedford, OH). Nitrogen and air are supplied by a nitrogen generator (Shiftletts Inc, East Wenatchee, WA) and Kaeser air compressor.

Fruit quality evaluation. Green color and relative loss of green color was determined by a handheld digital meter (DA meter; Sinteleia, Bolonga, Italy). Flesh firmness was quantified using a fruit texture analyzer (FTA, Güss Manufacturing Ltd, Strand, South Africa). Soluble solids content (SSC) readings were measured from fruit juice samples expressed from fruit (exluding core tissue and seeds) via a juicer (Champion Classic 2000 Juicer, Plastaket Manufacturing Inc., Lodi, CA, USA) with a hand-held digital refractometer (HI 96822; HANNA Instruments, Smithfield, RI, USA). Ten mL of this juice was diluted 1:1 with distilled water and pH and titratable acidity determined on a potentiometric titrator (Mettler-Todelo, Columbus, OH, USA). Fruit were also visually rated for senescent scald (peel) and senescent core breakdown (flesh); defect rating scales are indicated in table footnotes.

MAP bags. The modified atmosphere bags are LifeSpan brand (Amcor, Inc., Zurich, Switzerland). Use of a particular product does not imply endorsement by the USDA-ARS.

Atmosphere evaluations. Ethylene and CO_2 gas samples (0.5 mL) were injected in a gas chromatograph-flame ionization detector (model 8890, Agilent Technologies, Santa Clara, CA, USA) equipped with a multimode inlet (MMI) (Agilent Technologies), Porabond Q column (Agilent CP7350, 10 m x 320 µm x 5 µm) connected in series to a thermal conductivity detector (TCD) and flame ionization detector (FID). Oxygen samples from a static headspace were pumped (Gas Sampling Sensor Micro Pump kit, GasLab.com, Ormond Beach, FL) through an infrared type sensor (LOX-O2-F coupled MX300 chip, GasLab.com) and reported to software (Gaslab 2.1, Gaslab.com).

Statistics. Data analyses were performed in statistical software packages SAS (SAS Inc, Cary, NC) or R (https://www.r-project.org/).

Results and Discussion

Objective 1. Evaluate ultra-low oxygen in comparison to other CA regimes for long-term 'Bartlett' storage.

'Bartlett' fruit were obtained from two commercial harvests in the Hood River Valley, OR, August 17, 2024 (Odell, elevation ~700 ft above sea level), and August 31, 2024 (Parkdale, elevation ~1700 ft above sea level). A subset of fruit from each harvest is currently stored in the USDA-ARS Wenatchee and Hood River cold storages under the following CA conditions at 30 °F:

0.5% O_2, <0.5% CO_2
0.8% O_2, <0.5% CO_2
1.5% O_2, <0.5% CO_2
2.5% O_2, <0.5% CO_2
Control fruit (no CA)

For Odell fruit, (harvested August 17), CA conditions were established one week after harvest at USDA-ARS Wenatchee, and one month after harvest at Hood River. Parkdale fruit (harvested August 31) CA conditions were established in Wenatchee one week after harvest, and two weeks after harvest in Hood River. Initial fruit quality for both harvests is indicated in Table 1. There was a brief delay in fruit quality evaluations due to the timing of when the bin was obtained from the packinghouse relative to harvest. These fruit will be removed from CA and evaluated in February 2025.

Table 1. Fruit quality seven days (Odell) and ten days (Parkdale) after harvest. Fruit were maintained in 30 °F cold storage, with the exception of transport. No internal nor exogenous ethylene was detected for any fruit.

Harvest date and location	Weight per fruit (g)[1]		I_{AD} (DA meter)[2]		hue[3]		Firmness (lbf)		Brix		TA	
Aug. 17, Odell	199	B	1.81	ns	114.3	A	17.1	ns	11.1	ns	0.34	A
Aug. 31, Parkdale	248	A	1.79	ns	113.1	B	16.7	ns	12.2	ns	0.20	B
p -value	0.0025		0.5536		0.0330		0.2999		<0.0001		0.0002	

1. Values in a column followed by different letters are statistically different at $p < 0.05$ (two-tailed t-test); not statistically different = ns.
2. DA meter; an indicator of green color (lower values indicate less green); for more information, see Hanrahan and Roder in References.
3. Hue, measured by colorimeter (Konica Minolta); lower values indicate less green color.

Objective 2. Determine optimum maturity for long-term CA storage for 'Bartlett'.

In August 2024, 'Bartlett' pears were harvested at Oregon State University Mid-Columbia Research and Extension Center (OSU-MCAREC), Hood River, OR in three successive harvests (Table 1). Trees are 15 years old, on OHFx97 rootstock, and trained to a central leader. Commercial harvest timing was determined by a combination of the Hood River 'Bartlett' harvest maturity model (Chen, 2016), experienced fieldman recommendation, and flesh firmness. The first harvest (H1) on Aug. 6, 2024 (Table 2), was an 'early' harvest, with firmness at the time of harvest similar to high firmness fruit in University of California-Davis guidelines (Mitcham et al, 1996). The goal of the second harvest (H2), on Aug. 14, 2024, was to simulate present commercial harvest practices for standard CA conditions. The third harvest (H3), on August 21, was the "late" harvest, intended to represent fruit destined for earlier marketing, e.g. short-term CA or air storage. Based on firmness and size, these fruit were harvested earlier than a typical "late" harvest (Table 2).

Harvested fruit were cooled and two days later, one set (72 fruit) was placed in CA storage (1.5% O_2, ~0% CO_2) at USDA-ARS Wenatchee. A second set (~140 fruit) from each harvest was transported to a commercial cooperator for storage in CA one week after the last harvest, and a third set (~100 fruit) remains at USDA-ARS Hood River as an air-stored control. Beginning in January 2025, these fruit will

be removed from CA at their respective locations to determine relative storage longevity in CA in relation to harvest timing. Partial results may be presented at the research review (February 20, 2025) but are not available in this report due to the submission deadline.

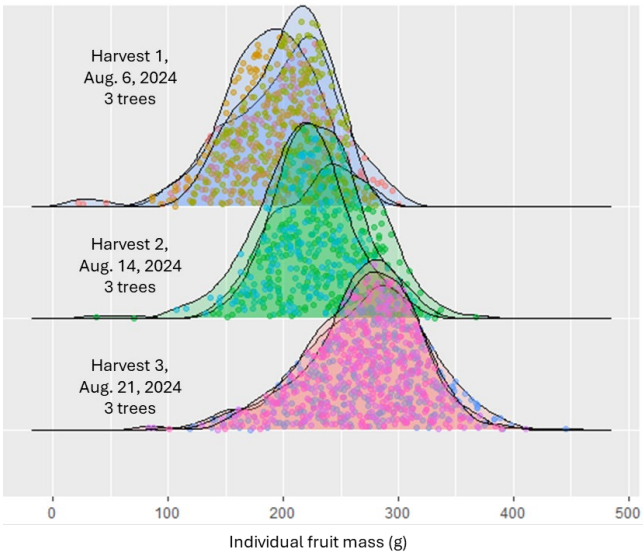
At harvest, the difference between treatment groups was most apparent for fruit weight, I_{AD} (DA meter), and firmness, while influence on hue, Brix, and titratable acidity were negligible (Table 2, Fig. 1). Brix to firmness maturity ratios for H1 and H2 aligned with California recommendations for higher firmness harvests (Mitcham et al., 1996). Average "indirect chlorophyll or green color indicator" (I_{AD}, obtained by a DA meter) values were lower than optimal I_{AD} values established by Wang et al. (2015), whose results indicated that 'Bartlett' fruit harvested at I_{AD} 2.1 to 2.2 were optimal for long-term (3-month air) storage of 'Bartlett'. In their study, fruit firmness at harvest for 'Bartlett' corresponded relatively linearly to I_{AD}; note that I_{AD} did not correlate well with firmness for other cultivars in the study, including 'Anjou'. Their study also indicated I_{AD} greater than 2.2 led to inferior postharvest eating quality, that is, fruit were not mature enough to ripen properly. There was also considerable variability in firmness in our study (data not shown) within each harvest, which has been documented in other studies, for example, Claypool (1973). This challenge may be addressed by ethylene conditioning (Villabos-Acuna and Mitcham, 2008).

Table 2. Fruit quality at harvest, from IFP block, OSU-MCAREC, 3005 Experiment Station Dr., Hood River, OR. No internal or exogenous ethylene was detected for any harvest.

Harvest	Weight per fruit (g)[1]	I_{AD} (DA meter)[2]	hue[3]	Firmness (lbf)	Brix (°Bx)	TA
H1	225 C	2.05 C	115.9 A	21.2 A	11.54 B	0.43 ns
H2	240 B	2.00 B	115.2 B	19.5 B	11.91 A	0.44 ns
H3	291 A	1.92 A	114.9 B	18.5 C	11.56 B	0.42 ns
Pr<F	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	0.2555

1. Values in a column followed by different letters are statistically different at $p < 0.05$.
4. DA meter; an indicator of green color (lower values indicate less green); for more information, see Hanrahan and Roder in References.
2. Hue, measured by colorimeter; lower values indicate less green color.

Figure 1. Individual 'Bartlett' fruit weight per successive harvests. Each distribution indicates the harvest from one tree.



The interconnected concerns for optimizing maturity for long-term storage of 'Bartlett' are 1) defining optimal maturity, and 2) establishing the ideal method(s) for determining maturity. To address this second

Table 3. Mass and estimated volume per tree according to harvest timing.

Harvest timing[1]	Total weight (g)[2]		Single fruit volume (cm^3)[3]	Percent increase[4]	box size (% of fruit in each size category) (44 lb box)[5]										
					50	60	70	80	90	100	110	120	135	150	165	180	195	210
H1 "early"	226	C	214 C	-	0	0	3	12	25	19	13	11	9	4	2	0	0	0
H2 "on-time"	240	B	247 B	17%	0	2	16	27	22	16	8	5	2	1	0	0	0	0
H3 "late"	291	A	287 A	38%	2	19	35	20	11	5	2	2	2	0	0	0	0	0
Pr<F	<0.0001		<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. Harvest timing per Hood River Bartlett Maturity model combined with pressure testing; n = three trees per harvest timing.
2. Values in a column followed by different letters are statistically different at $p < 0.05$.
3. Volume estimated per this equation established in-lab: volume (cm^3) = 1.005*mass in grams + 15.698. The equation has an r^2 of 0.9482.
4. Percent increase of average fruit mass (g) relative to Harvest 1
5. No statistical analysis performed.

concern, peel and flesh samples collected at each harvest will be analyzed to determine molecular maturity markers, or next generation maturity indicators (NGMIs); this portion of the study is led by the Honaas Research Group (USDA-ARS, Wenatchee).

A third consideration in optimizing harvest maturity for long-term 'Bartlett' CA storage is the additional mass accrued by delaying harvest and relative size distribution of fruit at each harvest (Table 3). H2 had a 17% average fruit mass increase relative to H1, and H3, 38% mass increase relative to H1. Additionally, for H1, 57% of fruit were categorized as size 90s, 100s, and 110s; H2, 81% as 70s, 80s, 90s, and 100s; and H3, 85% as 60s, 70s, 80s, and 90s. In addition to increased size, this data set suggests increasing uniformity in size with later harvest. The potential economic and marketing implications are beyond the scope of the present study.

Objective 3. Evaluate the influence of modified atmosphere packaging (MAP) on fruit quality post long-term CA storage.

In January 2024, fruit were obtained post-CA and post-packing from two packinghouses in the Columbia River Gorge vicinity. Fruit from Packinghouse 1 were post-CA ten days and size 100. Fruit from Packinghouse 2 were post-CA for an unknown amount of time and size 90. Fruit quality upon receipt is indicated in Table 4. Fruit were repacked from packinghouse materials into either perforated polyethylene bags (control), modified atmosphere (MAP) bags (LifeSpan, Amcor Inc., Zurich, Switzerland), or a subset of boxes held at either 30 °F or 45 °F (the latter simulating an extended broken cold chain). Periodic evaluation of fruit quality is indicated in Table 4 and Figure 2.

After two weeks at 45 °F, fruit that were not in a MAP bag deteriorated beyond edibility for both packinghouses. Based on mean internal breakdown severity, packinghouse was more significant than any treatment, time, temperature combination for internal breakdown, suggesting that handling prior to receipt at USDA-ARS was more impactful than experimental treatment. Packinghouse 1 fruit had been out of storage for ten days prior to receipt whereas Packinghouse 2 had been more recently removed from CA prior to packing.

Wang and Sugar (2013) focused on fruit packed in MAP immediately after harvest, while the present work focused on MAP post-CA; that is, fruit that had already been stored for over five months. The present results indicate the utility of MAP in cold chain breaks, conditions warmer than ideal holding temperatures (Table 4, Fig. 2, Table 5). When held at a low temperature (30 °F) for 6 weeks, most measures of quality did not statistically differ between fruit stored in perforated polyethylene bags vs. MAP.

Table 4. Fruit quality in response to MAP, temperature, and post-repacking (re-packing into MAP) duration, combined to include packinghouses. Fruit stored at 45 °F in

perforated polyethylene bags (control) were discarded at 2 wk post-packing prior to ripening and 4 wk post-packing due to deterioration. Average pre-experiment I_{AD} was 1.79, firmness 8.2 lbf.

	Bag type[1]	Temp. (°F)	Weeks post-re-packing	I_{AD} (DA meter[2])	Flesh firmness (lbf)	Senescent scald (peel defect)[3]	Senescent core breakdown (flesh defect)[4]
Unripened (day 0 post-experimental storages)	control	30	**2**	1.68 ab	11.5 a	0.61 cd	0 b
		30	**4**	1.64 ab	7.8 cd	0.86 abc	0 b
		30	**6**	1.60 bc	7.6 cd	0.30 d	0 b
	MA	30	**2**	1.76 a	8.3 bc	0.97 ab	0 b
		30	**4**	1.71 ab	7.4 de	0.77 bc	0.03 b
		30	**6**	1.60 bc	7.5 d	0.66 bc	0.03 b
	control	45	**2**	1.03 d	4.3 f	1.13 a	0 b
		45	**4**	-- --	-- --	-- --	-- --
	MA	45	**2**	1.63 bc	9.0 b	0.77 bc	0 b
		45	**4**	1.30 d	5.1 f	1.11 a	0.42 a
	Pr<F (Overall model, postharvest handling)[5,6]			<0.0001	<0.0001	<0.0001	<0.0001
Ripened (day 5 post-experimental storage)	control	30	**2**	0.63 ns	2.0 c	1.25 ab	0.03 d
		30	**4**	0.57 ns	2.1 bc	1.19 bc	0.53 c
		30	**6**	0.53 ns	2.2 bc	1.11 bc	0.58 c
	MA	30	**2**	0.56 ns	2.3 b	0.91 c	0.20 cd
		30	**4**	0.51 ns	2.8 a	1.11 bc	0.44 cd
		30	**6**	0.50 ns	2.6 a	1.13 bc	0.31 cd
	control	45	**2**	-- --	-- --	-- --	-- --
		45	**4**	-- --	-- --	-- --	-- --
	MAP	45	**2**	0.54 ns	2.1 bc	1.19 bc	1.19 b
		45	**4**	0.51 ns	2.2 bc	1.50 a	3.20 a
	Pr<F (Overall model Postharvest handling)[5,6]			0.4599	<0.0001	0.0175	<0.0001

1. Control = perforated polyethylene, MAP = LifeSpan brand.
2. DA meter; an indicator of green color (lower values indicate less green); for more information, see Hanrahan and Roder in References.
3. Senescent scald peel defect, surface area affected: 0 = none, 1 = >0-10%, 2 = 11-50%, 3 = 50%-100%)
4. Senescent core breakdown flesh defect: 0 = none, 1 = >0-10%, 2 = 10%-50%, 3 = 51-75%, 4 = 76-100% when cutting across the core horizontally.
5. Experimental factors were combined into a single term "handling" for a one-way ANOVA. Values in a column followed by different letters are statistically different at $p < 0.05$.
6. Day 5 of ripening was analyzed separately from day 0.

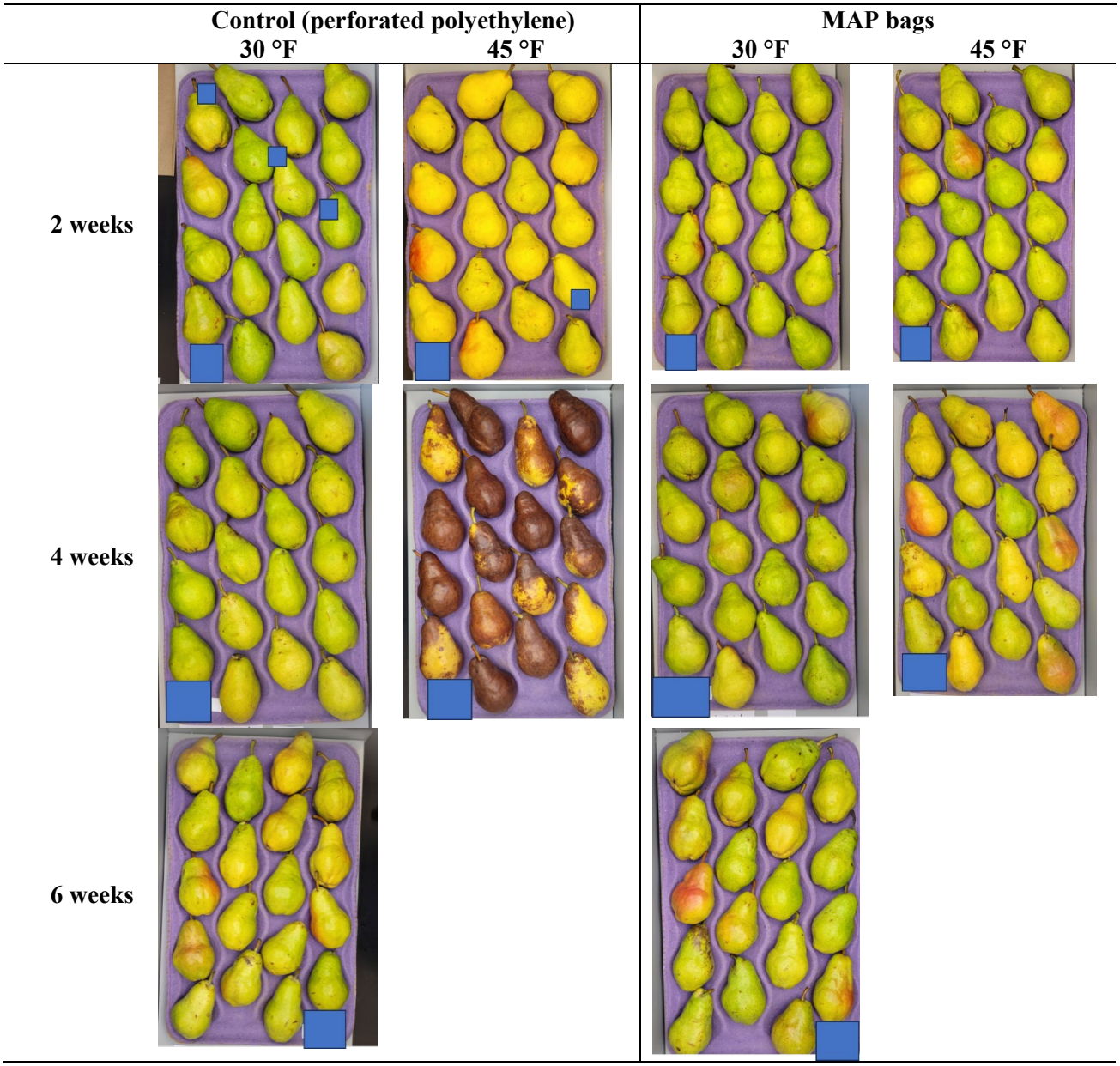


Figure 2. 'Bartlett' from Packinghouse 2, post-CA (approximately 5.5 months postharvest), packed into perforated polyethylene vs. MAP bags and held at 30 °F and 45 °F (extended cold chain break). Photos were taken upon removal from boxes in air storage. Blue boxes censor confidential information of the cooperator.

Table 5. Summary of MAP interaction with temperature and storage duration. In the present study (6 weeks in MAP, and post approximately 5.5 months CA storage) MAP was most useful for retaining fruit quality when there was a break in the cold chain.

	Quality attribute

	Color (DA meter)	Flesh firmness	Senescent scald	Senescent core breakdown
Summary	Warmer temperatures without MAP leads to loss of green in cold chain	Warmer temperatures without MAP increases firmness loss	Higher temperatures lead to higher incidence of senescent scald; effects of MAP on senescent scald unclear	Higher temperatures lead to higher incidence of senescent core breakdown; no discernable effect of MAP on senescent core breakdown

MAP bag atmospheres over the course of the experiment are indicated in Figure 3. Research by Wang and Sugar (2013) indicated that experimental MAP materials produced low oxygen conditions and relatively higher CO_2 2.2% O_2 and 5.7% CO_2) corresponded with greater incidence of internal browning after 3-4 months. Conversely commercial MAP materials with relatively higher oxygen (12.3% O_2 and 5.7% CO_2) allowed for fruit to be stored 3-4 months without issue. In the present study, at 45 °F, resulting CO_2:O_2 ratios far exceed recommendations established by Wang and Sugar (2013), but in short-term storage (<6 weeks), MAP at 45 °F (simulation of transport cold chain break) still led to superior quality outcomes relative to fruit in perforated polyethylene bags. The decrease of CO_2 and increase of O_2 for Packinghouse 2 at 45 °F correspond to fruit senescence, that is, dying fruit. It is important to note that following the termination of the experiment, CO_2 levels continued to increase and O_2 levels continued to decrease. Research in 2025 will extend the duration of fruit stored in MAP post-packing at 30 °F to address this outcome.

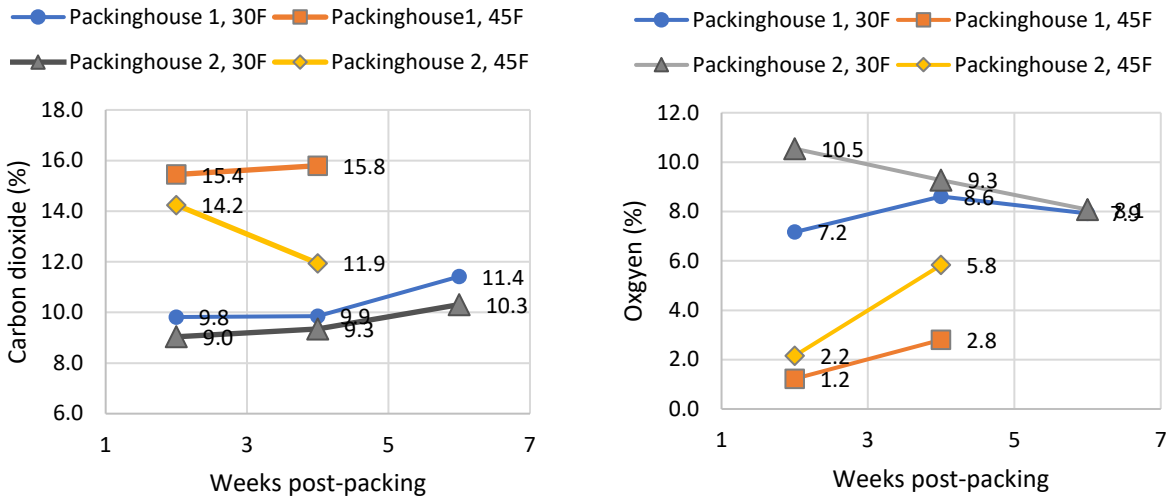


Figure 3. Carbon dioxide and oxygen levels in MAP bags at either 30 °F or 45 °F.

Ethylene levels built up in MAP at 45 °F (Figure 4) relative to MAP at 30 °F. Additionally, MAP appeared to initially suppress ethylene production relative to beginning levels at 30 °F. The apparent retention of ethylene in MAP bags warrants further study. This aspect of post-packing MAP will be expanded in 2025. Wang and Sugar (2013) did not report ethylene levels in MAP.

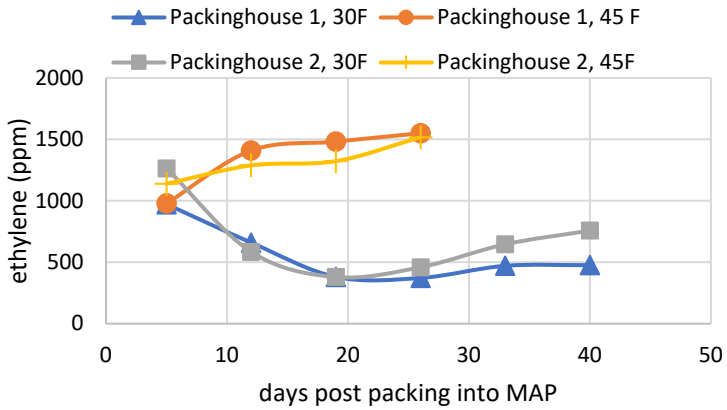
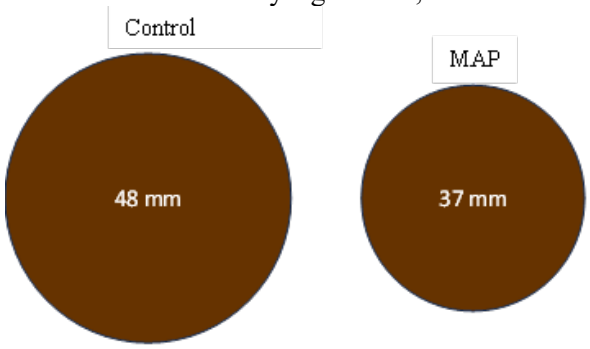


Figure 4. Ethylene levels in MAP bags, February – March, 2024.

The effect of MAP on Mucor rot (*Mucor pyriformis*) was also evaluated (Figure 5), indicating suppression of *M. pyriformis* growth rate.

Figure 5. Over the course of 4 weeks post-repacking (into MAP materials) and post-inoculation, MAP decreased Mucor lesion size (48 mm, control, vs. 37 mm, MAP), p <0.0001. While inoculated lesion size reduction is statistically significant, MAP does not eliminate rot issues.



References

Claypool, L.L., 1973. Further studies on controlled atmosphere storage of 'Bartlett' pears. J. Amer. Soc. Hort. Sci. 98: 289-293.

Chen, P. 2016. Pears. pages 471-480. *In* The Commercial storage of fruits, vegetables, and florist and nursery stocks. Available at: https://www.ars.usda.gov/is/np/CommercialStorage/CommercialStorage.pdf.

Hanrahan, I., Roder, S. No date. DA Maturity Indicator. Washington Tree Fruit Research Commission and Washington State University. Available at: https://treefruit.wsu.edu/article/da-meter-maturity-indicator/.

Mitcham, E.J., Cristoso, C.H., Kader, A.A., 1996. Pear (Bartlett): Recommendations for maintaining postharvest quality. University of California, Davis, Department of Plant Sciences, Davis, California. Pp. 1-2. Available at: https://postharvest.ucdavis.edu/files/259434.pdf and https://postharvest.ucdavis.edu/produce-facts-sheets/pear-bartlett.

Wang, Y., Sugar, D., 2013. Internal browning disorder and fruit quality in modified atmosphere packaged 'Bartlett' pears during storage and transit. Postharvest Biology and Technology 83: 72-82.

Wang, Y., Castagnoli, S., Sugar, D. 2015. Integrating I_{AD} index into the current firmness-based maturity assessment of European pears. Proc. XII International Pear Symposium. Eds. T. Deckers and J. Vercammen. Acta Hort. 1094: 525-532.

Project Title: Efficacy of Natamycin for Control of Mucor Rot in Pear Fruit
Report Type: Final Project Report
Primary PI: Dr. Rachel Leisso
Organization: USDA-ARS Tree Fruit Research Lab – Hood River Worksite
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Cooperators: Dr. David Felicetti and Dr. Christian Aguilar (AgroFresh/Pace International LLC). Shawn McMurtrey, Kartini Luther, Janet Turner, and Kevin Wang (USDA-ARS Hood River Worksite), Dr. Bob Spotts (Professor Emeritus, Oregon State University).

Project Duration: 1-Year
Total Project Request for Year 1 Funding: $29,955.80
Other related/associated funding sources: Awarded
Funding Duration: 2024 - 2025
Amount: $5,000
Agency Name: AgroFresh/Pace International LLC
Notes: AgroFresh/Pace International LLC funded $5,000 of this project and also provided product, residue testing, and in-kind technical support.

Budget 1
Primary PI: Rachel Leisso
Organization Name: USDA-ARS TFRL
Contract Administrator: Mara Guttman
Telephone: 510-559-5619
Contract administrator email address: Mara.Guttman@usda.gov
Station Manager/Supervisor: David Rudell
Station manager/supervisor email address: David.Rudell@usda.gov

Item	2024
Salaries	$ 22,162.00
Benefits	$ 1,695.00
Wages	
Benefits	
RCA Room Rental	$ 1,648.80
Shipping	$ 200.00
Supplies	$ 1,000.00
Travel	$ 1,500.00
Plot Fees	$ 1,750.00
Total	$ 29,955.80

Footnotes: Salary: 1.0 PTE Biological Science Technician (GS-5), (three 8-hr days per week) + 15 hours overtime.
Benefits: For Biological Science Technician (GS-5)
RCA room rental: per OSU-MCAREC fee book (cost per sq ft x time) (one room)
Shipping: Postage required to send fruit to Pace laboratory for residue testing.
Supplies: Harvest and storage supplies, pathogen culture and inoculation supplies.
Travel: To field sites and fruit transport (fuel).
Plot fees: 0.25-acre rental, OSU-MCAREC

Objectives

1. Compare the rates of different natamycin concentrations for prevention of post-packing Mucor rot on pears that have been in storage for six-months.
Determine the efficacy of natamycin for significant control of Mucor rot when applied as an aqueous dip post-storage in laboratory trials. This experiment will replicate and build upon previous baseline studies that have been conducted on citrus fruit but have not yet been tested on 'd'Anjou' pears (Kim et al. 2017, Saito et al. 2023).

2. Compare rates of different natamycin concentrations immediately post-harvest for preventative control of Mucor rot on pears.
Determine the amount of disease incidence incurred in 'd'Anjou' pears when the fungicide and inoculum are combined in an experimental dump tank in a lab setting. Results from this objective will provide insight into optimal concentrations for natamycin use on pear packing lines.

3. Evaluate efficacy of natamycin in combination with other postharvest chemicals for control of Mucor rot on pears at harvest.
Compare the efficacy of natamycin to control Mucor rot in pears at harvest with fruit that has been in long term storage. Demonstrate the best strategy for combinations of postharvest chemicals to be used with natamycin treatments. The data from this experiment will be beneficial to the industry as it will help to identify optimal chemical combinations for significant control of Mucor rot in pears.

Significant findings

- The postharvest timing of natamycin (BioSpectra 100SC) application and concentration of active ingredient influence treatment efficacy, with earlier application (shortly after harvest vs. five months postharvest) and higher concentration (highest label rate) generally leading to greater reduction of disease incidence.
- The 1000 ppm natamycin treatments resulted in numerically lower disease incidence in all experiments when compared to the 500 ppm natamycin treatments.
- Average Mucor rot lesion diameter was lower for 1000 ppm treatments (compared to 500 ppm treatments) when the near-harvest and at five-month postharvest trials were combined and averaged.
- In an experimental dump tank that contained the highest label rate of BioSpectra 100SC (1000 ppm natamycin) and *Mucor piriformis* spores, Mucor rot incidence was reduced by 66%.
- A low rate of propiconazole (Mentor 45WP, 0.3g/L) combined with the highest label rate of natamycin (BioSpectra 100SC, 1000 ppm) reduced Mucor rot by 22% in a preliminary trial.

Methods

The post-harvest fungicide BioSpectra 100SC (active ingredient natamycin) was evaluated for control of Mucor rot on 'd'Anjou' pears. Pears were wound inoculated with *Mucor piriformis* and then treated with the lowest label rate of BioSpectra 100SC (500 ppm natamycin) and the highest label rate of BioSpectra 100SC (1000 ppm natamycin). Untreated fruits were used as controls. The fruit were then placed on fruit trays, wrapped in a perforated plastic bag, and stored in cardboard boxes in regular air at -0.5°C ± 0.5°C (31°F). Lesion sizes were measured in two perpendicular directions using a digital caliper after three weeks. A total of 16 fruit samples (two experimental replicates of eight fruits per replicate) and two liquid samples per treatment were sent to AgroFresh/Pace in Wapato, WA for residue testing. These experiments were conducted four different times using fruit from two different growing seasons. For the first two experiments, fruit from the 2023 harvest that originated from two different growing locations was used to evaluate the efficacy of BioSpectra 100SC on 'd'Anjou' pears that had been stored in air storage for five months. For all other

experiments, fruit from the 2024 harvest was used. Fruit from the 2024 harvest originated from two different growing locations (Hood River, OR, and Cashmere, WA). For objective two, a laboratory experiment was conducted to determine the amount of disease incidence incurred when 'd'Anjou' pears were dipped in an experimental dump tank that contained BioSpectra 100SC and *M. piriformis* sporangiospores. The high and low label rates were used for the BioSpectra 100SC concentrations in the dump dank water. Four holes were punctured on two opposite sides of the fruit at the equator region. The fruit was then dipped into the BioSpectra 100SC and *M. piriformis* spore solution for 10 seconds and allowed to dry at room temperature. The control dump tank contained only water along with *M. piriformis* sporangiospores. After fruit was dipped into the experimental dump tank they were then placed on fruit trays, wrapped in a perforated plastic bag, and stored at room temperature for 6-7 days. The percentage of infected fruit and the percentage of infected wounds per fruit were calculated after 6-7 days.

A final small-scale trial was conducted testing the efficacy of BioSpectra 100SC alone or in combination with the fungicide Mentor 45WP against Mucor rot. Fruit were wound inoculated with *M. piriformis* using the previously mentioned methods, then treated with the following treatments: BioSpectra 100SC (114oz/100 gal or 8.9g/L)), Mentor (4oz/100 gal or 0.3g/L), and BioSpectra 100SC + Mentor. A total of 18 'd'Anjou' pears were used per treatment. Lesion sizes were measured in two perpendicular directions using a digital caliper after the fruit was left in regular air cold storage for 7 days and then room temperature for 7 days.

Fruit quality evaluations were conducted on one replicate of 18 pears per treatment for trials in fall 2024 and experimental dump tank trials. Fruit quality was assessed using standard fruit quality measures which included fruit weight, index of absorption difference (I_{AD}; DA meter), firmness, soluble solids contents, and titratable acidity. Fruit was also inspected for any evidence of phytotoxicity such as marking, russeting, or other potential fruit finish issues.

Results and Discussion

Disease incidence

The individual trial with the lowest disease incidence was observed in the 'd'Anjou' pears grown near Cashmere, WA and treated at the near-harvest time point. There was 0% infection rate three weeks after Mucor rot inoculation and 1000 ppm natamycin treatment (Fig. 1A and Table 1). However, these fruit had a 9% infection rate when inspected again at the four-week time point (Fig. 1B), which is an indication that natamycin may reduce the rate of Mucor rot growth (fungistatic) but not eliminate (fungicidal) a portion of Mucor rot propagules. The 500 ppm natamycin treatment corroborated this outcome with a 30% infection rate after three weeks and an 83% infection rate at four weeks (Fig. 1A, 1B, and Table 1).

These results are consistent with findings from Saito et al. (2023) who found that Mucor rot incidence increased after three and four weeks in storage. Through our testing we found that trials could not continue beyond five weeks in air storage due to control fruit (fruit with no natamycin treatment) deteriorating. Additional research efforts will utilize controlled atmosphere storage or modified air packaging to conduct longer term experiments or evaluate repeated applications.

Results were similar for the two separate experiments conducted on fruit five-months postharvest. For these trials, the lowest label rate of BioSpectra 100SC (500 ppm natamycin) reduced Mucor rot incidence by 43-46% whereas the highest label rate (1000 ppm natamycin) reduced Mucor rot by 54-59% (Table 1). The natamycin fruit residue values were considerably lower for these two trials (five months postharvest, performed in spring 2024) compared to the other reported natamycin liquid and fruit residues (Table 1). This was a result of initial fungicide application methods that were later modified leading to higher natamycin fruit residue values. Considering this, these two trials represent minimal optimal natamycin residue amounts on aged fruit. As expected with deliberate inoculation, the control treatments for these experiments had a disease incidence of 98-100% (Table 1). This represents a severe situation that typically would represent higher incidence than would occur

in a commercial setting. None-the-less, the 500 ppm and 1000 ppm natamycin dip treatments reduced Mucor rot by an average of 45-57% after three weeks.

Figure 1. 'D'Anjou' pears grown near Cashmere, WA in the natamycin application rate trial performed shortly after harvest. The small spots in the center of the fruit are the site of wound inoculation with the Mucor rot organism, *M. piriformis.* **A.** Comparison of one replicate of fruit treated with natamycin after three weeks at 31˚F. Results illustrate reduction in incidence and lesion size in fruit treated with 500 or 1000 ppm natamycin; this trial yielded the best overall results at the three-week evaluation timepoint. **B.** One replicate from this trial that was also inspected after four weeks at 31˚F illustrating lesion growth, even in natamycin-treated fruit.

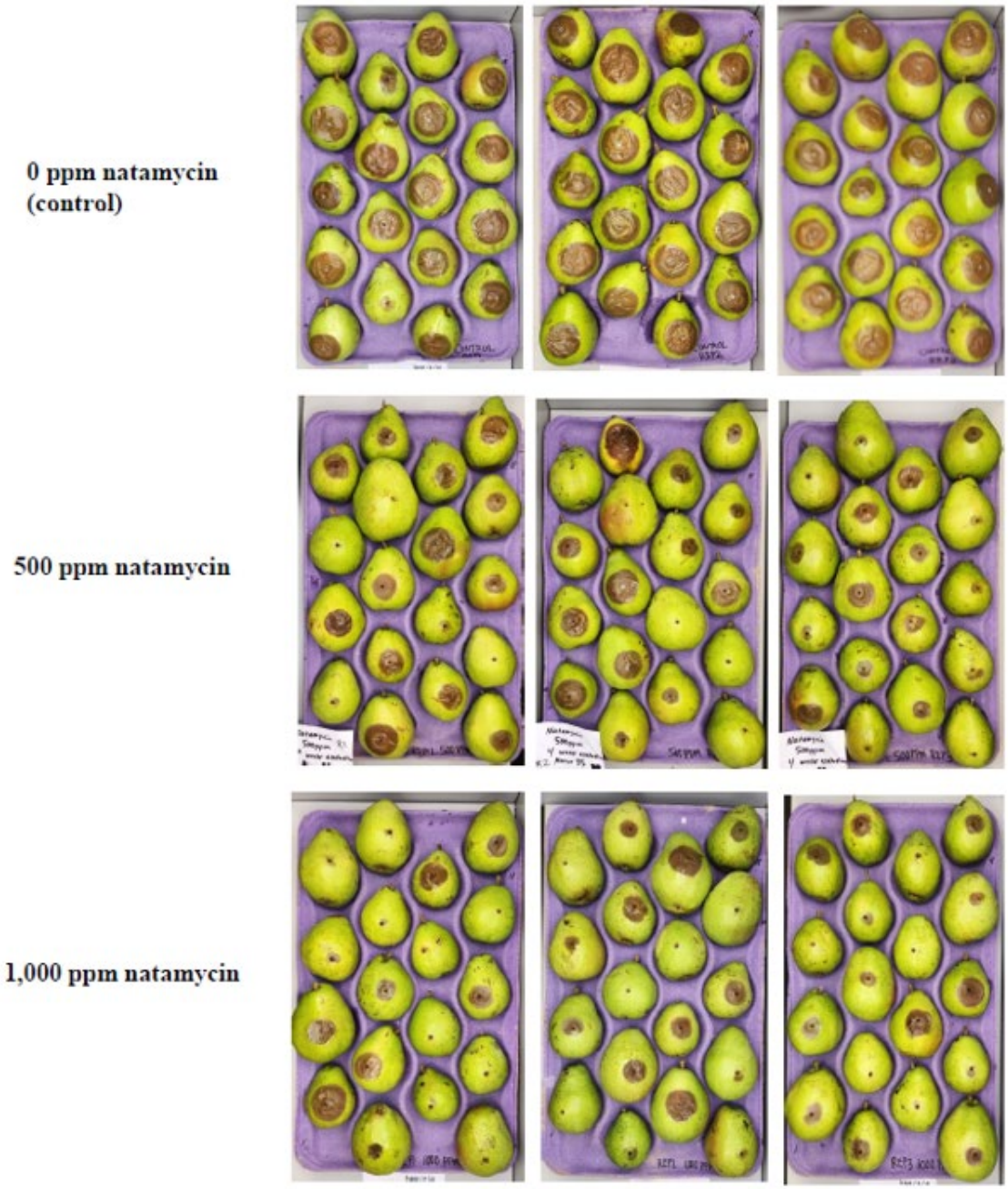

Figure 2. 'D'Anjou' pears from Hood River, OR in the natamycin rate application trial performed shortly after harvest; pictures were taken 3-weeks post-inoculation and subsequent treatment. The small spots in the center of the fruit are the site of wound inoculation with the Mucor rot organism, *M. piriformis*. Results illustrate reduction in disease incidence and lesion size in fruit treated with 500 or 1000 ppm natamycin.

Table 1. Natamycin liquid residue, fruit residue post-application, and infection rates of Mucor rot following *Mucor piriformis* inoculation and natamycin treatment for 'd'Anjou' pears from four separate experiments. Evaluations were conducted three weeks post-inoculation and natamycin treatment.

Location where fruit was grown	Time point (inoculation date)	Natamycin target rate (ppm)	Liquid residue (ppm)	Fruit residue (ppm)	Infection rate (%)[1]
Hood River, OR	At harvest (9/4/2024)	0	-	-	96 (n = 52/54) a
Hood River, OR	At harvest (9/4/2024)	500	391.74	2.99	70 (n = 38/54) b
Hood River, OR	At harvest (9/4/2024)	1000	781.57	5.52	54 (n = 29/54) c
Hood River OR	Five months (2/27/2024)	0	-	-	98 (n = 53/54) a
Hood River OR	Five months (2/27/2024)	500	-	0.30	54 (n = 29/54) c
Hood River OR	Five months (2/27/2024)	1000	-	0.42	46 (n = 25/54) c
Cashmere, WA	At harvest (9/17/2024)	0	-	-	100 (n = 54/54) a
Cashmere, WA	At harvest (9/17/2024)	500	457.61	0.75	30 (n = 16/54) cd
Cashmere, WA	At harvest (9/17/2024)	1000	927.80	1.29	0 (n = 0/54) d
Cashmere, WA	Five months (3/5/2024)	0	-	-	100 (n = 54/54) a
Cashmere, WA	Five months (3/5/2024)	500	-	0.31	57 (n = 31/54) c
Cashmere, WA	Five months (3/5/2024)	1000	-	0.77	41 (n = 22/54) c

1. Chi-square statistical tests. Numbers in columns followed by different letters are statistically different.

Although not statistically different, on average for all combined treatments, the fruit treated with natamycin (BioSpectra 100SC) shortly after harvest had a lower disease incidence (58%) when compared to the fruit that had been treated five-months postharvest (66%). The 1000 ppm natamycin treatments resulted in lower disease incidence in all cases when compared with the 500 ppm treatments. Based on these results, applying the highest label rate of natamycin as soon as possible after harvest may be beneficial for preventative control of Mucor rot, although the duration of control is unknown, as experiments terminated at 3 weeks post-inoculation. However, postharvest Mucor rot incidence varies year-to-year, with risk factors unclear and an area of research need. Additional research incorporating principals of microbial ecology may lend insight into microbially-based *M. piriformis* suppression; for example Silvestri et al. (2020).

Lesion diameters

The width of the rotted area of the fruit surrounding the Mucor-inoculated wound (mean lesion diameters) were generally smaller shortly after harvest, relative to fruit wounded and inoculated five-months after harvest (Fig. 3). The present study did not result in a statistical separation in lesion sizes in response to 500 ppm vs. 1000 ppm treatments with the exception of the Cashmere fruit near-harvest trial, although trends suggest that 1000 ppm treatments reduced lesion diameters relative to 500 ppm treatments; results were also variable given site and storage (Fig. 3 and Fig. 4). The control, 500 ppm, and 1000 ppm treatments had mean lesion diameters of 26 mm (1 inch), 12 mm (0.5 inch), and 10 mm (0.4 inch) for the combined near-harvest testing. The combined five-month post-harvest experiments resulted in average lesion sizes of 29 mm (1.1 inch), 19 mm (0.7 inch), and 19 mm (0.7 inch) for the control, 500 ppm, and 1000 ppm treatments respectively (Fig. 4). In summary, trends from these trials suggest that the highest label rate of natamycin (BioSpectra 100SC) reduced the rate of Mucor rot growth but there was not statistically significant differences in lesion sizes between the 500 and 1000 ppm label rates in most scenarios.

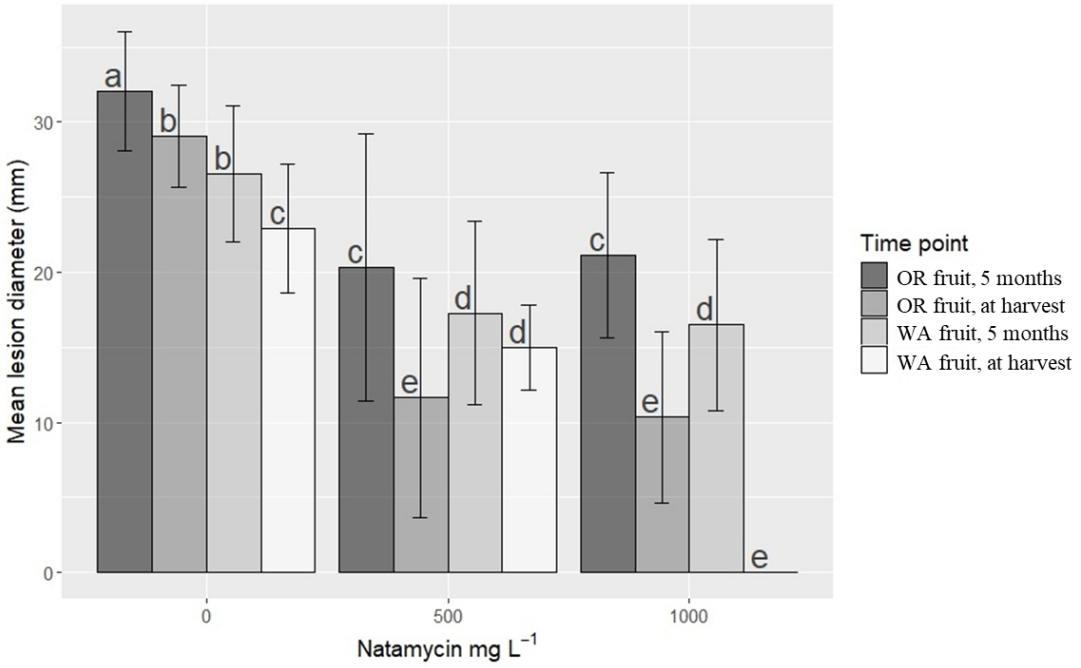

Figure 3. Mean lesion diameter of infected fruit inoculated with *M. piriformis* after three weeks in regular air storage at 31˚F. Values followed by the same letter are not statistically different according to Duncan's multiple range test ($p < 0.05$). 1 mg L^{-1} = 1 ppm.

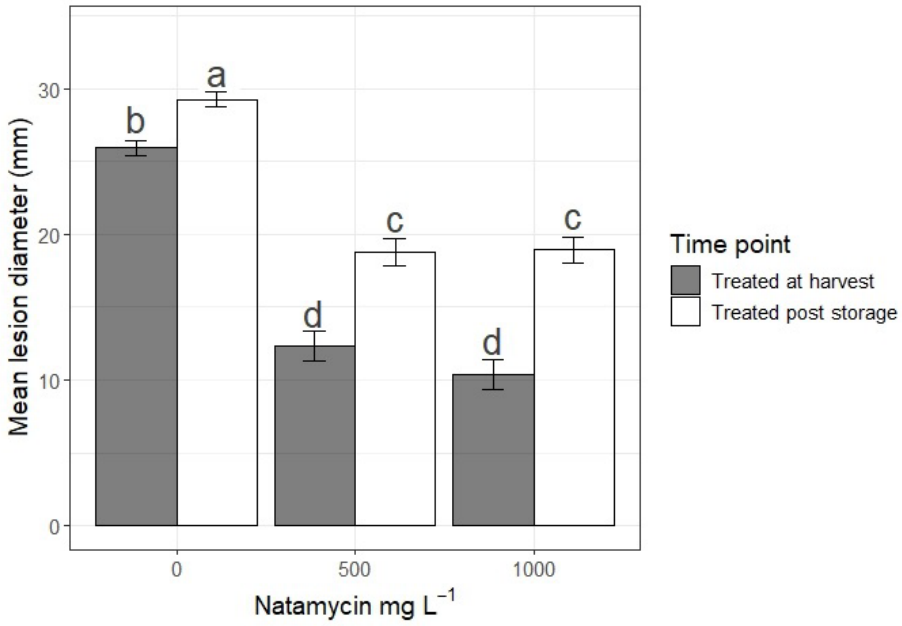

Figure 4. Mean lesion diameter among four combined experiments. Lesions were measured three weeks after inoculations. 'D'Anjou' pears were stored in air storage at 31˚F. Values followed by the same letter are not statistically different according to Duncan's multiple range test ($p < 0.05$). 1 mg L^{-1} = 1 ppm.

Experimental dump tank

The experimental dump tank trials were designed to simulate a pear packing line dump tank where natamycin was added to the water. Chlorine was not used within these trials as natamycin is chemically incompatible with chlorine (Adaskaveg et al. 2019). Further research is needed to determine the cost efficacy of 1000 ppm BioSpectra 100SC in dump tank water at pear packing houses; line spray applications or gas/vapor applications may be more economically feasible.

In agreement with the previous wound inoculation trials, the treatments with the lowest percent of infected fruit (17%) and the lowest percent of wounds infected (6%) occurred in the Cashmere, WA fruit that had been treated with 1000 ppm natamycin (Table 2). Aside from the controls, the fruit with the highest percent of infected fruit (65%) and the highest percent of wounds infected (31%) was observed in the fruit grown in Hood River, OR that was treated with 500 ppm natamycin (Table 2).

When the two dump tank trials were combined, the 500 ppm and 1000ppm natamycin treatments reduced the average percent of fruit infected by 53% and 72% respectively. Experimental dump tanks treated with 500 ppm and 1000 ppm natamycin decreased the average percentage of infected wounds by 82% and 86%, respectively.

Table 2. Average natamycin residues in experimental dump tank solutions, percent of fruit infected (any one or more of four wounds infected), and percent of wounds infected. Fruit were dipped in experimental dump tank for 10 seconds. Lesions were determined after an incubation for 6 days (Hood River, OR fruit) and 7 days (Cashmere, WA fruit).

Location where fruit was grown	Natamycin target rate (ppm)	Mean natamycin liquid residue (ppm)	Mean natamycin fruit residue (ppm)	Percent of fruit infected (%)[1]	Percent of individual wounds infected (%)[1]
Hood River, OR	0	-	-	100 (n=54/54) a	97 (n = 209/216) a
	500	434.93	0.48	65 (n = 35/54) ab	31 (n = 66/216) b
	1000	925.85	1.27	39 (n = 21/54) b	17 (n = 37/216) bc
Cashmere, WA	0	-	-	96 (n = 52/54) a	83 (n = 179/216) a
	500	547.43	0.75	30 (n = 16/54) b	11 (n = 23/216) c
	1000	967.09	1.60	17 (n = 9/54) c	6 (n = 12/216) c

1. Chi-square goodness-of-fit statistical tests. Numbers in columns followed by different letters are statistically different.

Natamycin combined with other postharvest chemicals

A small-scale trial was conducted testing natamycin in combination with the post-harvest chemical propiconazole (Mentor 45WP). More thorough trials testing propiconazole and other postharvest products with larger sample sizes and more detailed results will be presented at the research review (February 20, 2025) but are not available in this report due to the submission deadline. Testing from the original proposal was going to involve testing natamycin with postharvest wax however these plans were changed after learning that in some packinghouses post-harvest waxes are used on red ‘d’Anjou’ pears but are not commonly used on green ‘d’Anjou’ pears (industry communication). Furthermore, while the lab results have demonstrated a certain amount of initial Mucor rot suppression via natamycin (BioSpectra 100SC), high Mucor rot disease variability year-to-year, as well as the potentially limited term fungistatic nature of natamycin may necessitate additional disease control strategies. As a result, we propose to continue testing BioSpectra 100SC in combination with additional products such as BioSave, Mentor, and Aureo Shield. We will then move on to testing these products in combination with postharvest waxes to demonstrate the best strategy for combinations of post-harvest chemicals to be used for controlling Mucor rot.

Fruit quality

Within the fruit quality evaluations there were no statistically significant differences between the fruit weight and I_{AD} in response to treatment, and while there were effects on firmness and titratable acidity, result were not consistent throughout the experiments (Table 3, 4, 5, 6). Fruit quality near-harvest for fruit from Hood River, OR (Table 3) suggests a slight reduction in firmness in response to natamycin dip application, but this result is not consistent (Tables 4, 5, and 6). Similarly, titratable acidity was slightly lower for natamycin treated fruit near-harvest for the Cashmere, WA location (Table 4), but this result is also inconsistent (Tables 3, 5, and 6).

Fruit quality may have contributed to the statistically significant differences between the trial with the least amount of Mucor infection (Cashmere, WA at harvest) and the trial with the most amount of Mucor rot infection (Hood River, OR, at harvest) (Table 1). The fruit from Cashmere was smaller and firmer at harvest with an average weight of 206 grams and an average firmness of 13.3 lbs. at harvest. The fruit from Hood River, OR had an average weight of 217 grams with an average firmness of 14.4 lbf (Table 3, 4). Spotts (1985) reported that immature 'd'Anjou' pears are generally more resistant to decay, but the present results (two sites) do not conform to this observation, as the firmness of 13.3 lbf (less firm) would suggest that the fruit from Cashmere, WA were more mature at harvest. Additionally, 13% of the fruit from the Hood River, OR at harvest trial was reported to have had cork spot which likely also contributed to the advanced decay. There was no phytotoxicity observed within any of the fruit quality evaluations with less than 1% scald or scuff reported.

Table 3. Fruit quality for at harvest 'd'Anjou' pears treated with natamycin compared with untreated control. Fruit was grown at Hood River, OR and harvested on 9/4/2024. Natamycin treatments and day 0 fruit quality evaluations were conducted on 9/4/2024. Fruit was stored in cold storage at -0.5°C ± 0.5°C (31°F). Follow-up fruit quality evaluation was conducted on 9/25/2024.

Treatment	Days post-treatment	Weight (g)	I_{AD}[1]	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	0	223.8 ns[2]	1.9 ns	15.3 ac[3]	11.9 ns	0.36 a
	21	212.0 ns	1.8 ns	14.1 b	11.9 ns	0.37 a
Natamycin (500 ppm)	0	214.2 ns	1.9 ns	14.8 ab	11.9 ns	0.38 a
	21	209.9 ns	1.8 ns	13.9 c	11.9 ns	0.35 a
Natamycin (1000 ppm)	0	213.3 ns	1.9 ns	14.7 abc	11.9 ns	0.24 b
	21	214.5 ns	1.9 ns	13.8 c	11.9 ns	0.36 a

1. Higher values indicates more green.
2. ns, not significant.
3. Where the overall model was significant (p <0.05), Tukey's *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

Table 4. Fruit quality for at harvest 'd'Anjou' pears treated with natamycin compared with untreated control. Fruit was grown near Cashmere, WA and was harvested on 9/14/2024. Fruit was stored in cold storage at -0.5°C ± 1.5°C (31°F). Natamycin treatments and fruit quality evaluations were completed on 9/17/2024.

Treatment	Weight (g)	I_{AD}	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	207.4 ns[1]	1.7 ns	13.6 ns	12.8 ns	0.37 a[2]
Natamycin (500 ppm)	208.7 ns	1.7 ns	13.4 ns	13.1 ns	0.25 c
Natamycin (1000 ppm)	201.2 ns	1.7 ns	12.9 ns	12.8 ns	0.30 b

1. ns, not significant.
2. Where the overall model was significant (p <0.05), Tukey's *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

Table 5. Fruit quality evaluation for Hood River, OR fruit used in the experimental dump tank trial. The fruit was commercially harvested on 9/8/2024 and kept in cold storage at -0.5°C ± 1.5°C (31°F). Natamycin treatments occurred on 10/22/2024. Fruit was left at room temperature for one week with a follow up fruit quality evaluation conducted on 10/29/2024.

Treatment	Days post-treatment	Weight (g)	I_{AD}	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	0	220.3 ns[1]	1.8 ns	13.5 a[2]	6.9 a	0.30 a
	7	228.0 ns	1.6 ns	8.1 b	7.1 b	0.27 ab
Natamycin (500 ppm)	7	205.3 ns	1.6 ns	8.2 b	8.2 b	0.25 b
Natamycin (1000 ppm)	7	225.5 ns	1.7 ns	10.3 b	10.3 b	0.29 a

1. ns, not significant.
2. Where the overall model was significant (p <0.05), Tukey's *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

Table 6. Fruit quality evaluation for 'd'Anjou' pears grown near Cashmere, WA that were used in the experimental dump tank trials. Fruit was harvested on 9/14/2024 and was stored in cold storage at -0.5°C ± 1.5°C (31°F). Experimental dump tank trials were conducted on 11/5/2024. Fruit was left at room temperature with a fruit quality evaluation being conducted on 11/13/2024.

Treatment	Weight (g)	I_{AD}	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	168.1 ns[1]	1.5 ns	3.1 a[2]	2.9 a	0.25 a
Natamycin (500 ppm)	165.7 ns	1.5 ns	5.6 b	5.7 b	0.27 b
Natamycin (1000 ppm)	180.5 ns	1.6 ns	4.9 b	4.9 b	0.26 ab

1. ns, not significant.
2. Where the overall model was significant (p <0.05), Tukey's *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

References

Adaskaveg, J. E., Förster, H., & Chen, D. (2019). Positioning natamycin as a post-harvest fungicide for citrus. Citrograph, 10(4), 62-65.

Boonyakiat, D., Chen, P. M., Spotts, R. A., & Richardson, D. G. (1987). Effect of harvest maturity on decay and post-harvest life of ‘d'Anjou’ pear. Scientia Horticulturae, 31(1-2), 131-139.

Kim, Y. K., Kwak, J. H., Smilanick, J. L., & Fassel, R. (2017). BioSpectra 100SC™ 100SC: a new biorational fungicide to control postharvest diseases of fruits. In IV International Symposium on Postharvest Pathology: Next Generation Innovation and Commercial Solutions for Postharvest 1323 (pp. 111-118).

Saito, S., Wang, F., & Xiao, C. L. (2023). Baseline Sensitivity of Mucor piriformis to Natamycin and Efficacy of Natamycin alone and in Combination with Salt and Heat Treatments against Mucor Rot of Stored Mandarin Fruit. Plant Disease, 107(11), 3602-3607.

Silvestri, L., Sosa, A., Mc Kay, F., Vitorino, M. D., Hill, M., Zachariades, C., ... & Mason, P. G. (2020). Implementation of access and benefit-sharing measures has consequences for classical biological control of weeds. BioControl, 65, 125-141.

Sholberg, P. L., and G. R. Owen. (1991). Populations of propagules of Mucor spp. during immersion dumping of Anjou pears. Canadian plant disease survey, *71*(1), 33-35.

Spotts, R.A., (1985). Effect of preharvest pear fruit maturity on decay resistance. Plant Dis., 69: 388-390.

Executive summary

Title: Efficacy of Natamycin for Control of Mucor Rot in Pear Fruit

Keywords: ‘d’Anjou’ pears, BioSpectra 100SC, Mentor, natamycin, Mucor rot

Abstract:

Mucor rot is caused by the pathogen *Mucor piriformis*, which can be responsible for severe post-harvest decay in pears. For this study, the efficacy of BioSpectra 100SC (active ingredient natamycin) was tested in a laboratory setting. Testing was conducted at harvest and at five-months post-harvest. The most effective treatment was found to be the highest label rate of BioSpectra 100SC (1000 ppm natamycin) applied at harvest which reduced Mucor rot infection by 73%. Applying BioSpectra 100SC as soon as possible after harvest was more effective than applying later in the storage season. The 1000 ppm natamycin treatments resulted in lower numerical disease incidence and smaller average lesion diameters when compared to the lowest label rate of BioSpectra 100SC (500 ppm natamycin) when the at harvest and at five-month postharvest trials were combined and averaged. Additional laboratory trials were also conducted to determine the amount of disease incidence incurred in ‘d’Anjou’ pears when BioSpectra 100SC and *Mucor piriformis* inoculum are combined in an experimental dump tank. On average, the dump tanks with 500ppm and 1000ppm natamycin treatments reduced the average percent of fruit infected by 53% and 72% respectively. The fungicide Mentor 45WP combined with the highest label rate of BioSpectra 100SC reduced Mucor rot by 22% in a small preliminary trial. Further testing of BioSpectra 100SC in combination with other post-harvest chemicals is proposed for 2025-2026. This research has improved our understanding of the efficacy of natamycin for controlling Mucor rot on ‘d’Anjou’ pears in a laboratory setting. BioSpectra 100SC is currently the only commercially available postharvest fungicide that we are aware of that has been shown to help reduce Mucor rot incidence. Aside from the Mucor rot control, BioSpectra 100SC can be used as a rotational fungicide to help reduce fungicide resistance as according to the label BioSpectra 100SC also helps to control blue and grey mold.

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

Report Type: Final Project Report

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Cooperators: None

Project Duration: 3 Year

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

Other related/associated funding sources: Requested
Funding Duration: 2025 - 2029
Amount: $ 4,000,000+
Agency Name: USDA SCRI
Notes: Title: Integrating multidisciplinary and translational approaches to manage postharvest rots on apples and pears in major U.S. pome fruit growing regions. All three PIs are listed as co-PIs on this project.

Other related/associated funding sources: Requested
Funding Duration: 2025 - 2028
Amount: $640,000
Agency Name: USDA NIFA

Notes: Title: Leveraging diverse germplasm resources to develop breeding tools for postharvest rot resistance in pome fruit. PI Gottschalk is lead PI for this proposal and co-PI Collum is listed as co-PI.

WTFRC Collaborative Costs:

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

Footnotes:

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

Footnotes:

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

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

Footnotes:
Objectives: Our project has four objectives that complementarily address the evaluation of pear germplasm for post-harvest traits. The first objective is to evaluate the USDA Pear Collection for optimal harvest and storage time for 50 high-value genotypes. We proposed using two germplasm sources to acquire 50 genotypes: 1) USDA Pear Collection at the USDA ARS National Clonal Germplasm Repository (NCGR) in Corvallis, OR, which contains nearly 2,300 unique pear cultivars, breeding lines, and hybrids that represent 36 species and 2) the USDA ARS Appalachian Fruit Research Station (AFRS) breeding program in Kearneysville, WV. The aims are to evaluate the lines for harvest dates, storage requirements, and the presence/absence of post-harvest diseases. We are approaching the disease evaluations in a two-step process. First, evaluate the fruit for natural infections and the classification of pathogens present. Second, conduct resistance testing by inoculating the genotypes found to be free of natural infection for resistance to the identified pathogens. The second objective is to characterize the 50 high-value genotypes for fruit quality, attributes including total soluble solids, acidity, polyphenolic content, texture, peel and flesh color, and overall grade. This objective aims to characterize fruit quality traits using two approaches, destructive and non-destructive, correlate their measures, and develop models used to predict the destructive trait measurements using the non-destructive equipment in the future. The third objective is to challenge the 50 high-value genotypes in simulated supply-chain stress to document resistance to bruising, scuffing, and puncturing. This objective aims to identify germplasm that can withstand the intense forces that are exerted on the fruit during the supply-chain process. However, we have found that fruit received from NCGR pear collection undergoes shipping stress and upon receipt can exhibit real-life damage. We have deviated from our initial objective here to take qualitative measures from the NCGR fruit since it has already been subjected to the planned stresses. Genotypes that exhibit

damage are noted and the damage type is described. The fourth objective is to document and distribute findings through publications and presentations regarding the resistance of the 50 high-value genotypes to storage disorders and diseases. The aim here is to provide communication with the stakeholders and provide any products developed from the analyzes as impactful tools for evaluation of post-harvest traits in pear.

Significant Findings

Objective 1:

- Evaluated over 100 unique genotypes for harvest date and conditioning requirement.
- Evaluated 50+ genotypes for susceptibility to *Penicillium expansum* and *Colletotrichum fioriniae* (45 from NCGR and 46 from AFRS).
- Identified seven genotypes that were significantly less susceptible to *P. expansum* and *C. fioriniae* compared to 'Bartlett' and 'Gem' in 2024.

Objective 2:

- Identified numerous genotypes associated with large fruit size, high sugar content, and high acidity.
- Identified the range of tannic acid in *Pyrus communis,* including content levels associated with standard cultivars.

Objective 3:

- Conducted a first of its kind phenotyping assay to determine supply chain resilience, which identified a two potential sources of resiliency.

Methods

Objective 1: We identified high-value germplasm from historical texts, the USDA GRIN database, and recommendations from germplasm curators and previous breeders. The terms that were used as queries in the literature search for desirable genotypes included disease-resistance (fire blight, *Monolinia*, and post-harvest pathogens), ships well, excellent flavor, keeps well, fruit quality, acidic, phenolic (non-perry), early ripening, late-ripening, and tree-ripe. Following bloom and prior to the fruit ripening period, crop load was estimated from each tree to determine if the minimal fruit number need for all analyses was available. For harvest timing, our initial approach was to select five randomly selected fruit from each tree were collected weekly. Each fruit was cataloged for color development and underwent firmness testing using a penetrometer with a measurement taken from the sun-exposed and shaded side of the fruit following removal of the peel. A genotype will be determined as harvest-ready when firmness decreases to an average of 20 lbf, and color development has reached its peak. We have found that the simple approach of lifting the pear(s) on a branch from the bottom of the fruit, with a minimal force that resulted in release, the pear was determined as harvest ripe. Several of the AFRS breeding lines correlated with known harvest dates using this approach as opposed to decreases in firmness. Moreover, during the first year of harvest date phenotyping, we found many of the varieties when picked at 20 lbf did not ripen in storage to a sufficient lower firmness level (3 lbf). This result suggests that we were picking fruit too immature. We have modified our harvesting approach to using this more simplistic ease-of-release from the branch to indicate harvest timing. Potentially, this result is due to the hybrid (*Pyrus* spp.) origins of many of the breeding lines at AFRS. We have applied this approach to the NCGR sourced fruit as well which were collected and phenotyped during the 2023 and 2024 seasons.

Each genotype then had 75 fruits, or the maximum available, harvested and packed into 40 lbs fruit boxes and stored at USDA AFRS in a new cold storage unit. For the NCGR fruit, harvested pears were wrapped in a Styrofoam fruit wrapper and placed into trays and packaged into boxes for shipping. Overall, this approach maintained the integrity of many of the shipped genotypes. However, some genotypes were found to still be susceptible to the shipping forces (bruising, scuffing, and punctures) and were damage upon receipt even though significantly protected during the shipping

process. The boxes of fruit were kept in cold storage at 30˚F and 90-98% relative humidity. At ten days to biweekly intervals, starting at two weeks in storage to 12 weeks or until ripe, three randomly selected fruit will be taken out of storage and rested at room temperature for 24 - 48 hours. Following the acclimation period, the selected fruit was tested for firmness using a penetrometer. The genotypes were considered ripe when average firmness reaches 3 lbf or less.

A total of 91 pear genotypes (45 from NCGR and 46 from AFRS) were directly challenged with *Penicillium expansum* or *Colletotrichum fioriniae* using a wound inoculation method. Depending on fruit availability ten or twenty fruits from each genotype were inoculated with each pathogen. Fruits were harvested at maturity and inoculated within a week of harvest. On the day of inoculation, fruits were removed from cold storage and allowed to acclimate to room temperature. All fruits were surface sterilized with 70% ethanol and allowed to dry in a laminar flow hood. For *P. expansum* experiments, fruit was wounded with a 3 mm x 3 mm wounding tool and the plug was removed. A conidial suspension was prepared from a 7-day culture of *P. expansum* isolate MD-8 by flooding the plate with sterile distilled water plus Tween-20 and the concentration was adjusted with a hemacytometer to $1x10^4$ conidia/mL. 25 µl drops of the suspension were placed in the wounds with a repeating syringe. For *C. fioriniae* experiments, fruit was wounded with a 4 mm cork borer and the plug was removed. Corresponding plugs were punched from a 7-day culture of *C. fioriniae* isolate WV-223 with the same 4 mm cork borer. *C. fioriniae* plugs were placed mycelium side down into the fruit wounds. For all experiments, inoculated fruits were stored in covered fruit bins at room temperature and lesion diameters were measured at 3-, 5-, and 7-days post inoculation.

Objective 2: We originally proposed using twelve randomly selected pears from each genotype, that are identified as at an optimal eating quality following storage, to be used to evaluate fruit quality traits. However, limited crop loads, higher soft scald incidence, an outbreak of *Fabraea* leaf spot at AFRS, and longer cold condition sampling time points than anticipated required the decrease of the number of replicates to five for this objective. The five fruits first underwent size (length, diameter, and weight) and shape (qualitative) measures. Following non-destructive measurements, all five of the replicate fruit per genotype were analyzed using Near-infrared (NIR) Produce Quality Meter (Felix Instruments). After NIR measurement, each replicate pear was processed to extract juice using a Good Nature M-1 Fruit Grinder and Press. The extracted juice was frozen and underwent measurements for TSS (ATAGO PAL-1), TA and pH (Orion Star T910 Autotitrator), and total polyphenolic content (Folin-Cointreau; absorbance using a spectrometer) using industry-standard measurement methods during the winter months. The data obtained from the NIR meter and industry-standard methods will be inputted into Felix Instrument's model-building software to develop and validate models for the NIR meter for future use. Our initial plan was to use the NIR meter as the sole instrument used to determine all fruit quality metrics except for a juice extraction to determine polyphenolic content in years two and three. However, due to limited availability of fruit from each genotype consistent between years we continued to perform the destructive phenotyping. By collecting more of the ground truth measurements through destructive sampling will only increase our power in training accurate and predictive models using the NIR meter. Due to the limited replicate fruit, we were unable to conduct a sensory evaluation using a trained three-person panel consisting of staff at AFRS.

Objective 3: We will evaluate each genotype for resilience to stress associated with the supply chain including bruising, puncturing, and degradation severity estimation. This objective began during the 2024 season due to the limited fruit available during the 2022 and 2023 seasons and the need to identify the cold conditioning requirements for each genotype across two consecutive years to predict the timing more accurately for evaluations. Additionally, we have found the fruit shipped from NCGR is already subject to real-world shipping stress. As a result, we are modifying this objective to quantitively document damage to fruit received from NCGR. As for fruit obtained from AFRS, when a genotype has acquired one or two year of storage data, it will be selected for evaluation when excess

fruit is available. We were able to repair and utilize a robot arm to simulate container loading and unloading which would cause bruising along with puncture wounds. The robotic stress was applied by having the robot's arm traverse a horizontal space at speed setting that mimics truck movement on the roadway and a drop treatment that covers a distance of 600 mm in < 1 sec. The robotic-associated testing occurred at AFRS under the guidance of Dr. Amy Tabb who has performed similar simulations. To evaluate the fruit under these two conditions, eight replicated fruits were randomly selected for each genotype. Fruit was then placed into a cardboard box with a trimmed down cardboard fruit insert on both the top and bottom sides (clam shell). On top of the upper fruit insert, a sheet of one cm diameter bubble wrap was used to serve as an additional cushion. Fruit was then enclosed in the box with the packaging and placed onto the robot arm. The fruit was then subjected to 30 mins of the shaking and five simulated drops in succession. Following the stress, the fruit was rested for four to five days at room temperature and then evaluated for presence/absence of bruising, puncture, and degradation severity. The same quantitative measures were taken from the NCGR shipped fruit.

Objective 4: The results gained from Objectives 1-3 will be presented and distributed to the research community and stakeholders.

Results and Discussion

Objective 1: The identification of 50 high-value varieties from historic literature was successful. We additionally, were able to properly re-identify 60+ genotypes in the historic AFRS germplasm. Unfortunately, in year one, a minor frost in the spring of 2022 and biennial bearing habits extremely limited the fruit available for the NCGR. In 2022, we obtained harvest dates for 43 genotypes all sourced from AFRS. In 2023, we obtained harvest dates for 69 genotypes. Of those 69, 29 were collected from the NCGR and remaining 40 from AFRS. In 2024, we obtained harvest dates for 113 genotypes. Of those 113, 49 were collected from the NCGR and remaining 64 from AFRS. Across the three years of this project, 43 genotypes were collected for a single season and 76 genotypes were collected over two or three seasons. In total, we evaluated a combined 129 unique genotypes across the three years of this project. This result represents a 258% increase in the genotypes evaluated over what was proposed. The measurement and documentation of condition requirement was successful in each year of the project. However, the total number of genotypes with conditioning requirement evaluations done was less than what we obtained for harvest date. This limitation stems from complexities to ripening fruit. For example, some genotypes were unable to meet the target firmness to be called "conditioned" because they developed storage disorders such as scald and decomposition. In 2022, we documented conditioning requirements for 32 genotypes. In 2023, we documented conditioning requirements for 71 genotypes. In 2024, we documented conditioning requirements for 94 genotypes. Across the three years of this project, 52 genotypes have documented conditioning requirements based on a single season observation and 63 genotypes have documentation for two or three seasons. In total, we documented a combined 115 unique genotypes across the three years of this project. We were able to document condition requirement for 89% for the genotypes we harvested.

For the past three seasons, we have documented harvest date and cold conditioning requirements for >100 unique genotypes. We have documented a strong peak in harvesting dates for pears between 225 and 275 days into the calendar year (August 13th – October 2nd) (**Fig. 1A**). The mean harvest date for pears evaluated in this study was on the 245th day of the year (September 2nd). However, a few varieties were found to be harvesting after October 2nd and represent extremely late ripening genotypes. The cold conditioning requirements for the pears evaluated in this study ranged from as low as 9.5 days to a much as 125 days post harvesting (**Fig. 1B**). A peak in conditioning requirement was observed between 25 and 50 days. The mean requirement for conditioning was ~47 days. Although weakly correlated (Kendall's $\tau = 0.2$), harvest date was significantly associated with the cold conditioning requirement (**Fig. 1C**). We found many genotypes that were exceptions to this

trend - harvested relatively early yet required extensive condition time to reach desirable firmness. These genotypes include varieties such as ‘Talgarskaya Krasavitza’ and ‘Giant Seckel’ and breeding lines NJ Rock R27 T65 and US 84909-184. These genotypes could serve the purpose to breed for conventional harvest dates with long conditioning requirements, resulting in longer marketing window for pear. Alternatively, several genotypes were identified as having short conditioning requirements and represent more ideal material for direct-to-market applications and breeding objectives. These genotypes included varieties such as ‘Bell’, ‘Mac’, ‘Summercrisp’, and breeding lines such as NY 10355 and US 84907-078. Furthermore, varieties such as ‘Passe Crassane’ and ‘Marie Louise’ could be used to target late harvest dates but low conditioning requirements. Testing if harvest date and conditioning requirement is predictively inherited needs to be tested.

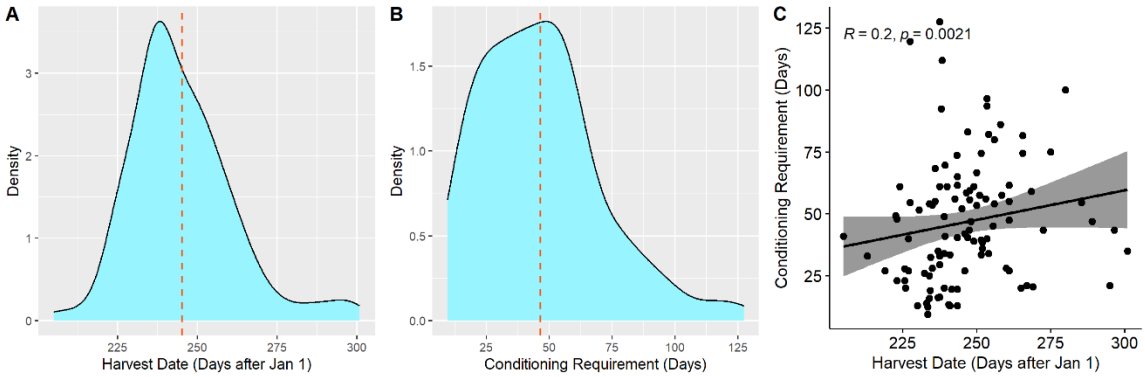

Figure 1. A) Variation in harvest date for pear germplasm. B) Cold conditioning requirements in pear germplasm. C) Correlation plot between harvest and cold conditioning requirement. Red dashed lines indicate the mean. Correlation significance test conducted using Kendall's τ test.

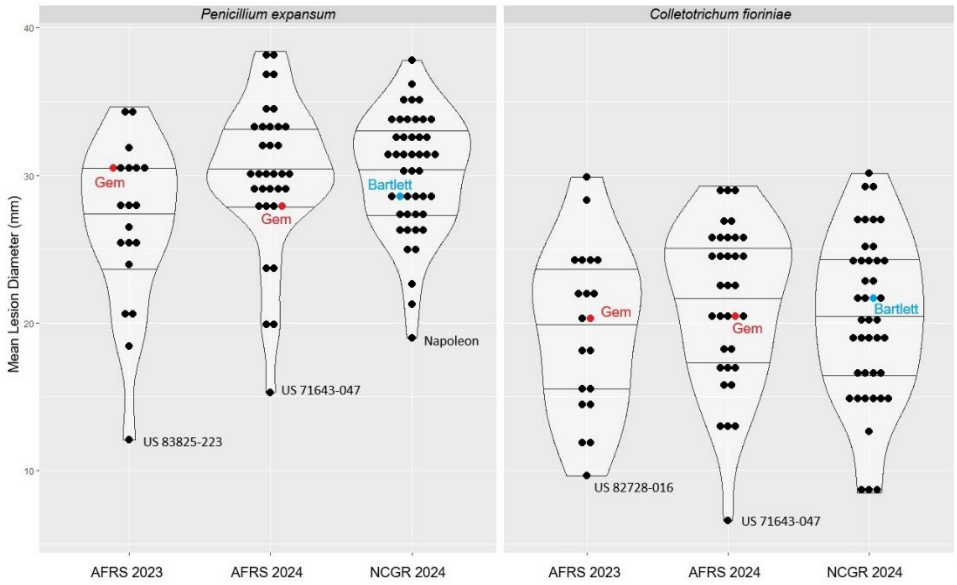

Figure 2. Variation in mean decay lesion diameter in pear germplasm 7 days after inoculation with *Penicillium expansum* or *Colletotrichum fioriniae*. The mean lesion diameter of each pear genotype tested in 2023 and 2024 is represented by a dot. Lines indicate the lower quartile, median, and upper quartile. Control genotypes ‘Gem’ and ‘Bartlett’ are indicated by red and blue dots respectively.

A total of 91 genotypes (45 from NCGR and 46 from AFRS) were directly challenged with *P. expansum* or *C. fioriniae*. Eight genotypes from AFRS were tested in both 2023 and 2024. 'Gem' and 'Bartlett' were included as controls that are highly susceptible to both *P. expansum* and *C. fioriniae*. The mean decay lesion diameter after 7 days ranged from 12.05 mm to 38.40 mm for *P. expansum* and 6.58 mm to 30.11 mm for *C. fioriniae* (**Fig. 2**). Location where pear germplasm was harvested (NCGR or AFRS) and year tested (AFRS 2023 vs. AFRS 2024) did not have a significant effect on mean decay lesion diameters (**Fig. 2**). We found seven genotypes had significantly reduced lesion sizes when challenge with *P. expansum* compared to 'Bartlett' in 2024. These included three from NCGR ('Golden Spice', 'Riehl Best', and 'Napoleon') and four from AFRS (US 71643-047, US 79439-004, US 83825-223, US 99422-202) (**Fig. 3**). All genotypes that were identified as significantly less susceptible to *P. expansum* in 2024 were also significantly less susceptible to *C. fioriniae* except for Napoleon which was not challenged with *C. fioriniae* due to limited fruit. Napoleon has previously been reported to be resistant the pathogen *Monilinia.* Two of the identified genotypes (US 83825-223 and US 79439-004) were also tested the previous year. In 2023, US 83825-223 was also significantly less susceptible to *P. expansum* and *C. fioriniae* compared to 'Gem' and 'Bartlett', while 79439-004 was significantly less susceptible to *C. fioriniae* but not *P. expansum*. We plan to retest genotypes that had significantly less decay in 2023 or 2024 again in 2025 to generate at least 2 years of data for each genotype.

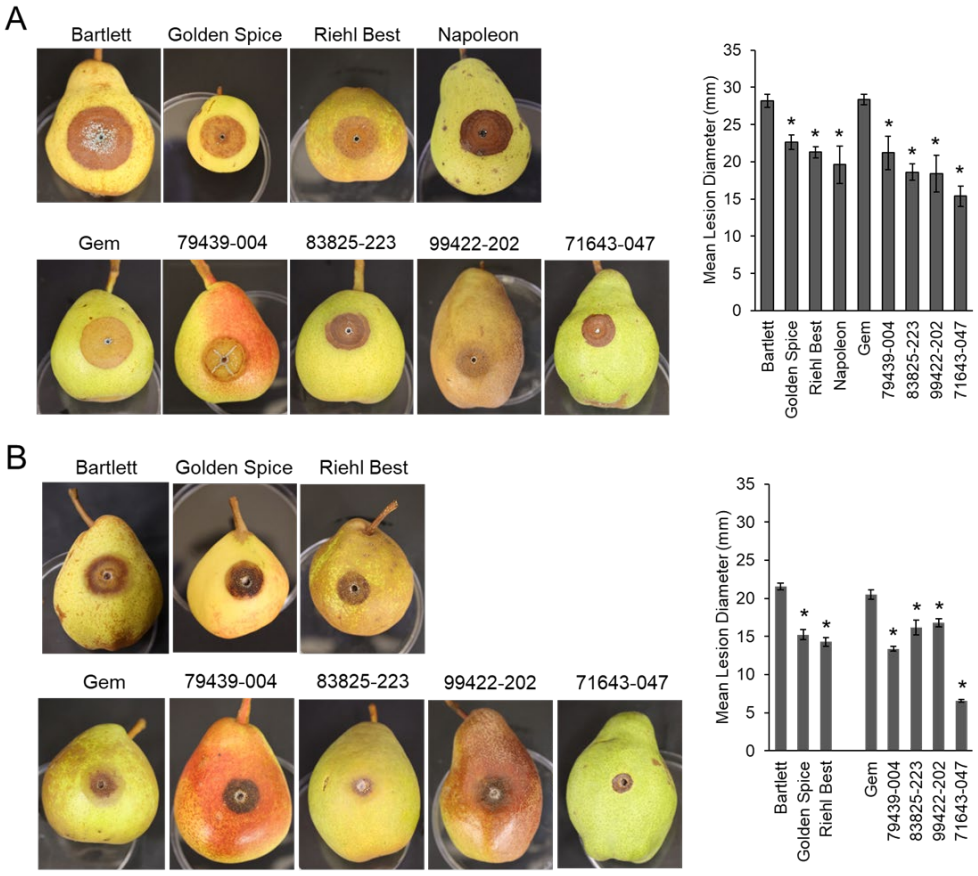

Figure 3. Wound inoculation of pear genotypes with *Penicillium expansum* (A) or *Colletotrichum fioriniae* (B). A representative image of lesion development 7 days post inoculation is shown for selected pear genotypes inoculated in 2024. Bars represent the mean lesion diameter (mm) ± standard error. A * indicates a significant difference of $p < 0.05$ compared to Bartlett using a one-way ANOVA followed by post-hoc Dunnett's test.

Objective 2: We have documented fruit size measurements for all three years of this study. These measurements included length, diameter, and weight. As expected, we observed a correlation between weight and the other two measurements (**Fig 4**). We have successfully selected a wide range of variation in these measurements within the germplasm. The longest genotypes we've identified are breeding lines US 71643-047 and US 67251-045. We also observed that breeding lines with more recent hybridization with Asian species tend to be larger in diameter and weight such as NJ 12, NJ 15, and ILL-2ON-028. Additional breeding lines were identified as being relatively high in weight and more similar in length vs diameter measurements (symmetrical) such as advanced selection US 84907-166. Regarding NCGR genotypes, we observed varieties with desirable measurements that could be used to breeding for size. For example, 'Beurré Clairgeau' and 'Marie Louise' are relatively long, 'Bergamotte Arsene Sannier' is large in diameter and weight. Conversely, varieties such as 'Merricourt' and 'Zelinka' are small and then to be elongated, whereas 'Golden Spice' is small but round (**Fig. 4**).

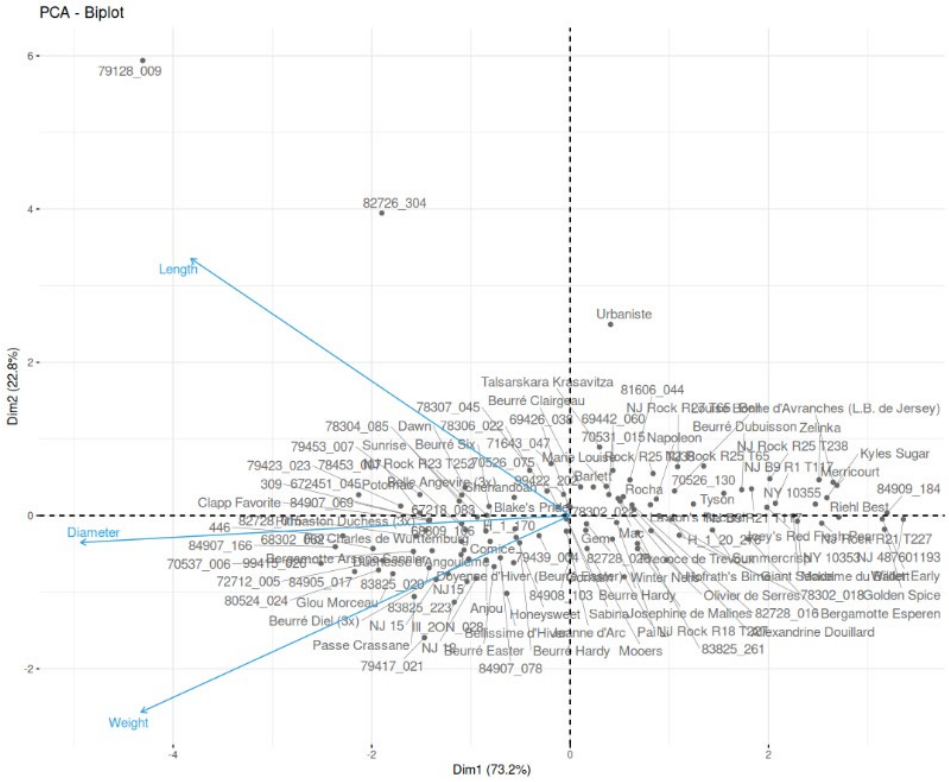

Figure 4. PCA plot of fruit size measurements. Blue arrows represent the direction of the variable as it increases in measurement.

Since 2022, we have analyzed 117 unique genotypes of pear for fruit quality traits. These traits are represented as replicated measurements on a per replicate pear basis for juice yield, total soluble sugar (TSS) content (ºBrix), pH, titratable acidity (TA), and total phenolic content (bitterness). For 67 genotypes, we have documented fruit quality across two or three seasons. The remaining 50 genotypes have only a single season representation. Within this germplasm, we found that the average juice yield was ~89 mL/fruit (**Fig. 5A**). The juiciest varieties were found to be breeding lines including US 99415-026 at 171 mL/fruit. For sweetness, we identified several historic varieties that were nearly two-fold higher than the germplasm's average of ~13 Brix (**Fig. 5B**). These high TSS varieties include 'Louise Bonne d'Avranches' (25.98º Brix), 'Urbaniste' (21º Brix), 'Riehl Best' (20.68º Brix), and 'Olivier de Serres' (19.8 ºBrix). The highest TSS value in the USDA breeding program was US 78302-022 at 14.63º Brix. These historic varieties could serve as new germplasm resources for increasing sweetness in the breeding program. Titratable Acidity (TA) within the germplasm averaged 3.83 g/L **(Fig. 5D**). However, we identified three breeding lines that were three- to four-fold higher than the average (US 70531-015, US 71643-047, and US 82726-304).

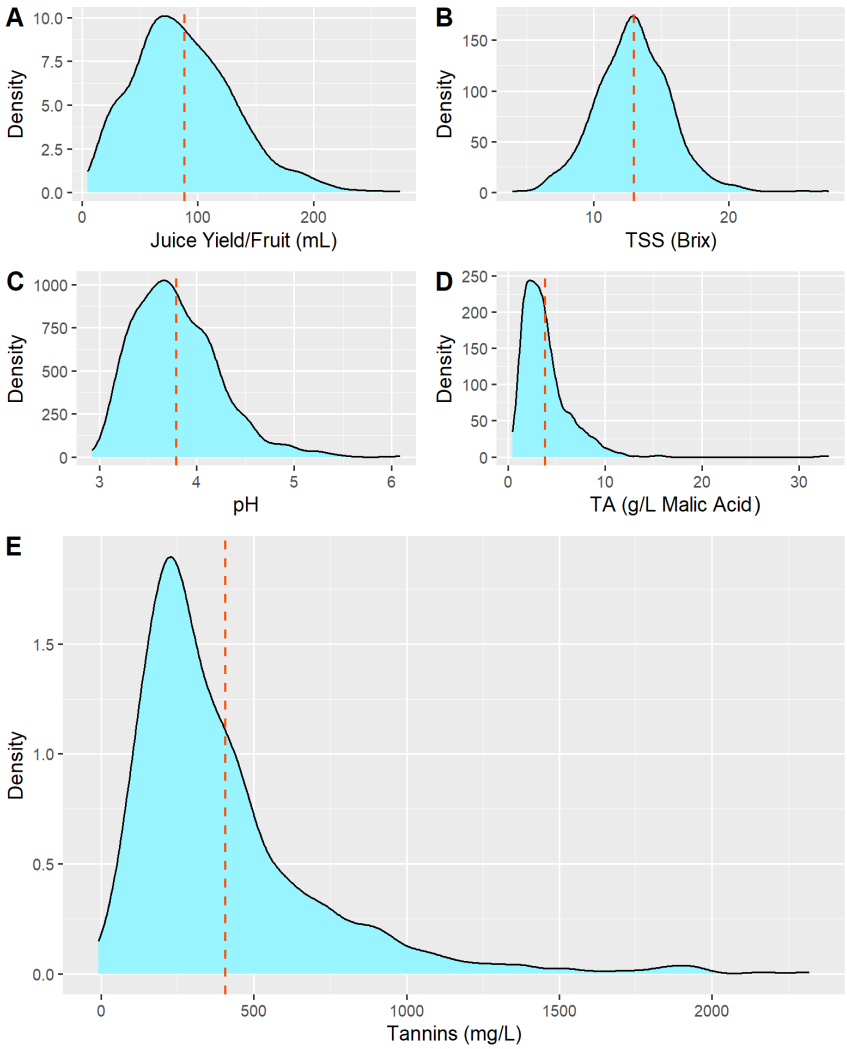

Figure 5. Density plots pear fruit quality metrics A) juice yeild in mL/fruit, B) TSS (Brix), C) pH, D) TA (g/L Malic Acid), and E) Tannins (mg/L).

Higher acidity has been correlated with consumer preference for increased flavor and, thus, represent desirable genetics already present in the USDA breeding program. Lastly, we documented the variation in tannins (i.e. phenolic) content in this germplasm. Tannins contribute to the bitter and astringent flavor profiles and are generally undesirable. We found the average to be ~400 mg/L within the germplasm (**Fig. 5E**). The highest recorded tannin contents belong to historic varieties 'Bellissime d'Hiver' and 'Hofrath's Birne' at >1300 mg/L. A breeding line that is only a generation or two removed from an interspecific hybridization NJ Rock R21 T227 was the second highest at 1326 mg/L and is recognized as the most bitter fruit in the breeding germplasm. Generally, many of the breeding lines were (35 genotypes) were found to have below the average tannin content. This group included the advance testing line US 79439-004 at 197 mg/L. Other industry standard varieties were also identified as containing <400 mg/L of tannins ('Comice', 'Clapp Favorite', 'Gem', and 'Bartlett'). This information on fruit quality is invaluable to aiding in the improvement of pear flavor within the breeding program.

Objective 3: Resilience to physical damage during shipping and handling is a critical trait for pear breeding moving forward. Many of the standard varieties currently produced are highly susceptible to scuffling, punctures, and bruising while they traverse the logistics pipeline. To overcome those limitations, we set out to phenotype germplasm to simulated and actual shipping and handling stress. The simulated stress was conducted using the robot arm programmed to simulate trucking (shaking) and drop forces. Here, we evaluated eight replicated fruits from 38 different genotypes. We evaluated the average damage (bruises/fruit, punctures/fruit, and percent compromised/damage severity) for each genotype (**Fig. 6A**). Over 28 of the evaluated genotypes exhibited some to high susceptibility to damage. However, ten genotypes were found to be resistant with no recorded damage. Of those ten, five were from the same cross/family; US 78302, which is a hybridization of US 56112-146 [US 309 open pollinated] × 'Madame Ernest Baltet'. We fortunately have one of the grand parents in our

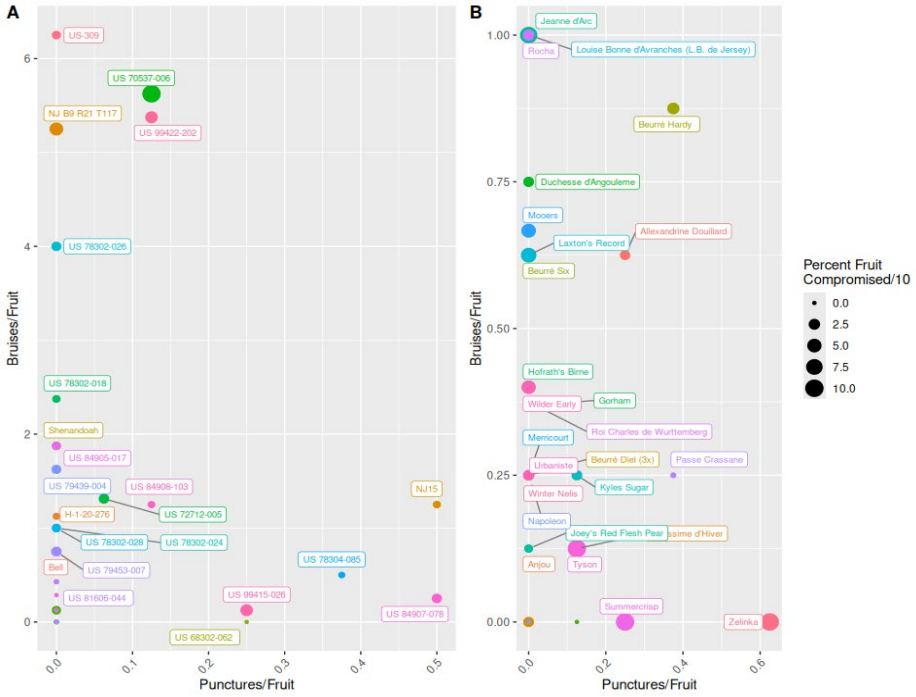

Figure 6. Quantification of buises, punctures, and percent fruit compromised from A) simulated and B) actual shipping stress. Percent fruit compromised is scaled to 10% of acutal values. Fruit with variety names or numbers listed indicate susceptiblity to shipping stress.

germplasm (US 309), which exhibited the highest bruising rate observed (**Fig. 6A**). This result suggests that either the unknown pollen parent of the US 56112-146 or ‘Madame Ernest Baltet’ is providing this resistance, if it’s proven to be genetically controlled/influenced. Further study of this important result is needed. We additionally evaluated 34 of the varieties shipped from USDA NCGR for real-world shipping damage (**Fig. 6B**). Of the 34, only six were found to be free of damage. These included ‘Burre Dubuisson’, ‘Buerré Easter’, ‘Clapp Favorite’, ‘Josphine de Malines’, ‘Marie Louise’, and NY 10353. These varieties represent germplasm material that should be further evaluated for genetic potential in providing shipping and handling resiliency.

Objective 4: Publication and dissemination of these results are forthcoming. We anticipate preparing two or three publications that summarize the results of objectives 1 and 2 in the Fall of 2025. One publication will solely focus on the results obtained related to the disease resistant screening. The other one or two publications will focus on the harvest date, conditioning requirements, and fruit quality metrics. All works will be published in open-access journals and notification of publication will be shared with the respective funding associations and committees. Preliminary results from the natural disease incidence and plant pathogen inoculation testing in Objective 1 were presented at the American Phytopathological Society Annual Plant Health Meeting in July 2024, the American Society for Horticultural Science Annual Conference in September 2024, and at the Cumberland-Shenandoah Fruit Workers Conference in December 2024.

Executive Summary

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

Key words: pears, germplasm characterization, breeding, disease resistance, supply chain resiliency

Abstract: The USDA pear breeding program has spent 100-plus years breeding for increased disease resistance often to the detriment of fruit quality. Having established strong disease resistance in the breeding program, new breeding directions for the program are to improve fruit quality, post-harvest disease resistance, and resiliency to the supply chain. However, information is lacking on these traits in the breeding program and at the USDA pear collection in Corvallis, OR. To fill this gap in knowledge, we set out to evaluate 50 high potential germplasm lines for harvest date, cold conditioning requirement, post-harvest disease resistance, fruit size and quality, and supply chain resiliency. We surpassed our goals and identified harvest date and conditioning requirements for over 100 historic varieties and breeding lines, resulting in the identification of desirable late harvesting and cold conditioning genotypes. We additionally screen over 50 of those genotypes for susceptibility to *Penicillium expansum* and *Colletotrichum fioriniae.* Seven genotypes were found to be significantly resistant compared to commercial varieties ‘Bartlett’ and ‘Gem’. We have also evaluated over 100 genotypes of pear for fruit size and fruit quality. We identified several varieties of pear with sugar content nearly two-fold higher than the average in the germplasm (~20 or more °Brix). Furthermore, we found the USDA breeding program contains several breeding lines with extremely high acid content, a desirable trait for more flavorful eating experience. Lastly, we evaluated over 50 genotypes for supply chain resiliency. We found a family of breeding lines that were all highly resistant to damage in simulated shipping conditions. The pedigree of that family suggests a historic variety - ‘Madame Ernest Baltet’ - could be a source for that resiliency. These results, ultimately, will guide the breeding program in determining desirable crosses for improving fruit quality and postharvest traits.

Project Title: Ultra-low O_2 CA strategies to reduce d'Anjou storage disorders

Report Type: Continuing Report

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Cooperators: Dr. DoSu Park; Emmi Klarer

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 68,937
Total Project Request for Year 2 Funding: $ 70,395
Total Project Request for Year 3 Funding: $ 71,910

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2024
Amount: $ 236,147
Agency Name: USDA-ARS
Notes: In-house project with complimentary objectives. Funds (over 3 years) for ½ storage maintenance and costs ($12,000), supplies and materials ($9000), travel ($3000), and 0.2 FTE (PI and Co-PI) and 0.5 FTE Postdoctoral research associate ($113,742).

WTFRC Collaborative Costs: None.

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Item	2022	2023	2024
Salaries*	27000	28080	29203
Benefits	9437	9815	10207
Wages	5000	5000	5000
Benefits			
Equipment			
Supplies	5000	5000	5000
Travel			
Miscellaneous **	12500	12500	12500
Plot Fees			
Total	58937	60395	61910

Footnotes: *0.5 FTE WSU postdoc at WSU benefits rate. **1/5 of instrument service contract to be used for project activities.

Budget 2
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Item	2022	2023	2024
Salaries			
Benefits			
Wages	10000	10000	10000
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total	10000	10000	10000

Footnotes: Part-time wages to perform fruit quality analysis

Objectives:
1. Identify temperature/atmospheric combinations that reduce superficial scald without causing other disorders.
2. Determine what post-storage ripening and scald controls can be used following ultra-low oxygen (ULO) controlled atmosphere (CA).
3. Evaluate tests that indicate disorder control effectiveness during ULO CA.

Significant Findings:
1. ULO (0.5% O_2) controlled superficial scald.
2. Temperature management is critical; conventional low temperature (31 ºF) controlled internal browning better than 33 ºF.
3. CO_2 management is critical (<0.5%); elevated CO_2 (5%) in ULO caused severe internal browning.
4. Post-long-term ULO CA storage 1-MCP treatment improved scald and quality outcome after a 2-month post-storage cold chain; effective rate depended on storage duration and atmosphere.
5. Black speck is a significant problem historically linked with ULO CA; the disorder has not developed on any pears in our tests to-date. Earlier work indicates disorder causes are season/site specific and should be considered before using ULO.

METHODS

Objective 1. Identify temperature/atmospheric combinations that reduce superficial scald without causing other disorders.

For year 2, 'd'Anjou' pears were harvested from a Cashmere, WA, orchard 1 week prior to, at commercial maturity, and 1 week following commercial maturity. Pears were transported to the Tree Fruit Research Laboratory in Wenatchee, WA, sorted, analyzed for maturity, and placed storage atmospheres comprising 0.5% CO_2 and 0.5, 1.0, or 1.5% O_2 at 31 °F or 33 °F. Each combination was initially represented by 72 pears. Pear quality and maturity (imaged, firmness, soluble solids, titratable acidity (TA), ethylene production, internal and external appearance) were analyzed at 3, 6, and 8 months. The remainder of the pears were placed in a simulated post-storage cold chain where they were stored in air at 33 °F for an additional 4 or 8 weeks, assessing quality/maturity immediately upon removal as well as following 7 days at 68 °F. The evaluation immediately after 8 months storage simulates fruit condition for distribution and following 4 weeks plus 7 days at shelf temperature, the ultimate quality on the retail display/consumer table.

As superficial scald and browning disorders can be influenced by location, in year 3, 'd'Anjou' pears were harvested from 9 different orchards (Hood River, OR; Dryden, WA; Yakima, WA; Orondo, WA; Cashmere, WA (4); White Salmon, WA) at commercial maturity. Pears were transported to the Tree Fruit Research Laboratory, sorted, analyzed for maturity, and placed in controlled storage atmospheres comprised of 0.5% O_2 and 2.5 or 5% CO_2 at 31 °F or 33 °F (ongoing). Each combination was initially represented by 144 pears per location. Fruit will be removed from CA after 8 months of storage and run through a simulated post-storage cold chain where they will be stored in 33 °F for an additional 4 weeks, then 7 days at 68 °F. Quality and maturity will be assessed upon removal from CA, 33 °F storage, and after 7 days at 68 °F. An additional 72 fruit of each treatment will be evaluated for disorders at the end of the stimulated cold chain.

Objective 2. Determine what post-storage ripening and scald controls can be used following ULO CA.

In year 2, pears were harvested from an orchard near Hood River, OR, at commercial maturity and transported to the Tree Fruit Research Laboratory, sorted, and analyzed for harvest maturity/fruit

quality. To test the impact of delayed 1-MCP treatment on ripening capacity, ‘d’Anjou’ pears were placed in ULO CA (0.5% O_2; 0.5% CO_2) at 33 °F and stored for 8 months. At 8 months, a subset of the pears were treated with 150 ppb or 1000 ppb 1-MCP for 12 hours in air. Pear fruit quality/maturity (image, firmness, soluble solids, TA, ethylene production, internal and external appearance) was analyzed, and fruit was placed in a simulated post-storage cold chain where they were stored in air at 33 °F for an additional 4 or 8 weeks, sampling quality/ripeness immediately upon removal as well as following 7 days at 68 °F.

In year 3, ‘d’Anjou’ pears were harvested from an orchard near Cashmere, WA, at commercial maturity and transported to the Tree Fruit Research Laboratory, sorted, and analyzed for harvest maturity/fruit quality. Pears were immediately placed in CA comprised of 0.5% CO_2 and 0.2, 0.5, or 1.0% O_2 at 31 °F and will remain in storage for 8 months (ongoing). After removal from CA, half of the pears will be treated with 1000 ppb 1-MCP while the other half will remain untreated as a control. Evaluation will consist of a simulated post-storage cold chain where pears will be stored in 33 °F for an additional 4 weeks, then 7 days at 68 °F. Quality and maturity will be assessed upon removal from CA, 33 °F storage, and after 7 days at 68 °F. An additional 72 fruit of each treatment will be evaluated for disorders at the end of the stimulated cold chain.

Additionally in year 3, ‘d’Anjou’ pears from the same orchard near Cashmere, WA, were placed in CA comprised of 0.5% O_2 and 0.5% CO_2 at 33 °F. After 2, 4, and 6 months of storage (ongoing), a subset of pears will be removed from CA. Half of each subset will be treated with 1000 ppb 1-MCP while the other half will remain untreated for control. The pears will be run through a simulated post-storage cold chain where they will be stored in 33 °F for an additional 4 weeks, then 7 days at 68 °F. Quality and maturity will be assessed upon removal from CA, 33 °F storage, and after 7 days at 68 °F. An additional 72 fruit of each treatment will be evaluated for disorders at the end of the stimulated cold chain.

Objective 3. Evaluate tests that indicate disorder control effectiveness of ULO CA.

In year 2, peel and cortex of a subset of pears stored at different temperatures and O_2 percentages from the Cashmere orchard in Objective 1 were sampled at 0, 1.5, 3, 6, and 8 months to track changes in levels of natural chemicals associated with disorder risk in pears. Tissue was processed, stored, and analyzed using 3 in-house analyses for natural chemicals, including those associated with superficial scald and CO_2 sensitivity. These analyses also include those directed towards confirming links between pithy brown core and natural peel chemicals in an earlier study.

In year 3, peel and cortex of a subset of pears from each of the 9 orchards stored at different temperatures and O_2 percentages from the Cashmere orchard in Objective 1 were/will be sampled at 3, 6, and 8 months to track changes in levels of natural chemicals associated with disorder risk in pears. Tissue will be processed, stored, and analyzed using 3 in-house analyses for natural chemicals, including those associated with superficial scald and CO_2 sensitivity.

RESULTS AND DISCUSSION

ULO (0.5% O_2) controls superficial scald

ULO atmosphere (0.5% O_2: 0.5% CO_2) nearly eliminated scald following long term storage (8 months), 1 month post-storage cold chain, and 7 day simulated retail shelf during the first 2 years of the project. Scald developed on 'd'Anjou' stored in 1% and more so on those stored in 1.5% O_2 on pears from different locations and harvest maturities (Fig. 1). Lower storage temperatures slightly improved scald outcome albeit O_2 % had the most substantial impact.

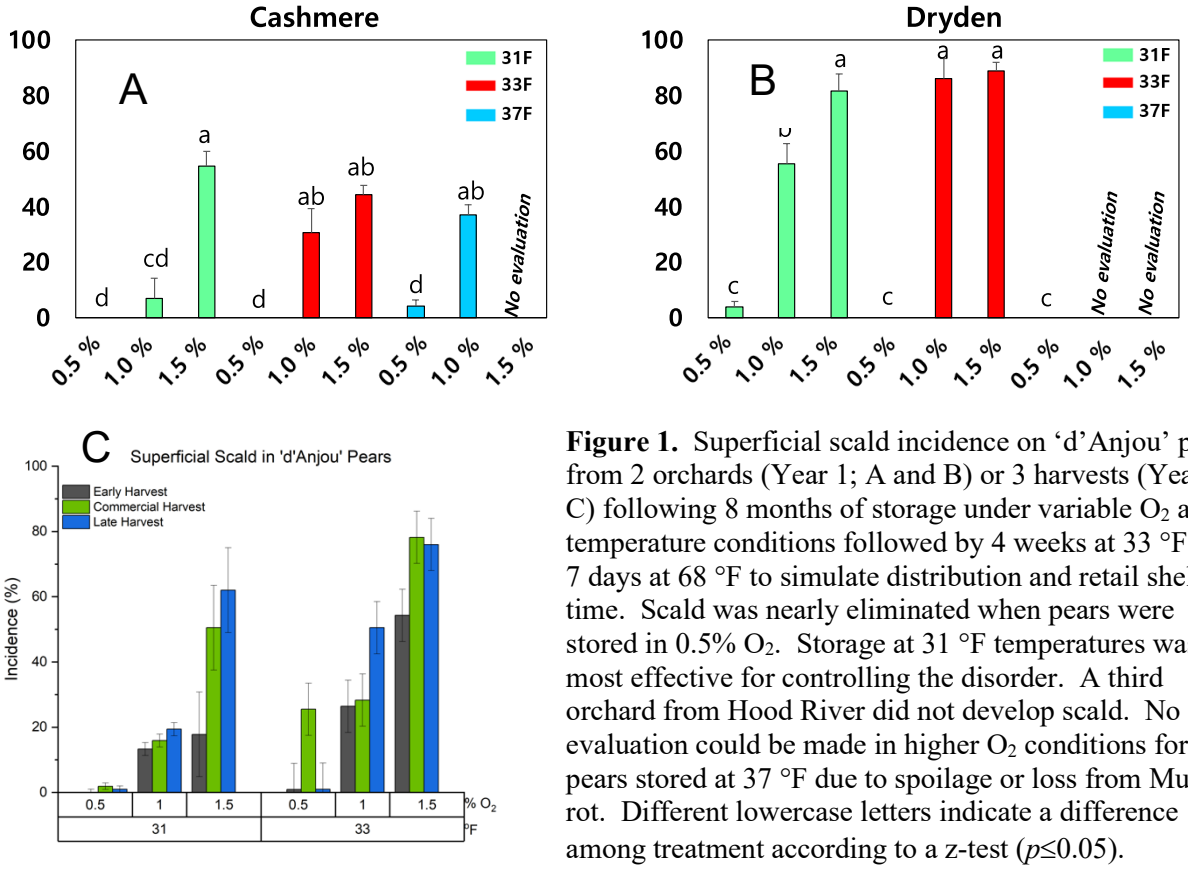


Figure 1. Superficial scald incidence on 'd'Anjou' pears from 2 orchards (Year 1; A and B) or 3 harvests (Year 2; C) following 8 months of storage under variable O_2 and temperature conditions followed by 4 weeks at 33 °F and 7 days at 68 °F to simulate distribution and retail shelf time. Scald was nearly eliminated when pears were stored in 0.5% O_2. Storage at 31 °F temperatures was most effective for controlling the disorder. A third orchard from Hood River did not develop scald. No evaluation could be made in higher O_2 conditions for pears stored at 37 °F due to spoilage or loss from Mucor rot. Different lowercase letters indicate a difference among treatment according to a z-test ($p \leq 0.05$).

Lower storage temperature retains the most fruit integrity for handling and distribution

Firmness was impacted most by storage temperature in both seasons. The rate of softening was not impacted by storage atmosphere in most cases. An exception is in year 1, where 37 °F was not sufficient to control softening and storing the pears from the Cashmere and Dryden orchards at 0.5% O_2 reduced softening (not shown). This storage temperature was eliminated from Year 2 and 3 activities. As expected, pear maturity impacted softening more than storage temperature or atmosphere in Year 2 (Table 1). For both seasons, pears stored in 0.5% O_2 at 31 °F remained firm during a 4-week post-CA storage period in 33 °F air and ripened fully when held at 68 °F for 7 days regardless of orchard or harvest maturity.

Table 1. Firmness of 'd'Anjou' pears from 3 harvests (Cashmere, WA) after controlled atmosphere (CA) storage with different oxygen and temperature condition combinations in Year 2. Pears

evaluations were conducted throughout a simulated cold chain: upon removal of CA storage at 8 months followed by 4 weeks of air storage at 33 °F and 7 days at 68 °F. Letters indicate significant differences in firmness ($p \leq 0.05$).

			Firmness (lbs)		
Harvest	**°F**	**% O_2**	**8 mo**	**8 mo + 4 wk**	**8 mo + 4 wk + 7 d**
Early	31	0.5	14.20 b	13.73 b	2.39 a
		1	14.16 b	13.60 b	2.36 a
		1.5	14.44 b	12.60 b	1.94 a
	33	0.5	15.24 b	13.66 b	1.96 a
		1	14.71 b	12.92 b	2.24 a
		1.5	13.70 b	11.28 bc	2.27 a
Middle	31	0.5	11.93 bc	12.29 b	2.00 a
		1	12.83 b	12.08 bc	2.23 a
		1.5	12.81 b	12.27 b	2.37 a
	33	0.5	12.96 b	10.74 bc	2.45 a
		1	12.93 b	11.58 bc	2.66 a
		1.5	12.63 b	12.10 b	3.19 a
Late	31	0.5	-	10.67 bc	2.52 a
		1	-	10.71 bc	2.95 a
		1.5	-	9.80 c	2.48 a
	33	0.5	-	9.73 c	2.67 a
		1	-	9.60 c	2.75 a
		1.5	-	9.88 c	2.83 a

Higher storage temperatures resulted in elevated levels of pithy brown core and internal browning

We expected a combination of low temperature and O_2 levels to increase incidence of internal disorders. Instead, in all 3 orchards, higher temperature led to higher rates of pithy brown core by the final evaluation at 8 months plus simulated distribution/retail shelf life, especially in fruit from the Hood River orchard, with no consistent relationship with O_2 percentage in any case (Fig. 2). Only insignificant levels of the disorder occurred earlier than this final evaluation (not shown). Pithy brown core did not develop in fruit used for any of the Year 2 activities.

Disorder risk appears to be orchard-specific or, potentially, related to harvest maturity. The association with maturity and higher storage temperature indicates that the disorder is associated with CO_2 sensitivity, a condition that can be also orchard-specific in apple and other pear cultivars like ‘Conference’. Accordingly, the relationship between ULO and CO_2 sensitivity was evaluated in Year 3 incorporating 9 orchards across the growing regions to account for site variability.

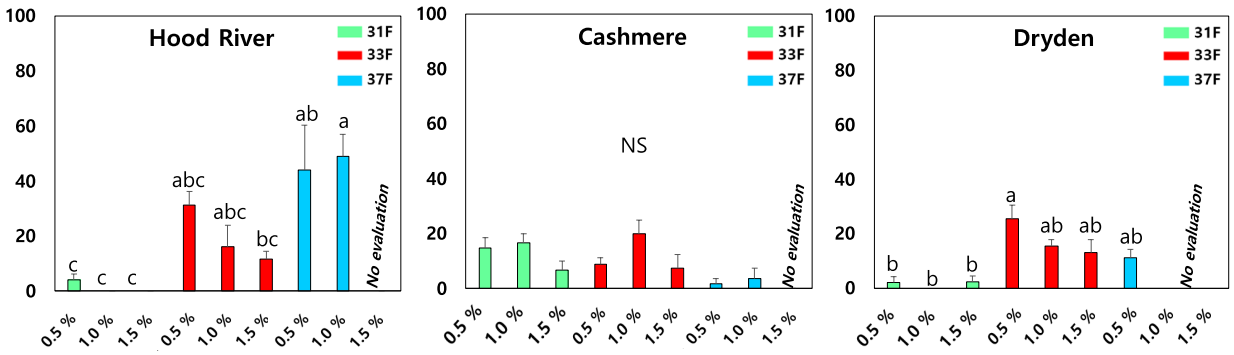


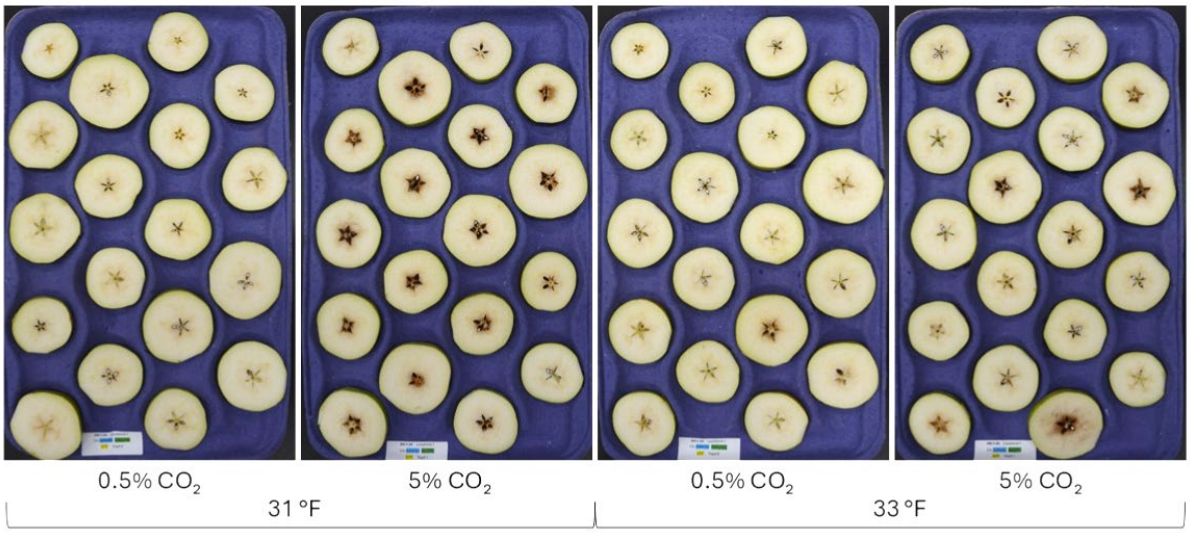
Figure 2. Pithy brown core incidence in 'd'Anjou' pears from 3 orchards following 8 months storage under variable O_2 and temperature conditions followed by 4 weeks at 33 °F and 7 days at 68 °F to simulate distribution and retail shelf time. Pithy brown core was reduced by lower temperature or remained the same in pears from 2 orchards. No evaluation could be made in higher O_2 conditions for pears stored at 37 °F due to spoilage or, mostly, loss from mucor rot. Different lowercase letters indicate a difference among treatment according to a z-test ($p \leq 0.05$).

CO_2 management is critical to reduce or eliminate pithy brown core

To determine the relationship between disorders developing in ULO and CO_2 sensitivity, in Year 3, we harvested 'd'Anjou' from 9 orchards, stored them at 33 °F in atmospheres comprising 0.5% O_2 and either 0.5 or 5% CO_2. Our earlier work with 'd'Anjou' using the same storage conditions failed to cause internal disorders of any type. However, early storage data (3 months) confirms that pithy brown core of 'd'Anjou' is linked with elevated CO_2 as symptoms only developed in pears from all orchards stored under 5% CO_2 (Fig. 3). Symptoms were severe in some cases and extended into the flesh. Disorder severity was orchard dependent indicating CO_2 sensitivity is orchard dependent as with many apple and pear cultivars.

Figure 3. Pithy brown core/internal browning incidence (%) of 'd'Anjou' pears from 9 different orchards after 3 months of storage in ULO CA with varying temperature and CO_2 conditions (and 0.5% O_2). Included images (bottom): Cross sections of 'd'Anjou' pears from Orchard 7. Different lowercase letters indicate statistical separation within a column.

	31 °F		33 °F	
Location	**0.5% CO_2**	**5% CO_2**	**0.5% CO_2**	**5% CO_2**
1	0 a	11.1 ab	0 a	44.4 cd
2	0 a	33.3 bc	5.6 a	72.2 de
3	5.6 a	55.6 cd	11.1 ab	55.6 cd
4	5.6 a	55.6 cd	0 a	50.0 cd
5	0 a	22.2 abc	0 a	44.4 cd
6	0 a	61.1 cde	0 a	5.6 a
7	0 a	88.9 e	11.1 ab	38.9 bcd
8	5.6 a	38.9 bcd	5.6 a	50.0 cd
9	0 a	66.7 de	0 a	11.1 ab



One possible reason for this is a relative difference in flesh tissue density among orchards and seasons as has been reported for 'Conference' pears. Elevated temperatures would also exacerbate this as pears respire more at higher temperatures, producing more CO_2 that accumulates in the flesh tissue. The CO_2 is held in denser flesh tissue longer. This highlights the importance of CO_2 and temperature management when using ULO conditions, especially during the first months of CA storage.

Post-long term ULO CA storage 1-MCP treatment can improve scald/quality outcome

In Year 1, we tested the impact of low rate 1-MCP applications (150 ppb) at harvest and after 1, 2, and 8 months 0.5% O_2, 0.5% CO_2 CA storage at 31 °F to determine if post-storage application in combination with ULO would improve the scald/quality outcome after 8 months storage and a 4 week cold chain (33 °F air) and still ripen fully after 7 days at 68 °F. Earlier applications failed to ripen and the 8-month application had no impact (not shown) indicating the combination of the earlier application and ULO storage conditions reduced ripening capacity too much while the later application at that low rate was entirely ineffective.

To account for this, in Year 2 we stored 'd'Anjou' from Hood River for 8 months in 1.0% O_2, 0.5% CO_2 CA storage at 33 °F as the ULO storage conditions used in Year 1 may entirely control scald and not provide a contrast to test if post-storage 1-MCP treatment afforded better scald control. Upon removal from storage, pears were treated with 150 or 1000 ppb 1-MCP. Scald incidence was cut by more than a half after an 8-week cold chain (33 °F air) plus 7 days at 68 °F when treated with 1000 ppb 1-MCP compared with the 150 ppb rate (Fig. 4). Pears treated with 1000 ppb at 8 months remained firm during the simulated distribution period (cold air storage) but still ripened fully even after a 4-week cold chain (Table 2).

Results-to-date confirm the potential to reduce superficial scald and quality loss for 'd'Anjou' pears during longer post-storage distribution schemes following long-term CA storage. However, considering our Year 1 results, the effective application rate is different depending on storage atmosphere used and storage duration. If applied at a high rate too early in the storage period or following a storage regime that halts ripening to a large degree, such as ULO, then the pears may never ripen. Conversely, if 1-MCP is applied at too low a rate after a long storage period or after a storage period that does not control ripening as effectively, then the crop protectant may not be effective. In effect, the application rate should be considered variable given these conditions. The effective rates remain to be determined. Our Year 3 activities include examinations of post-storage 1-MCP application following storage at different O_2 % as well as different storage durations to directly assess the influence of these variables on the effective post-storage application rate.

Table 2. Firmness of 'd'Anjou' pears after controlled atmosphere (CA) storage followed by different rates of 1-MCP. 1-MCP at a rate of 1000 ppb but not 150 ppb improved the scald/quality outcome. Evaluations were conducted throughout a simulated cold chain: upon removal of CA storage (8 mo) followed by 4 or 8 weeks of air storage at 33 °F (4 or 8 wk) and 7 days at 68 °F (7 d). Different letters in a column indicate statistical separation in firmness ($p \leq 0.05$).

	Firmness (lbs)				
1-MCP (ppb)	**8 mo**	**8 mo + 4 wk**	**8 mo + 4 wk + 7 d**	**8 mo + 8 wk**	**8 mo + 8 wk + 7 d**
150	13.06 a	7.93 b	1.70 d	6.81 b	1.77 d
1000	12.83 a	13.09 a	3.50 c	12.14 a	1.58 d

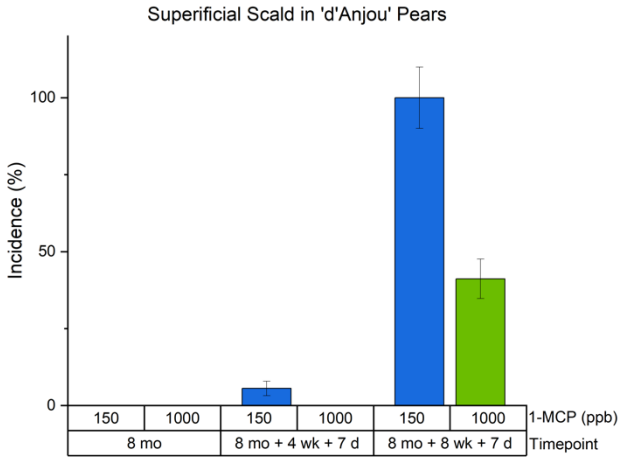


Figure 4. Superficial scald incidence of 'd'Anjou' pears following 8 months of controlled atmosphere storage followed by treatment of either 150 ppb or 1000 ppb 1-MCP, then 4 weeks or 8 weeks at 33 °F and 7 days at 68 °F to simulate cold storage, distribution, and retail shelf time.

Natural chemical levels associated with superficial scald and pithy brown core risk

In Year 1, conjugated trienol (CTOL) levels increase with superficial scald risk in apple and pear peel prior to symptom development. This held true in the current study where the highest levels at 1-8 months (prior to symptom development) were associated with the highest O_2 percentages (not shown). CTOL levels in peel of pears stored in 0.5% O_2 changed the least compared with the initial values (typically undetectable in apple or pear peel before storage). An analysis protocol for this natural chemical to determine superficial scald risk in apple peel has been published (Blakey and Rudell, 2017) and is currently in use by some regional apple producers. We have subsequently identified another class of compounds, phytosterols, that are associated with changes in plant cellular membranes (the envelopes holding cell components in the correct place that must remain fluid at all temperatures). Phytosterols were identified earlier in association with superficial scald of apples and pears and, more recently, with soggy breakdown and CO_2-related disorders of apple. Links with a ratio of 2 of the phytosterols (ASG/SE) increased in peel with O_2 percentage, as did scald incidence. However, the link was not as clear with regard to temperature in pears stored at 37 °F which had a low ratio compared with those stored at lower temperatures, yet similar scald incidence (not shown). Our future analyses will focus on Year 3 data where substantial incidence of pithy brown core and internal browning has developed, providing prime contrasts to determine whether monitoring these natural chemicals yields potentially actionable information regarding superficial scald or CO_2-sensitivity risk. The tests also incorporate fruit from 9 orchards which is expected to provide further confirmation of the risk assessment value of these natural chemicals.

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

Report Type: Final Project Report

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Project Duration: 3 Year + No Cost Extension

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

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

WTFRC Collaborative Costs: none

Budget 1
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Item	2021	2022	2023	2024
Salaries	$22,250.00	$22,850.00	$48,279.00	$0.00
Benefits	$8,455.00	$8,687.00	$18,346.00	$0.00
Wages				
Benefits				
RCA Room Rental				
Shipping				
Supplies	$2,210.00	$2,200.00	$2,200.00	$0.00
Travel				
Plot Fees				
Miscellaneous				
Total	$32,915.00	$33,737.00	$68,825.00	$0.00

Footnotes: 1 Biological Science Technician = Half funding for 100% FTE (salary+benefits) technician for years 1 and 2, and full funding for year 3.
2 Supplies: RNA/DNA extraction, tissue culture, greenhouse, molecular supplies and consumables.
If project duration is only 1 year, delete Year 2 and Year 3 columns.

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

Major improvements in and understanding of adventitious shoot regeneration in Bartlett, OHxF 87 and OHxF 97. Our findings on plant responses to different mineral nutrients and hormones in the regeneration, micropropagation, and rooting between cultivars are important inputs for the development of nursery protocols for tissue culture-based propagation.

Successful transformation of callus tissue in 3 cultivars. Our success in the initial phases of the transformation process bring us one step closer to introducing tools like rapid cycle breeding, or any other biotechnology-based tools, which will be important for breeding programs and future research leading to the development of new rootstocks.

Built connections and collaboration with Strauss Lab at Oregon State University to test different strains of Agrobacterium that can enhance adventitious shoot regeneration from transformed callus tissue. Similar to the transformation of callus, this will be helpful for introduction of biotechnological tools for more difficult cultivars that don't respond as well to tradition Agrobacterium strains. This has potential to aid the use of more varied cultivars in a rapid cycle breeding system.

Results

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

1a. Selection of germplasm to be transformed

In Year 1, we were able to obtain 'OHxF 87', 'OHxF 97' (recently confirmed to actually be 'Old Home' x 'Bartlett' crosses by [1]), and 'Bartlett' tissue and initiated these into tissue culture. Successful micropropagation has continued successfully. In years 3 and 4, contamination events temporarily reduced population numbers, however measures were taken to deep clean spaces and purchase newer equipment when necessary to maintain sterility. Additionally in year 3, we obtained the 'Conference' cultivar, as this has been transformed successfully in other labs, as it is particularly amenable to shoot regeneration, even in the presence of agrobacterium [2]. In year 4, we have begun to use 'Conference' in transformation experiments.

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

In year 1, we obtained the original RCB construct from the Cutler lab at UC Riverside, which contained the *FLOWERING LOCUS T (FT)* gene from Arabidopsis, a red fluorescence marker (RFP),

and the necessary proteins to make the flowering gene inducible (Inducibility machinery) (Fig. 1A). We modified the construct to contain an antibiotic resistance gene (*NptII*, conferring resistance to Kanamycin), and one of two flowering genes that have been used for early flowering previously in apples and pears (*CiFT* from citrus, and *BpMADS4* from birch [3, 4]) (Fig. 1B). In year 2 we made an additional version, replacing the Kanamycin resistance gene for a Hygromycin resistance gene, as we had found examples in the literature of varying sensitivities to Kanamycin across plant species (Fig. 1C) [5-7]. In year 4, we sequenced this version of the construct to confirm it is correct, and plan to use it in transformation experiments in the future.

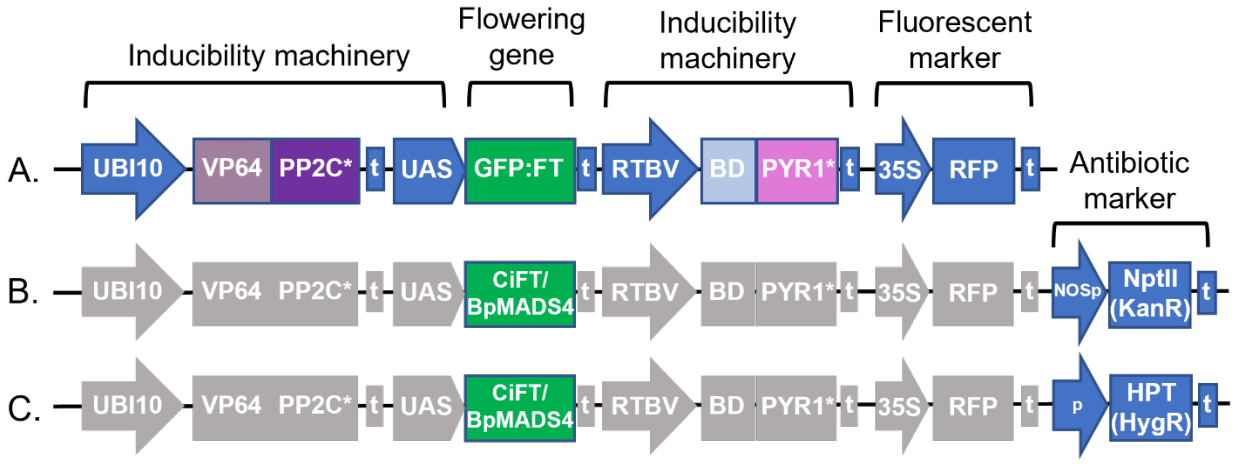

Figure 1. RCB construct development. A. Original construct received from Cutler lab. B. Construct developed in Year 1, containing flowering genes for pear and a Kanamycin-resistance gene (NptII). C. Construct developed in Year 2 containing a Hygromycin-resistance gene (Hpt), replacing KanR.

1c. Transform germplasm

In year 2 we confirmed that the RCB construct was functional and transformable by transforming Arabidopsis and obtaining seeds with the construct inserted (Fig. 2A and B). We further showed that pear callus tissue was successfully being transformed, as indicated by glowing red tissue resulting from the fluorescent marker included in the construct (Figs. 1 and 2C-F). Pear callus is the tissue formed in response to wounding and hormone inputs, and acts as an intermediate tissue from which new adventitious shoots can regenerate, given the ideal hormone inputs and growth conditions. Throughout year 3 we continued transformation trials, altering experimental parameters to improve callus transformation and determine protocols for shoot regeneration from this callus. Our initial base protocol used the following parameters:

Agrobacterium containing the RCB construct was grown overnight until saturation, then diluted in the morning and grown to an optical density of OD600 = 0.8. Growth media contained 100uM acetosyringone to stimulate agrobacterium virulence. Young leaves, just fully expanded, were excised from tissue culture-propagated plantlets and soaked in liquid NN69 media [8] containing and hormones (22uM TDZ as the cytokinin, and 10uM NAA as the auxin) for 60 minutes to avoid oxidative browning and stimulate callus production. Leaves were transferred to media-moistened filter paper and 4mm biopsy punches were used to cut leaf discs from the petiole-end of the leaves (2 leaf discs per leaf), with each leaf disc containing midrib tissue. The biopsy punches introduce wounding around the entire edge of each disc, and we included midrib tissue, as it tends to be more competent to develop callus and adventitious shoots. Leaf discs were moved to inoculation media containing the agrobacterium, acetosyringone, and 30g/L sucrose, and left to soak for 60 minutes. Control leaf discs were soaked in identical media without agrobacterium added. Leaf discs were then moved to liquid co-cultivation NN69 media containing 30g/L sucrose and hormones (22uM TDZ and

10uM NAA) and kept in the dark for 4 days at 20C, to allow growth of both the agrobacterium and the callus tissue. After 4 days, antibiotics were added to the liquid media (300mg/L Cefotaxime and 200mg/L Timentin) and left to culture overnight to eliminate the agrobacterium and prevent overgrowth. Leaf discs were then transferred to solid NN69 media, containing 50mg/L Kanamycin, 30g/L sucrose, and hormones (22uM TDZ and 10uM NAA), and grown in darkness at 20C for 2 weeks. After 2 weeks, plates were moved to unlit shelves, maintained at room temperature, and checked weekly for red fluorescence and adventitious shoot regeneration. Callus transformation, indicated by red glowing spots (# of red spots/total # leaf discs transformed), was reported at 4 weeks, and adventitious shoot regeneration is reported at 8 weeks. Leaf discs were transferred to fresh media every subsequent 4 weeks.

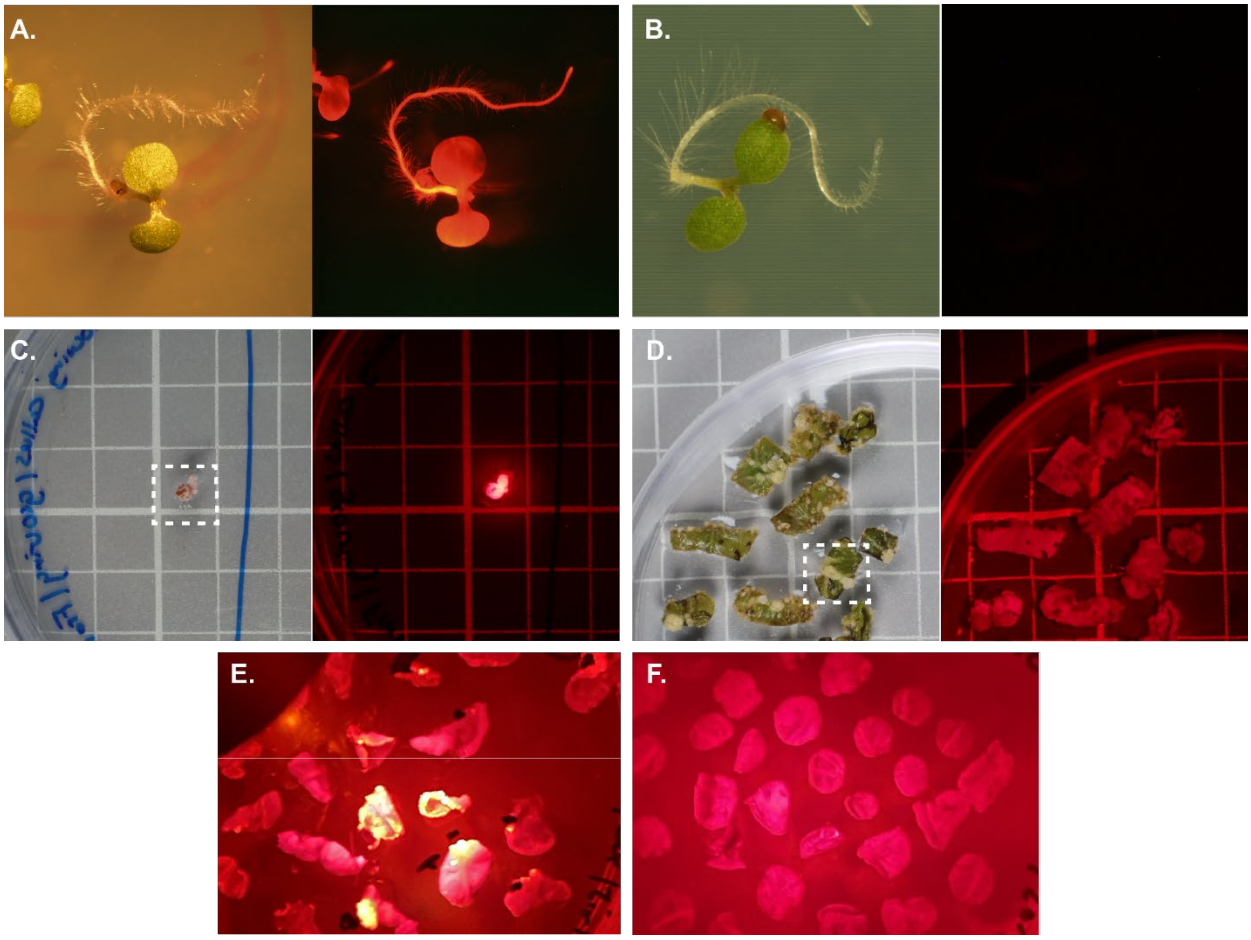

Figure 2. Red fluorescent marker indicates transformation of Arabidopsis and pear callus tissue. A. Arabidopsis seedlings that have been successfully transformed with the RCB construct and selected on Kanamycin, in white light (left) and green light to excite the red fluorescence (right). B. Arabidopsis seedlings that have not been transformed, for reference. Chlorophyll fluoresces to a low level, but the bright red of the fluorescent marker is absent. C. Transformed pear callus that has been isolated from a leaf, in white light (left) and green light (right). D. Non-transformed (control), pear leaf squares growing callus, not showing the bright red of the marker. E-F. Additional images of transformed and glowing callus (left) and non-transformed control callus (right) on leaf discs generated in year 3.

Table 1 contains results from trials throughout years 1-4, where we varied different parameters identified from the literature to be beneficial for regenerating different plant species from transformation events [9-14]. In these trials, we tested leaf tissue wounding methods (slicing whole

ID/ Date	Experimental parameters tested	Cultivars	Callus transform.	Takeaways
211210	First run with protocols from Kearneysville	Bartlett	Unknown	Method of Agrobacterium inoculum prep is difficult to control - switching to sub-culturing.
220328	Agro conc. (OD600=0.3, 0.6)	Bartlett	Unknown	No regen
220519	Agro conc. (OD600=0.1, 0.3)	Bartlett	Unknown	No regen
220614	Inoculation (soak+cut, vacuum)	Bartlett	Unknown	No regen, review of more protocols points to need for AS earlier.
220615	Agro conc. (OD600=.008, 0.3) Inoculation (soak+cut, vacuum)	Bartlett	Unknown	Vacuum infiltration does not seem to be an improvement.
220803	Inoculation (soak+cut, vacuum)	Bartlett	Unknown	Agrobacterium overgrowth shortened experiment - different antibiotics needed.
221028	Tested multiple Kanamycin-Resistance-containing vectors, including RCB construct	Bartlett	Unknown	Regeneration of controls (without Agro) is improved on NN69 media. Antibiotics helped with knocking down overgrowth.
221110	Agro conc. - tested OD600=0.8 Tested multiple vectors again	Bartlett	Unknown	One leaf formed and later died, but briefly had a transformant. Still some issues with Agro overgrowth.
221205	Using biopsy punches Agro removal - testing pre-selection	Bartlett	65/150= 0.43	Biopsy punches improve time and get similar callus growth.
221215	Same as RCB_221205	Bartlett	35/173= 0.23	
230315	Carbohydrate source - sucrose vs. sorbitol	Bartlett	Suc. 22/50= 0.44 Sor. 14/49= 0.29 1:1 15/49= 0.30	No significant difference in callus transformation with different carbohydrate sources.
230316	Cultivar type, protocol same as 221028 and 221110	OHxF97	97 62/101= 0.61	New standard protocol yields highest rates so far.
230622	Cultivar type, protocol same as 221028 and 221110	Bartlett OHxF87	B 56/90= 0.62 87 53/85= 0.62	New standard protocol yields highest rates so far.
230714	Pre-culture callus before inoculating	Bartlett	0/75= 0	Callus growth prior to inoculation appears to block transformation.
230928	Microprop. Media (DKW, PM2, QL)	Bartlett	n/a	Lost to fungal contamination
231016	Microprop. Media (DKW, PM2, QL, varying CK)	Bartlett	D+BA 64/55= 1.2 Q+mT 54/69=0.78 Q+BA 22/56=0.39	DKW media with BA as cytokinin and QL media with mT give highest rates.
241007	Following protocol from NZ group	Conference Bartlett OHxF 97	n/a	Published protocols must be missing info. Leaves did not produce callus.
241108	Following protocol from NZ group, using LBA4404 Agro	Conference Bartlett	n/a	Bacterial contamination and no callus growth.

Table 1. Experimental comparison, outcomes, takeaways from standard transformation trials. Abbreviations: AS – acetosyringone, NN69 – Nitsch and Nitsch 69 media, MS – Murashige and Skoog media, DKW – Driver Kuniyuki Walnut media, Q or QL – Quoirin and Lepoivre media, mT – meta-Topolin (cytokinin), BA – 6-Benzylaminopurine (cytokinin).

leaves vs. cutting into squares or circles with biopsy punches), leaf tissue soaking to avoid oxidation (with and without hormones included), liquid versus solid media during the co-cultivation stage, Agrobacterium concentration and strain, carbon source (sucrose vs. sorbitol), multiple different hormone combinations, micropropagation media prior to transformation, and testing published protocols from other labs. Thus far, we found that for 'OHxF87' and 'OHxF97', our current standard protocol (written above) resulted in the highest amount of transformed callus, determined by the number of red fluorescent spots in leaf callus, and for 'Bartlett', this protocol combined with plant growth on DKW media prior to leaf excision worked best (Table 1).

In year 4, we began conducting transformation trials using an additional strain of Agrobacterium, obtained from the Strauss lab at Oregon State University [15]. This Agrobacterium strain, called S82, has been used in combination with standard Agrobacterium (which contains the construct of interest) to enhance transformation rates of very difficult-to-transform cultivars of eucalyptus and poplar [16]. Briefly, the S82 strain contains phytohormone-biosynthesis genes (for plant auxins and cytokinins) that a wild strain of Agrobacterium would have, meaning that cells transformed with S82 are able to biosynthesize these hormones and signal to surrounding cells to divide and grow. When the S82 strain transforms plant cells, those cells divide and grow into callus tissue, but never appear to regenerate their own adventitious shoots. Instead, they signal to surrounding callus tissue to regenerate. Thus, when plant tissue is transformed with both S82 and Agrobacterium containing a construct of interest, like our RCB construct, the S82-transformed callus should signal to the nearby RCB-construct-transformed callus to develop adventitious shoots. Additionally, the S82 cells have a green fluorescent marker, so we can track the number and location of transformed callus (Fig. 3). Table 2 contains results from trials with S82 and Agrobacterium containing our RCB construct.

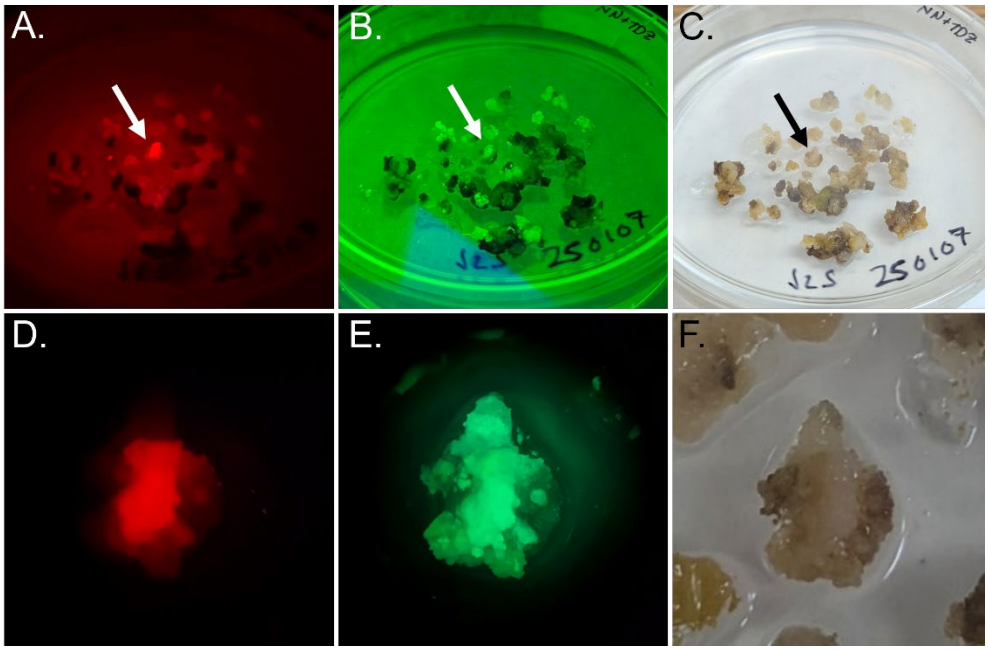

Figure 3. Callus transformed with S82 nearby to callus transformed with RCB construct. A-C. A plate of callus developed from leaf discs inoculate with S82- and RCB-construct-containing Agrobacterium, shown with red fluorescence (A.), green fluorescence (B.) and bright field (C.). Arrow indicates callus zoomed in on in D-F. Green fluorescent marker indicated cell transformed with the S82 strain, and red indicates cells transformed with the RCB construct.

For transformation, we follow a similar protocol to before, but with the following modifications: Excision, soaking, and wounding of leaves remains the same. Leaves are inoculated in a ratio of

RCB-containing to S82-containing agrobacterium for 20 minutes and then co-cultivated on solid Nitche & Nitche 69 (NN69) media for 5 days at 25C under dark conditions. Leaf discs are washed with sterile milli-Q water 3 times followed by a single wash in Nitche& Nitche 69 containing 200 mg/L Timentin, 300 mg/L cefotaxime, 50 mg/L carbenicillin and 600 mg/L Rifamcin (TCRcarb) then cultured on Nitche & Nitche69 agar plates containing 2% sucrose, TCRcarb antibiotics with no hormones for 7 days 'rest' period at 25C under dark conditions. After 7 days leaf discs are cultured on plates containing NN69 but with the addition of 50 mg/L kanamycin in addition to the TCRcarb antibiotic cocktail at 25C in the dark for 2 weeks then moved into the light, as the S82-transformed callus tissue will produce the hormones needed for adventitious shoot regeneration. Leaf discs are checked for fluorescence and adventitious shoots every 2 weeks after being moved to 16:8 light conditions.

Following this protocol, we have tested multiple different ratios of S82:RCB-construct-containing Agrobacterium (25:1, 10:1, 5:1, 2.5:1, and 1:1). We found that 2.5:1 gives the highest numbers of callus transformation (Table 2). Further, we have varied the "rest" period, which is the period after co-cultivation that leaf discs are allowed to grow on media without Kanamycin, to allow callus containing S82 to grow. We found that having no rest period results in no callus transformation, while a period of 7 days allows for substantial S82-callus growth. In our most recent trial, we are adding no Kanamycin in the initial phase until regenerant form, then we will move these onto Kanamycin plates and look for red fluorescence.

ID/ Date	Experimental parameters compared/tested	Cultivars	Agro. Ratio RCB/S82	Callus transform. RCB	Callus transform. S82	Regen
240119	Comparing ratios of Agro containing RCB construct and S82	Bartlett	1:1 5:1 10:1 25:1	1:1 22/95= 0.23 5:1 40/95= 0.42 10:1 22/80= 0.28 25:1 4/107= 0.04	1:1 49/95= 0.52 5:1 52/95= 0.55 10:1 0/80= 0.00 25:1 3/107= 0.03	22
240206	Comparing ratios, removed "rest period"	Bartlett	5:1 10:1 25:1	n/a	n/a	n/a
240508	Focus on 5:1 ratio with "rest period" returned	Bartlett	5:1	20/107= 0.19	40/107= 0.37	29
241028	Microprop. Media (MS and QL for Bartlett, WPM for OHxF 97, all with 5uM mT)	Bartlett OHxF97	2.5:1	MS 93/74= 1.25 QL 40/47= 0.85 WPM 46/68= 0.68	MS 198/74= 2.68 QL 162/47= 3.45 WPM 196/68= 2.88	1

Table 2. Experimental outcomes from S82 transformation trials. Callus transformation here is reported as the number of callus cell clusters fluorescing from the red or green marker (indicating cells are transformed with the RCB construct or S82, respectively), over the number of total leaf discs in the experiment. The "Regen" column refers to the number of adventitious shoots regenerated, none of which contained the RCB construct at the time of this report. In the first 3 trials, all plants were micropropagated on QL media with 5uM meta-Topolin as cytokinin.

While we have not yet confirmed any adventitious shoots transformed with the RCB construct (only callus thus far), we have gotten adventitious shoots that are not transformed (Regen, Table 2). This is to be expected, as there is plenty of callus growing that has not been transformed and does not glow with either the red or green fluorescent marker. This also signifies that the S82-transformed callus cells are behaving as they should and sending signals to surrounding callus to regenerate into shoots, we just have not yet had enough RCB-construct-containing callus cells near enough to the S82-

containing cells to receive substantial signal. Future trials will focus on trying to increase RCB-construct-containing cells to improve these chances.

In year 4, we have also tested transformation of our RCB construct into 'Conference', using published protocols designed to work for this cultivar specifically [2, 17]. While 'Conference' is not our target germplasm, it has been identified as a cultivar that is easy to regeneration and amenable to transformation [13, 18], making it a good control. In our initial attempt, we followed the protocol outlined in the recent Tomes et al. 2023 paper [17], but we saw no callus transformation (Table 1). This was in part due to bacterial contamination, likely endophytes coming from the plants themselves. However, before bacterial contamination became a major issue, we also found that callus developed was quite slow or not at all, suggesting that the protocols also did not contain all necessary details. We are currently initiating another transformation attempt with 'Conference', following protocols modified by our collaborators in Kearneysville, which have been successful previously with this cultivar.

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

2a. Rescue transformants, confirm presence of construct

In year 3, we attempted to regenerate plant tissue from the callus that has been transformed. Early in the year we had one regenerant with a red fluorescent-glowing leaf, however this regenerant appeared to have lost the cells containing the shoot apical meristem tissue, and thus never continued to grow. In addition, we found several regenerants that continued to grow on Kanamycin (RCB_230622, Table 1), suggesting they contain the transgene, but their tissue does not glow red when we looked at fluorescence. However, continued growth on Kanamycin led to these regenerants dying. In the future, regenerants that show positive PCR results will be sequenced to confirm the location of the transgene within the genome. Confirmed plants that reach sufficient size will be rooted, acclimated, and moved to soil before moving on to characterization. While we were previously concerned about ability to root these cultivars, in year 2 we tested rooting protocols and saw success for 'Bartlett', 'OHxF 87', and 'OHxF 97'.

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. While we were not able to reach this objective thus far, we hope that this subobjective will begin to be addressed in the coming year.

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

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

While we have not reached this objective yet, when we have confirmed transformed plants, our plans are as follows: Confirmed transformed plants will be allowed to grow until branches can support fruit weight. At this point we will characterize flowering gene expression and flowering responses to chemical induction in more detail. After spraying leaves with Mandipropamid, we will collect leaf and bud tissue and use quantitative PCR to determine gene expression levels compared with control genes and control tissues. We will observe timing of flowering as well as inflorescence and flower morphology. In citrus, the Cutler lab and collaborators have seen high levels of gene expression in response to chemical induction, as well as flowering occurring in the axillary bud associated with

leaves sprayed after about 2-3 weeks. We will perform experiments to determine the optimal chemical doses (varying concentrations), the best way to deliver the chemical (varying addition of surfactant/wetting agents), and how timing of flowering and flower morphology respond to these different factors. Given difficulties in regenerating plants from transformed tissue (Obj. 1c), this work may begin towards the end of coming year.

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. Further, we expect that with more normal phenotypes, we will be able to identify more lines in a shorter amount of time that have functional bud and flower development. We will induce multiple flowers per plant and observe stages of pollination, fruit set, fruit and seed development, and seed viability. In citrus, these tests were able to be performed in 1 year old transformed trees. This work will take place once we induce and characterize flowers, in Obj. 3a.

3c. Begin crossing with germplasm containing other desirable traits.
Once stable lines have been optimized and characterized, we will begin performing crosses with desirable germplasm. Initially, we will cross with fire-blight resistant germplasm identified in Objective 1a, containing additional sources of resistance to OHxF backgrounds. Because there are multiple sources of fire-blight resistance [19-21], 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.

Discussion

Breeding for tree fruit crops can be a very lengthy process, in part due to the length of the tree's juvenility period, which is the time it takes for the tree to bear fruit. In European pears, this can take up to ~10+ years, depending on cultivar. Our goal is to develop a system in pears that shortens the length of this process and allows for more rounds of breeding crosses in a shorter time. In other tree crops, including apple, plum, and citrus, rapid cycle breeding (RCB) systems have been developed and used to drastically shorten this time by introducing flowering genes, typically through overexpressing them. While ultimately useful, overexpression of flowering genes can lead to many abnormal phenotypes, such as early bud termination and weak branches, and often many lines need to be screened to find RCB transformants that are viable. Here, we are trying to introduce an inducible flowering gene, which will allow us to have normal plant phenotypes until flowers are needed, and hopefully decrease the number of plant lines needing to be screened to avoid phenotypes like early termination.

Here we report continued successful transformation of pear callus tissue (Fig. 2). As mentioned above, pear callus is the intermediate tissue that develops in response to wounding and hormones, and from which adventitious shoots can regenerate, given the optimized conditions. It is well understood in the literature that adventitious shoot regeneration in response to hormone inputs is highly cultivar-dependent. While callus transformation has been successful, we have yet to regenerate shoots from

this callus. This tells us that we have identified conditions and hormones that allow for cells to be transformed by Agrobacterium carrying our RCB construct, which is encouraging. This also tells us that we have yet to find conditions in which adventitious shoot regeneration occurs in our cultivars of interest. It is common that rates of regeneration after transformation are much lower than regeneration rates in total absence of Agrobacterium, however it is not well understood why this occurs. Since starting this project, other groups have had success with transforming other cultivars, such as 'Conference', underlining the cultivar-specificity of plant responses to transformation and regeneration treatments. In addition to development of important tools for breeding, our work in understanding the difference between cultivar responses will be very useful for future work in applying biotechnology to more cultivars. Further, our findings may lead to improved predictability for regeneration, transformation, micropropagation, and rooting across cultivars in the future.

Over the timeline of this grant, we were also able to improve callus transformation efficiency in 'Bartlett', 'OHxF 87' and 'OHxF 97'. We have developed a base protocol that when applied to 'OHxF 87' and 'OHxF97', has reached callus transformation efficiencies of 0.62 and 0.61 red fluorescent spots/total leaf discs, respectively (230316 and 230622, Table 1). These efficiency calculations were very similar to 'Bartlett' under the same conditions (230622, Table 1). 'Bartlett' could be additionally improved by growing plants on DKW media supplemented with 4.4uM BA (231016, Table 1), which we have not yet applied to the OHxF cultivars. This was very encouraging, as it suggests that the transformation of cells is working well and similarly for all cultivars tested. This further underlined that adventitious shoot regeneration from transformed callus tissue seems to be the step that varies more widely between cultivars and remains the bottleneck.

We were also able to identify parameters that did not have strong effects on callus transformation in these cultivars. Carbon source, comparing sucrose, sorbitol, and a mixture of the two, showed no significant differences in callus transformation, and did not lead to adventitious shoot regeneration from transformed callus, despite reports that it improve regeneration in 'OHxF333' (cite). Further, based on communications with collaborators at UC Davis, we tested whether pre-culturing callus would improve transformation and regeneration (230714, Table 1), and found that no callus was transformed. We hypothesize allowing the callus to form ahead of time may have actually blocked the Agrobacterium from entering the cells. Early on, we found no difference in the effect of inoculation methods (whether soaking and cutting leaves with a scalpel or vacuum infiltration them in the Agrobacterium inoculum) on adventitious shoot regeneration. However, we did not have a method to check for the red fluorescent marker at the time, so the effect on callus transformation is unknown and worth investigating further.

Late in 2023, a New Zealand group reported development of an RCB tool in 'Conference' pear (cite Tomes). They used a construct that overexpresses the MADS4 flowering gene from Birch (BpMADS4) that has been used to develop these tools in apples (cite Malnoy). The 'Conference' cultivar has been cited in the literature as very easy to regenerate (cite that paper without hormones), which makes it a good starting point, however it is not target germplasm for rootstock research. They also used a different Agrobacterium strain, LBA4404, which we began a trial with, however we faced bacterial contamination and will need to make another attempt (Table 1). We have attempted to follow their protocol exactly as published to transform 'Conference' (as a control), 'Bartlett', and 'OHxF97', however leaves did not produce callus and eventually died. This may suggest the protocol is missing key information to reproduce results, or that other unknown metadata (i.e. water quality, light spectra, etc.) are influencing growth. As mentioned earlier, our next steps are to compare this protocol to the protocols used successfully in Kearneysville for work with 'Conference' and determine if we can transform it as a proof of principle. This would also help us determine whether our difficulties with adventitious shoot regeneration in 'Bartlett', 'OHxF87' and 'OHxF97' are due to cultivar-specific differences.

Recent work with the S82 Agrobacterium strain shows an increase in callus transformation with both constructs, as well as adventitious shoot regeneration, although this is so far from tissue that hasn't contained the RCB construct. This is very promising and we feel that we are very close to transforming these cultivars. We also plan to use the 'Conference' cultivar in future S82 trials, as the literature suggests it is quick to regenerate adventitious shoots. This would further demonstrate cultivar-specific differences, and help us to better understand, and hopefully work toward predicting, what causes these different responses.

Throughout the timeline of this project thus far, we have successfully built a strong tissue culture program in the lab, and have learned much about micropropagation and rooting, in addition to transformation and adventitious shoot regeneration. As stated throughout the report, there are many, many variables that can be altered and tested to optimize each one of these processes. In addition, we have learned a great deal about the need for controlled environmental inputs. In Year 1 and part of 2, plants were grown the in lab, and we found that pear cultures are far more sensitive to the fluctuating ambient temperatures of our building than our colleagues' apple cultures. Purchase of controlled growth chambers aided greatly in growth consistency. In Years 3 and 4, we experience large scale contamination events, due to the failure of cold room near the lab, which when warmed up, grew a large amount of mold. We were able to decontaminate, repaint, and replace the condensers, and this reinforced the need for our sterile spaces to be rearranged, which we have now done. Finally, we learned the scale and time commitment of this types of work, and the need for personnel. Personnel changes, loss, and rehiring presented a largely unavoidable challenge, however this reinforced for us the importance of developing highly detailed and reproducible protocols, such that new lab members can easily learn. We further hope to publish many of these for the use of the community as well.

References

1. Montanari, S., Postman, J., Bassil, N.V., and Neale, D.B. (2020). Reconstruction of the Largest Pedigree Network for Pear Cultivars and Evaluation of the Genetic Diversity of the USDA-ARS National Pyrus Collection. G3 (Bethesda) *10*, 3285-3297.
2. Mourgues, F., Chevreau, E., Lambert, C., and de Bondt, A. (1996). Efficient Agrobacterium-mediated transformation and recovery of transgenic plants from pear (Pyrus communis L.). Plant Cell Rep *16*, 5.
3. Matsuda, N., Ikeda, K., Kurosaka, M., Takashina, T., Isuzugawa, K., Endo, T., and Omura, M. (2009). Early Flowering Phenotype in Transgenic Pears (Pyrus communis L.) Expressing the CiFT Gene. Journal of the Japanese Society for Horticultural Science *78*, 410-416.
4. Flachowsky, H., Peil, A., Sopanen, T., Elo, A., and Hanke, V. (2007). Overexpression of BpMADS4 from silver birch (Betula pendula Roth.) induces early-flowering in apple (Malus×domestica Borkh.). Plant Breeding *126*, 137-145.
5. Fu, Q.T., Li, C.Q., Tang, M.Y., Tao, Y.B., Pan, B.Z., Zhang, L., Niu, L.J., He, H.Y., Wang, X.L., and Xu, Z.F. (2015). An efficient protocol for Agrobacterium-mediated transformation of the biofuel plant Jatropha curcas by optimizing kanamycin concentration and duration of delayed selection. Plant Biotechnol Rep *9*, 405-416.
6. Matsuda, N., Gao, M., Isuzugawa, K., Takashina, T., and Nishimura, K. (2005). Development of an Agrobacterium-mediated transformation method for pear (Pyrus communis L.) with leaf-section and axillary shoot-meristem explants. Plant Cell Rep *24*, 45-51.
7. Ming, M.L., Long, H.J., Ye, Z.C., Pan, C.T., Chen, J.L., Tian, R., Sun, C.R., Xue, Y.S., Zhang, Y.X., Li, J.M., et al. (2022). Highly efficient CRISPR systems for loss-of-function and gain-of-function research in pear calli. Hortic Res-England *9*.
8. Nitsch, J.P., and Nitsch, C. (1969). Haploid plants from pollen grains. Science *163*, 85-87.

9. Dimitrova, N., and Nacheva, L. (2021). An Optimized Micropropagation Protocol by Ex Vitro Rooting of Pear Rootstock OHF 333 (Pyrus communis L.). Acta Agrobotanica *74*, 1-9.
10. Sharma, R., Modgil, M., Sharma, P., and Saini, U. (2012). Agrobacterium-mediated transfer of chitinase gene in apple (Malus x domestica Borkh.) rootstock MM106. Indian J Hortic *69*, 1-6.
11. Maheshwari, P., and Kovalchuk, I. (2016). Agrobacterium-Mediated Stable Genetic Transformation of Populus angustifolia and Populus balsamifera. Frontiers in Plant Science *7*.
12. Kim, M.-S., Klopfenstein, N.B., and Chun, Y.W. (1997). Agrobacterium-mediated Transformation of Populus Species. In Micropropagation, genetic engineering, and molecular biology of Populus, Volume General Technical Report RM-GTR-297, N.B. Klopfenstein, Y.W. Chun, M.-S. Kim and M.R. Ahuja, eds. (Fort Collins, CO: USDA Forest Service), pp. 51-59.
13. Bell, R.L., Srinivasan, C., and Lomberk, D. (2009). Effect of nutrient media on axillary shoot proliferation and preconditioning for adventitious shoot regeneration of pears. In Vitro Cell Dev-Pl *45*, 708-714.
14. Reed, B.M., Denoma, J., Wada, S., and Postman, J. (2013). Micropropagation of pear (Pyrus sp.). Methods Mol Biol *11013*, 3-18.
15. Laboratory, F.B. (2023). Presentations. Forestry Biotechnology Laboratory, https://biotechlab.forestry.oregonstate.edu/presentations, Accessed 12 Jan 2024.
16. Strauss, S., and Goralogia, G.S. (2023). Altruistic transformation with novel Agrobacterium genes (talk). Forestry Biotechnology Laboratory, https://biotechlab.forestry.oregonstate.edu/sites/default/files/StraussGoralogia_October2023.pdf, Accessed 12 Jan 2024.
17. Tomes, S., Gunaseelan, K., Dragulescu, M., Wang, Y.Y., Guo, L., Schaffer, R.J., and Varkonyi-Gasic, E. (2023). A MADS-box gene-induced early flowering pear (*Pyrus communis* L.) for accelerated pear breeding. Frontiers in Plant Science *14*.
18. Leblay, C., Chevreau, E., and Raboin, L.M. (1991). Adventitious Shoot Regeneration from Invitro Leaves of Several Pear Cultivars (Pyrus-Communis L). Plant Cell Tiss Org *25*, 99-105.
19. Montanari, S., Perchepied, L., Renault, D., Frijters, L., Velasco, R., Horner, M., Gardiner, S.E., Chagné, D., Bus, V.G.M., Durel, C.-E., et al. (2016). A QTL detected in an interspecific pear population confers stable fire blight resistance across different environments and genetic backgrounds. Molecular Breeding *36*.
20. Peil, A., Bus, V.G.M., Geider, K., Richter, K., Flachowsky, H., and Hanke, M.V. (2009). Improvement of Fire Blight Resistance in Apple and Pear. International Journal of Plant Breeding *3*, 1-27.
21. Zurn, J.D., Norelli, J.L., Montanari, S., Bell, R., and Bassil, N.V. (2020). Dissecting Genetic Resistance to Fire Blight in Three Pear Populations. Phytopathology *110*, 1305-1311.

Executive Summary

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

Keywords: agrobacterium-mediated pear transformation, inducible flowering, RCB

Abstract:

Traditionally, breeding for desirable fruit tree traits is a very long process, in large part due to long juvenility periods, resulting in waiting years for trees to bear flowers for crossing. In several tree crops - for example, apple, plum, citrus, poplar - rapid cycle breeding (RCB) systems have been developed that allow for multiple rounds of crossing within a much shorter time frame, allowing for rapid stacking of desirable traits. We aim to develop an RCB system for European pears, such that rapid breeding can occur to integrate traits like dwarfing (from Dr. Evans' WSU pear rootstock breeding program) and fire blight resistance (connecting with Dr. Bassil's work at the NCGR on Old Home and other genetic sources of resistance), to highlight a few examples. RCB systems in the past have been built on the overexpression of flowering genes, transformed into initial germplasm of interest. However, this constant overexpression of flowering genes can result in undesirable phenotypes, such as weak branches and early termination of flower buds, and require the need to screen many lines to find plants with the right level of expression of flowering. To avoid this, we aimed to transform pears with an inducible-flowering construct, developed by the Cutler lab at UC Riverside and successfully used in citrus. Through the project thus far, we have run numerous trials to transform this inducible-flowering RCB construct into 'Bartlett', 'OHxF 87' and 'OHxF 97'. We included 'Bartlett', as it is common PNW cultivar with a sequenced genome, allowing for future understanding of the genetics underlying transformation. We included 'OHxF 87' and 'OHxF 97', as they have genetics for fire blight resistance and semi-dwarfing. We have had success with transformation of callus tissue, which is the tissue formed in response to wounding and hormone inputs that acts as an intermediate tissue from which new adventitious shoots can regenerate, given the ideal hormone inputs and growth conditions. Further, we have steadily optimized and improved transformation of callus tissue with the RCB construct, which we have been able to monitor through the red fluorescent marker in the construct. However, we have not yet obtained adventitious shoots carrying the inducible-flowering RCB construct. We hypothesize that the 'Bartlett' and 'OHxF' germplasm is more difficult to regenerate and transform that many of the genotypes used in existing literature, due to unknown cultivar-specific requirements. Currently, we are conducting trials with an Agrobacterium strain called S82, which has been used successfully by the Strauss Lab at Oregon State University to transform difficult poplar genotypes by co-inoculation with a construct of interest (in our case, with the RCB construct). We have seen major improvements in callus transformation and are continuing to monitor trials for adventitious shoot regeneration. Once our inducible-flowering RCB construct is transformed into our germplasm, it will be tested for successful inducibility, as well as functional pollination and fruit growth. When these tests are complete, we can use these trees to induce flowering within a year of planting seeds and perform crosses with germplasm of interest to aid in the development of improved rootstocks for the U.S. pear industry.

Project Title: Development of a transgene-free gene editing system in European Pear

Report Type: Final Project Report

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Cooperators:

Project Duration: 1-Year

Total Project Request for Year 1 Funding: $ 8667

Total Project Request for Year 2 Funding: $
Total Project Request for Year 3 Funding: $

Other related/associated funding sources: Awarded
Funding Duration: 2022-23, 2023-24, and 2024-25
Amount: Awarded $8994 (2022-23), $8289 (2023-24), $8234 (2024-25)
Agency Name: California Pear Advisory Board
Notes: We were awarded funding for this project for the past three years from CPAB. The majority of funds from CPAB went to the Brown Lab at UC Davis.

WTFRC Collaborative Costs: none

Budget 1
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Item	2023		
Salaries			
Benefits			
Wages	$6,658.00		
Benefits	$509.00		
RCA Room Rental			
Shipping			
Supplies	$1,500.00		
Travel			
Plot Fees			
Miscellaneous			
Total	$8,667.00		

Footnotes:

OBJECTIVES

Long-term objective: To establish a system for gene editing in pear, to allow the future development of germplasm with dwarfing, fire blight resistance, and other desirable traits.

Objective 1: Optimize shoot tissue regeneration from leaf discs of 'OHxF 87' and 'OHxF 97'.
Objective 2: Optimize methods for isolating and culturing pear protoplasts from in vitro micro shoots.
Objective 3: Design and generate gene-editing machinery.

Significant Findings

Growth of 'Bartlett' and 'OHxF 97' on rooting media or addition of a dark/etiolation period prior to leaf excision do not strongly influence adventitious shoot regeneration alone. However, regeneration rates improved overall from previous years. These findings suggest that our current base protocol has been further optimized, but that the addition rooting media and dark periods likely need to be combined with other inputs to increase shoot regeneration. These are important findings for the development of tissue culture-based propagation, both in research labs and the nursery industry.

Published pear protoplast isolation protocols led to overdigestion of tissue. This tells us that these protocols need refinement and updating, as they are likely missing information on variables that were not focused on in the publications (i.e. environmental factors, micropropagation media and conditions). Improvement of these protocols is an important step to obtain the protoplast cells needed to perform gene-editing without introducing transgenes, which will aid in the improvement of rootstocks.

Target CRISPR sequences within the Phytoene Desaturase (PDS) target gene were identified. PDS is an important target gene for testing whether a gene editing system is functioning properly, and identification of these target sequences means that we will have everything ready for editing once protoplast isolation, culturing, and adventitious shoot regeneration protocols are optimized and ready.

Results

Objective 1: Optimize shoot tissue regeneration from leaf discs of 'OHxF 87' and 'OHxF 97'.

Two major bottlenecks in developing and using transgene-free gene-editing systems are: 1) delivery of DNA or RNA into plant cells without use of Agrobacterium and without permanent DNA incorporation into the plant genome and; 2) regeneration of tissue, and then plants, from those cells. One way to achieve transgene-free, or DNA-independent, editing is to use protoplasts. This method has been developed in other woody crops [1, 2]. Protoplasts are plant cells with their cell walls removed, and thus can take up DNA or RNA directly and temporarily without relying on Agrobacterium. However, to generate protoplasts and optimize the process (described in Obj. 2), and to eventually recover plants from protoplasts, true-to-type callus tissue is needed, as well as a system to regenerate single cells into plants. In this objective, we focused on optimizing adventitious shoot regeneration in 'OHxF 97' and 'Bartlett'. We planned to optimize regeneration in 'OHxF 87' as well, however a contamination event this year drastically reduced our numbers of 'OHxF 87' plantlets.

In the previous year, we identified multiple parameters that increased regeneration rates in *in vitro*-grown Bartlett plantlets in preliminary experiments: 1. specific hormone combinations in the regeneration media; 2. growing plantlets on rooting media prior to excising leaves for regeneration; 3. subjecting plantlets to a period of darkness (etiolation) prior to excising leaves. This year, we aimed to further test and optimize these treatments.

Briefly, our protocols for these tests were as follows: 'Bartlett' and 'OHxF 97' plantlets were trimmed and transferred to either a multiplication or rooting media and placed in the dark for 1 week. At the beginning of week 2, all plants were removed from dark and places in the light for an additional week. At the beginning of week 3, half of the plants grown on rooting media were placed back in the dark for 2 weeks. All other treatments remained in light for the same duration. At the end of week 4, shoot regeneration protocols were performed: leaf discs were cut or punched from the midrib region of young, expanding leaves from each treatment and placed on regeneration media. For 'Bartlett', full leaves were also excised and wounded by stabbing the leaves with forceps. Regeneration media consisted of full-strength MS media (for 'Bartlett') or NN69 media (for 'OHxF 97', [3]) with 15uM TDZ, and 5uM NAA. OHxF 97 leaves were also pre-soaked in liquid media prior to placement on plates to avoid oxidative browning, which has been an issue in the past for this cultivar. 'Bartlett' plates contained 9-12 leaf discs each, each plate was considered one replicate, with 3 replicate plates per treatment, and two full runs of the experiment separated by a week. 'OHxF 97' plates contained between 15-25 leaf discs, with 3 replicate plates per treatment. Plates were kept in darkness for 3 weeks and moved to light. Both total shoots and numbers of discs with regenerating shoots were counted at 4, 5, and 6 weeks and are reported below. A Student's t-test was used to determine significant differences between the means of different treatments. Media comparisons can be found in Table 1. Treatment comparisons can be found in Table 2.

Table 1. Media for Etiolation and Rooting experiment, prior to leaf excision:

	Multiplication Media	Rooting Media
MS media containing Gamborg's Vitamins (M404)	4.44g (1x)	2.22g (0.5x)
Hormones	5uM BAP, 0.5uM K-IBA	5uM K-IBA
Sucrose	30g (3%)	15g (1.5%)
Agar (A111)	6g	6g
pH	5.5	5.8

Table 2. Treatments compared in etiolation and rooting experiment

Media prior to leaf excision	Dark treatment (W1-W2-W3-W4)	Cut method
Multiplication Media	Dark-Light-Light-Light	Leaf disc/square
Multiplication Media	D-L-D-D (OHxF 97 only)	Leaf disc/square
Rooting media	D-L-L-L	Leaf disc/square
Rooting media	D-L-L-L	Full leaf, random stabs (Bartlett only)
Rooting media	D-L-D-D	Leaf disc/square
Rooting media	D-L-D-D	Full leaf, random stabs (Bartlett only)

At weeks 4, 5, and 6 after leaves and leaf discs were placed on regeneration media, we observed and calculated regeneration efficiencies, measured as the percentage of discs that had at least one regenerating shoot (Figure 1A, week 6 reported), as well as average shoots per disc, measured as the total number of regenerating shoots divided by the number of discs with at least one regenerated shoot (Figure 1B, week 6 reported). Regeneration on the multiplication media used in this experiment ranged from 14.8% to 25.9% (Figure 1A), which shows a marked improvement from 3% last year when the same base media was used. Statistical comparisons were made between different medias (MM vs. Root) for the same light conditions and cut methods, as well as different light conditions

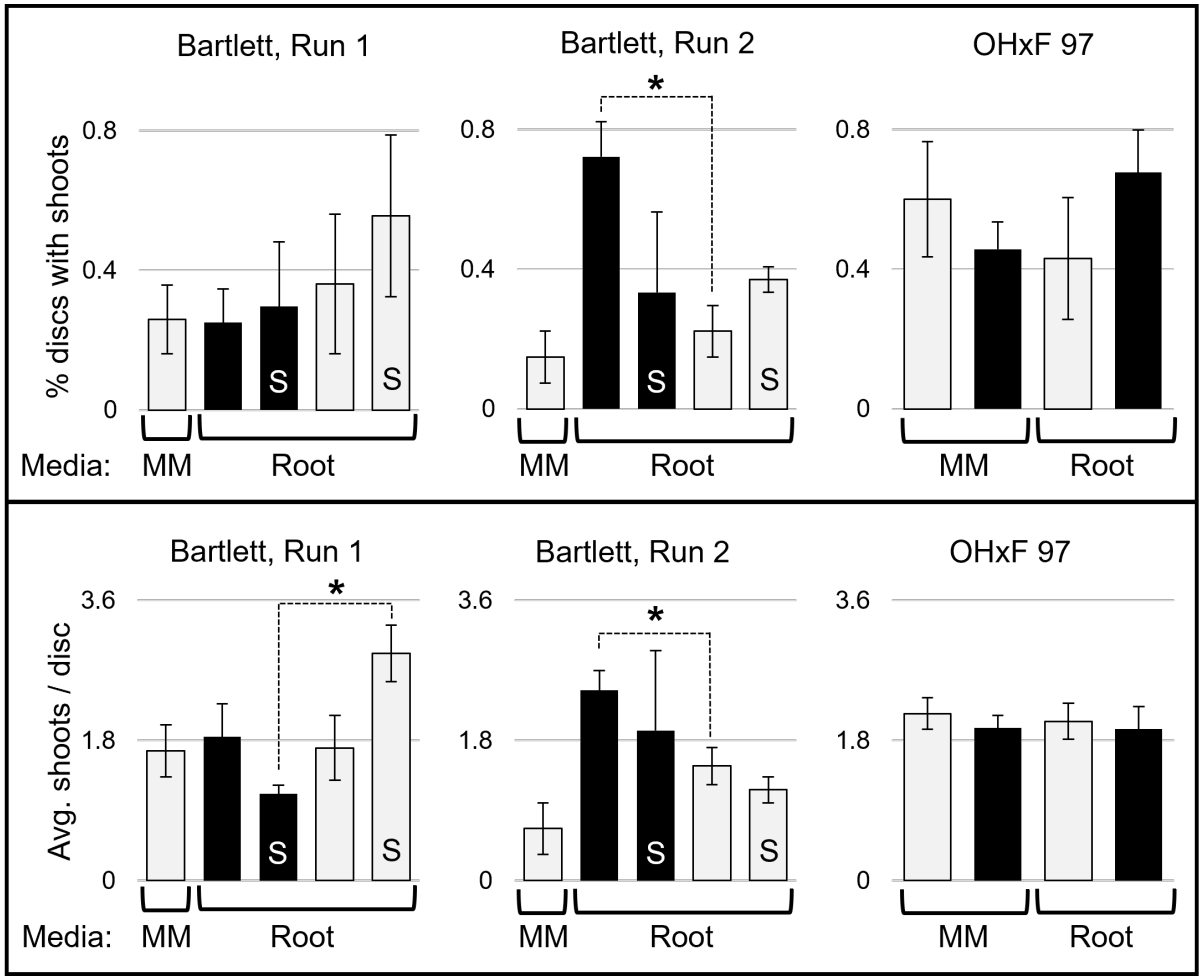

Figure 1. Regeneration efficiencies and shoot organogenesis averages at week 6 in leaf material from plantlets grown in different media and light conditions. A. Regeneration efficiencies, measured as the percent of leaf discs with at least one regeneration shoot, and B. the total number of regenerated shoots divided by the number of regenerating leaf discs are reported for 'Bartlett' and 'OHxF 97'. A fully replicated experiment was run twice for 'Bartlett' leaves, spaced one week apart. Prior to leaf excision and placement on regeneration media, in vitro plantlets were grown on either multiplication (MM) or rooting (Root) media, and subjected to either an extended dark period or kept in light. Light gray bars represent light treatment, and black bars represent dark treatment. "S" represents leaves that were wounded via stabbing with forceps. Student's t-tests were used to determine significant differences in two-way comparisons of the means of regeneration results from plants grown on different medias, in different light conditions, or using different cut methods. Asterisks indicate significance levels of $p<0.05$.

(DLLL vs DLDD) for the same media and cut methods, and few significant differences were found. In the first experimental run for 'Bartlett', no significant differences were found in regeneration efficiencies. However, in excised leaves that were wounded via stabbing with forceps, growth in darkness resulted in fewer shoots per leaf, but this effect was not seen in the second run of the experiment. In the second 'Bartlett' run, a significant improvement could be seen in regeneration efficiency for plant material that had received the dark treatment, compared to light (Figure 1A, middle graph). Further, in the same run, a large increase in regeneration efficiency could be seen when both dark treatment and rooting media were used, as compared to multiplication media in the light (Figure 1A, middle graph). However, these differences were not seen in the first run (Figure 1A, left graph). This suggests a potential role for an unknown parameter that differed between the two runs. In past years, we have seen regeneration rates from 'OHxF 87' and 'OHxF 97' reach ~35%. While we did not see significant differences between rooting media or darkness treatments for 'OHxF 97' in this experiment, we calculated regeneration efficiencies between 43% and 67.7%, which shows improvement and suggests that this combination of media and hormones is beneficial for regeneration in this cultivar. Overall, we cannot conclude that the addition of rooting media and/or a dark treatment can improve regeneration rates alone, and we will continue to test these parameters together with other inputs. Further, we saw several improvements to regeneration rates with the methods used in this study over results from previous years.

Objective 2: Optimize methods for isolating and culturing pear protoplasts from in vitro micro shoots.

One of the most common methods used for DNA-free gene-editing in woody plant species is polyethylene glycol (PEG)-mediated transformation of protoplasts, followed by regeneration of protoplasts into in vitro shoots that carry the edited gene. Before testing transformation, we've been working towards developing a reliable protocol for isolating and culturing protoplasts from common U.S.-grown genotypes.

In previous years, the Brown lab was able to isolate protoplasts using a modified protocol from similar experiments in grapes [4]. This year in the Waite lab, protoplast isolations followed a similar protocol, with modification of enzyme concentrations and using the media outlined in previous experiments with pears [5]. Briefly, 0.3-0.5g of recently unfurled, fully expanded leaves from Bartlett in vitro plantlets were harvested into CPW 13M media (recipe in Table 3), cut into 1-2mm strips, and soaked for 1 hour to plasmolyse the cells. During this hour, the enzyme solution was made fresh by adding 1.0% Cellulase Onozuka RS, 0.1% Pectolyase Y-23, 5mM 2-(N-morpholino) ethanesulfonic acid (MES) solution, and 1.0% Polyvinyl Pyrrolidone (PVP) to 20mL CPW 13M media. Leaf strips were then transferred into dishes containing the enzyme solution and shaken at root temperature (25C) in very dim light at 40rpm. Digestions were carried out for 16 hours and 18 hours. Tissues were then run through a nylon sieve to remove cellular debris, and centrifuged at 100xg for 10 minutes. Protoplasts at the meniscus were then resuspended into 21% sucrose, re-centrifuged at 100xg

Table 3. Media for protoplast isolations:

CPW 13M	mg/L
KNO3 (Potassium nitrate)	101
KH2PO4 (Potassium phosphate)	27.2
CaCl2.2H2O (Calcium chloride dihydrate)	1480
MgSO4.7H2O (Magnesium sulfate heptahydrate)	246
KI (Potassium iodide)	.16
CuSO4.5H2O (Cupric sulfate pentahydrate)	.02
Mannitol	130g

for another 10 minutes, and observed on the microscope. Building on experiments from last year, we also added an antioxidant mixture to the digestion solutions.
Our initial attempts this year results largely in incompletely digested cell walls. We also found that after collecting the digested tissues and filtering out the cells, protoplasts could be found in both the meniscus (top layer of the solution) and the bottom of the tubes after centrifugation, possibly due to that incomplete digestion. We next tested three additional enzyme mixtures from published pear protocols, outlined in Table 4, both for 16h and 18h duration. We found that these enzyme mixtures and digestions times resulted in overdigestion and leaf material showed a high level of oxidative browning, despite adding antioxidants to the digestion solution (Fig. 2). No characteristic band of protoplasts could be seen in the filtered solutions, in contrast to the previous year (Fig. 2). In future trials, we will test different levels of antioxidants, varying concentrations of enzymes, and varying digestions times with these mixtures.

Table 4. Enzyme mixtures tested for cell wall digestion and protoplast isolation.

Protocol publication	Media	Maceroenzyme R-10	Onozuka Cellulase R-10	Hemicellulase	Pectolyase Y-23
Revilla et al., 1987 [6]	CPW 13M	-	1.0%	1.0%	0.1%
Ochatt and Powers, 1988 [5]	CPW 13M	-	1.0%	-	0.1%
Ochatt and Powers, 1992 [7]	CPW 13M	0.2%	1.0%	1.0%	0.1%

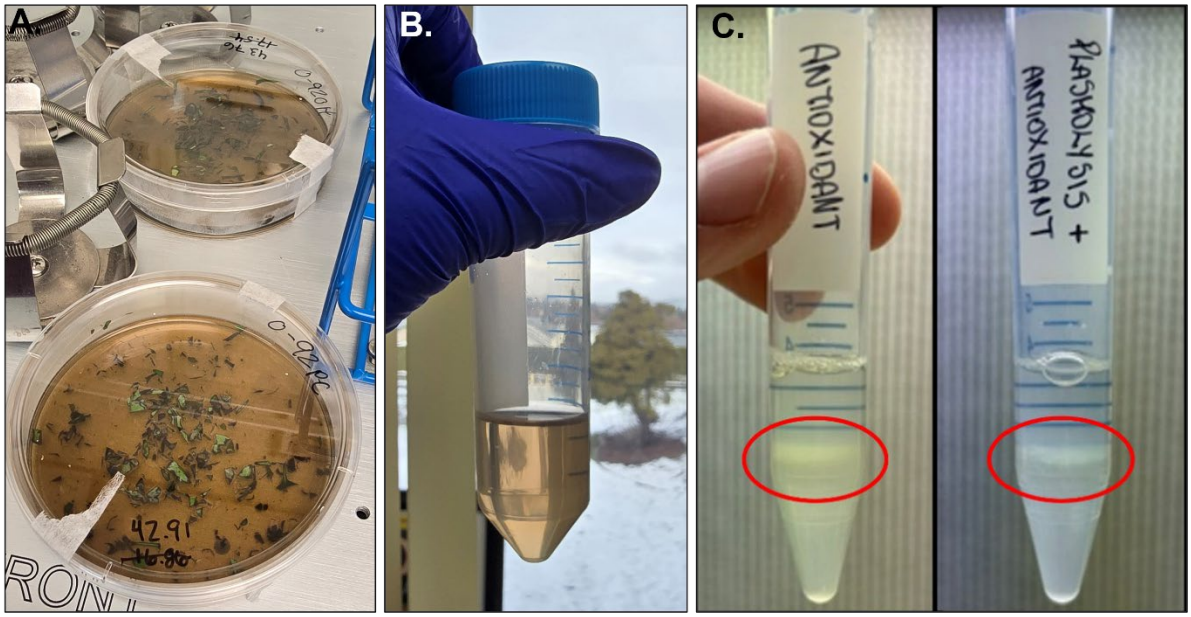

Figure 2. Overdigestion and oxidation during protoplast isolation experiments. A-B. Overdigested and browned tissues were seen after 16-18hours of treatment with all enzyme mixtures tested. Characteristic protoplast layer was no seen in B., compared to treatments from 2023 in C.

Objective 3: Design and generate gene-editing machinery.

In previous years, we determined that the use of Ribonucleoproteins (RNPs), a complex of pre-formed gene editing enzymes and guide RNAs, would be ideal for delivery of the gene editing machinery into plant cells via protoplast transformation. This year, we determined the sequences that would need to be purchased, such that once we develop a reliable protoplast regeneration system we will be ready to test transformations. We searched the genome for the correct sequences of the PHYTOENE DESATURASE (PDS) gene that we identified previously as a strong initial proof-of-concept target, as editing of this gene results in bleached tissues. To determine the guide RNA sequences that will be needed to target the CRISPR-Cas9 protein to the correct locations in the genome, we first identified all copies of the PDS gene, as well as homologous genes, in the Bartlett genome using the BLAST tool (Genome Database for Rosaceae, BLAST+ tool), and used the program JBrowse to determine that the genes were correct and expressed [8]. Next, we used the CRISPOR program to determine ideal guide RNA sequences that will be used to guide the editing machinery to the precise location in the gene [9]. Upon entering a desired sequence (in our case, the first two exons of the PDS gene) into the program, CRISPOR scans the sequence and the rest of the Bartlett genome to identify sequences that are likely to be high efficiency and have low chances of editing off-target sites. Table 5 contains the target gene ID, as well as the guide RNA sequences that will be included to guide the gene editing enzymes to the correct locations. The table also includes the number of predicted off-target sites, which are sequences elsewhere in the genome that share some similarity with the target. The number of mismatches in these potential off-target site correlates with the likelihood of being edited, such that 3-4 mismatches is less likely than 1-2 mismatches. Once edited, these sites will be sequenced to select for plants in which no off-target sites have been edited.

Table 5. Guide RNA sequences for targeting pear PDS gene for gene-editing

Gene description	Gene ID	Possible guide RNA sequences	# of potential off-target sites
Phytoene Desaturase	04g02050 - Exon 1	TTGGCAGCTCAAGTTAGCAGCGG	4 (w/ 3 mismatches) 11 (w/ 4 mismatches)
		AAAGAAAAGGCATCGCATCGGGG	2 (w/ 3 mismatches) 22 (w/ 4 mismatches)
		AAGCTGTTTATAGAAGGCCCAGG	1 (w/ 3 mismatches) 6 (w/ 4 mismatches
Phytoene Desaturase	04g02050 - Exon 2	GTACTGTCAAGGTCTGGTCTTGG	7 (w/ 4 mismatches)
		TTAGCAGTACTGTCAAGGTCTGG	2 (w/ 3 mismatches) 5 (w/ 4 mismatches)
		TTTAACGGCTTGGTTGGGCGAGG	17 (w/ 4 mismatches)

Discussion

Over the years of this project, funded by both the Fresh and Processed Pear Committees and the California Pear Advisory Board, we have learned a great deal and made significant improvements to callus production and phenotypes, adventitious shoot regeneration (in the absence of Agrobacterium),

and protoplast isolation in ‘Bartlett’, ‘OHxF 87’ and ‘OHxF 97’ germplasm. This foundational knowledge is absolutely crucial for building biotechnological tools like a transgene-free gene-editing system. Tissue culture-based techniques like micropropagation, adventitious shoot regeneration, transformation, protoplast isolation, and rooting were developed decades ago. However, work with these techniques in tree crops like pears has been done by relatively few researchers, and many protocols have not been revisited for a long time. Until quite recently in this field of *in vitro* biology, institutional knowledge was not often published and much has been lost as researchers retire. Further, many older articles were published before high-quality photographs were typically included in journals, which makes it more difficult to reproduce protocols. Thus, building this knowledge anew, and especially with cultivars relevant to the U.S. pear industry, has helped us to take great steps toward developing gene-editing in pears.

References

1. Malnoy, M., Viola, R., Jung, M.H., Koo, O.J., Kim, S., Kim, J.S., Velasco, R., and Kanchiswamy, C.N. (2016). DNA-Free Genetically Edited Grapevine and Apple Protoplast Using CRISPR/Cas9 Ribonucleoproteins. Frontiers in Plant Science *7*.
2. Najafi, S., Bertini, E., D'Inca, E., Fasoli, M., and Zenoni, S. (2023). DNA-free genome editing in grapevine using CRISPR/Cas9 ribonucleoprotein complexes followed by protoplast regeneration. Hortic Res *10*, uhac240.
3. Nitsch, J.P., and Nitsch, C. (1969). Haploid plants from pollen grains. Science *163*, 85-87.
4. Tricoli, D. (2019). Renewal Progress Report for CDFA Agreement Number# 18-0397.
5. Ochatt, S.J., and Power, J.B. (1988). Plant-Regeneration from Mesophyll Protoplasts of Williams Bon Chretien (Syn Bartlett) Pear (Pyrus-Communis L). Plant Cell Reports *7*, 587-589.
6. Revilla, M.A., Ochatt, S.J., Doughty, S., and Power, J.B. (1987). A General Strategy for the Isolation of Mesophyll Protoplasts from Deciduous Fruit and Nut Tree Species. Plant Science *50*, 133-137.
7. Ochatt, S.J., Chevreau, E., and Gallet, M. (1992). Organogenesis from 'Passe Crassane' and 'Old Home' pear (Pyrus communis L.) protoplasts and isoenzymatic trueness-to-type of the regenerated plants. Theor Appl Genet *83*, 1013-1018.
8. Jung, S., Lee, T., Cheng, C.H., Buble, K., Zheng, P., Yu, J., Humann, J., Ficklin, S.P., Gasic, K., Scott, K., et al. (2019). 15 years of GDR: New data and functionality in the Genome Database for Rosaceae. Nucleic Acids Res *47*, D1137-D1145.
9. Concordet, J.P., and Haeussler, M. (2018). CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. Nucleic Acids Res *46*, W242-W245.

Executive Summary

Title: Development of a transgene-free gene editing system in European Pear

Keywords: adventitious shoot regeneration, protoplasts, DNA-free transformation

Abstract:

Gene editing has a strong potential to be useful for clonal crop species like pears. This is in part because it allows for the ability to make precise DNA changes without breeding, which gives us an additional tool for introducing traits into the germplasm. However, traditional gene-editing relies on the integration of transgenes into the plant's genome. Methods for the removal of transgenes often require additional rounds of breeding, especially for clonal species, which counteracts many of the benefits. In the past decade, researchers have begun developing methods for transgene-free gene editing in many crop plants, in which gene-editing machinery is introduced into plant cells without integrating any foreign genetic material into the plant's DNA. This reduces the need for additional rounds of breeding to address regulatory concerns. One of the most common ways this has been achieved is through introducing gene-editing machinery into protoplasts cells to edit the DNA, allowing these edited cells to grow into callus tissue, and then subsequently regenerating adventitious shoots from those cells. Protoplasts are plant cells which have had their cell walls digested, allowing for easier movement of the gene-editing machinery into the cell to reach the nucleus. The most difficult steps in this process are isolation of protoplasts and culture into callus, and adventitious shoot regeneration from that callus. This is in part due to each pear genotype having specific and distinct responses to media additives like nutrients and hormones. This year, we aimed to improve upon adventitious shoot regeneration protocols for ‘Bartlett’, ‘OHxF 87’ and ‘OHxF 97’ genotypes, optimize protoplast isolations by testing more digestion parameters, and finish designing the gene editing machinery. We focused in on one of our highest-producing shoot regeneration experiments from the previous year – plant growth on rooting media and dark treatment prior to leaf excision – and found that when performed on a larger scale, these treatments alone did not improve adventitious shoot regeneration. However, our efficiency rates were generally higher than they have been in the past, signifying that overall, our base protocols have improved. Protoplast isolation trials were run to test enzyme concentrations, combinations, and digestion times from three previously-published protocols for pears, and all led to overdigestion and oxidation. In future trials, we will expand the concentrations and digestion times with these enzymes, as well as vary the antioxidant concentrations in the media. Finally, specific guide-RNA target sequences of the Phytoene Desaturase gene were identified to be used with the gene-editing machinery once a protoplasts isolation and regeneration system are established. Future work will continue to focus on developing and optimizing protocols for these more difficult steps, such that genotypes important for the U.S. pear industry can be edited for important trait-associated genes.

Project Title: Pear Rootstock Breeding PR-22-102

Report Type: Final Project Report

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Cooperators:
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Project Duration: 3 Years

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

Other related/associated funding sources: Awarded
Funding Duration: 2022
Amount: approximately $6,000
Agency Name: USDA ARS
Notes: Summer intern (Edwin Polanco) "FACT: Research Experience for Undergraduates on Phenomics Big Data Management." (PI: Sankaran). Award covered stipend plus travel and housing.

Funding Duration: 2022 - 2025
Amount: various
Agency Name: Pome fruit breeding program royalties
Notes: apple royalties used to supplement for e.g. conference travel costs, publication fees, equipment, collaborative genetics/genomics research with cooperator Waite including graduate student Ramesh Pilli.

Funding Duration: 2023
Amount: $147,827
Agency Name: NNII
Notes: orchard (CV) infrastructure and equipment which will benefit both pear rootstock and apple scion breeding programs

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Kate Evans
Organization Name: WSU-TFREC
Contract Administrator: Waylan Safranski
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Contract administrator email address: wski@wsu.edu

Item	2022	2023	2024
Salaries	$53,144.00	$55,270.00	$57,481.00
Benefits	$17,507.00	$18,207.00	$18,936.00
Wages	$6,955.00	$7,233.00	$7,522.00
Benefits	$4,365.00	$4,539.00	$4,721.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$12,890.00	$9,890.00	$5,890.00
Travel	$3,080.00	$3,080.00	$3,080.00
Plot Fees	$2,651.00	$3,182.00	$3,395.00
Miscellaneous	$0.00		
Total	$100,592.00	$101,401.00	$101,025.00

Footnotes: Salaries for research assistant professor (Teh) and then Research Associate (Cain) as the point person for pear rootstock; Wages for time-slip labor for orchard management and trait phenotyping; 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. Conduct marker-trait association for rootstock-conferred traits in seedling populations
3. Validate stability/repeatability of preliminary dwarfing locus
4. Maintain a relevant pear rootstock parent germplasm
5. Evaluate B × A and B × C selections

A major bottleneck to high-density pear production in the Pacific Northwest is the lack of dwarfing, precocious, cold-hardy rootstocks. Such rootstocks can transform the industry enabling high-density pear production. A foundational project (PI: Evans; "Pear Rootstock Breeding"; PR-09-905) established a collection of diverse parental germplasm for use as crossing parents. The first pear rootstock seedling populations were produced in PR-15-105 "Pear Rootstock Breeding" (PI: Evans) (see Fig 1 for proposed breeding timeline). Evaluation of these populations began in project PR-19-108, which also included the first steps toward establishing necessary genotyping resources to inform breeding for dwarfing. This project aimed to build on recent (and concurrent) research to further develop a long-term dedicated pear rootstock breeding program at the WSU Tree Fruit Research and Extension Center, Wenatchee, WA. Research effort focused on evaluating current seedling populations for selection and collecting robust phenotypic data that could be integrated with existing genotypic information to facilitate efficient future selection of desirable rootstocks.

SIGNIFICANT FINDINGS

- Approximately 2,000 *Pyrus* seedlings were evaluated for scion and rootstock vigor traits in 2022-2025.
- Ten precocious seedlings that were previously micropropagated (10 replicates per seedlings) are being maintained in the WSU TFREC hoop house to add to the parent germplasm set.
- Forty-two seedlings from the 2016 seedling families (including some of the previously micropropagated individuals) were selected for propagation for further evaluation and possible Phase 2 inclusion.
- Two dwarfing loci were identified (on chromosomes 5 and 15); analysis was completed to characterize their haploblocks and their relative contributions to vigor reduction.

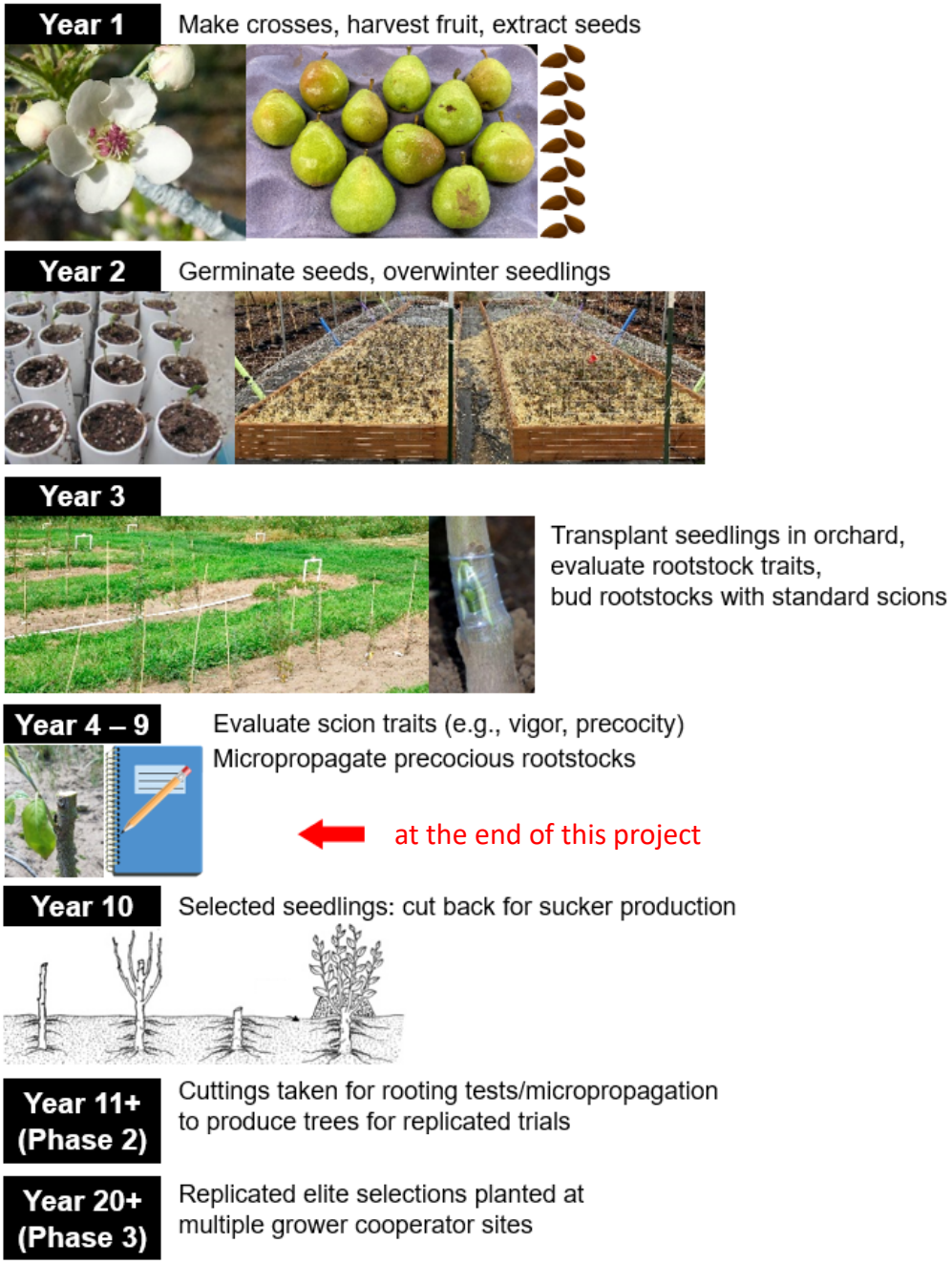

Figure 1: Timeline overview of the WSU pear rootstock breeding program in developing new dwarfing pear rootstocks.

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 (2020-22)	Branch angle (2020-22) Floral bud count (2021-24) Internode length (2020-22) Scion growth (2020-22) Trunk diameter (2020-23) Budbreak (2021-23)
2017	~320	Branch angle (2020) Presence of spine (2020)	Branch angle (2023-24) Floral bud count (2023-24) Internode length (2022) Scion growth (2022-24) Trunk diameter (2022-24) Budbreak (2022-24)
2019	~1,000	Branch angle (2022) Presence of spine (2022)	Branch angle (2024) Floral bud count (2023-24) Internode length (2023) Scion growth (2023-24) Trunk diameter (2023-24) Budbreak (2023-24)

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

Bloom data was collected for all populations each spring, however due to risk of fire blight infection, once bloom was recorded, it was removed.
Evaluation of the 2016 seedling families was completed and forty-two individuals were selected in summer 2024, moving ahead of the expected timeline shown in Figure 1. Tissue was sent to Qualterra for micropropagation. In addition, seedling trees were cut back to below the graft union and mounded with sawdust to encourage production of rooted suckers. The aim is for sufficient material to test for fire blight resistance in the greenhouse and to establish a subset of individuals into a Phase 2 replicated trial in a subsequent project.
Approximately 1400 new pear seeds produced in 2023 are currently germinating and will be screened for resistance to fire blight in the greenhouse in spring 2025.

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

Two dwarfing loci were identified (on chromosomes 5 and 15) in two of the 2016 seedling families. Further analysis was completed to characterize their haploblocks and their relative contributions to vigor reduction. [A haploblock is a section of DNA that tends to be inherited as a unit rather than frequently be rearranged during meiosis.] Each dwarfing haplotype accounted for 30% to 50% reduction in vigor ($p < 0.05$). Combined haplotype analysis

showed that one dwarfing locus was sufficient to significantly reduce vigor. Presence of two dwarfing haplotypes further reduced vigor by a total of 50% to 70% ($p < 0.05$).

Objective 3: Validate stability/repeatability of preliminary dwarfing locus
We are continuing to collaborate with Dr. Waite (USDA-ARS, Wenatchee) to add precision to the DNA region associated with dwarfing, using a new computational tool (Khufu) to identify genetic variants in our data set. Progress has been slower than expected with Hudson Alpha, however we recently started collaborating with Dr. Gottschalk (USDA-ARS, Kearneysville) and hope to leverage his experience with similar data sets.

Objective 4: Maintain a relevant pear rootstock parent germplasm
The existing rootstock parent germplasm continues to be maintained at WSU Sunrise orchard. No new material has been added although ten precocious seedlings from the 2016 families have been micropropagated with a view to add to the parent collection.

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. Musacchi for his advice on training these trees.
Fruit yield and quality data has been collected for both the 2023 and 2024 season, which together with vigor data should enable final selection in spring 2025.

Executive Summary

Project title: Pear Rootstock Breeding

Key words: dwarfing; precocious; *Pyrus*

Abstract:

The lack of dwarfing, precocious, cold-hardy rootstocks is a major bottleneck to high-density pear production in the Pacific Northwest. Such rootstocks can transform the industry enabling high-density pear production. This project aimed to build on recent (and concurrent) research to further develop a long-term dedicated pear rootstock breeding program at the WSU Tree Fruit Research and Extension Center, Wenatchee, WA. Research effort focused on evaluating current seedling populations for selection and collecting robust phenotypic data that could be integrated with existing genotypic information to facilitate efficient future selection of desirable rootstocks.

Approximately 2,000 *Pyrus* seedlings were evaluated for scion and rootstock vigor traits over the 2022-2025 period. Forty-two seedlings from the oldest seedling families (2016) were selected for propagation for further evaluation, with a view to including some of them in a future replicated Phase 2 trial. Final selections on an earlier small set of potential rootstocks (B × A and B × C trees) will be made in spring 2025; these would also need to be evaluated in a full replicated trial.

Data from the oldest seedling block was used to identify two dwarfing loci (areas of the genome on chromosomes 5 and 15); analysis was completed to characterize their relative contributions to vigor reduction. Further data mining is on-going to add precision to the regions identified as they contain many genes, the first steps toward establishing necessary genotyping resources to inform breeding for dwarfing.

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

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

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

Other funding sources: **None**

WTFRC Budget: ***None***

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

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

Footnotes:
[1] Benefits were calculated from actual OPE rates (20% of OSU technician). An annual increase of 5% was applied to years 2 and 3.
[2] Wages are for part-time employee to help with general maintenance during the season; 190 hours at $15/hr. Part-time employee benefits are calculated at 10%.
[3] Travel is to cover mileage to plot for measurements and one trip per year (4 days) for Einhorn (total $1,500) to travel to plots to perform pruning and training tasks and meet with K. Galimba and S. Musacchi and grower

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

Budget 2
Organization Name: WSU **Contract Administrator:** Kathy Roberts, Shelli Tompkins
Telephone: (509) 293-8803 **Email:** katy.roberts@wsu.edu, shelli.tompkins@wsu.edu
Station Manager/Supervisor: **Email Address:**

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

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

Budget 3
Organization Name: North American Plants, Inc. **Contract Administrator:Yongjian Chang**
Telephone: 503-474-1852 **Email address:** ychang@naplants.com
Station Manager/Supervisor: **Email Address:**

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

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

Significant Findings:

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

- **Based on growth habit, vigor, canopy balance, precocity and production during the first four cropping years (2023 was the 4th crop), roughly half of these rootstocks continued to perform well. Genetic testing of all accessions indicated that several of the 20 accessions (this includes the newly propagated accessions in Obj 2) have a high degree of genetic similarity (via finger printing analysis). These analyses were performed on leaves sampled from suckers at the Entiat, WA site and tissue cultured rootstocks at NAP which were derived from shoot tips collected at the Clonal Germplasm Repository in Corvallis, OR.**
- **Our multi-site trial facilitated a comparison between two very different sites (soil and climate) on tree growth and production. The shorter, relatively cooler growing region of Parkdale with heavier more fertile soils produced trees that were 50-100% larger than their counterparts in WA.**
- **High performing 'D'Anjou' trees on size controlling quince rootstocks in both OR and WA produced between 20 and 40 bins per acre in 2023. This was nearly double the yields of 2022. Production is based on 1210 trees/acre which is the planting density of the trial. Fruit size for these combinations ranged from poor (152 g) to excellent (271 g) and varied considerably between and within sites. Most accessions produced box counts of 80s to 90s in OR and 90s to 100s in WA.**
- **2023 yields of high-performing Bartlett trees on size controlling quince were slightly higher than D'Anjou in WA, resulting in ~ 30 to 50 bins per acre. Tree density was the same as Anjou. In OR, Bartlett yields were lower; trees produced roughly ~50 fruit per tree (on average) equating to 30 bins per acre. Fruit size was generally good (220 g and 190 g on average in OR and WA, respectively).**
- **In the existing trials, Comice serves as the interstem between the quince rootstock accessions and the scions (Anjou or Bartlett). Comice is regarded as having good compatibility with quince rootstocks, in general; however, pear scions do differ in their relative compatibility with quince. Thus, the poor performance of a few rootstocks could be attributed to interstem issues (i.e., incompatible with Comice). This is further supported by their differential behavior when direct-grafted to either Bartlett or Anjou. For example, 99.002 had more vigor in OR for both pear scions without an interstem as compared to trees with Comice interstem.**
- **Trees are maintainable in their 3 ft in-row spacing, even with Amjou in the fertile Parkdale site, with the exception of a few accessions. Pruning of Bartlett trees was mostly by short-pruning. Anjou trees received a combination of short and long pruning techniques. Trees were trained to a spindle architecture with very narrow canopies that were slightly pyramidal in form. All large limbs (~50% of the trunk diameter) were removed with renewal cuts to encourage weak replacement shoots.**

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

- **All cold hardy quince selections that were not previously tissue-cultured were successfully micropropagated from shoot tips in 2022. These represent diverse germplasm of cold hardy and plausibly dwarfing pear rootstocks and include the three hardiest quince taxa of the entire germplasm collection. Rooting of a sufficient number**

of each selection to facilitate new tree production for future field-performance trials is underway.

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.

Confirming the genetic identity of selections (i.e., true to type)

I would like to begin with an accounting of the philosophy and methodology applied to this project which, like any other germplasm exploration, comprises an inherently high degree of risk. The original research that defined the cold hardiness of quince accessions at the National Clonal Germplasm Repository in Corvallis, OR was conducted by PI Einhorn many years ago. From that research, ~20 quince accessions were selected based on their performance over three consecutive years throughout their dormancy transitions (September through April). The NCGR furnished material of these selections to NAP. NAP was successful in micropropagating half of the accessions, initially. These tissue-cultured and subsequently rooted explants were then supplied to Helios nursery. Helios nursery planted these in an OR liner field and grafted them with Comice interstems, later budded Bartlett and Anjou, raised the trees for two years, dug them and sent them to Einhorn in OR. Einhorn divided the trees and sent half to Musacchi in WA. The trees were then planted in their respective sites with appropriate experimental designs. Performance during the first few years of some accessions (dwarfing, growth habit, precocity, yield, and fruit size) showed very good potential. We remained cognizant, however, of the many potential issues facing quince, in addition to cold tenderness, when used as pear rootstocks. Several of these horticultural challenges (decline, incompatibility, fire blight, iron deficiency/chlorosis, etc.) can require many years of field testing (possibly beyond the timeframe of this project) before enough confidence could be gained to advance any promising selections to a subsequent round of testing (i.e., small scale commercial plots). Based on our collective experience with quince and the timeline of this project, we were purposeful not to prematurely 'release' promising selections to commercial entities; an approach intended to avoid scenarios that would cost the industry far more money/resources than the funding already received for the project or the interest we collectively share for identifying a dwarfing, productive pear rootstock. After observing strikingly similar performance and growth of scions on several rootstock selections over several years, we decided to collect leaves from rootstocks suckers in WA as well as from all tissue culture jars at NAP, which originated from the NCGR, to confirm their genetics. Material was sent to an external molecular laboratory specialized in fingerprinting by SSR markers in a blind experimental design that included standard quince rootstocks (i.e., Quince A [from two sources; US and Europe], Quince C, Quince BA29C, Quince Sydo, etc.). The CYD accessions 57.001 and 65.001 were reported to have a high level of genetic similarity (i.e., they are likely identical). 22.001, another promising rootstock, tested as genetically similar to Quince MA, a standard quince. A second year of fingerprinting provided confidence as to individual accession origin, as far as could be traced to the NCGR. All accessions from the project are listed in Table 6 with an explanation of their origin and our recommendation based on fingerprinting and/or performance data to stop future development or continue trialing. Despite issues in traceability back to the NCGR collection in Corvallis, there were no mixups in the field plantings of these trials, despite the many transfers of material from the

inception of tissue culturing to the field trials. This was confirmed by having representation of several replicates of each treatment in the assays.

Mortality

Mortality has been documented in previous reports as the average percent survival for each combination. 68.002 had the highest proportion of dead trees with both scions after approximately 4 years from planting (~50%). The accessions 118.001 and 99.002 also experienced mortality between 35 and 60%. For high-performing combinations, additional mortality after that observed in the establishment year was not observed, at either site. Regarding combinations without an interstem, Anjou/99.002 (direct graft) had the highest incidence of tree failure (83%), while Bartlett/99.002 (direct graft) had 0% mortality in WA. Detailed mortality data from WA is shown in Table 2. These data support a future evaluation of compatibility in order to determine the optimal pear interstem for these rootstocks.

Pruning

Dormant pruning of the Entiat, WA and Parkdale, OR plots was conducted in March and April 2023, respectively. The same methodology as reported in the previous years was executed in each plot. For Anjou, some significant differences emerged when comparing the average pruning weights (as kg per tree) among the 9 combinations in trial with Comice as interstem; Anjou/Comice/99.002 had greater than 2 kg per tree of pruned wood (Table 1), which was significantly greater than all other combinations and agrees with trunk measurements (Table 2) and results from previous years. At the other extreme, Anjou/Comice/68.002 and 67.001 produced $1/6^{th}$ of the pruning weights; these data also aligned with the tree size (as measured by trunks). In OR, pruning weights and trunk size were also the lowest for this combination. For Bartlett, no differences among combinations were observed for average pruning weight in 2023 as shown previously, but clear differences emerged for cumulative pruning weights over the life of the planting (Table 1) and was supported by trunk measures (Table 2). Most combinations had good vigor and produced a similar weight of pruning wood with Bartlett/Comice/118.001 and 68.002 having markedly lower vigor both in pruning weight and trunk size (Tables 1 and 2). At either end of the spectrum, similar observations were seen in OR, suggesting that despite vast differences in climate, very vigorous and very weak genotypes were performing similarly.

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

Table 1. Pruning wood weight (kg/tree) on March 8th, 2023 and cumulated 6-year pruned wood weight (kg/tree) from 2018 to 2023. All trees are scions of d' Anjou or Bartlett with a Comice interstem and roots belonging to the quince accessions for the present study. Direct graft combinations of scions on quince rootstocks were excluded. The means are averages of 3 replications per combination. Significance, *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. ***The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow in ALL talbes).***

Cultivar	Rootstock	N=	Pruned wood weight (kg/ tree) in 2023		Pruned wood weight (kg/ tree) in the past 6 years (∑2018-2023)	
Anjou	22.001	3	0.93	BC	3.60	BC
Anjou	23.001	3	0.69	BC	3.65	BC
Anjou	57.001	3	0.83	BC	3.95	B
Anjou	65.001	3	0.82	BC	4.02	B
Anjou	67.001	3	0.37	C	2.60	BCD
Anjou	68.002	3	0.42	C	1.43	D
Anjou	70.001	3	1.18	B	3.96	B
Anjou	99.002	3	2.43	**A**	6.35	**A**
Anjou	118.001	3	0.57	BC	1.89	CD
Significance			***		***	
Bartlett	22.001	3	0.69		4.28	AB
Bartlett	23.001	3	0.45		3.56	AB
Bartlett	57.001	3	0.75		4.56	AB
Bartlett	65.001	3	0.84		5.13	**A**
Bartlett	67.001	3	0.40		2.80	AB
Bartlett	68.002	3	0.26		1.54	B
Bartlett	70.001	3	0.97		4.35	AB
Bartlett	99.002	3	0.81		4.06	AB
Bartlett	118.001	3	0.13		1.48	B
Significance			NS		**	

Table 2. Trunk cross section area, TCSA and mortality (%) for d' Anjou and Bartlett in January and October 2023. All trees are scions of Bartlett or Anjou with a Comice interstem and roots belonging to the quince accessions for the present study. The means are averages of N trees per combination (N is varied in the experiment). Significance, *=p<0.05, **=p<0.01, ***=p<0.001. NS, not significant. The mortality, shown as percentage, were performed arcsin() transformation, before performing AOV analysis and post-hoc. Post-doc letters separations are by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. Note, one tree from Comice/99.002 and two from 118.001 were excluded due to the data missing or incorrect TSCA measurements, recorded Oct 2023.

Cultivar	Rootstock	N (for Jan 2023)=	N (for Oct 2023*)=	TCSA (cm^2) on Jan 2023		TCSA (cm^2) on Oct 2023		Tree growth (cm^2) between Nov 2021 to Jan 2023		Tree growth (cm^2) between Jan 2023 to Oct 2023		Mortality (%) on Oct 2023	
Anjou	22.001	22	22	23.47	BC	28.93	BCD	5.00	BC	5.45	AB	0%	B
Anjou	23.001	12	12	22.37	BC	26.09	BCD	4.48	BC	3.73	ABC	0%	B
Anjou	57.001	16	16	26.88	B	32.82	B	5.87	BC	5.94	AB	0%	B
Anjou	65.001	17	17	28.25	B	34.30	B	5.84	BC	6.05	AB	0%	B
Anjou	67.001	12	12	17.99	BC	20.54	CDE	3.03	BC	2.55	BC	0%	B
Anjou	68.002	5	5	12.01	C	12.81	E	1.29	C	0.80	C	64%	**A**
Anjou	70.001	29	29	24.00	BC	30.62	BC	6.84	BC	6.61	AB	26%	**A**
Anjou	99.002	17	17	43.05	**A**	51.11	**A**	13.37	**A**	8.06	**A**	60%	**A**
Anjou	118.001	7	7	16.60	BC	18.81	DE	2.20	BC	2.21	BC	36%	**A**
Significance				**		***		***		***		**	
Bartlett	22.001	24	24	17.50	AB	20.01	AB	1.90		2.51	AB	0%	D
Bartlett	23.001	12	12	19.05	AB	21.97	AB	1.22		2.92	AB	0%	D
Bartlett	57.001	15	15	19.93	AB	22.76	AB	2.09		2.84	AB	0%	D
Bartlett	65.001	17	17	22.07	**A**	25.60	**A**	1.91		3.53	**A**	0%	D
Bartlett	67.001	13	13	14.00	BC	15.59	BC	1.12		1.59	AB	0%	D
Bartlett	68.002	7	7	11.07	C	12.02	C	1.33		0.95	B	56%	**A**
Bartlett	70.001	25	25	18.89	AB	22.06	AB	2.53		3.17	AB	14%	C
Bartlett	99.002	**36**	35	20.19	AB	23.55	AB	2.13		3.02	AB	35%	B
Bartlett	118.001	**25**	23	8.69	C	9.68	C	0.85		0.72	B	34%	B
Significance				**		***		NS		***		***	

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

Bloom

The number of Anjou flower clusters per tree counted in spring 2023 was considered excellent, with most combinations having between 200 and 300 clusters per tree in WA (Table 3). Clusters in OR were much lower ranging from 50 to 120 per tree, which was more than half observed in 2022. Bartlett had fewer clusters than Anjou in WA, ranging from 35-80 per tree (Table 3), which was similar to OR (data not shown). No significant differences emerged among the 9 combinations, irrespective of cultivar, for bloom. The phenological status, full bloom (FB) and petal fall (PF) was recorded for both cultivars on May 3rd, 2023. Bartlett showed to be more advanced in its phenological stage, with six combinations of Bartlett in PF stage having 33% or more of the trees than Anjou (Table 3). For Anjou, all combinations were scored as at full bloom (FB) on May 3rd, 2023. There was no significant difference in phenological stage across all nine combinations for either Anjou and Bartlett.

Table 3. Flower clusters number and phenological status for Anjou and Bartlett were recorded on April 17th, and May 3rd 2023, respectively. All trees are scions of d' Anjou or Bartlett with a Comice interstem and roots belonging to the quince accessions for the present study. Direct graft combinations of scions on quince rootstocks were excluded. Significance, *=p<0.05, **=p <0.01, ***=p <0.001. NS, not significant. The count data fit normal distribution, then AOV analysis was applied. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow).

Cultivar	Interstem	Rootstock	N=	Flower cluster counts/tree 2023	Full bloom (% trees in that stage) 2023	Petal fall (% trees in that stage) 2023
Anjou	Comice	22.001	3	307	100	0
Anjou	Comice	23.001	3	297	100	0
Anjou	Comice	57.001	3	366	100	0
Anjou	Comice	65.001	3	267	100	0
Anjou	Comice	67.001	3	277	100	0
Anjou	Comice	68.002	3	322	100	0
Anjou	Comice	70.001	3	186	100	0
Anjou	Comice	99.002	3	250	100	0
Anjou	Comice	118.001	3	283	100	0
Significance				NS	NS	NS
Bartlett	Comice	22.001	3	38	100	0
Bartlett	Comice	23.001	3	55	67	33
Bartlett	Comice	57.001	3	80	100	0
Bartlett	Comice	65.001	3	50	67	33
Bartlett	Comice	67.001	3	66	67	33
Bartlett	Comice	68.002	3	63	33	67
Bartlett	Comice	70.001	3	77	67	33
Bartlett	Comice	99.002	3	35	33	67
Bartlett	Comice	118.001	3	76	100	0
Significance				NS	NS	NS

Productivity

2023 was the fourth cropping year from orchard establishment. Anjou was harvested in WA on 8/29 (roughly 2.5 weeks earlier than 2022) and October 3 in OR. Production of Anjou was higher than the previous year due to frost events and poor pollination conditions of 2022 (discussed in 2022 report). High performing 'D'Anjou' trees on size controlling quince rootstocks in both OR and WA produced between 20 and 40 bins per acre in 2023 (see WA yield data in Table 4; OR data are still being prepared). This was nearly double the yields of 2022. Production is based on 1210 trees/acre which is the planting density of the trial. While yield did not differ significantly among accessions, the mumber of fruit per tree did (Table 4). The largest and smallest fruit number/tree for Anjou was 95 pears for Comice/57.001 and 29 pears for Comice/99.002, respectively. Recall that 99.002 had the largest volume of pruning wood and produces an extremely vigorous tree; thus, even on quince rootstocks precocity and productivty can be compromised by roots imparting too much vigor to the scion. In the case of 57.001 (one of the four similar genotypes) there is a very nice balance of viogor (as seen by pruning weight or trunk size data) and productivity.

For Bartlett, 2023 yields of high-performing quince were slightly higher than D'Anjou in WA, resulting in ~ 30 to 50 bins per acre, based on 1210 trees per acre. In OR, Bartlett yields were lower; trees produced roughly ~50 fruit per tree (on average) equating to 30 bins per acre. Numerical but nonsignificant differences were observed among the accessions in the number of fruit per tree, ranging from Comice/65.001 with 84 pears per tree to Comice/68.002 having 52 pears per tree.

The average fruit weight was affected by the rootstock combination in Anjou showing an expected negative relationship with the number of fruit/tree; trees with higher crop load had smaller pears (Table 4). Anjou/Comice/57.001 and Anjou/Comice/23.001 had the lowest average fruit weights (152 g), while Anjou/Comice/99.002 had the largest fruit weight (246 g). However, no significant difference in average fruit weight in 2023 was found among Bartlett combinations. Fruit sizes in OR tended to be ~1 box size larger than WA (data will be presented at the review).

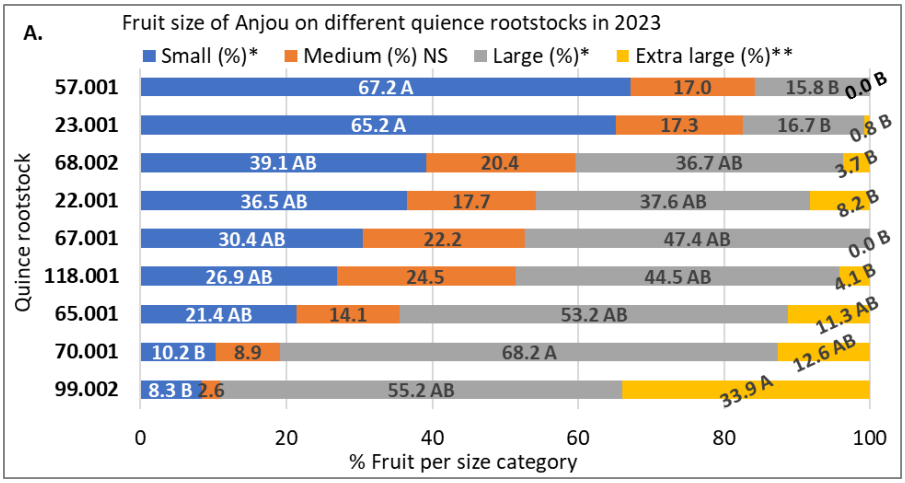
Table 4. Yield parameters for Bartlett and Anjou, with a Comice interstem grafted on quince accessions, on August 24th and August 29th, 2023 (harvest dates for Bartlett and Anjou, respectively). The means are averages of 3 trees per combination (N= 3). Significance, *p=<0.05, **p=<0.01, ***p=<0.001. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow).

Cultivar	Rootstock	N=	Fruit number per tree at harvest		Net tree yield (kg per tree)	Fruit weight (g)		Mton per acre	Mton per hectare	Average number of bins per acre
Anjou	22.001	3	57	AB	10.59	190	BC	12.82	30.93	32.11
Anjou	23.001	3	73	AB	10.53	152	C	12.74	30.75	31.92
Anjou	57.001	3	95	A	14.30	152	C	17.30	41.75	43.34
Anjou	65.001	3	44	AB	9.06	203	ABC	10.96	26.46	27.46
Anjou	67.001	3	71	AB	12.86	183	BC	15.56	37.54	38.97
Anjou	68.002	3	79	AB	13.73	181	BC	16.61	40.08	41.61
Anjou	70.001	3	49	AB	10.65	220	AB	12.89	31.10	32.28
Anjou	99.002	3	29	B	7.11	246	A	8.61	20.77	21.56
Anjou	118.001	3	81	AB	15.21	188	BC	18.40	44.41	46.11
Significance			*		NS	**		NS	NS	NS
Bartlett	22.001	3	74		14.14	188		17.11	41.28	42.85
Bartlett	23.001	3	75		13.53	182		16.37	39.51	41.01
Bartlett	57.001	3	70		12.03	191		14.55	35.12	36.46
Bartlett	65.001	3	84		17.35	209		20.99	50.65	52.58
Bartlett	67.001	3	68		12.18	183		14.74	35.57	36.92
Bartlett	68.002	3	52		9.59	201		11.60	27.99	29.06
Bartlett	70.001	3	77		16.40	213		19.85	47.90	49.72
Bartlett	99.002	3	64		11.40	178		13.79	33.28	34.55
Bartlett	118.001	3	57		9.53	176		11.53	27.83	28.89
Significance			NS		NS	NS		NS	NS	NS

Fruit quality

2023 pear grading by size with Aweta sorting line (WA): After harvest in 2023, pears from both varieties were sorted by an Aweta sorting machine, based on fruit weight (Fig. 1). Bartlett and Anjou fruits were both sorted on September 7, 2023 (9-14 days after harvest for Bartlett and Anjou, respectively). The size categories are small (<166 g, ≥ 120 pears/box), medium (166 g to 182 g, 110 pears/box), large (183-260 g, 80-100 pears/box, the optimum size preferred by the market) and extra-large (>260 g, ≤70 pears/box). For Anjou, small size fruit were found in higher proportions in combinations, Anjou/Comice/57.001 (67.2%) and Anjou/Comice/23.001 (65.2%), then followed by Anjou/Comice/68.002 (39.1%). The lowest proportion of small pear size was observed in Anjou/Comice/70.001 (10.2%) and Comice/99.002 (8.3%, Table 8). On the contrary, Anjou/Comice/99.002 had more extra-large size fruit (33.9%), followed by the combination Anjou/Comice/70.001 (12.6%,) and Anjou/Comice/65.001 (11.3 %, Figure 1A). The combination reporting the highest proportion of large fruit size (the optimum size), was Anjou/Comice/70.001 got more large-size fruit (68.2%), then followed by 99.002 (55.2%) and 65.001 (53.2%, Figure 1A). On the contrary, the lowest proportion of large fruit was found in Anjou/Comice/23.001 (16.7%) and

Anjou/Comice/57.001 (15.8%). No significant difference in the proportion of pear in the medium size was found across all nine accessions for Anjou (Figure 1A). In general, at least 80% of fruits for Anjou/Comice/70.001 and Anjou/Comice/99.002 were in the large and extra-large grade. However, this higher ratio of large size and extra-large size fruit in Anjou could be related to the crop load (49 fruits for Comice/70.001 and 29 fruits for Comice/99.001 in 2023 harvest). No specific rootstock effect was found for the proportions of pear fruit in each size category for Bartlett ($p>0.05$, Figure 1B). However, the highest proportions of large size fruit was found in Bartlett/Comice/65.001 (58.1%), followed by Bartlett/Comice/70.001 (48.9%), and Bartlett



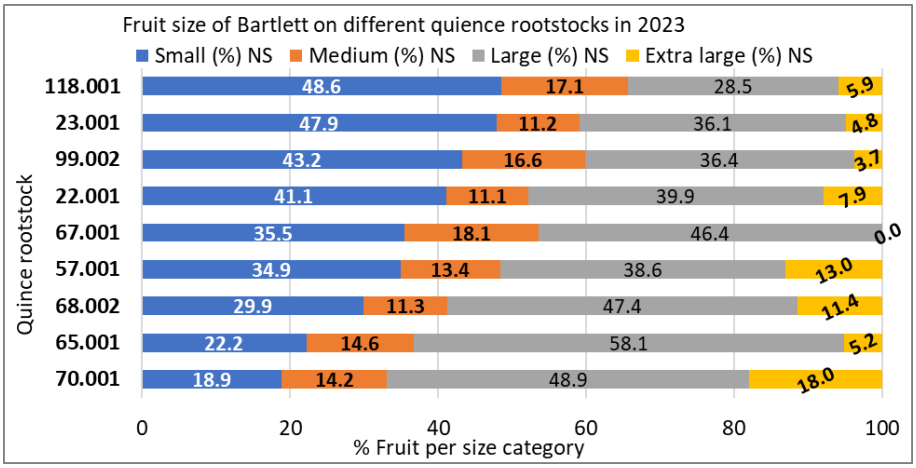


Figure 1. Fruit size distribution for 2023 harvest: **A.** Anjou with Comice interstem grafted on quince accessions, **B.** Bartlett with Comice interstem grafted on quince accessions. Fruit was harvested on Aug 24th, 2023 for Bartlett and Aug 29th, 2023 for Anjou and sorted on September 7th by the use of Aweta sorting machine. 'Small' means fruit weight < 166 g, 'Medium' between 166 g and 182 g, 'Large' between 183 g and 260 g, and 'Extra large' > 260 g. The percentage represents the average of three replications per combination (N= 3). Significance, *=p<0.05, **=p<0.01, ***=p<0.001. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each 'fruit size' parameter. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity further investigations on 2023 samples are ongoing.

Pear fruit internal quality (2023 harvest)

After seven-day ripening at room temperature, Bartlett IAD was measured then destructive analysis was carried out on October 3rd and October 4th, 2023 (Table 5). Compared with 2022 harvest, 2023

harvest had a lower IAD values on the sorting day and after the ripening process (Table 5). The IAD value after the ripening process was low, ranging from 0.01 to 0.02 for the combinations, Bartlett/Comice/65.001, Comice/23.001, Comice/57.001, and Comice/67.001. The highest IAD mean after ripening was measured as 0.08 in Bartlett/Comice/68.002 (Table 5). Bartlett/Comice/68.001 showed the highest IAD values after the ripening in both years (Table 5). Fruit firmness values were higher in four combinations: Bartlett/Comice/67.001 and Bartlett/Comice/57.001, followed by Bartlett/Comice/118.001. Both Comice/67.001 and Comice/57.001 showed among the largest values for firmness in the two consecutive years in Bartlett (Table 5). SSC did not show a clear discrimination in 2023 for Bartlett (Table 5).

On October 11th and October 12th, 2023, Anjou was measured for all parameters as reported for Bartlett. Unlike 2022 harvest, no significant difference was found in IAD at sorting and IAD after seven days of ripening for 2023 harvest (Table 5). IAD values of some rootstock combinations decreased more after the ripening process, however, the IAD drop did not show differences between combinations, nor did firmness SSC showed significant differences among the Anjou combinations and, in particular, A/Comice/68.002 and A/Comice/118.001 confirmed to produce poor quality fruit in terms of SSC in both years.

Table 5. Internal quality fruit analysis for Anjou and Bartlett, crop 2023, index of absorbance difference (IAD), IAD after seven days, and IAD drop after the ripening process, firmness, and soluble solid content (SSC). All trees are scions of Anjou or Bartlett with a Comice interstem and roots belonging to the quince accessions under evaluation. Direct graft combinations of scions on quince rootstocks were not included in the quality analysis. Significance: *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters within each column identify similar means for each parameter. Harvest days: Bartlett 8/24/23 and Anjou 8/29/23, Sorting days: Bartlett and Anjou, 9/26/23-9/27/23. Anjou selected pears of size 65-70 mm with an IAD ranging between 1.81 and 1.94. Bartlett selected pears of size 55-65 mm with an IAD ranging between 0.6 and 1.86. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow).

Cultivar	Interstem	Rootstock	N=	I_{AD} at sorting	I_{AD} after 7-day ripening		I_{AD} drop		Average firmness (kg)		SSC (Brix, %)	
Anjou	Comice	22.001	3	1.80	1.68		0.12	A	7.53		16.35	AB
Anjou	Comice	23.001	3	1.80	1.69		0.11	A	7.47		16.73	AB
Anjou	Comice	57.001	3	1.80	1.67		0.13	A	6.78		17.41	A
Anjou	Comice	65.001	3	1.86	1.74		0.13	A	7.47		16.59	AB
Anjou	Comice	67.001	3	1.86	1.74		0.12	A	6.50		16.10	AB
Anjou	Comice	68.002	3	1.78	1.71		0.07	B	6.32		15.48	B
Anjou	Comice	70.001	3	1.77	1.68		0.09	B	7.07		15.64	B
Anjou	Comice	99.002	3	1.86	1.78		0.08	B	7.92		16.03	AB
Anjou	Comice	118.001	3	1.87	1.78		0.09	B	6.74		15.54	B
significance				NS	NS		***		NS		*	
Bartlett	Comice	22.001	3	1.47	0.04	AB	1.44		0.69	B	14.22	
Bartlett	Comice	23.001	3	1.31	0.01	B	1.29		0.88	AB	15.57	
Bartlett	Comice	57.001	3	1.19	0.02	B	1.17		1.00	A	14.88	
Bartlett	Comice	65.001	3	1.19	0.01	B	1.18		0.87	AB	15.31	
Bartlett	Comice	67.001	3	1.20	0.02	B	1.18		1.00	A	14.65	
Bartlett	Comice	68.002	3	1.33	0.08	A	1.25		0.92	AB	15.50	
Bartlett	Comice	70.001	3	1.27	0.07	AB	1.21		0.73	B	14.89	
Bartlett	Comice	99.002	3	1.55	0.06	AB	1.50		0.73	B	15.62	
Bartlett	Comice	118.001	3	1.24	0.03	AB	1.21		0.96	A	15.36	
significance				NS	**		NS		**		NS	

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

After several attempts (2021 and 2022) to establish cultures, NAP has successfully cultured all of the missing accessions where material still exists at the NCGR in Corvallis, OR (10 of 11 original accessions) in sufficient numbers to begin generating trees for future rootstock trials. These include the top three cold hardy accessions previously not propagated due to challenges with media/material. Objective 2 is on schedule; tissue cultured accessions have been rooted so that tree generation of ~200 liners per accession can be produced and grafted/budded in a nursery for future trials.

Table 6. Summary Table of all quince rootstocks trialed in the field during the duration of this project or developed in tissue culture for future trialing. We recommend actions based on our findings and informed via genetic finger printing analysis according to 4 classification groups: A, Advance to next trial phase; B, begin new testing trial; C, cease all activities and discard tissue cultures due to unknown genetics as explained in the table and report; and, D, discard material due to poor trial performance, mislabeling or duplication.

ID as reported throughout the project	**Tissue cultures at NAP**	**Field Trialed During this Project**	**Classification Group**	**Grouping Reason/comments** **Recommendations**
57.001/65.001	Yes	Yes	A (Advance to S3 phase, collect data for 6 to 10 years)	Performance interesting; the two accessions are identical
22.001	Y	Y	A	Performance interesting; this can advance but it is not novel, it is equivalent to Quince MA
67.001	Y (reintroduced from root suckers collected from the Entiat trial; no longer at NCGR	Y	A (Advance to S3 phase, collect data for 6 to 10 years)	Performance interesting; Appears to be unique
68.002	No	Y	D (Discard)	Performance poor; High mortality; survivors generally very weak
118.001	N	Y	D (Discard)	Moderate mortality levels, overly vigorous, low yield, *NAP cultures are not genetically similar to source at NCGR and field trees are not the same as either NAP or NCGR material.

70.001	Y	Y	*D (Discard)	Inconsistent performance between sites; low production in WA, high in OR. Genetics from all 3 sites (NAP, NCGR, Entiat) matches.
99.002	Y	Y	D (Discard)	High mortality levels, overly vigorous, low yield, is not genetically similar to source at NCGR
23.001	*Y	Y	C (Cease activities and start over from the source material; retest hardiness)	Performance interesting, but tissue cultures and NCGR material do not match- they are genetically different; potentially same as 29.001. *Therefore, the NAP cultures are not what was trialed in the field.
126.001	Y	N	B (Begin testing in new Phase 2 trial)	Test in Phase 2 small-scale plots 6-10 years then move to Phase 3 test for 6-10 years if warranted, then commercialize if promising
67.004	Y	N	B	
64.001	Y	N	B	
32.002	Y	N	B	
13.001	Y	N	B	
128.001	Not clear	N	B	

29.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
34.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
66.001 and 75.001	Y	N	C	These two are genetically identical and neither of the tissue cultures matches the NCGR material
67.002	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
71.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
104.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
120.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different

Executive Summary

A three-year evaluation comprising three cropping seasons (4th through 6th leaf) evaluated the agronomic performance of 9 cold-hardy quince rootstocks with 'd'Anjou' and 'Bartlett' scions and 'Comice' insterstem at two sites, Parkdale OR and Entiat WA. Four of these rootstocks produced dwarf trees with good vigor to fruit balance and were horticulturally managed within a three foot in-row spacing after 7 years in the field. These accessions produced between 20-50 bins of fruit per year, depending upon year, site, cultivar and rootstock accession, and had relatively low mortality. In general, trees in OR were ~50% larger than in WA for any given accession. Poorly performing rootstocks behaved similarly between sites. In addition to these field trials, previously selected cold hardy quince accessions that were not previously tissue-cultured were successfully micropropagated from shoot tips in 2022 and 2023. An exhaustive, two-year genetic fingerprinting analysis was undertaken to confirm the identity of the rootstock accessions tested and propagated within this proposal to ensure that each accession was true to type and could be traced back to its original progenitor tree at the NCGR. For each of the nine field-tested accessions, material was collected from several replicate trees of a given rootstock at the Entiat WA field trial site (via sucker material), NAP (from tissue culture jars) and NCGR (from the original tree). In some cases, the original tree no longer exists. For the additional 15 accessions tissue-cultured during the project timeline, but not yet field tested, material was selected at NAP (from tissue culture jars) and the NCGR (from the original tree). Analyses revealed two or more accessions to be genetically similar (i.e., duplicates) ***and*** several accessions to have genetic dissimilarity between/among locations (e.g., Entiat, WA, NAP and/or the NCGR), precluding our ability to identify them and link the present material to the original cold hardiness status. Based on these data, we can recommend how each of the 25 rootstock accessions should be handled to avoid severe economic consequences that will inevitably arise if the industry propagates, trials and/or commercializes material of unconfirmed origin. First, the nine accessions field tested for 6 years, including 3 years of cropping: 1) Discard **five** accessions (57.001 ***or*** 65.001 [these are the same], 68.002, 118.001, 99.002, 70.001) due to the duplication, poor vegetative and/or yield performance, high mortality, and/or mislabeling; 2) re-evaluate the cold hardiness of **one** accession (23.001) for which no root suckers could be sampled and material at NAP and NCGR differed from one another (making it impossible to determine if the trees in field trials are the same as the original material evaluated for its cold hardiness); and, 3) advance **three** accessions (57.001 ***or*** 65.001 [these are the same], 22.001 and 67.001) to a Phase 3 trial. Phase 3 trialing should comprise an additional 6-10 years of small-scale commercial plot testing. Rootstocks deemed promising upon completion of phase 3 trialing, based on tree health, cropping, canopy balance, and fruit quality could be candidates for potential commercialization. Between 200 and 600 rooted plants were tissue cultured for each accession during the timeline of the project to facilitate Phase 3 trialing. For the **15** rootstocks accessions previously selected for their cold hardiness, **14** were successfully established in tissue culture. Our recommendation for the handling of these rootstocks is as follows: 1) the genetic identity of **six** accessions was confirmed (13.001, 32.002, 64.001, 67.004, 126.001, 128.001). These were rooted and are ready for grafting (100-500 plants depending on the accession) using interstems and scions to assess their performance in field trials, a minimum of 6 years and preferably 8-10 to include five cropping cycles. Rootstocks that performed well following the completion of these trials could be advanced to a phase 3 trial (as described above). The remaining **eight** accessions (29.001, 34.001, *66.001 and 75.001* [these are the same], 67.002, 71.001, 104.001, and 120.001) were not genetically similar to the mother tree at NCGR. Thus, we recommend discarding the tissue cultures and reanalyzing the cold hardiness of the original NCGR tree to compare with early hardiness data. Those that are hardy can be cultured and rooted for future field trials.