2024 NW Pear Research Review

A bee pollinates Pear blossoms in a pear block in Wapato, Washington.

Photo Source: Paige Beuhler

February 15, 2024

Hybrid Format
Yakima, WA

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

Report Type: Final Project Report

Primary PI: Jacqueline Serrano
Organization: USDA-ARS
Telephone: 509-454-4461
Email: jacqueline.serrano@usda.gov
Address: 5230 Konnowac Pass Road
Address 2:
City/State/Zip: Wapato, WA 98951

Co-PI: W. Rodney Cooper
Organization: USDA-ARS
Telephone: 509-454-4461
Email: jacqueline.serrano@usda.gov
Address: 5230 Konnowac Pass Road
Address 2:
City/State/Zip: Wapato, WA 98951

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

Project Duration: 3 Year **(**+NCE)

Total Project Request for Year 1 Funding: $ 30,000
Total Project Request for Year 2 Funding: $30,000
Total Project Request for Year 3 Funding: $6,000

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: W. Rodney Cooper
Organization Name: USDA-ARS
Contract Administrator: Mara Guttman
Telephone: 509-510-5619
Contract administrator email address: Mara.guttman@usda.gov

Item	2020	2021	2022
Salaries	$8,650.00	$8,866.00	$0.00
Benefits	$2,768.00	$2,837.00	$0.00
Wages	$0.00	$0.00	$0.00
Benefits	$0.00	$0.00	$0.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$17,582.00	$16,797.00	$5,000.00
Travel	$0.00	$500.00	$0.00
Plot Fees	$1,000.00	$1,000.00	$1,000.00
Miscellaneous	$0.00	$0.00	$0.00
Total	$30,000.00	$30,000.00	$6,000.00

Footnotes:

Salary and benefits for Biological Science Technician that conducted laboratory bioassays and assisted with field trial.

Supplies for volatile collections and analyses (bags, glassware, pumps, tubing, fittings, solvent, gases, chemicals), field trials (traps, posts, lures, chemicals), bioassay materials (glassware, fittings), and general lab supplies (gloves, pipette tips, GC parts, vials)

OBJECTIVES: Recap, Goals, and Activities:

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

Preliminary results from caged bioassays were promising and suggest that pear tree volatiles may be attractive to winterform psylla. However, the results were not significantly different, likely due to flaws in the bioassay methods. We had difficulty hiring a technician who was meant to conduct bioassays in first two years of project. In Year 3, we collected winterform pear psylla from evergreens at the Moxee farm to use for laboratory bioassays, but due to mechanical failures in the building, the collected insects died before use. We have repeated collections for bioassays to be conducted in Year 4. Both Y-tube and caged bioassays were conducted with pear psylla to determine their attraction to host plants. For all bioassays, we examined variation in responses of winterform and summerform males and females to pear and an overwintering evergreen host. In Year 4, we also conducted a field trial, testing whether volatiles identified from pear were attractive to winterforms.

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

No volatile collections were conducted during Year 1 of funding, due to the timing of the project (February-March) and when research funds were received (late summer 2020). We designed a method to allow us to perform simultaneous collections from multiple trees, which incorporated powerful air and vacuum pumps and manifolds. These materials were purchased and used to build the collection system for implementation in Year 2. The volatile collectors that were used in the collections were purchased as a prefabricated item and were found to be contaminated. Therefore, we had to create our own volatile collectors that have been determined to be free of contaminants. The new collectors were used in Year 3, with the system to sample several trees in the field. However due to undetectable differences in the volatiles of the trees, a different volatile sampling method was in Year 4 so that collections took place in a smaller glass container.

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

Due to delays in completing research for other objectives, this objective was eliminated.

SIGNIFICANT FINDINGS

- Preliminary caged bioassays suggested that pear tree volatiles are attractive to winterform psylla.
- Winterform pear psylla were attracted to juniper volatiles and preferred to settle on juniper shoots over pear shoots, but summerforms did not respond to volatiles from juniper.
- Attraction to pear and juniper volatiles varied by season, tree phenology, and psyllid physiology.
- Prefabricated volatile collectors were found to be contaminated with several chemicals, which prevented volatiles emitted by pear trees to be properly analyzed. New, cleaner, and cheaper collectors were made for volatile collections.
- Whole tree volatile collections were too large in volume to detect any volatiles emitted by pear trees before and after budswell.

- Volatile collections with cuttings and pruned stems in 1L jars also revealed minimal results. A more sensitive sampling technique will be needed to sample volatiles from small growing pear leaves. Groups of whole stems and pruned pieces from overwintering trees revealed a small amount of a single major volatile, β-myrcene and (*E*)-4,8-dimethyl-1,3,7-nonatriene, respectively. It is unclear if either play a role in psylla attraction.
- Field trials with two volatiles emitted pear, were not successful in capturing significant amounts of pear psylla, which could be attributed to trap type or lure release rate. Additional studies are needed to optimal combination of trap and lure type.

METHODS

Insect Collection

Psylla were collected from pear trees located at the ARS facility in Wapato and the USDA experimental farm near Moxee. For assays with post-diapause winterform psylla, insects were held overnight in a growth chamber maintained at 41°F with 12:12 (L:D) h photoperiod. Summerform psylla were held overnight at 50°F with a 16:8 (L:D) h photoperiod. Psylla females were dissected, and reproductive development was ranked based on Krysan and Higbee (1990) with ovarian scores ranging between 0 and 7 where 0 indicates no reproductive development and 7 indicates full reproductive maturity.

Plant Collection

Pear shoots were collected from Bartlett pear trees grown in a commercial orchard near Wapato, WA or in an experimental orchard located at the USDA research farm near Moxee, WA. Pear phenology (Larsen 2023) was monitored at both locations to record dates of bud swell (scale separation) and bud burst. Pear seedling whips were removed from cold storage and used for dormant pear in assays with summerform psylla. Juniper shoots were collected from ornamental trees located near Wapato, WA that typically harbor large populations of overwintering pear psylla (Cooper et al. 2019). Each shoot was thoroughly rinsed in water and cut to 10 cm length, and the cut end of each shoot was placed in water.

Laboratory Bioassays

Y-tube olfactometer experiments compared choice of spring winterform, summerform, and autumn winterform psylla to three treatments: 1) pear versus blank, 2) juniper versus blank, or 3) pear versus juniper. The Y-tube olfactometer was setup as described in Horton and Landolt (2007) (Figure 1). Male and female pslla were assayed separately and each insect was observed for 5 min. When an insect entered an arm of the Y-tube, it was considered a choice.

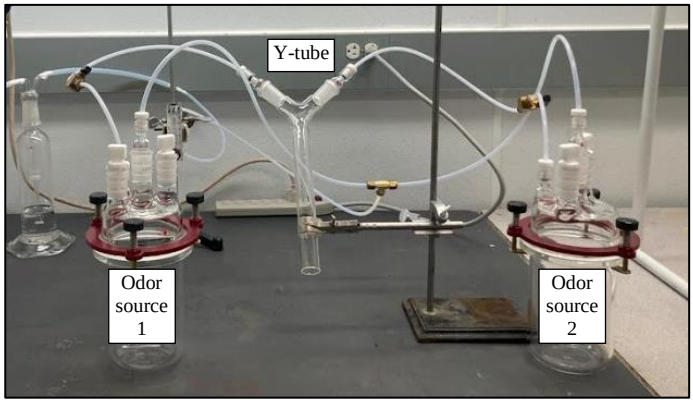


Figure 1. Example of Y-tube olfactometer set up.

Choice preference assays were used to examine attraction of psylla to plant shoots in a greenhouse setting. Treatments included dormant pear, active pear collected directly before the assay, and juniper Three plant shoots (one per treatment) were arranged in each of six cages within a greenhouse. Ten psylla (equal numbers of males and females) were released into the center of each cage. Plant shoots were examined after 24 hours and the number of psylla choosing a shoot was recorded. Assays were then repeated with new plant shoots that were covered with a mesh sleeve cage to prevent psylla from landing on the shoot, and to reduce visual cues.

Collection and Analysis of Pear Volatiles

Volatiles were collected from Bartlett pear trees during the dormant phase through the bud-swell phase when psylla re-entry is known to occur. These collections took place semiweekly as the trees exit dormancy. Phenological growth stage of the tree will also be recorded, following the BBCH identification keys of pome fruit trees.

In 2021 and 2022, volatiles were collected from 5 trees in orchards in Moxee, WA. Briefly, branches were wrapped in polyethylene bags (Figure 2A) that were fitted with an inlet and outlet for filtered air flow to be introduced using vacuum and air pumps. A charcoal filter was attached to the air pump (before the manifold) to introduce clean air into the inlet of the bag (Figure 2B). A volatile collector was connected to the outlet and to the manifold of the vacuum line (Figure 2B). The tubing that is connected to the inlet and outlets of each bag is fitted with a flow meter to ensure constant flow over the trees (Figure 2C). Each collection was conducted over four hours during peak daylight hours (approximately 10:00-14:00). Once the volatile collections were complete, the collectors were extracted with high purity solvent, which were stored in a freezer until analyses.

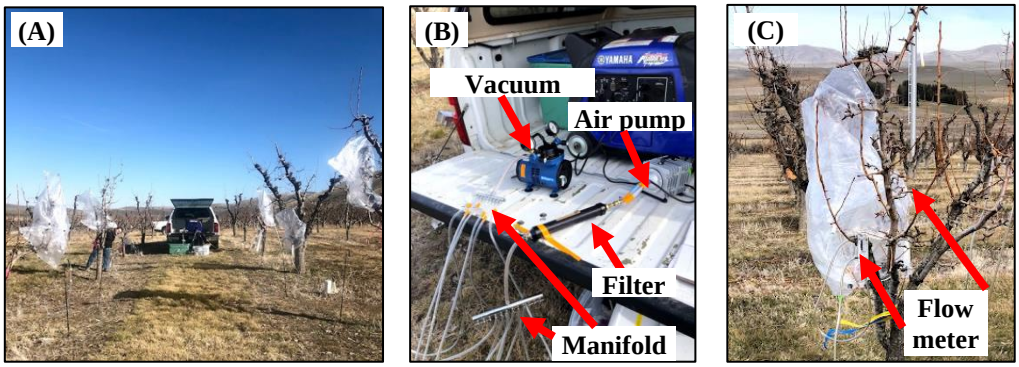


Figure 2. Example of volatile collection set up: (A) Volatiles being collected from 5 Bartlett pear trees at the USDA experimental farm in Moxee; (B) air pump, vacuum pump, and tubing set up; (C) up close image of volatile collection set up on pear tree.

In our second attempt to collect volatiles from trees in the field, we did not detect any differences in volatiles between the tree and the control (bag with no tree) and we also did not see any seasonal differences in volatiles emitted by post dormant trees. We believe because the bags are such a large sampling area in comparison to the small buds or leaf clusters, that it is hard to detect small quantities of volatiles using this collection method. Therefore, we attempted to collect volatiles from cuttings of pear twigs (whole or cut into pieces) by placing them in 1 liter glass containers (Figure 3). The glass containers will also be used to house plants that were also used for Y-tube bioassays.

The extracts were analyzed by coupled gas chromatography-mass spectrometry (GC-MS) to tentatively identify compounds present in the volatile profile of the trees (via mass spectra interpretation). The identification of the compounds was confirmed, when possible, by comparisons or retention times and mass spectra with those of authentic standards. Qualitative and quantitative comparisons were made between extracts of volatiles from pear trees present throughout the duration of the collections. These comparisons were made within and between samples, across difference phenological growth stages.

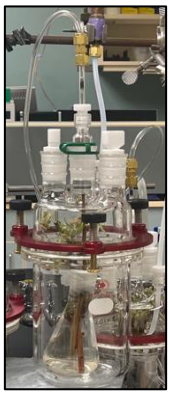


Figure 3. Example of volatile collection set up: 1L glass container with an inlet and outlet port so that volatiles can be collected using laboratory vacuum and onto charcoal collectors.

Field Trial

There were two compounds tested that were identified from pear trees in two recently published papers. The first compound was β-caryophyllene, which was the most abundant compound identified from trees at the BBCH 32-33 stages (Gallinger et al. 2023). The second compound was (*Z*)-3-hexenyl acetate, a compound found to be present on pear in high abundance, with or without the presence of pear psylla (Valle et al. 2023). We tested lures containing each compound individually, and a binary blend of the two. Lures were attached to clear sticky traps (AlphaScents), and each trap was fixed to a wooden stake at least 1 meter from the ground (Figure 4). Four replicates of traps were placed in the periphery of pear orchards, i.e. in Wapato surrounding the USDA-ARS facility and at the USDA-ARS farm in Moxee. Psylla captured on traps were sexed and counted in the laboratory. Lures were replaced biweekly and were made in-house using 4 mL plastic vials.

Figure 4. Example of clear sticky trap used in field assays testing plant volatiles. Lures were suspended from the top of the binder clips, so that they were level with the top of the trap.

RESULTS AND DISCUSSION

Bioassays

Results from preliminary caged bioassays (conducted in 2019) were promising and suggest that pear tree volatiles may be attractive to winterform psylla (Figure 5). However, the results were not significantly different, likely due to flaws in the bioassay methods. In short, a dual choice assay was conducted in a small cage, where 40 psylla were introduced and presented with two traps, one containing an untreated piece of filter paper, and the other containing filter paper treated with volatiles collected from pear trees. Although the results, were not significantly different, they do suggest that the pear psylla may be attracted to pear volatiles.

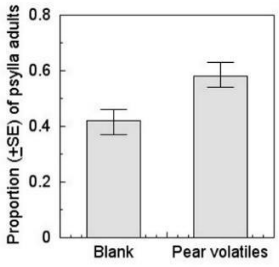


Figure 5. Mean (±SE) number of pear psylla caught in traps baited with a nontreated piece of filter paper ("Blank") and pear volatiles.

We have conducted similar caged bioassays, presenting psylla with juniper, dormant pear, and nondormant pear. In early assays, significantly more spring-collected winterforms settled on exposed juniper shoots compared with dormant or active pear, and it later assays there was a switch, showing significantly more psylla settled on active pear shoots than on other treatments (Figure 6A). Similar results were seen when assays were performed with covered shoots (Figure 6B) indicating that psylla may be using odors to locate preferred plants. Significantly more summerform psylla settled on exposed active pear shoots than on either juniper or dormant pear

shoots (Figure 7) and in assays with covered shoots, about equal numbers of psylla settled on active pear and juniper shoots suggesting that visual or gustatory cues have a significant role in host settling (Figure 7). Results with autumn collected winterforms were dependent on collection date. In both exposed and covered assays, significantly more pear psylla settled on pear shoots than on juniper shoots in assays conducted in November when pear psylla females exhibited ovarian development (Figure 8). In contrast, more psylla settled on juniper shoots than on pear in December when no ovarian development was observed (Figure 8).

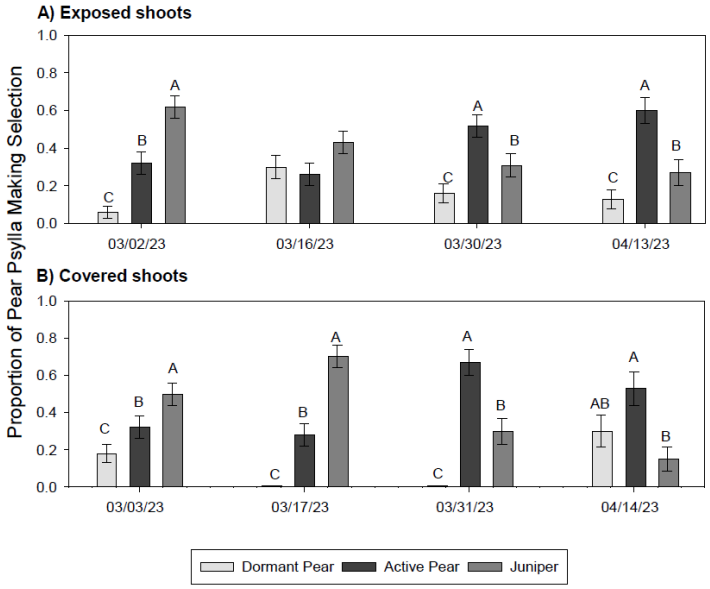


Figure 6. Results of choice preferences studies using spring-collected winterform pear psylla. Shoots were either (A) exposed or (B) covered with a mesh sleeve cage to prevent gustatory cues and to reduce visual cues. Different letters denote significant differences (α=0.05) among means within each date.

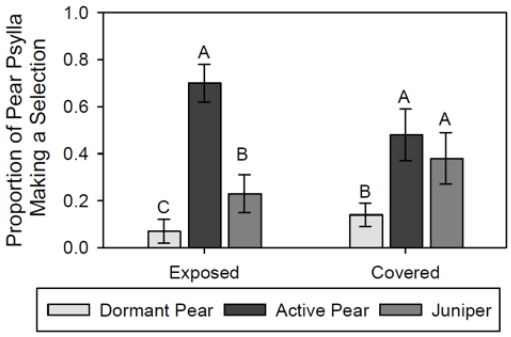


Figure 7. Results of choice preferences studies using summerform pear psylla. Shoots were either exposed or covered with a mesh sleeve cage to prevent gustatory cues and to reduce visual cues. Different letters denote significant differences (α=0.05) among means within each date.

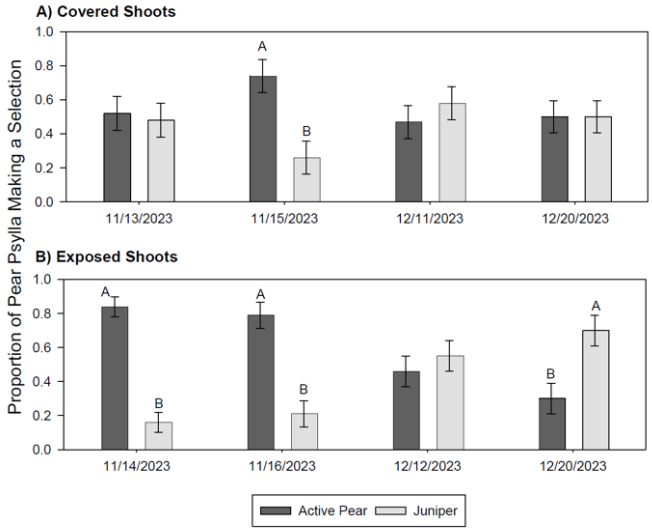


Figure 8. Results of choice preferences studies using autumn-collected winterforms. Shoots were either (A) exposed or (B) covered with a mesh sleeve cage to prevent gustatory cues and to reduce visual cues. Different letters denote significant differences (α=0.05) among means within each date.

Results from Y-tube olfactometer assays are presented in Figure 9. Spring-collected winterforms preferred juniper odors and pear odors over odor blanks in Y-tube preference assays (Figure 9a). When pear psylla were provided a choice between juniper and pear, males and females exhibited different host odor preferences, and results show that females preferred pear odor while males preferred juniper odors (Figure 9A). Assays with summerforms (Figure 9B) indicate that they do not respond to juniper odors, but both sexes chose pear odors significantly more often than odor blanks and juniper odors. Psylla collected in November, showed a strong preference for juniper odors over odor blanks and pear odors over odor blanks (Figure 9C) and males preferred juniper odors over pear odors, but females did not show a preference for either odor source. All psylla collected in November exhibited external characteristics consistent with the winterform morphotype including dark coloration and large body size. However, ovarian development was observed in about 50% of females. By December, we did not observe ovarian development in dissected females. Psylla assayed in mid-December showed a strong preference for juniper odors over both odor blanks and pear odors and chose pear odors and odor blanks equally (Figure 9D).

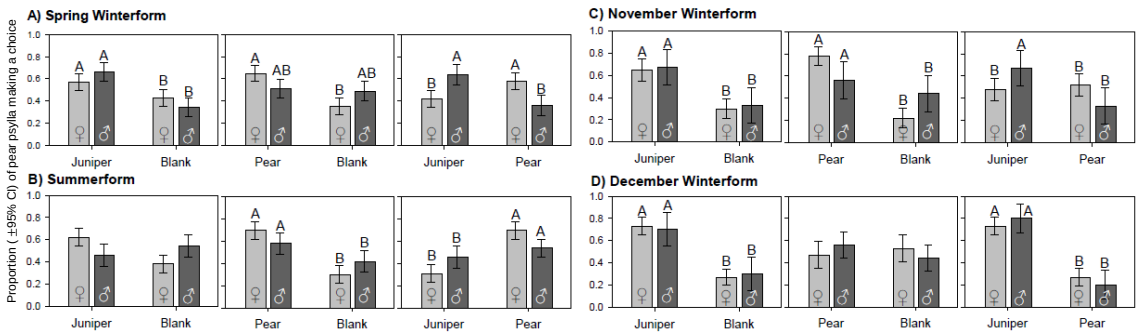


Figure 9. Results of pear psylla Y-tube olfactometer assays with (A) spring-collected winterforms, (B) summerforms, (C) winterforms collected in November, and (D) winterforms collected in December. Different letters denote significant differences among combinations of odors and psylla sexes.

Observation of ovarian development in autumn-collected winterform psylla was highly unexpected and led to interesting bioassay results. Previous studies show that wild pear psylla remain in complete diapause with ovarian development scores rarely exceeding 2 or 3 until February (Krysan and Higbee 1990, Horton et al. 1998, Horton and Landolt 2007). In contrast, 33% of females of the winterform morphotype dissected in early December had ovarian development scores as high as 6 or 7 (mature eggs). Females dissected in mid- to late-December were characterized by a lack of ovarian development consistent with previous studies on pear psylla diapause (Krysan and Higbee 1990, Horton et al. 1998, Horton and Landolt 2007). This shift in physiological status did not correspond when any obvious trends in temperature, however there are several possibilities to explain ovarian development in autumn-collected pear psylla. One possibility is that the strength of diapause varies depending on autumn climates. If this is true, we may expect ovarian development to occur more frequently in autumn winterforms in lower latitudes, and for the frequency of autumn ovarian development to increase in the Pacific Northwest in response to climate change. Regardless, ovarian development was not observed in females by mid-December, and the fate of these females remains unknown. It is possible that reproductively maturing winterforms died by mid-December, dispersed for sampling locations, or reabsorbed eggs. These observations warrant further research on the current state of pear psylla diapause in the Pacific Northwest.

Analyses of volatiles

In March 2019, preliminary volatile collections were conducted with a Bartlett pear tree at the USDA-ARS farm in Moxee, using methods described above. As a control, volatiles were sampled from a collection bag that did not contain a pear tree. Collected volatiles were then extracted and analyzed via GC-MS. Results from this analysis showed that there were differences in volatile profiles between the pear tree and the control, especially during the earlier minutes of the analysis. Additional samples were collected from one tree on a semi-weekly basis during March 2020, and analyzed via GC-MS. Compounds detected in 2019 analyses, were not detected in any of the samples taken in March 2020 (data not shown). During the analyses, there appeared to be issues with old GC-MS instrument used for analyses.

In 2021, a new GC-MS was purchased and installed in the lab and all extracts of volatiles from 2021 were analyzed on the new instrument. It appeared that each of the analyzed extracts contained many peaks/compounds. However, compound identifications revealed that the extracts contained several contaminants, including some related to plastics. Through careful analyses of solvents and collectors, we determined that the collectors were the source of contamination. Because the source of contamination were the volatile collectors, a newer collector needed to be developed and used. The collectors that were used are similarly made to the previous used collectors in that glass tubing was used to house the adsorbent. However, the adsorbent was changed from Porapak Q to thermally desorbed charcoal and there were no plastic components (Figure 10).

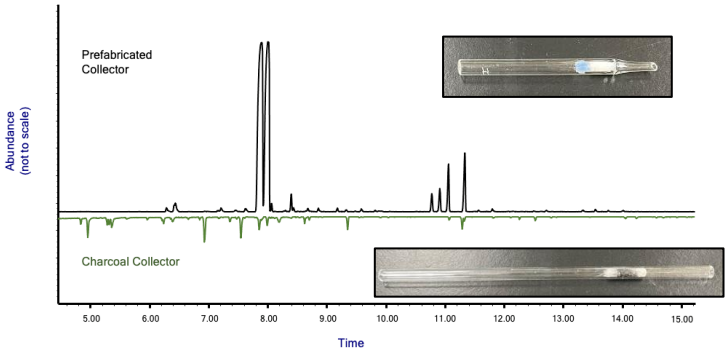


Figure 10. Representative chromatograms of an extract from an unused collector (top trace) and the extract from the new charcoal collectors (inverted trace). The trace representing the extract of the charcoal was scaled up for demonstration purposes.

In 2022, we repeated the volatile collections with the charcoal collectors, and conducted biweekly collections from the end of February through the end of March. It appeared that each of the analyzed extracts contained many peaks/compounds. However, these peaks were present in pear extracts and the controls, which indicates that these compounds are not unique to the trees (Figure 11). In addition, we also saw minimal differences in volatiles emitted by the trees from dormancy through bud burst, approximately BBCH10 (Figure 12). Our results suggest that the area, i.e. size of the bags, is too large to detect the small quantities of volatiles that are emitted by the post dormant trees. We made another attempt to sample volatiles, using custom 1L glass jars, from cuttings taken from pear when it was passed bud break and leaves were beginning to separate (approximately BBCH9-10). This method seemed promising because it was successfully used to sample cherry leaves. However, we found that samples of groups of pear cuttings contained the same compounds as the control flask of water), although sometimes at different abundances (Figure 13). The difficulties in obtaining detectable plant volatiles from early stages of pear growth, indicate that non-destructive sampling methods need to be highly sensitive to detect volatiles from small leaves, such as headspace sampling that can be directly injected into a GC-MS.

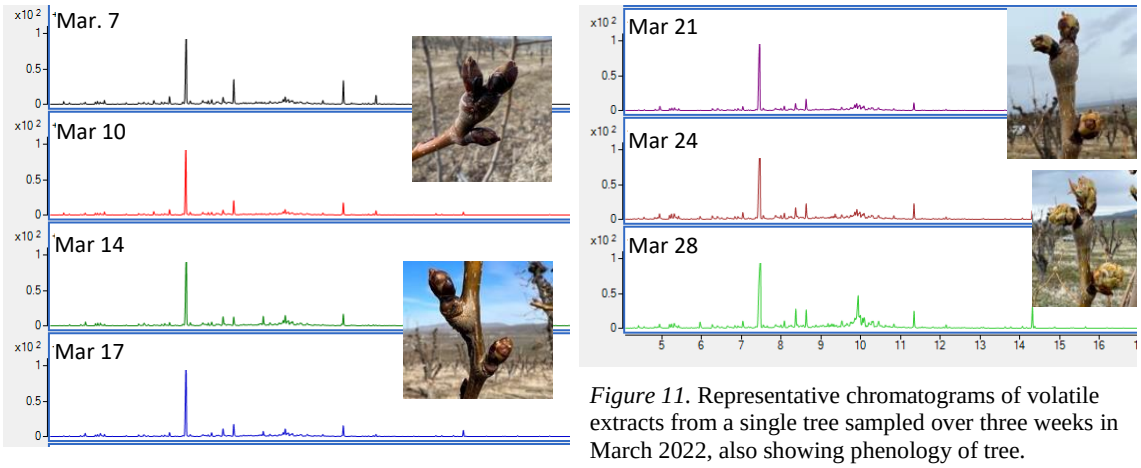


Figure 11. Representative chromatograms of volatile extracts from a single tree sampled over three weeks in March 2022, also showing phenology of tree.

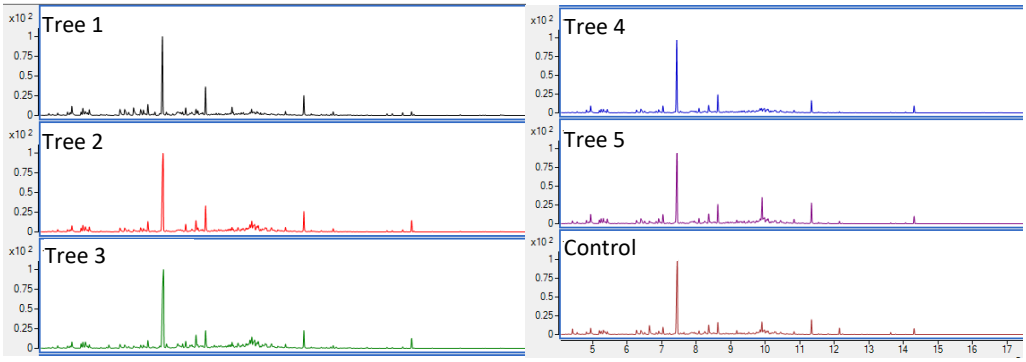


Figure 12. Representative chromatograms of volatile extracts from five different single trees and an empty bag control, sampled on when trees were past bud break, approximately BBCH10.

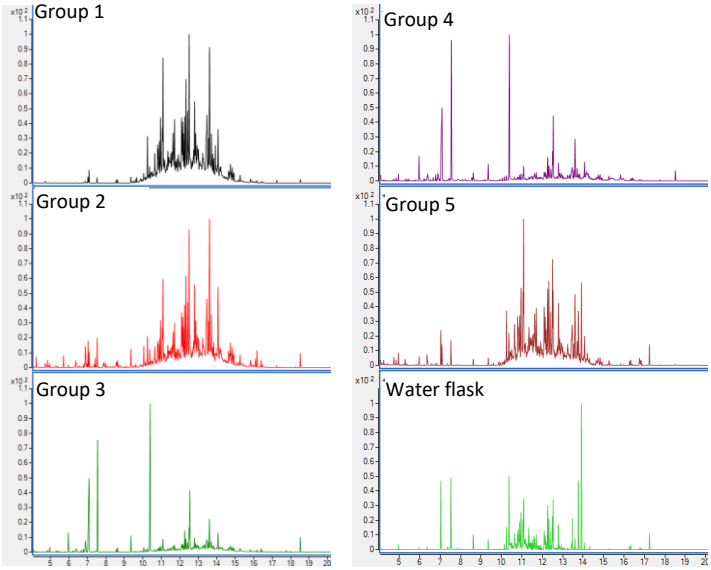


Figure 13. Representative chromatograms of volatile extracts from five different groups of pear cuttings placed in a flask of water, and control (only a flask of water), that were sampled in 1L glass jars (see Figure 3).

Volatiles were also collected from pear that was used for Y-tube bioassays by sampling whole twigs or twigs that were cut into 1-inch pieces (to simulate winter pruning). There was not a significant amount of plant volatiles detected in the sample compared to the background. The major volatile produced from intact pear branches was determined to be β-myrcene, however that compound was not detected in the sample of the cut branches, where the major volatile was another common plant compound, (*E*)-4,8-dimethyl-1,3,7-nonatriene. The importance of these compounds in the attraction of pear psylla is yet to be determined.

Field Trial

Traps baited with volatiles found in two different stages of pear (shoot development or later) did not attract significant numbers of pear psylla at either location. The highest overall capture was recorded on April 4th, for traps baited with β-caryophyllene. There were no obvious trends over time, and most treatments were not different from the control, except for β-caryophyllene on April 4th. An effective psylla trap may need a more complex blend of host plant volatiles, a visual cue like color, or vibration signals used by the psylla for courtship (Jocson et al. 2023), but more field research is needed to determine the best methods to trap pear psylla for pest management purposes.

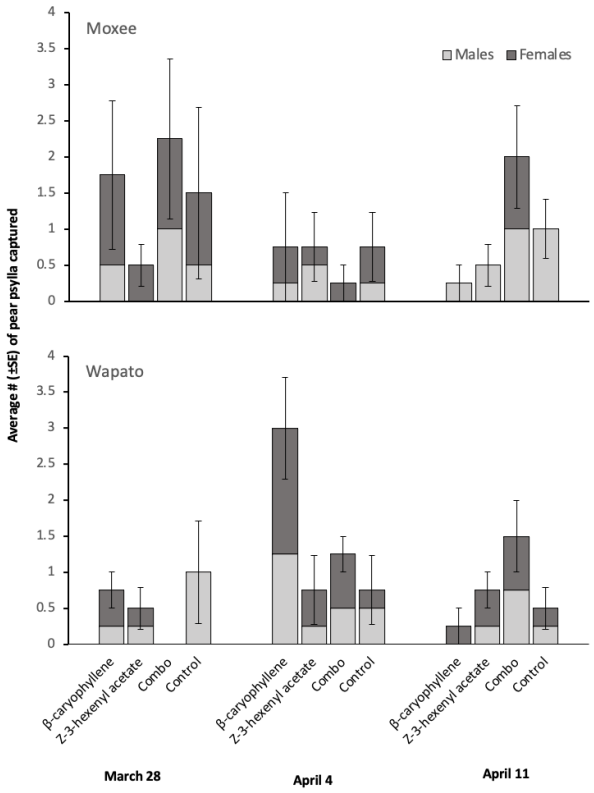


Figure 14. Mean (± 1 SE) numbers of winterform pear psylla caught on clear sticky traps baited with lures containing β-caryophyllene, (*Z*)-3-hexenyl acetate, a combination of the two, or the control. Trap captures are grouped by the dates they were checked, and proportions of males and females are also shown within average counts of the four replicates.

References

Cooper, W. R., D. R. Horton, M. R. Wildung, A. S. Jensen, J. Thinakaran, D. Rendon, L. B. Nottingham, E. H. Beers, C. H. Wohleb, D. G. Hall, and L. L. Stelinski. 2019. Host and non-host 'whistle stops' for psyllids: Molecular gut content analysis by high-throughput sequencing reveals landscape-level movements of Psylloidea (Hemiptera). Environ. Entomol. 48: 554-566.

Gallinger, J., M. Rid-Moneta, C. Becker, A. Reineke, and J. Gross. 2023. Altered volatile emission of pear trees under elevated atmospheric CO2 levels has no relevance to pear psyllid host choice. Environ. Sci. Pollut. Res. 30: 43740–43751.

Horton, D. R., and P. J. Landolt. 2007. Attraction of male pear psylla, *Cacopsylla pyricola*, to female-infested pear shoots. Entomol. Exp. Appl. 123: 177-183.

Horton, D. R., D. A. Broers, T. Hinojosa, and T. M. Lewis. 1998. Ovarian development in overwintering pear psylla, *Cacopsylla pyricola (*Homoptera: Psyllidae): Seasonality and effects of photoperiod. Can. Entomol. 130: 859-867.

Jocson, D. M. I., M. T. Gonzales, D. R. Horton, L. B. Nottingham, E. H. Beers, L. C. Oeller, and D. W. Crowder. 2023. Characterizing Substrate-Borne Vibrational Mating Signals Produced by Pear Psylla, *Cacopsylla pyricola* (Hemiptera: Psyllidae). J. Insect Behav. https://doi.org/10.1007/s10905-023-09839-2

Krysan, J. L., and B. S. Higbee. 1990. Seasonality of mating and ovarian development overwinter *Cacopsylla pyricola* (Homoptera: Psyllidae). Environ. Entomol. 19: 544-550.

Larsen, H. 2023. Cold effects on fruit and bud phenology, pp. 182. In M. Murray and M. Evans (eds.), Intermountain Tree Fruit Production Guide, vol. 2023. Utah State University.

Valle, D., V. Mukica, and A. Gonzalez. 2023. Herbivore-Dependent Induced Volatiles in Pear Plants Cause Differential Attractive Response by Lacewing Larvae. J. Chem. Ecol. 49:262-275

EXECUTIVE SUMMARY

Project title: Identification of pear tree volatiles attractive to winterform psylla
Key words: pear psylla, host odor, bioassay, behavior, attractant.

Abstract:

Before the start of this project, it was discovered that winterform pear psylla migrate to other hosts, such as evergreen trees, during the fall months where they would overwinter. As temperatures warm, pear psylla break their diapause and disperse from shelter plants and return to pear orchards to begin egg-laying. It is unknown how post-diapause winterform pear locate pear hosts in early spring. Volatile cues seem likely because research from the 1990s demonstrated that psylla do not respond to color in early spring before leaf development on pear. Over the course of the study, we wanted to determine if winterform pear psylla would show behavioral preferences to pear (before and after budbreak) and juniper, a known winter shelter plant. Additionally, we wanted to determine if there were any volatiles emitted by pear as trees emerged from dormancy that could serve as attractive cues to pear psylla. Results of bioassays performed in the lab demonstrate that pear psylla are attracted to plant volatiles emitted by pear and juniper, but behaviors varied by season, tree phenology, and psyllid physiology. We observed a shift in plant settling from juniper to pear in spring months, corresponding with an increase in ovarian development in female psylla and the initiation of pear bud swell, however, it is not clear from our data whether this shift in plant preference is due to changes in pear phenology, insect physiology, or both. Results from volatile collections indicate that more sensitive methods are need to sample budding pear, because passive sampling of cuttings in large glass containers or plastic bags over tree sections did not provide detectable plant volatiles. Field trials with two pear volatiles found in other studies, were not successful in capturing significant amounts of pear psylla, but more research is needed to determine the best trap type and lure device. Our study provides a foundation for further research on chemical ecology and overwinter biology of pear psylla. Additional research is needed to identify specific compounds that elicit behavioral responses by pear psylla and to determine whether they would have practical use for pear psylla management.

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

Report Type: Final Project Report

Primary PI: Rebecca Schmidt-Jeffris
Organization: USDA-ARS
Telephone: 509-454-6556
Email: rebecca.schmidt@usda.gov
Address: 5230 Konnowac Pass Rd
City/State/Zip: Wapato, WA 98951

Co-PI 2: Louis Nottingham/Robert Orpet
Organization: Washington State University
Telephone: 509-293-8756
Email: louis.nottingham@wsu.edu, robert.orpet@wsu.edu
Address: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

Cooperators: Steve Arthurs (BioBee); Chuck Weaver (G.S. Long & Parabug); Rudy Prey; Justin Ellgen (Simplot) [note: apple grower cooperators are specified in apple report]

Project Duration: 2 Year

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

Other related/associated funding sources:
Awarded

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

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

Funding Duration:	2021-2022
Amount:	$29,968
Agency Name:	Western IPM Center, project initiation grant
Notes:	This project expanded the efforts in this grant by providing support to conduct grower input sessions and a needs assessment survey. The WIPMC grant was also used to start a grant team and stakeholder

advisory group that submitted the WSARE grant (below).

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

Requested
Funding Duration: June 2024 – May 2027
Amount: $350,000
Agency Name: Western Sustainable Agriculture Research and Education (WSARE)
Notes: This project proposal used the data gathered from "Tactics to improve natural enemy releases in tree fruit" to develop targeted questions that will allow for the creation of best management practices for lacewing releases in apples.

Funding Duration: June 2024 – May 2027
Amount: $81,139
Agency Name: Washington Tree Fruit Research Commission (ACP)
Notes: The WSARE proposal above includes funding for one lead technician's salary and extension activities. Due to budget limitations, we were unable to request salary for additional research support. Therefore, this funding request is for an assistant for the lead technician so that the research can be completed. We will be informed of the funding decision in March.

Funding Duration: 2024-2026
Amount: >$15,000
Agency Name: BioBee
Notes: In-kind match for the above WSARE project; commercial insectary lacewings (Awarded: will receive if the above is funded)

Funding Duration: 2024-2026
Amount: ~$7,500
Agency Name: Zirkle Fruit
Notes: In-kind match for the above WSARE project; commercial insectary lacewings and drone pilot labor/fees (Awarded: will receive if the above is funded)

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 results from Obj. 1 of this project and other projects in pears.

WTFRC Collaborative Costs: None

Budget 1*

Organization Name: USDA-ARS **Contract Administrator:** Mara Guttman
Telephone: 510-559-5619 **Email address:** mara.guttman@usda.gov
Station Manager/Supervisor: Rodney Cooper **Email Address:** rodney.cooper@usda.gov

Item	2021	2022
Salaries[1,4]	$17,458	$17,894
Benefits[1,4]	$5,587	$5,726
Wages	$0	$0
Benefits	$0	$0
Equipment	$0	$0
Supplies[2]	$6,500	$6,500
Travel[3]	$0	$0
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	**$29,545**	**$30,120**

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

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

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

Footnotes:
[1]Nottingham salary ($7,612.50/mo × 12 mo × 2% FTE = $1,827 Year 1, Year 2 reflects 4% COLA increase) + Postdoc salary ($4,250/mo × 12 mo × 100% FTE = $51,000 Year 1, Year 2 reflects 4% COLA increase). Nottingham to supervise data collection efforts in pear in the Wenatchee area and advise on project methods and data summary. WSU Postdoc will be based at the USDA-ARS facility in Wapato, WA and supervised by Schmidt-Jeffris. The postdoc will be responsible for leading data collection and summarizing project results. Due to difficulties in finding a qualified postdoc candidate, we have expanded our search to also include an associate in research, which would have a similar salary, but be hired at the M.S. level. The associate in research (Daniel Hausler) was hired in early 2022.
[2] Benefits rate for Nottingham is 29.9% ($547 Yr 1, $569 Yr 2). Benefits rate for postdoc is 35% ($17,826 Yr1, $18,539 Yr2).
[3]Summer technician at $15/hr×8 hr/wk ×10 wks, 9.4% benefits rate, salary includes 4% COLA increase in Year 2
*50% by WTFRC Apple Crop Protection, 50% by FPC/PPC Pear

Note: This report primarily contains pear-related content. Apple results are presented in detail in the Apple Crop Protection report.

OBJECTIVES

Obj. 1. Improve retention of released natural enemies. We tested whether commercially available food supplements (Artemia cysts on tape, *Ephestia* eggs on cards) and lures (methyl salicylate) increased retention of released natural enemies and also examined whether they recruited resident natural enemies and decreased pest populations. Only Artemia cysts were used in 2023 (*Ephestia* eggs were dropped). All fieldwork and pest/natural enemy counts are completed and analyzed for this project, but the molecular work is not yet complete. Several factors caused significant delays, including a move to a new lab space (which needed repairs before use) and the need to change our gut content protocols; we determined that neither pear psylla nor orchard aphid pests amplify well with COI universal primers. To overcome this, a colleague (B. Ohler) designed a pear psylla primer and we adapted aphid primers from another lab – these must be run as a separate PCR from the COI primers, increasing the number of samples we are running. Finally, the need to identify lacewings using molecular techniques (see below) added many additional samples to our workflow. The molecular work will be completed before the project term date (June 2024).

Obj. 2. Determine cost-effectiveness and efficacy of natural enemy release by drone. In 2022, this we tested releases of *Orius insidiosus* and lacewings (*C. plorabunda*) by ground and drone. We determined that the 0.25-acre plot trials were not an adequate method for testing drone releases and instead focused entirely on various ground-based releases in 2023. An objective specifically testing lacewing releases by drone at a large scale was included in the proposed WSARE project (see other/related funding sources). This grant will help determine if drones are a viable tactic for releasing natural enemies in orchards more generally (not just lacewings in apples).

SIGNIFICANT FINDINGS

Releases of insectary natural enemies for pear psylla control did not show any potential in this study. Recovery of released natural enemies was generally lower in the pear trials than in apple trials and the releases did not decrease pear psylla abundance. These results have allowed us to confidently advise growers to not use either *O. insidiosus* or lacewing releases for pear psylla control. Since we began these trials, a new natural enemy, the whirligig mite ("Crazee mite"), has become commercially available. Preliminary work conducted by colleagues indicates that it has strong potential for pear psylla control. Additionally, we informed the insectary industry that it does not appear that currently available natural enemies (with the potential exception of the new "Crazee mite") are not effective for pear psylla. As a result of consultations, one insectary is currently exploring their ability to rear a pear psylla natural enemy (details forthcoming, currently confidential).

Tactics for retaining and recruiting natural enemies had highly variable results between sites and years. In general, methyl salicylate lures showed some promise for recruiting lacewings (in apples only), *Campylomma*, and *Stethorus*. Food supplements may have increased *O. insidiosus* retention.

Lacewing identification became a critical component of this project. We determined that the "*Chrysoperla carnea*" we purchased for trials in 2021 were actually *C. externa* (purchased as larvae) and *C. plorabunda* (purchased as eggs). *Chrysoperla externa* can be separated from other lacewings visually under magnification, but to distinguish between "resident" lacewings and the released *C. plorabunda*, we had to develop molecular methods. We determined that the COI gene, which we are using in our gut content analysis, can also be used to separate resident from released lacewings. It is important to note that the lacewing species present in orchards that is often referred to as "*C. plorabunda*" is likely *C. johnsoni* and therefore a different species that what is commercially

available. However, *C. plorabunda* is native to Washington (found outside of orchards) and therefore likely to be a better climate match that *C. rufilabris*.

***Orius insidiosus* releases** were performed as part of the retention experiments for aphid and pear psylla control, but data from these trials also allowed us to access the efficacy of this predator for thrips control. One release of *O. insidious* (2,000/acre) reduced adult thrips on sticky cards by 50% in both apple trials and by >50% in one of the pear trials. Evaluations of thrips damage did not occur as part of this work, but should be included in future studies. More frequent releases (at lower rates) may be more effective and economical.

Whirligig mite was found in abundance on beat trays in some of our study locations. The role of this predator in North American orchards has received little attention, but research from Ireland and preliminary work from other projects suggest that it may be an important orchard natural enemy. It recently became available for purchase in the U.S. (Oregon only).

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

RESULTS AND DISCUSSION

Obj. 1. Improve retention of released natural enemies

The study was conducted two commercial pear orchards (organic: Leavenworth, WA in 2022 and soft conventional: Wapato, WA in 2023). The organic orchard in 2022 had a relatively low pear psylla population, so we switched locations in 2023 to better assess differences between treatments. In 2022, releases were conducted on June 3rd and monitored for three weeks post-release. In 2023, releases were conducted on May 25th and monitored for four weeks post-release.

There were five treatments consisting of combinations of lure use (Predalure, methyl salicylate), food supplements (Artemia, brine shrimp cysts on tape Fig. 1 + *Ephestia* eggs on cards), and releases (100,000 "*C. carnea*" lacewing eggs + 2,000 *Orius insidiosus* per acre): (1) Predalure + Foods + Release, (2) Predalure + Release, (3) Food + Release, (4) Release only, and (5) No-release control. In 2022, the "Food" treatment only used Artemia tape (the *Ephestia* eggs were dropped). Rates for the food treatments and lures were: 1 lure/plot, 50 m Artemia tape/plot, and 35,000 *Ephestia* eggs/plot (1 card/30 tags). Each combination was replicated in

Fig. 1. Ladybeetle feeding on Artemia tape

the orchard 5 times in 0.25-acre plots. Pear psylla, mites, lacewings, and minute pirate bugs were counted prior to release and then once weekly after release. Pear psylla and mites were sampled by collecting a 30-leaf brush sample in each plot. Beat tray samples were collected from the 9 center trees of each plot and all natural enemies from the tap counts were collected and stored in ethanol for identification and use in molecular gut content analysis. Two sticky cards were also hung in each plot to monitor adult natural enemies. The "*C. carnea*" sold by the insectary were tentatively identified as *C. plorabunda*. Final determination of the lacewing species using song analysis will be done by a lacewing biologist (K. Taylor, University of Maryland) this spring.

In the six trials (2 commercial apple, 2 research apple, 2 pear), only 8 *O. insidious* were recovered. However, the consistent decrease in thrips counts in plots where *O. insidiosus* were released indicates that this predator remained in plots long enough to reduce pest populations. Although it was most commonly found 1-2 weeks post-release, in the 2022 commercial apple and 2023 research apple orchards, *O. insidiosus* were found over a month after release. This species is not native to Washington and has never been found in an area where it was not recently released, therefore all recovered *O. insidiosus* are from that year's releases. Of the few *O. insidiosus* found, 75% of them were recovered from plots with supplementary foods. The two individuals recovered from plots without foods were found one month post-release, when the foods were likely completely consumed/decayed. Therefore, there is some evidence that the Artemia tape increased retention of *O. insidiosus* in the field. In future studies examining efficacy of *O. insidiosus* for thrips control in apples, the use of releases in combination with Artemia tape should be explored.

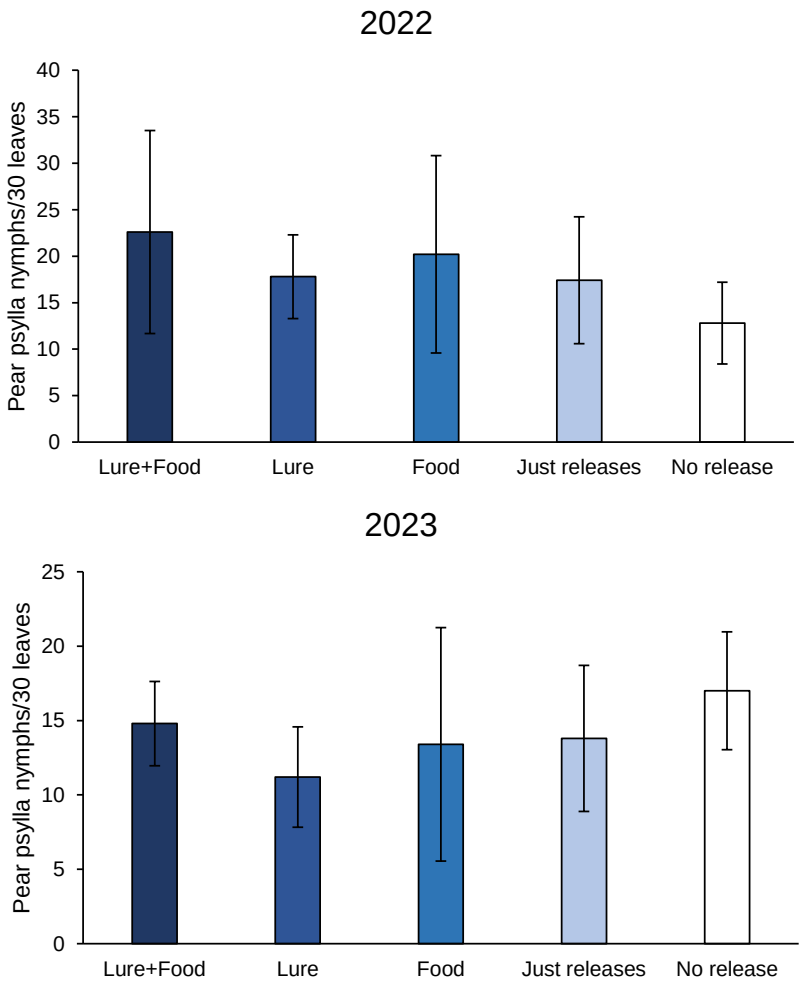


Fig. 2. The releases and the retention treatments did not improve pear psylla control. Seasonal sums.

Molecular identification of the *carnea*-group lac ewings recovered from the retention trials is ongoing. All samples have been processed and sequenced. Sequences have been aligned and we are currently constructing computationally-intensive phylogenetic trees to determine which collected individuals "match" the controls directly removed from insectary bottles. This analysis is anticipated to be completed in February 2024. Based on preliminary analysis, no

treatment increased retention of released lacewings. However, applications of methyl salicylate lures timed for approximately when released lacewings become adults (as opposed to during the release) may increase the likelihood that the adults remain in the orchard.

In 2022, None of the treatments in our study differed from each other in pear psylla abundance (Fig. 2); releases of *C. plorabunda* and *O. insidiosus* did not reduce pear psylla counts and lures and food supplements did not alter treatment efficacy. We were able to recover our released predators: two *O. insidiosus* were found one week post-release and two *C. carnea* larvae were found three weeks post-release. Releases significantly decreased thrips abundance (Fig. 3). This effect was also seen in some of the apple trials and indicates *O. insidiosus* releases should be further investigated for their potential to reduce thrips damage to susceptible fruit varieties.

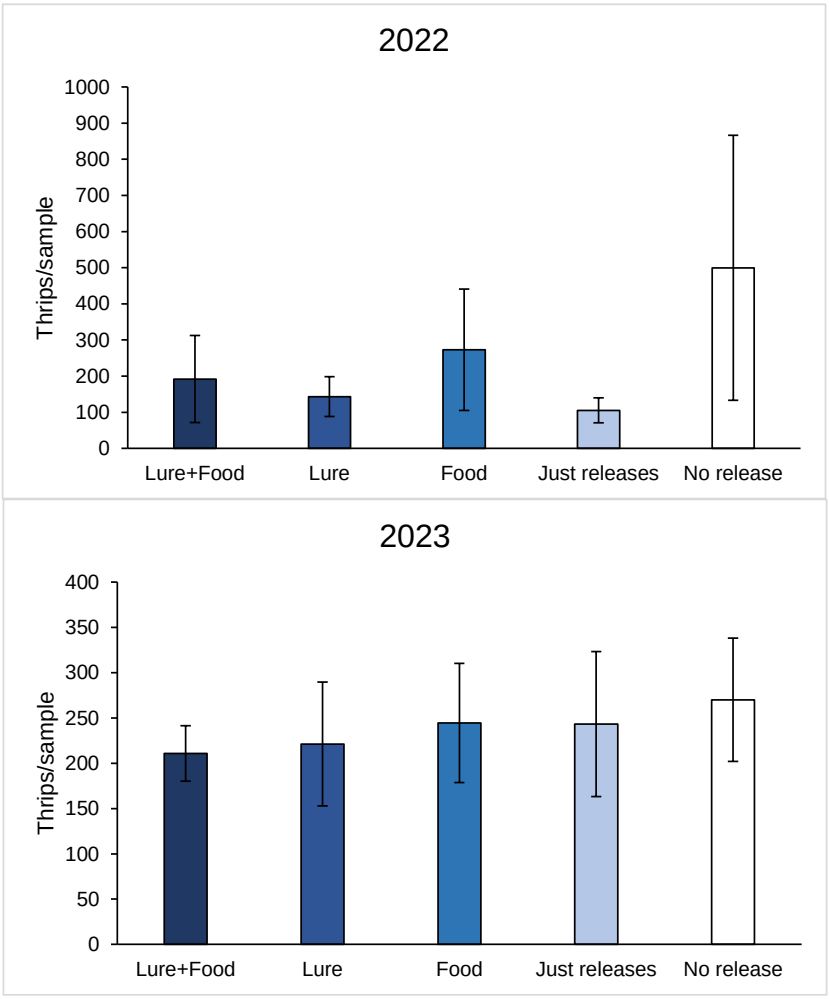


Fig. 3. In 2022, releases decreased thrips. This effect was not seen in 2023. Seasonal sums.

Lures increased *Campylomma* abundance (Fig. 4). Across our samples, the most prevalent natural enemies were *Campylomma*, whirligig mites, and spiders. *Deraeocoris* and lacewings were also present, but far less abundant.

In 2023, none of the treatments affected pear psylla abundance (Fig. 2). No *O. insidiosus* were found post-release and only one lacewing larvae was found. We also found lacewing larvae pre-release, so we are conducting genetic analysis to determine if the single recovered larvae is “released” or “resident”. Unlike the 2022 trial, releases did not decrease thrips abundance (Fig. 3). This may be because the *O. insidiosus* were not found post-release. The lure treatment was associated with an increase in *Campylomma*, but the trend was less dramatic in this trial compared to 2022 (Fig. 4). *Camplyomma* counts were also higher in the lure treatments in the 2023 apple trial. Unlike the 2022 trial, *Stethorus* were abundant in this orchard and we found that lures increased *Stethorus* abundance (Fig. 5). This effect was also observed in some of the apple trials. Methyl salicylate lures should be further investigated for their ability to increase *Stethorus* and *Campylomma* populations. We generally observed unusually high numbers of *Stethorus* throughout the season in a variety of crops in 2023; if *Stethorus* continues to be abundant, it may play a more important role in spider mite control in pears and other crops.

These samples are being used to conduct PCR-based gut content analysis to determine (1) which predators are most commonly found to have consumed pear psylla, (2) if any predators consumed the food supplements, and (3) if any pear psylla predators commonly eat each other (intraguild predation). This will provide growers with better recommendations on which natural enemies to focus on as part of conservation efforts. We are particularly excited to find whirligig mites in abundance; this is an important natural enemy of potato psyllids in weedy hosts near potato fields (Fig. 6). It is likely to also be an important pear psylla predator. Currently, whirligig mites are available for purchase in Canada and Oregon, but not Washington.

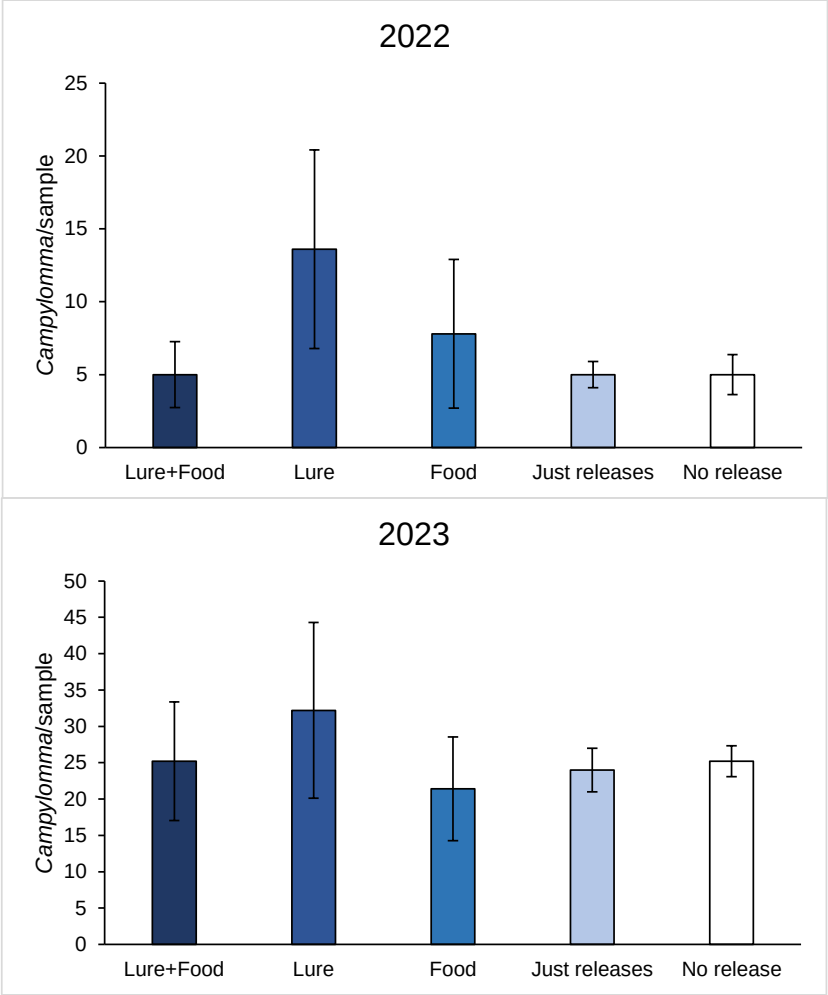


Fig. 4. In 2022, lures used alone significantly increased *Campylomma*. This effect was also seen in 2023, but was not statistically significant. Seasonal sums.

Between sites and across years, there was very little consistency in the effects of the treatments. Taken in combination with the apple data, *Stethorus* generally increased in plots with lures and may exert control on mites while rapidly moving between plots. Lacewings also showed a similar, although weak, trend. Because natural enemies interact with each other and pests over time, it is difficult to discern if changes in natural enemy abundance due to treatments are due to predation amongst themselves or changes in pest densities. The gut content work, which should be completed by June 2024, may provide additional information about these relationships.

Obj. 2. Determine cost-effectiveness and efficacy of natural enemy release by drone

In 2022, we tested releases of *C. plorabunda* eggs and *O. insidiosus* adults by ground (sprinkled) and by drone at a rate of 100,000 and 2,000 per acre, respectively. Because of the lack of observed effects on pear psylla in 2023 (and in the Obj. 1 trials), in 2022 we focused exclusively on the ground-based treatments. The trials were conducted in the same orchards each year as the Obj. 1 trials. In 2022, releases were conducted on June 10th and monitored for three weeks post-release. In 2023, releases were conducted on May 25th and monitored for four weeks post-release.

None of the treatments resulted in a decrease in pear psylla abundance in either year of the

study (Fig. 7). We were also unable to recover any of our released *O. insidiosus* and *C. plorabunda* in either year. A limited number of resident green lacewings were found in both trials. In 2022, due to time limitations, we were unable to release the natural enemies until a week after arrival (they were kept at 50 °F). It is possible that the quality of the natural enemies declined during storage, although we did confirm that they were alive prior to release. However, even in the retention trial and the 2023 efficacy trial, when natural enemies were immediately released, no effect was observed on pear psylla. Natural enemies that are currently commercially available are likely not appropriate for pear psylla management. However, the newly available "Crazee mite" shows potential for pear psylla control and should be investigated in future studies.

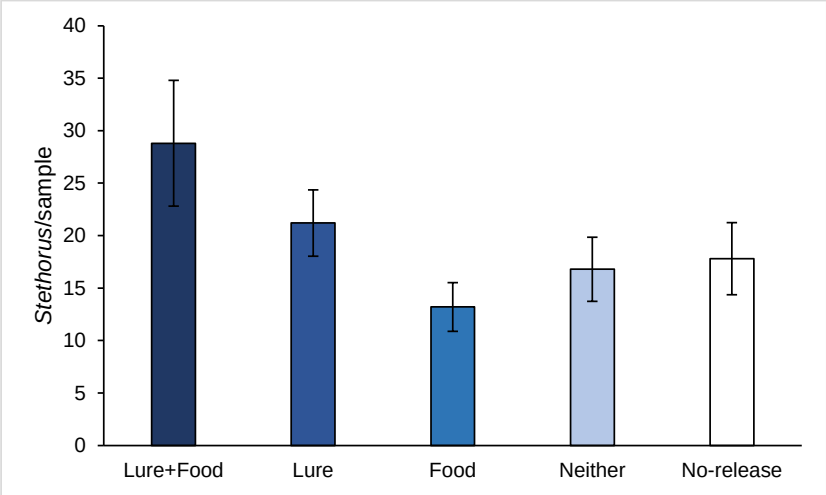


Fig. 5. In 2023, lures increased *Stethorus*. They were not present in the 2022 trial. Seasonal sums.

Fig. 6. Whirligig mite eating a pear psylla.

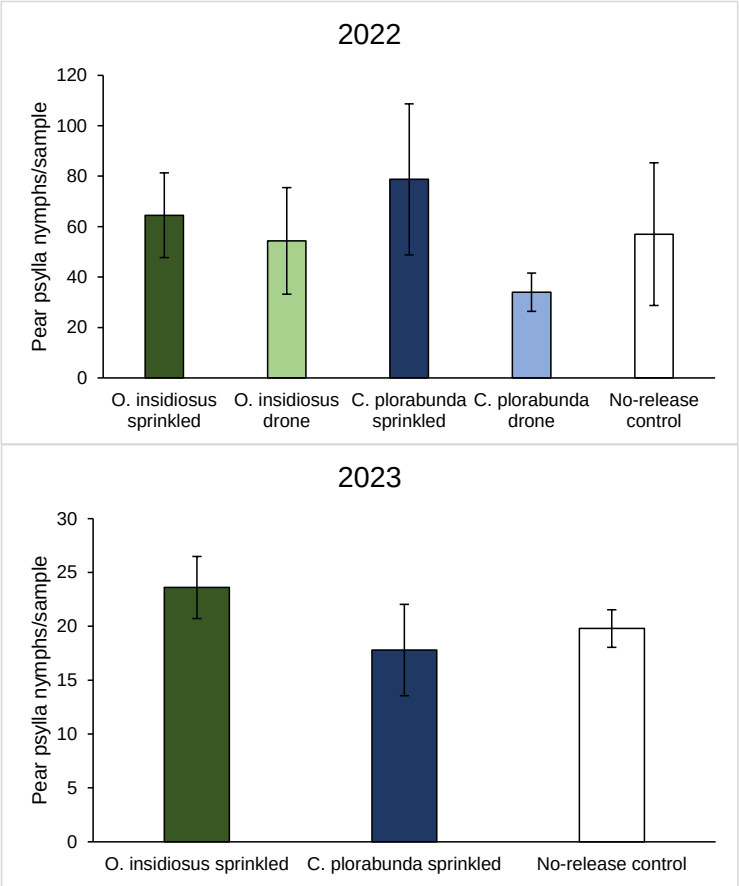


Fig. 7. In 2022 and 2023, releases of *O. insidiosus* or *C. plorabunda* did not affect pear psylla abundance. Seasonal sums.

EXECUTIVE SUMMARY

Project title: Tactics to improve natural enemy releases in tree fruit

Key words: lacewing, *Chrysoperla plorabunda*, *Orius insidiosus*, lures, supplementary foods

Abstract:

Growers have experimented with releases of natural enemies to control pests in tree fruit, but there are currently no best practice recommendations for releases in orchards. The purpose of this project was to determine which natural enemies and release methods showed the most promise for controlling orchard pests, with the pear work focusing on pear psylla. We also examined the potential of lures and supplementary food products for recruiting resident natural enemies and retaining released natural enemies. Tactics for retaining and recruiting natural enemies had highly variable results between sites and years. In general, methyl salicylate lures showed some promise for recruiting lacewings in apples and *Stethorus* in apples and pears. There was also a slight trend for increases of *Camplyomma* in lure-based treatments. In 2023, *Stethorus* was unusually abundant throughout the season in many crops and the importance of this natural enemy in reducing pest mite abundance in pears may need to be re-evaluated. Food supplements may have increased retention of released *O. insidiosus* and subsequently reduced thrips abundance. The use of lures after a lacewing release should be investigated to determine if they encourage released lacewings to remain in the orchard after they develop into adults. None of the release treatments decreased pear psylla abundance and retention of the released natural enemies in pear trials was much lower in than in apple trials. Commercially available natural enemies appear to be unsuitable for pear psylla control. However, the whirligig mite became available for purchase in Oregon in 2023 and has shown promise in preliminary research. As new natural enemies enter the market, they should be evaluated for their ability to control pear psylla. Augmentation with commercially available natural enemies may be particularly helpful in pear orchards in transition to organic or IPM spray programs.

Project Title: What factors impact mite outbreaks in pear?

Report Type: Continuing Project Report, NCE

Primary PI: Rebecca Schmidt-Jeffris
Organization: USDA-ARS
Telephone: 509-454-6556
Email: rebecca.schmidt@usda.gov
Address: 5230 Konnowac Pass Rd
City/State/Zip: Wapato, WA 98951

Co-PI 2: Louis Nottingham/Robert Orpet
Organization: Washington State University
Telephone: 509-293-8756
Email: robert.orpet@wsu.edu
Address: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

CO-PI 3: Chris Adams
Organization: Oregon State University
Telephone: 248-850-0648
Email: chris.adams@oregonstate.edu
Address: 3005 Experiment Station Dr.
City/State/Zip: Hood River, OR 97031

Project Duration: 2 Year

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

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
Telephone: 510-559-5619
Contract administrator email address: mara.guttman@usda.gov
Supervisor: Rodney Cooper
Supervisor email address: rodney.cooper@usda.gov

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

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

Budget 2

Primary PI: Louis Nottingham/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
Telephone: 541-737-3228
Email address: charlene.wilkinson@oregonstate.edu
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.
- Yakima Valley had much higher rust mite populations than the other two regions.
- Weed washes in alcohol were an effective method for detecting spider mites and phytoseiids in the ground cover.
- While phytoseiids ("typhs") were found in the survey, they were much less common than in apple orchards. This suggests that in pear orchards where pest mites do not flare, other natural enemies are responsible for biological control.
- The composition of the orchard natural enemy community varied dramatically by region.

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 will also conduct beat samples on 5 trees spaced roughly evenly throughout the orchard block. Any small predatory insects (of the appropriate size to eat mites) 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.

All 2022 arthropod counts are completed. 2023 brush counts and sticky card counts are completed and the tap counts and leaf wash samples are currently being processed.

We determined that typical molecular gut content analysis using universal COI primers is not suitable for this study. Our pest mites do not amplify with this primer. Instead, we are using species-specific primers for each predator collected in the study to determine whether it has 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. This will provide a bonus of determining which predators regularly consume pear psylla. There is no existing primer available for pear rust mite, so we are currently designing one. Extracting high quality DNA for sequencing pear rust mite has proven challenging. We attempted extractions in 2022, which did not yield useful sequences. We collected additional rust mites for sequencing and primer design this winter. We anticipate completely primer testing/design by June 2024 and completing the extraction/PCR for all molecular work by December 2024. In addition to these logistical delays, a lab space move and subsequent renovation in late 2022 also delayed the molecular portions of this research.

Landscape surrounding the orchard will be quantified using Cropscape and QGIS analysis procedures. We have requested pesticide records from growers for the two growing seasons and have nearly completed collecting this information. We have also collected data on mowing frequency and asked growers to indicate if they consider their orchard "dusty". In 2024, we will use the arthropod counts, management information, and weather data (WSU AgWeatherNet) to determine through statistical modelling which factors most strongly impact spider and rust mite populations. Model building procedures will be similar to those used in Schmidt-Jeffris et al. 2015. This will allow us to (1) determine if "bad mite years" can be predicted, (2) identify which practices are associated with mite flareups so growers can avoid them, and (3) identify the most important natural enemies of spider and rust mites so appropriate conservation methods can be implemented. Our data will also allow us to determine which management factors most impact abundance of key pest mite natural enemies; this information can be used to better conserve these predators.

The granted no-cost extension will allow us to complete the labor-intensive molecular work and computationally-intensive modelling needed to draw conclusions from this research.

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. New sites were selected in 2023 to ensure at least some Hood River locations had spider mites. Yakima Valley dropped three sites and added one in 2023, whereas all the Wenatchee Valley sites remained the same between both years.

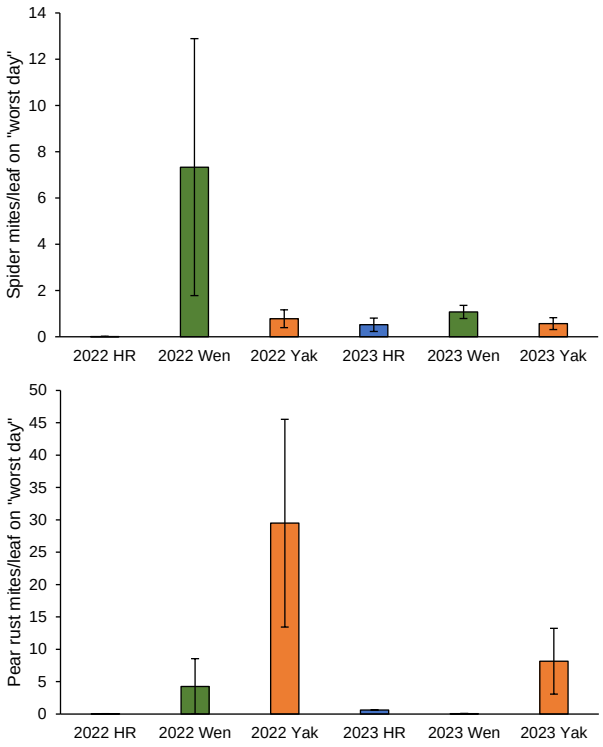


Fig. 1. Mean pest mites per leaf on an orchard's "worst mite day"

Wenatchee Valley sites had much higher twospotted spider mite populations than the other two regions, with Yakima Valley intermediate. To compare sites and regions, we plotted region's "mite peak" for both twospotted spider mite and pear rust mite (Fig. 1). Phytoseiids were generally uncommon. 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. High phytoseiid counts always correlated with spider mite outbreaks, but not all orchards with high populations of spider mites also had high phytoseiid levels. The vast majority of the phytoseiids found in 2022 brush samples were *G. occidentalis*. Phytoseiids from 2023 have not yet been identified to species.

Alcohol weed washes were effective at detecting spider mites in the ground cover in 2022 (2023 currently being processed). These were nearly always twospotted spider mite. In Yakima, a weed wash sample would typically contain 0-2 mites per sample date. In Wenatchee, as many as 63 twospotted spider mites were found in one sample. Phytoseiids were also found in the weed wash samples. Species other than *G. occidentalis* were more common in weed wash samples than in brush samples.

Across regions in 2022, the most common mite natural enemies in beat tray samples (2023 currently being processed) were *Deraeocoris*, spiders, *Stethorus*, and *Campylomma*. *Stethorus* and *Hippodamia convergens* were the most common ladybeetles collected. Surprisingly few natural enemies were captured on beat trays in Hood River, especially given that natural enemies were abundant on sticky cards. Natural enemies collected on beat trays are currently undergoing gut content analysis to detect the DNA of twospotted spider mite and pear psylla. Once a pear rust mite primer is developed, samples will also be screened for pear rust mite DNA.

In general, *Trechnites* made up a larger portion of the sticky card natural enemy community in Wenatchee compared to the other two regions (Fig. 2) – likely because pear psylla are also more abundant in this area. *Chrysopa* lacewings were more common in Wenatchee, whereas *Chrysoperla* and brown lacewings were more common in Yakima and Hood River. *Deraeocoris* was more abundant in Wenatchee, whereas *Campylomma* was more abundant in Yakima and Hood River (Fig. 2).

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 1-2). In Hood River, clover, dandelion, and mallow were also the three most common weeds (Table 1-2). 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.

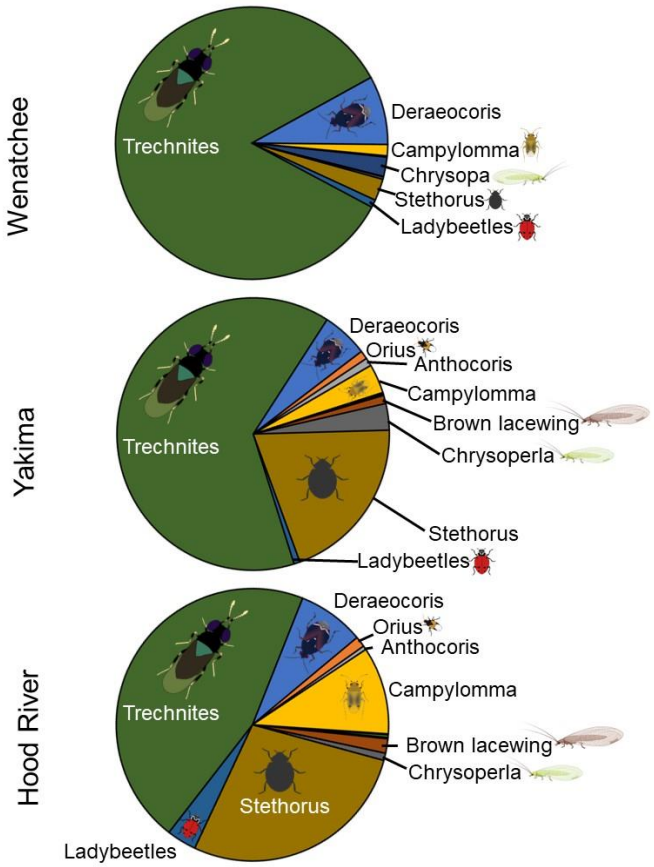


Fig. 2. Proportion of natural enemy community represented by each group found on sticky cards, separated by pear growing region.

Table 1. 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 2. 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

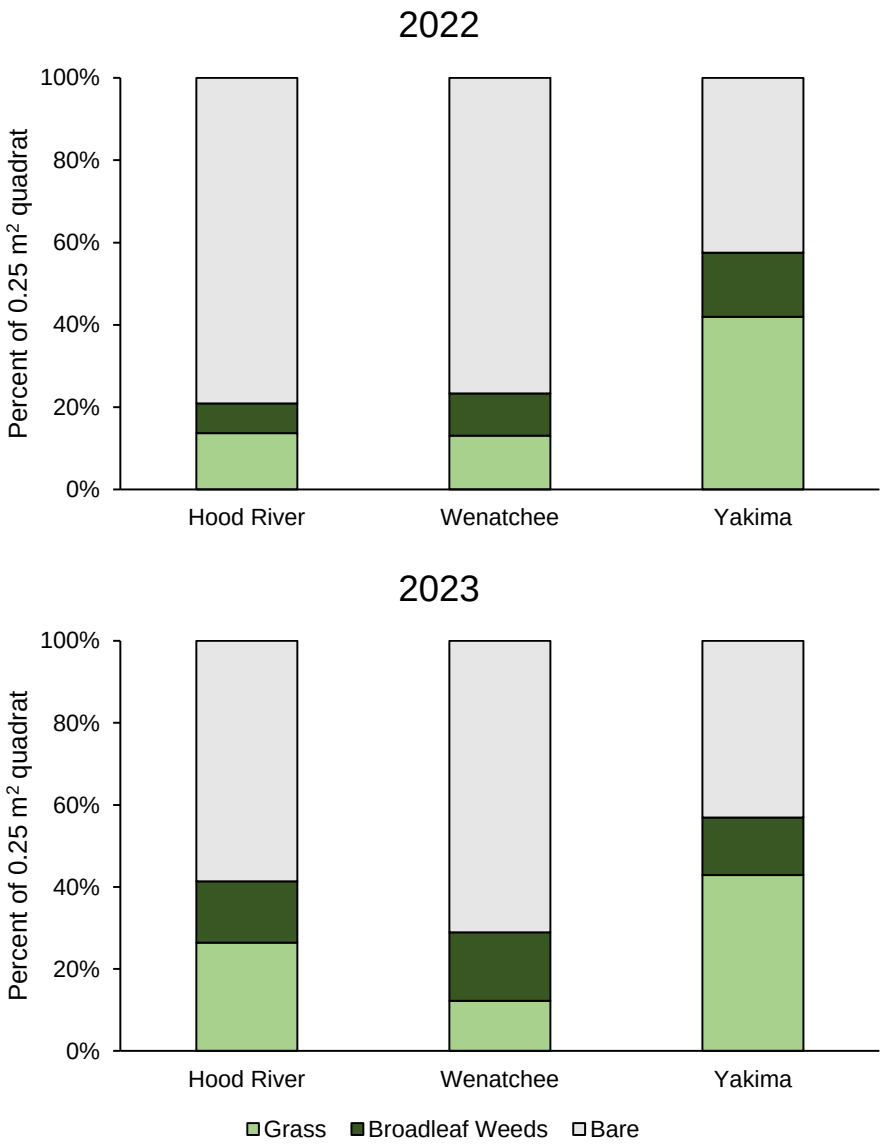


Fig. 3. Ground cover composition 0.5 m into the row from the base of the tree.

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

Report Type: Final Project Report

Primary PI: Christopher Adams
Organization: OSU
Telephone: 248-850-0648
Email: chris.adams@oregonstate.edu
Address: 3005 experiment station drive
City/State/Zip: Hood River, OR 97031

Co-PI 2: Rebecca Schmidt-Jefferies
Organization: USDA-ARS
Telephone: 509-454-6556
Email: Rebecca.schmidt@usda.gov
Address: 5230 Kennowac Pass Rd.
City/State/Zip: Wapato, WA 98951

Co-PI 2: Robert Orpet
Organization: WSU
Telephone: 509-293-8756
Email: robert.orpet@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee, WA 98801

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, and re-applying
Funding Duration: 2024 - 2027
Amount: $339,668
Agency Name: WSARE
Notes: We applied for this grant last year and were highly rated but not funded. We are re submitting the grant this spring 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

Organization Name: USDA-ARS

Contract Administrator: Mara Guttman

Telephone: 510-559-5619

Contract administrator email address: mara.guttman@usda.gov

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
Telephone: 509-293-8803
Contract administrator email address: shelli.tompkins@wsu.edu
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 through 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 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)

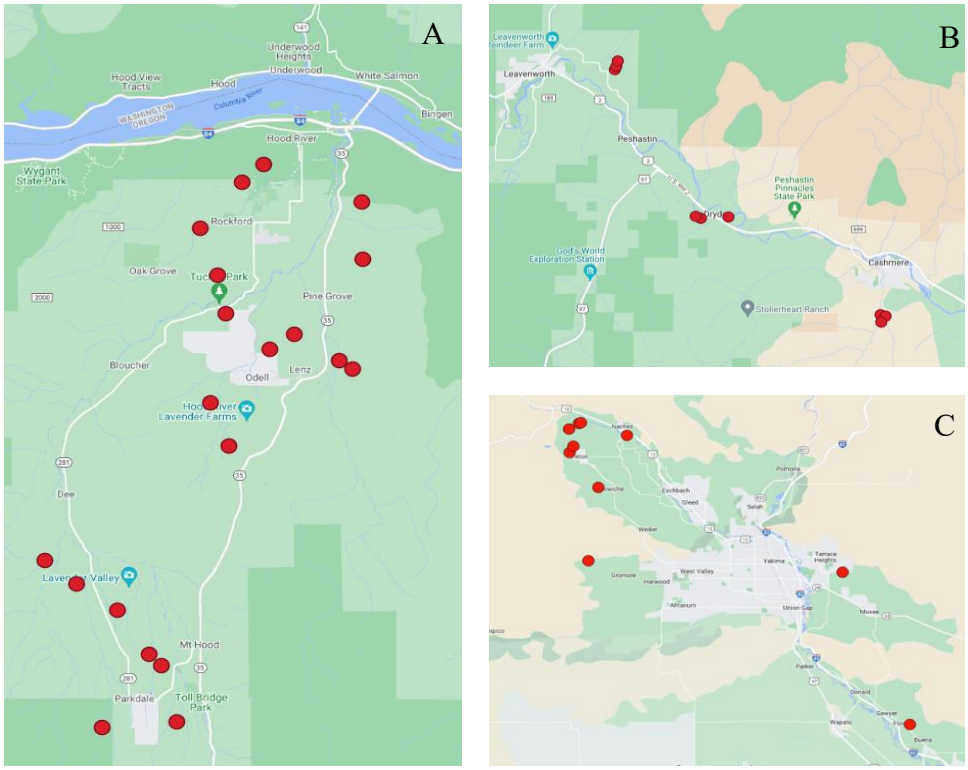
Lure baited yellow sticky cards effectively collected 12 key natural enemies season long and represent significant time savings over scouting the orchards with beat trays. In Addition, lure baited yellow sticky cards collected insects not typically collected in beat trays such as

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. This tool will allow for improved management decisions and better-timed sprays.

Researchers have been working on this objective for fifty years. This same question was Larry Gut’s Master’s degree, 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 average. One trend we have seen is that a steady drop in deraeocoris over three years that corresponded with a huge surge in pear psylla this past summer, despite huge numbers of lacewings being present all three years. We still have great variability between sites within each year, and we don’t find clear cause and effect. 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 data is informative to them.

Washington collaborators could not start work in the first year because of the off-set timing of funding. They will complete their final summer of collection this year. Rebecca Schmidt-Jeffris may need a no cost extension to manage the billing to her account. I did not make an extension request extension because I thought she would be able to move those funds into a spendable account. She is in the process of working out those details.



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.

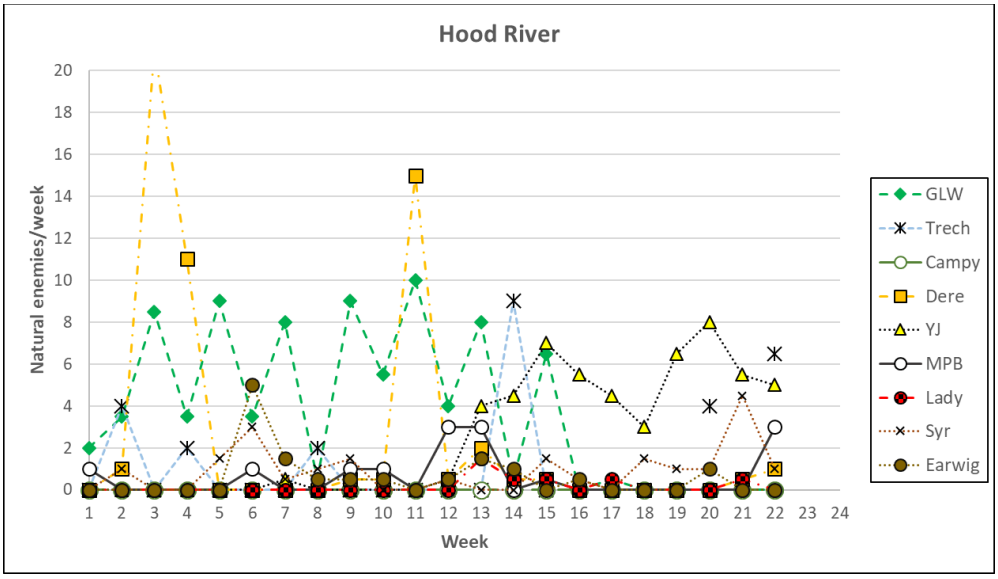


Figure 2. An example of the average natural enemy counts found in the Hood River region, sent out weekly to growers and crop consultants in 2021 - 2023. These area-wide averages were used by crop consultants, in conjunction with local trapping, to make decisions. Although crop consultants could not agree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

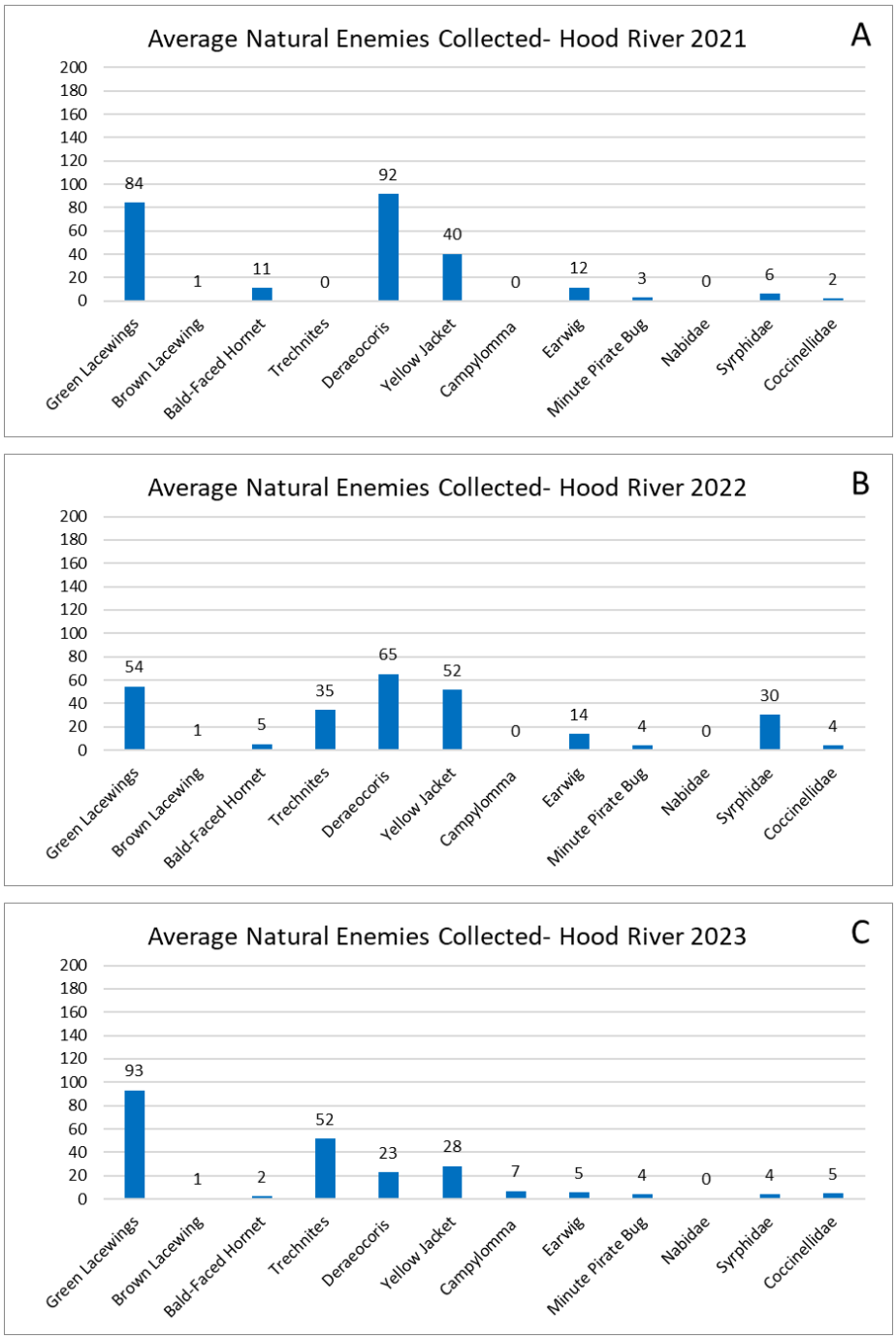


Figure 3 (A-C). Average natural enemy capture in Hood River by year shows a multi-year decreasing trend in deraeocoris that correlates with last high year's pear psylla counts. No other insect has shown a clear correlation.

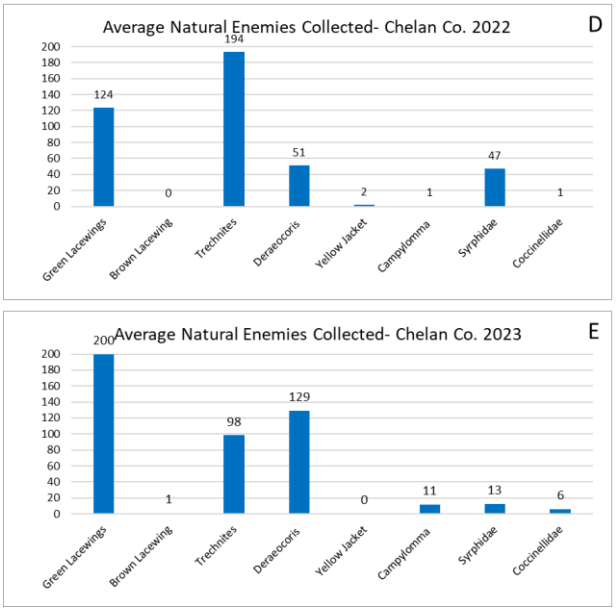


Figure 4 (D &E) Average natural enemies Chelan CO in 2022 (D) and 2023 (E).

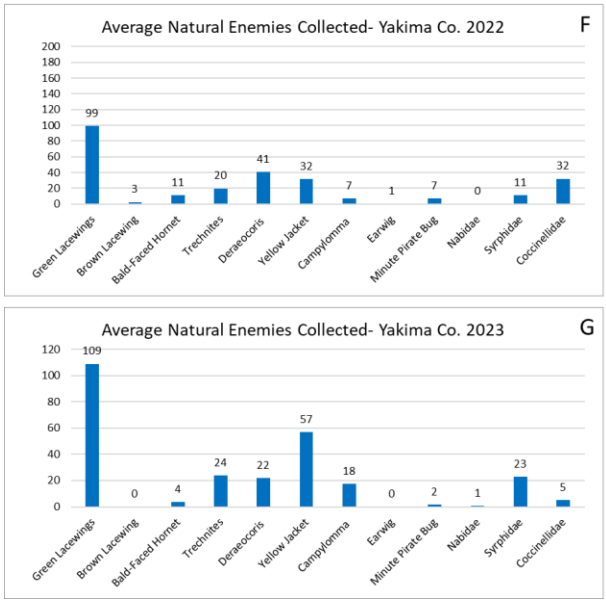


Figure 5 (F & G) Average number of natural enemies collected Yakima Co. in 2022 (F), 2023 (G).

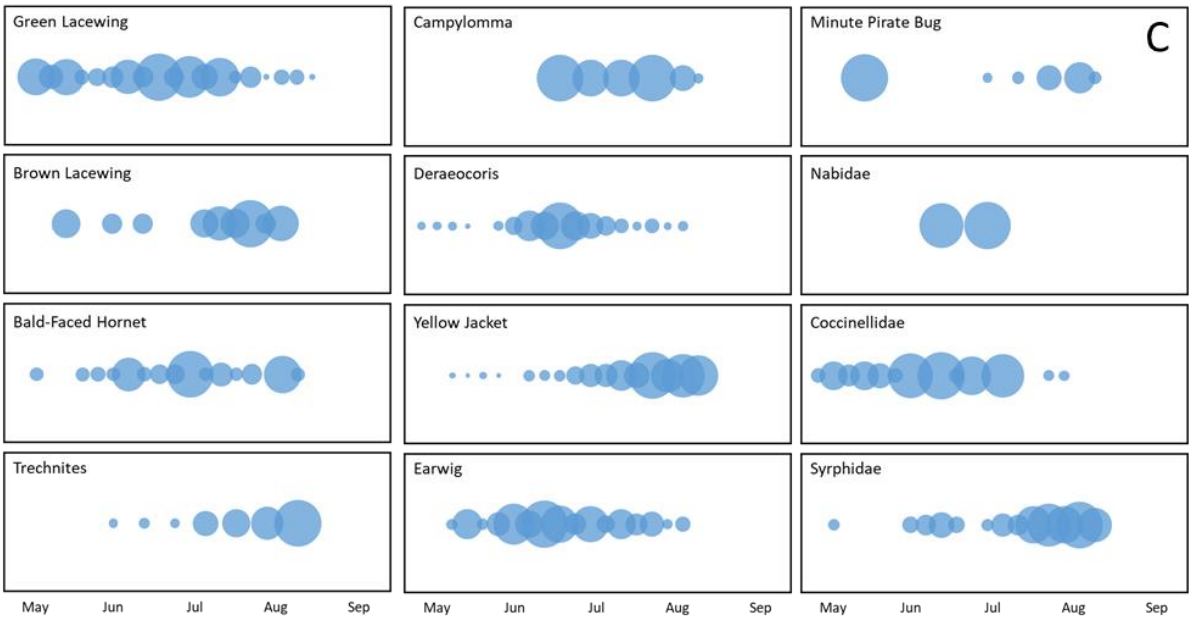


Figure 6. The relative abundance of natural enemies throughout the season in Hood River illustrates timing of natural enemy occurrence.

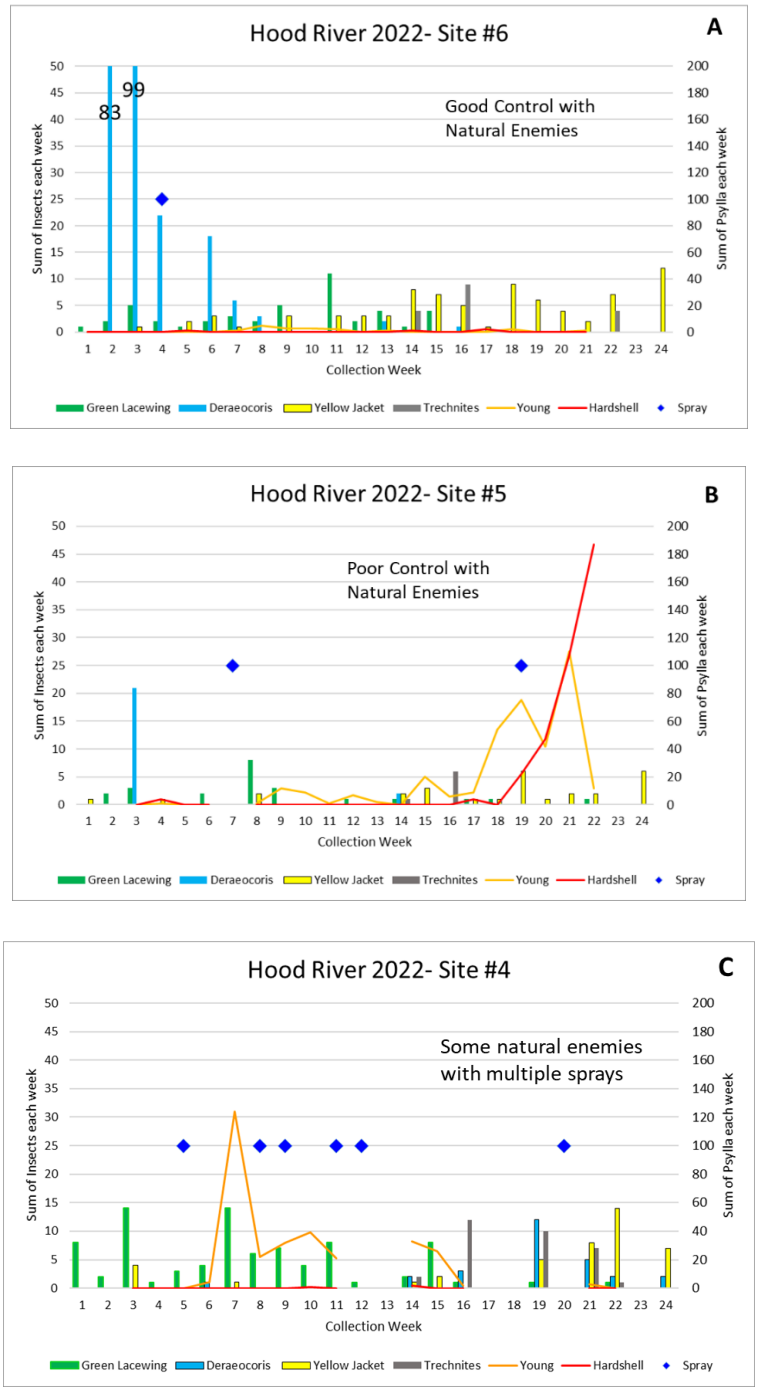


Figure 7. Representative orchards showing season long catch. Counts of natural enemies, young pear psylla nymphs (young), and mature psylla nymphs (hard-shell) at select sites in Hood River Co. Figure A shows ideal natural enemy control. Figure B shows lack of natural enemy control. And Figure C shows insufficient natural enemy control with multiple sprays.

Project Title: Biological control of BMSB using Trissolcus japonicus

Report Type: Final Project Report

Primary PI: Christopher Adams
Organization: OSU
Telephone: 248-850-0648
Email: chris.adams@oregonstate.edu
Address: 3005 experiment station drive
City/State/Zip: Hood River, OR 97031

Co-PI 2: Nik Wiman
Organization: OSU
Telephone: 541-250-6762
Email: nik.wiman@oregonstate.edu
Address: 15210 NE Miley Rd
City/State/Zip: Aurora, OR 97002

Cooperators:

Project Duration: 3 Year

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

Other related/associated funding sources: Awarded ($30,324)
Funding Duration: 2022 - 2023
Amount: $30,324
Agency Name: CGFG
Notes:

WTFRC Collaborative Costs:

Item	2021	2022	2023
Salaries [1]	$7,975.00	$8,215.00	$8,461.00
Benefits	$5,575.00	$5,742.00	$5,914.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies [2]	$2,000.00	$2,000.00	$2,000.00
Travel [3]	$1,000.00	$1,000.00	$1,000.00
Plot Fees			
Miscellaneous			
Total	$16,550.00	$16,957.00	$17,375.00

Footnotes:

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

[2]Research consumables

[3]Travel to field plots

Budget 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]	$9,100.00	$9,373.00	$9,654.00
Benefits	$3,900.00	$4,017.00	$4,138.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$1,000.00	$1,000.00	$1,000.00
Travel [2]			
Plot Fees			
Miscellaneous			
Total	$14,000.00	$14,390.00	$14,792.00

Footnotes:

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

[3]Travel to field plots

Budget 2

Co PI 2: Nik Wiman

Organization Name: OSU

Contract Administrator: Charlene Wilkinson

Telephone: 541-737-3228

Contract administrator email address: Charlene.wilkinson@oregonstate.edu

Station Manager/Supervisor:

Station manager/supervisor email address:

Recap of Original Objectives

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

1. **Raise and release Tj for release in key locations. (every year)**
   A colony of *T. japonicus* was established in 2021, and new wasps were reared from BMSB eggs collected from the MCAREC lab colony. Releases of the wasps occurred weekly from August 12th- October 7th at 12 sites in 2021, June 1st- October 3rd, 2022 at 12 sites, and June 9th- September 20th, 2023 at 12 sites
2. **Measure establishment using sentinel egg masses and yellow sticky traps (years 2 & 3)**
   Sentinel egg masses were placed at the 2021 release sites and left for 24 hours on 6-Jul and 20-July, 2022. Three yellow sticky cards were placed at each site and left for two weeks on 6-July, 20-Jul, and 1-Aug. In 2023 two yellow sticky cards were placed at each site and left for two weeks on 25-July, 17-Aug, and 1-Sep.
3. **Describe the habitats where wasp establishment is most successful (years 2 & 3)**
   The sites that appear to have successful establishment from the 2021 releases were bordered by mixed oak and conifer forest. This habitat provides the brown marmorated stink bug additional host plant resources, as well as refugia for both the stink bug and Tj from pesticide sprays applied in the pear orchards.
4. **Measure the effectiveness of Tj biocontrol for preventing fruit damage (years 2 & 3)**
   BMSB populations will be measured with lure baited (congregation pheromone) traps to measure BMSB populations in year zero (before releases of wasps) and then during each subsequent year, to measure change in populations. Growers hosting release sites will be asked to share cull reports from the packing houses.

Significant findings / outcomes

- **Other:** As part of these efforts, we have been sending out weekly reports of BMSB captured across the network of traps. This report allows stakeholders to see BMSB numbers across the entire growing region and see populations numbers are changing through time.
- **Objective 1:** A total of 72,234 Tj wasps were released during this project. A total of 8,434 Tj were reared at the MCAREC insectary and released at 15 pear orchards (14 pear and 1 peach) located throughout Hood River County in 2021. A total of 44,200 Tj were reared at the MCAREC insectary, and released at 12 orchards (11 pear and 1 peach) located throughout Hood River County in 2022. A total of 16,500 Tj were reared at the MCAREC insectary, and released at 12 pear orchards (11 pear in Hood River Co. and 1 apricot in Wasco Co.) in 2023.
- **Objective 1:** We collaborated with the Oregon Department of Agriculture and helped them release 1,400 Tj from their colony at our release sites in Hood River in 2021, and an additional 1,700 Tj in 2022.
- **Objective 2:** Tj were recovered on yellow sticky traps at 4 out of 14 of the 2021 release sites in 2022**.** In 2023 Tj were collected from 11 out of 12 release sites. The number of Tj found on yellow sticky cards ranged from 2-55 specimens at these 11 sites. Recovery of wasps at 28% (2022) and 92% (2023) of release sites is extremely encouraging.
- **Objective 3:** The 2021 sites where Tj was successfully recaptured were surrounded by mixed oak and conifer forest bordering the pear orchard.
- **Objective 4:** There was no correlation between wasp release site and reduced BMSB capture. Measurable impact may take several years.

Methods

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

We currently have a dozen cages of stink bugs housing about 30 insects each that regularly produce several hundred eggs per week (Figure 5). Stink bugs require daily fresh food and water, colony maintenance, and egg collection, totaling serval hours per day 7 days per week. Stink bug eggs are collected daily, and newly emerged wasps are placed in small cup containers with fresh stink bug eggs (Figure 5). Releases occurred every week from August through October at 15 sites in 2021, June- October at 12 sites in 2022, and June- September at 12 sites in 2023 (Figure 1). Weekly release numbers varied in 2021, depending on the number of wasps available each week. In 2022, 200-300 wasps were released at each site each week. In 2023, 50-200 wasps were released at each site each week.
To maintain colony heath, wild caught Tj wasps and wasps from other regional rearing programs will occasionally be added to our colony to prevent genetic drift within the colony.

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

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

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

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

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

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

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

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

Year zero stink bug populations were measured using pyramid traps containing the Trécé BMSB dual pheromone lure to measure the abundance of BMSB within each orchard. Pheromone baited traps will be maintained at each release site and traps checked weekly. Abundance of stink bugs will be used as one measure of effectiveness of biocontrol. Packing house cull report will be gathered from each grower to see how fruit damage changes from year to year.

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

Results and Discussion

We successfully established and maintained a colony of BMSB large enough to produce a steady supply of eggs. These BMSB eggs were used to establish and maintain a colony of Tj wasps, and to date we have released **72,234 wasps at 39 locations** across the Hood River growing region from this colony. In collaboration with Oregon Department of Agriculture's state-wide Tj distribution program an additional 3,100 Tj were released in Hood River Co. This collaboration added 1,400 wasps in 2021 and 1,700 wasps in 2022. In addition, we are assisting Dr. Nik Wiman's PhD student with her Tj wasp release in the Hood River area. Her project added another 1,200 wasps to the total released. The combination of these three efforts resulted in a total of 73,434 Tj released in the Mid-Columbia region between the years of 2017-2023.

In 2022 we began trapping efforts to look for establishment of the wasp in these locations. Sentinel egg masses were placed at the 2021 release sites and left for 24 hours on 6-July and 20-July, 2022. None of the recovered egg masses were parasitized, and so we discontinued the use of egg mases. Three yellow sticky cards were placed at each site and left for two weeks on 6-July, 20-July, and 1-Aug resulting in catch of Tj at 4 sites. A total of 100 wasps were collected at these four sites (n= 1, 3, 25, and 71). Another 41 wasps were captured that appear to be another *Trissolcus* species from 7 other sites. These established Tj wasps will continue to increase their populations in and around these orchards over time.

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

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

In 2023 the egg production of the BMSB colony was lower than the previous year but still very productive. We had hoped that our 2023 release numbers would exceed 2022's numbers, and even hired a high school aged student to spend more time with the colony. Unfortunately, for reasons that are not clear, our colony (and wasp production) was less in the 3rd year than in the previous year, but still respectable.

It is worth noting that the number of wasps we were able to produce in this small project far exceeded the total number of wasps produced by the Oregon Department of Agriculture program over the same period.

It may take years to detect measurable impact on BMSB numbers. However, getting wasps established throughout the fruit growing region now, *before* BMSB numbers grow to levels seen on the East coast, will improve our chance of slowing the BMSB populations. In addition, biocontrol techniques are more effective at reducing populations of BMSB off site where pesticides cannot be applied and where BMSB are building their numbers.

The cost of this biocontrol works out to be less than $1.00 per wasp to rear and release this insect across the valley. It appears that it was successful in establishing itself in and around pear orchards in Hood River. This initial investment in biocontrol should pay dividends in the future in the form of free biological control of the invasive BMSB as the wasps reproduce and spread.

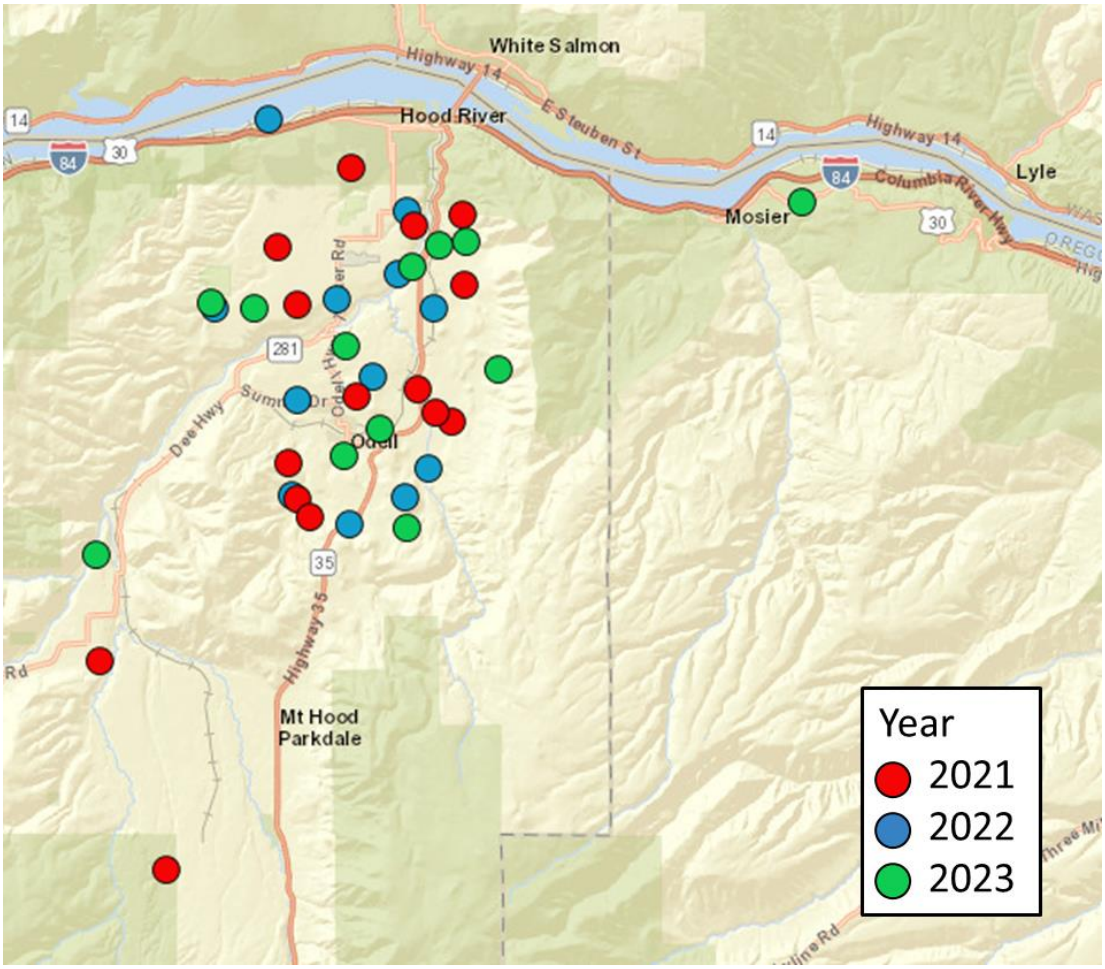


Figure 1: 2021-2023 *Trissolcus japonicus* release sites.

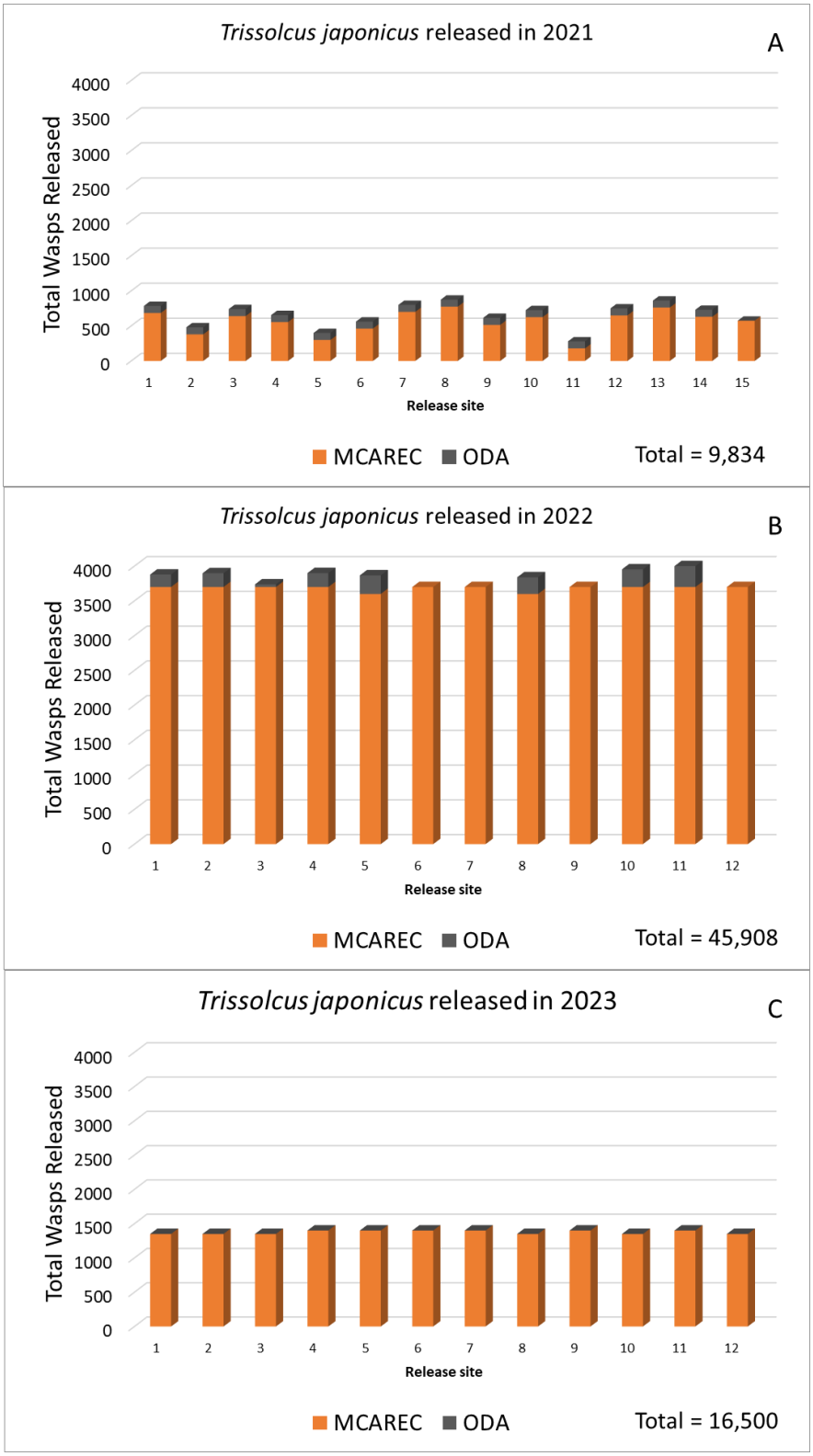


Figure 2. Number of *T. japonicus* released at each site reared by MCAREC and ODA in 2021 (A), 2022 (B), and 2023 (C). 2022 was an exceptionally good year and I had high hopes that we would continue at that rate into year three. Rearing insects can be challenging, it is not clear why we struggled in 2023.

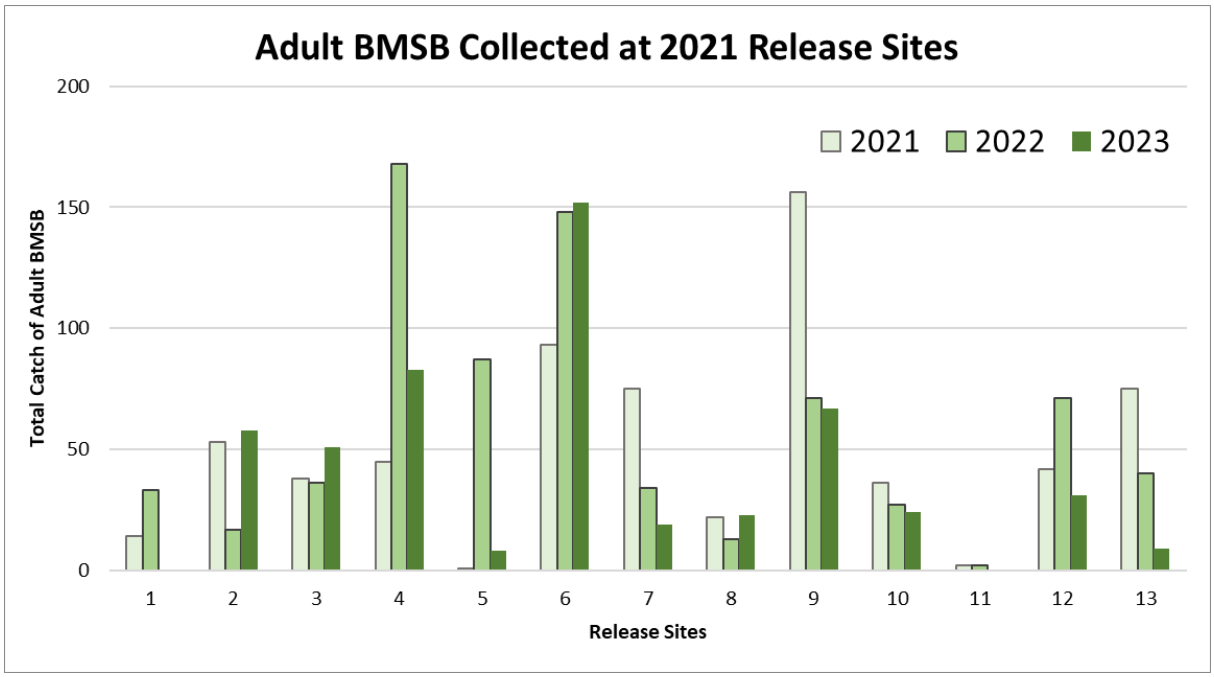


Figure 3. Total of BMSB adults collected in 2021-2021 at 2021 Tj release sites.

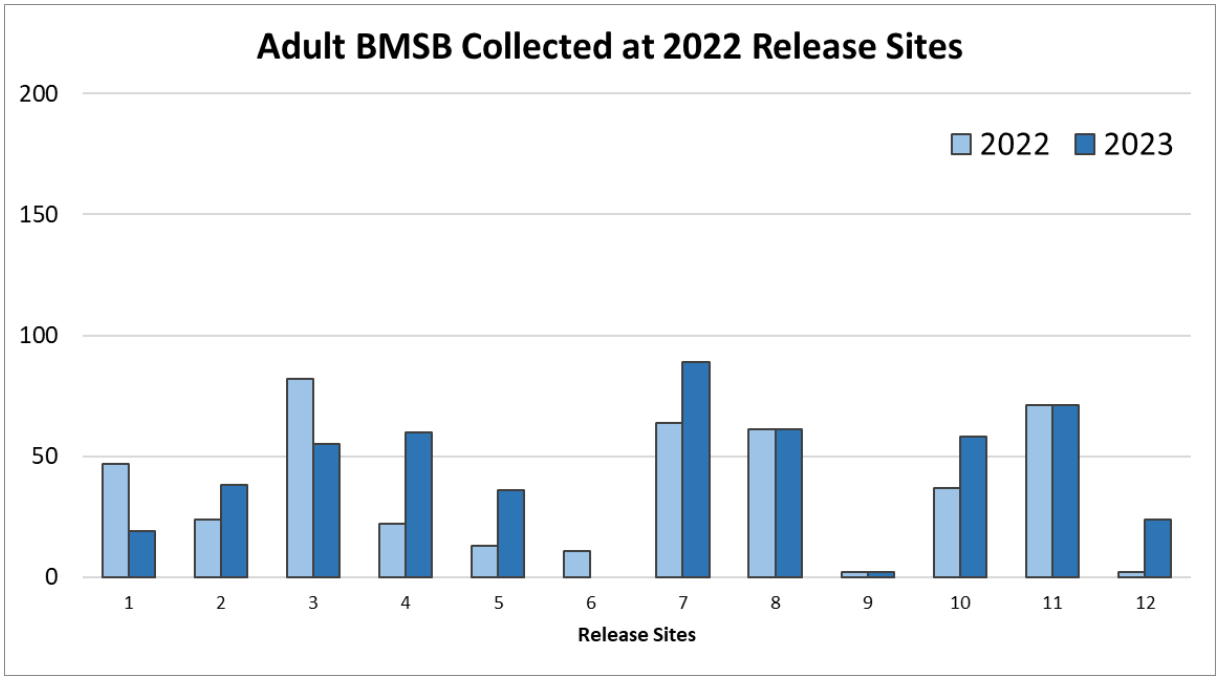


Figure 4. Total of BMSB adults collected in 2022-2023 at 2022 Tj release sites.

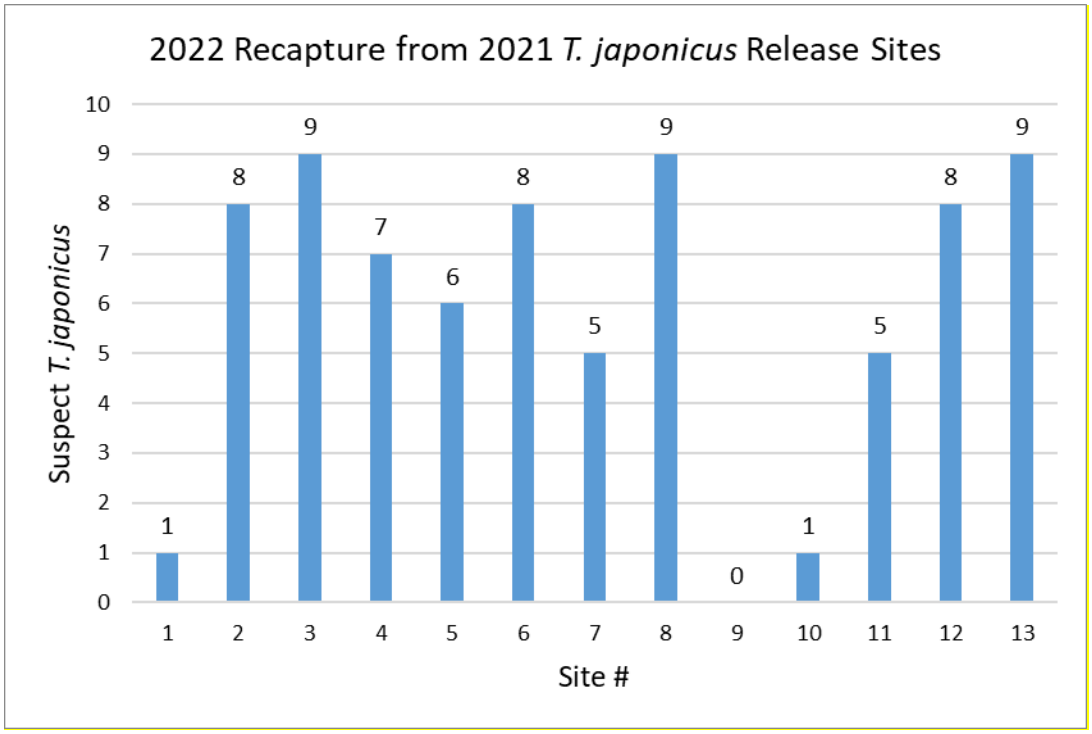


Figure 5. Total number of suspect Tj captured on sticky cards in 2022 at the 2021 release sites, used to measure successful establishment.

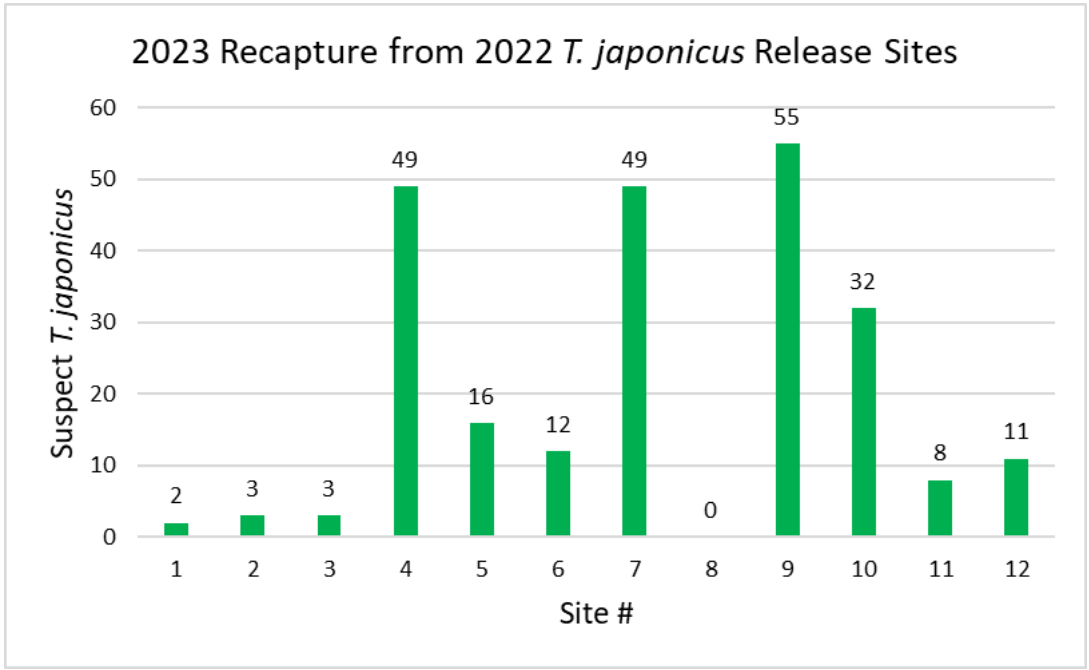


Figure 6. Total number of suspect Tj captured on sticky cards in 2023 at the 2022 release sites, used to measure successful establishment.

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

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

Project Title: Assessing and supporting effective areawide pear pest management

Report Type: Continuing Project Report

Primary PI: Dr. Robert Orpet
Organization: Washington State University
Telephone: 509-293-8756
Email: robert.orpet@wsu.edu
Address: 1100 N Western Ave
City/State/Zip: Wenatchee/WA/98801

Co-PI 2: Dr. Rebecca Schmidt-Jeffris
Organization: USDA-ARS
Telephone: 509-454-6556
Email: rebecca.schmidt@usda.gov
Address: 5230 Konnowac Pass Rd
City/State/Zip: Wapato/WA/98951

Co-PI 3: Dr. Chris Adams
Organization: Oregon State University
Telephone: 248-850-0648
Email: chris.adams@oregonstate.edu
Address: 3005 Experiment Station Dr
City/State/Zip: Hood River/OR/97031

Co-PI 4: Tianna DuPont
Organization: Washington State University
Telephone: 509-293-8758
Email: tianna.dupont@wsu.edu
Address: 1100 N Western Ave
City/State/Zip: Wenatchee/WA/98801

Co-PI 5: Dr. Jessica Goldberger
Organization: Washington State University
Telephone: 509-335-8540
Email: jgoldberger@wsu.edu
Address: WSU Pullman, 385 Clark, PO Box 646420
City/State/Zip: Pullman/WA/99164

Cooperators: Louis Nottingham, RT Curtiss, 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: *Proposal submitted*
Funding Duration: 2024
Amount: $15,000
Agency Name: Western IPM Center – Planning Document Grant
Notes: Funds are requested to support Obj. 1 of this proposal. PI: Orpet

Other related/associated funding sources: *Proposal submitted*
Funding Duration: 2024
Amount: $40,000
Agency Name: Washington IPM Center – Outreach and Implementation Grant
Notes: Funds are requested to support Obj. 2 of this proposal. PI: Orpet

Other related/associated funding sources: *Proposal being drafted*
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

Budget 1
Primary PI: Robert Orpet
Organization Name: Washington State University
Contract Administrator: Stacy Mondy
Telephone: 509-335-4563
Contract administrator email address: arcgrants@wsu.edu
Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@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% + Postdoc salary: $5,457 x 12 mo x 9.0% FTE for year 1 x 1.04 for each additional year benefits at 35.5%
[2]Supplies: weather stations = 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
Telephone: 541-737-3228
Contract administrator email address: charlene.wilkinson@oregonstate.edu

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: none.

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

- In the Wenatchee Valley, pear IPM was successful during 2023.
  - At six pairs of commercial orchards, estimated average percentage of Anjous downgraded from pear psylla marking was 0.5% for IPM vs. 4.2% for conventional.
  - Average materials costs were estimated $998/acre for IPM vs. $1,390/acre for conventional.

- Current grower cooperators in the Wenatchee Valley intend to expand IPM acreage in 2024, and other growers intend to begin trials in 2024.

- IPM guidelines are being shared more widely – subscriptions to *Pear Entomology Weekly* reached 160 people in December 2023 after advertisement at 12 extension presentations and 8 treefruit.wsu.edu newsletter articles during 2023. Partnership between co-PIs DuPont and Sayles added an additional three public discussion groups, one study circle, and a project field day.

Methods

Objective 1. First, previous Washington and Oregon surveys on pear pest management (Beers and Brunner 1991, Brunner et al. 2003, Goldberger and Lehrer 2016) were collated and reviewed by PI Orpet and co-PI Goldberger. Then, a new survey was drafted and designed to allow for comparison to these older surveys. The major portion of the survey is in reporting a sample spray program for a representative pear block and reporting on other IPM practices like honeydew washing.

Scientific survey distribution depends on first defining a sample population. The two older surveys from 1990 and 2000 had been mailed to pear growers and orchard managers based on a random sample from the subscription list of *Good Fruit Grower* in 1990 (1,086 mailed and 331 valid completed and returned) or from a "statewide industry organization" in 2000 (863 mailed, 129 valid completed and returned). The 2011 survey had used a list of growers provided by Pear Bureau Northwest (1001 mailed, 360 valid completed and returned). For the new survey, we seek to reach Washington and Oregon pear growers, managers, and consultants. Therefore, we have contacted the Pear Bureau, and they have agreed to endorse the survey and provide their list of growers to the WSU Social & Economic Sciences Research Center (WSU-SESRC). Their list will only be handled by the WSU-SESRC and will not be seen the research team to protect confidentiality. To obtain lists of consultants, PI Orpet is similarly enquiring with chemical distributors.

The new survey is currently under review by the Washington State University Institutional Review Board for determination of exemption from federal regulations on human subjects research. This is a required step for any research involving data collection from human subjects. The research is expected to be determined exempt from federal regulation due to low risks to participants. If review can be completed early enough, the survey will begin distribution by February 1, 2024, or else it will be distributed at the end of this field season to avoid overlap with the start of spray season.

Objective 2. For part 2a, the PI Orpet has continued to collaborate with nine pear growers and four consultants in the Wenatchee Valley to evaluate pear IPM guidelines and share data publicly. Scouting data were shared weekly via an online newsletter *Pear Entomology Weekly* for six IPM, six conventional, and 3 organic pear orchards that were commercially managed by cooperators. In addition, data were shared from one IPM and one conventional orchard managed by WSU in Rock Island. Co-PI Schmidt-Jeffris also shared scouting data from seven Yakima pear orchards, and co-PI DuPont shared a weekly summary from a related Scouting Network project funded by WSDA. The data were displayed alongside phenologically appropriate pear IPM management guidelines—i.e., selective spray options for dominant pear psylla life stages, tips on tree washing, and reminders on codling mating disruption.

For part 2b, pear psylla damage and management cost data were collected from the study orchards. Downgrade evaluations were conducted within a week of harvest for Bartlett and Anjou cultivars at each orchard by inspecting and categorizing 100 fruit at each location. Spray records were collected for all sites in Wenatchee. Co-PI Schmidt-Jeffris will provide Yakima spray programs for analysis. Materials costs for insecticides, miticides, and codling moth mating disruption were calculated for all spray records using a cost list collected by DuPont and Strohm in 2021. The list needs updating to reflect current materials costs, so co-PI Orpet will gather data from chemical distributors to accomplish this during 2024, but draft materials costs have been calculated to compare management.

Objective 3. An analysis correlating landscape with pear psylla and biocontrol outcomes was conducted for the Wenatchee Valley orchards from Objective 2. Data were available for 2023 from the current project, and data from most of the same sites during 2022 were available from previous work funded by Fresh Pear and Processed Pear Committees (Nottingham and Orpet 2023). A larger dataset from Tianna DuPont, who scouted pear orchards during 2023, will be provided for analysis in 2024. In addition, data from Yakima and Hood River regions will be collected for analysis in 2024.

Landscapes surrounding the project orchards were used as an explanatory variable for analysis. Landscapes were quantified as the percentage of an orchard's perimeter that does not border any other orchard within 20 meters (65 ft). The 20-meter distance means that an orchard across a county road or canal counts as bordering, but larger strips of non-orchard land on hills or open fields are considered to break up the pear monoculture. The hypothesis was 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.

Percentage Anjou downgrades and cumulative natural enemy counts pre-July were chosen as response variables for analysis. Raw natural enemy numbers across the season are correlated with pear psylla populations because higher pear psylla populations provide more food for predators to grow. Therefore, high natural enemy numbers is not necessarily a good metric for biocontrol success. Pear damage was chosen as one metric of biocontrol (if biocontrol is successful, pear damage would be low). For a second metric, cumulative natural enemy counts pre-July were summarized for each site. These natural enemy numbers may represent early movement of natural enemies into pear orchards and initial reproduction just before the second generation of pear psylla nymphs, which is

the generation where biocontrol tends to mainly build up. Natural enemies included in summations were: *Deraeocoris, Campylomma, Trechnites,* lacewings (adults and immatures), and ladybugs (adults and immatures).

To analyze correlations, the landscape metric was plotted by each biocontrol metric for year 2022 and 2023 datasets.

Results and Discussion

Objective 1. The pear pest management survey has not yet been distributed, so no new results have been collected yet. Review of older surveys indicate that most Washington pear acreage in 1990 and 2000 used broad-spectrum insecticides including Abamectin, Guthion, and pyrethroids. By 2011, Guthion use had declined, but a range of pesticides disruptive to natural enemies continued to be common in both Washington and Oregon, such as spinetroram, abamectin, and neonicotinoids. Relatively selective sprays like pyriproxyfen, kaolin, and spirotetramat were available but less commonly used. New data will show to what extent biocontrol-compatible spray programs are currently used in different regions and whether spray programs and Washington and Oregon are as divergent as researchers have recently assumed.

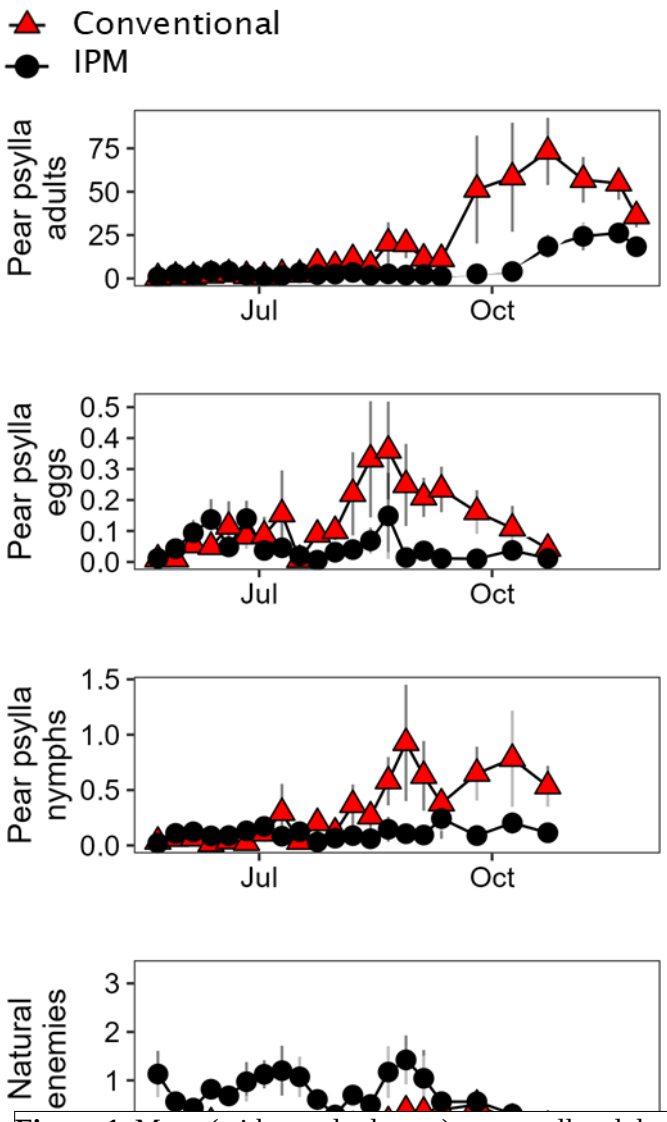


Figure 1. Mean (with standard error) pear psylla adults (per tray), pear psylla eggs (per leaf), pear psylla nymphs (per leaf), and natural enemies (Deraeocoris, Campylomma, Trechnites, lacewing larvae, lacewing adults, ladybug adults, and ladybug larvae). N = 7 conventional and 7 IPM orchards (6 commercial pairs and one WSU pair).

Objective 2. Scouting of orchards undergoing IPM trials showed success of the program during 2023. Average population dynamics of pear psylla adults, pear psylla eggs, pear psylla nymphs, and pear psylla natural enemies are shown in Figure 1. Pear psylla nymphs were slightly more abundant in IPM than in conventional orchards before July, but then pear psylla tended to be more abundant in conventional orchards for the rest of the season. Minimal fruit damage occurred in IPM orchards whereas outbreaks in some conventional orchards led to considerable fruit marking (Table 1). At the six pairs of commercial orchards, estimated average percentage of Anjous downgraded from pear psylla marking was 0.5% for IPM vs. 4.2% for conventional. Insecticide and miticide materials costs were lower in IPM than conventional orchards. Average materials costs were estimated $998/acre for IPM sites vs. $1,390/acre for conventional sites.

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

		Bartlett		Anjou	
Site	**Management**	**Date**	**% US1**	**Date**	**% US1**
Rock Island (WSU)	Conventional	8/14	100	9/11	90
	IPM	8/14	100	9/11	99
Monitor	Conventional	8/15	99	9/12	100
	IPM	8/22	100	9/12	100
Cashmere	Conventional	8/22	99	9/19	96
	IPM	8/15	99	9/05	100
	Organic	8/22	100	9/19	97
Dryden	Conventional	8/23	100	9/21	99
	IPM	8/23	95	9/21	97
	Organic	8/23	97	9/13	100
Peshastin	Conventional	8/31	100	9/21	100
	IPM	8/31	100	9/21	100
	Organic	8/31	100	9/21	100
HWY 97	Conventional	8/24	99	9/28	98
	IPM	8/24	100	9/07	100
Leavenworth	Conventional	8/31	96	9/28	82
	IPM	8/31	100	9/14	100
Average	**Conventional**	**-**	**99.0**	**-**	**95.0**
	IPM	**-**	**99.1**	**-**	**99.4**
	Organic	**-**	**99.0**	**-**	**99.0**

Although leaf sampling ended during fall, this project monitored pear psylla adults with beat trays continuously after harvest (Figure 1). This monitoring shows a very large increase in pear psylla adults in conventional orchards just before October. Pear psylla adults increased in IPM orchards rapidly during late October. The delay in this increase in IPM orchards, corresponding to the timing of decreasing adults in conventional orchards, suggests that areawide movement and mixing of pear psylla populations occurs during winter dispersal.

Extension activities integrated into this research were successful. Of the six growers on the project trialing IPM, two have adopted IPM for all their pear acreage, two are intending to increase acreage in 2024, and two are considering increasing their acreage. On a December 13 ‘Study Circle’ organized by co-PI DuPont, there were 50 in-person an 25 online attendees. Twenty-nine filled out a survey. On an open-ended question about what they intended to do differently as a result of attending, six people explicitly stated that they intend to begin or increase use of pear IPM. Others people reported similar intentions such “spray less” or “monitor my spray program more closely”.

Objective 3. There was no clear correlation between percentage of natural border with Anjou fruit damage or natural enemy counts for IPM or conventional orchards (Figure 2). Orchards on the project represented a good spread of landscape diversity, ranging from 0–100% natural border of orchards. This suggests landscape context is not an important factor determining whether IPM will work, but the results should be interpreted cautiously. The simple metric of percentage orchard border does not take into account the quality of habitat, management of neighboring orchards (organic, IPM, or conventional), the acreage of the subject orchard, or larger-scale landscape effects. Discussion at extension events suggests that some practitioners have observed that larger continuous areas of IPM or organic management work better due to areawide abundance of biocontrol and pear psylla suppression. In addition, scales larger than a 20-meter border may be important. A more refined

analysis with a larger dataset to be contributed by co-PI DuPont is planned to incorporate these factors into a statistical model. Additional years of data will also help assess stability of a potential relationship. An effect of landscape on biocontrol may be present some years and not others.

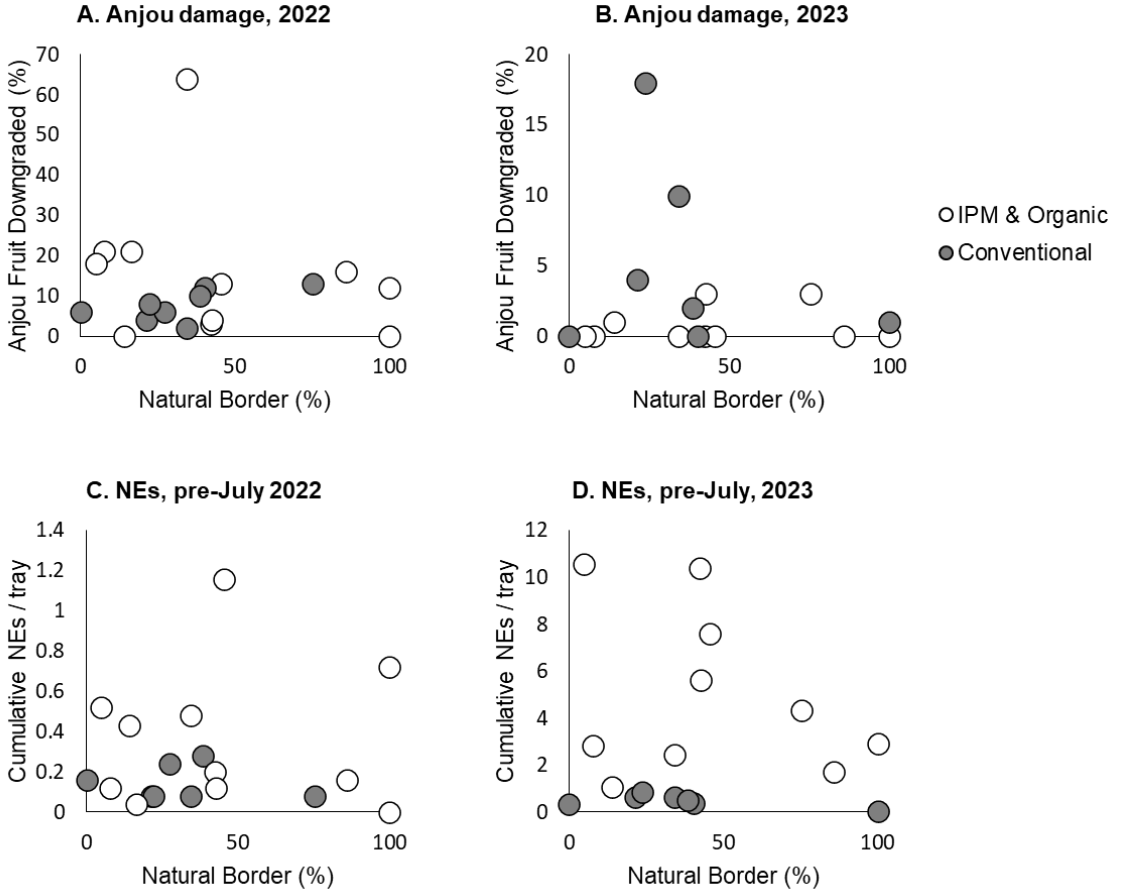


Figure 2. Correlations between percentage natural borders of pear orchards (natural border was defined as length of an orchard edge not within 20 meters of another orchard) with Anjou fruit downgrades in 2022 (A), and 2023 (B), and cumulative natural enemies of pear psylla per beat tray pre-July in 2022 (C) and 2023 (D).

References cited

Beers, EH, and JF Brunner. 1991. Washington State Apple and Pear Pesticide Use Survey. Washington State Apple and Pear Pesticide Use Survey 1989-90. A report submitted in fulfillment of a grant by USDA/National Agricultural Pesticide Impact Assessment Program, September 30, 1991.

Brunner, JF, et al. 2003. Pesticide Use and IPM practices in Washington's Pear and Cherry Orchards. Agrichemical and Environmental News 208.

Goldberger, JR, and N Lehrer. 2016. Biological control adoption in western U.S. orchard systems: Results from grower surveys. Biological Control 102: 101–111.

Nottingham, L, and R Orpet. 2023. Developing a phenology-based management program for pear psylla. Final Project Report. URL: https://treefruitresearch.org/report/developing-a-phenology-based-management-program-for-pear-psylla/

Proposal Title: OPTIMIZATION OF HONEYDEW WASHING SYSTEMS IN PEAR ORCHARDS

Report Type: Continuing Report

Primary PI: **RT Curtiss**
Organization: Washington State University - TFREC
Telephone: (917) 685-1546
Email: rcurtiss@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee, WA 98801

CO-PI 2: Robert Orpet
Organization: Washington State University - TFREC
Telephone: 509-293-8779
Email: robert.orpet@wsu.edu
Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee WA, 98801

Co-PI 3: Louie Nottingham
Organization: Washington State University - NWREC
Telephone: 540-798-2044
Email: louis.nottingham@wsu.edu
Address: 16650 State Route 536
City/State/Zip: Mount Vernon, WA 98273

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

Budget 1
Primary PI: RT Curtiss
Organization Name: Washington State University
Contract Administrator: Stacy Mondy
Telephone: 509-335-4563
Contract administrator email address: arcgrants@wsu.edu
Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@wsu.edu

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 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

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

Objective 3 – 2022-2023 key findings

- The surfactant tested did not improve washing efficacy

Objective 4 – 2022-2023 key findings

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

METHODS

<u>Objective 1: Monitor seasonal honeydew deposition to understand when washing should be applied</u>

Weekly through both years, at least nine commercial study sites located in the Wenatchee River Valley, and planted primarily with d'Anjou and Bartlett varieties, will have pear psylla and natural enemy populations, and honeydew levels monitored. Study sites will have one of three management systems: organic-, conventional- and IPM-based pest management. Plots will be monitored for natural enemies from March to October using beat trays, rolled cardboard traps, and yellow sticky cards with volatile lures. Pear psylla populations will be monitored by beat tray and leaf sampling. Honeydew will be monitored on leaves with a method to measure BRIX, and on fruit with visual inspection. Beat tray methods call for 30 samples of canopy dwelling arthropods to be conducted in each plot weekly. Each beat tray sample (one 'tray') involves holding an 18 ×18-inch (45 × 45 cm) white sheet 12-18 inches (30-45 cm) underneath a horizontal branch and striking it three times with a stiff rubber hose to dislodge insects in the tree onto the tray, which will then be counted by project personnel. Randomly selected branches for sampling will be 3-6 feet (1-2 meters) above ground. Natural enemies will likely include 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).

Predator and parasitoid populations will be further measured in pear orchards using yellow sticky cards baited with attractive lures containing plant volatiles and rolled cardboard traps. Traps will be placed in trees in a single transect crossing each orchard plot diagonally at a distance of at least 30 meters (98 ft) apart. Trees with sticky card traps will also contain one rolled cardboard earwig trap for convenience when monitoring. All lures will be replaced at six-week intervals. All traps will be checked once per week and the number of insects will be counted.

Pear psylla nymphs will be monitored by leaf samples that will include 100 leaves collected from branches throughout 10 randomly selected trees distributed throughout each plot weekly. In the early season, five spur fruiting bud leaves will be collected from each of the lower and upper canopies of each tree. Lower canopy leaves will be selected from branches in the inner, middle, and outer scaffold limbs four to six feet from the ground. Early in the season, upper canopy leaves will be collected from two fruit clusters using an extendable pole pruner. During summer, leaves will be selected from both fruit clusters and shoots. Collected leaves will be kept cool and returned to the laboratory to be processed using a leaf brusher. Leaves will be brushed between two motorized brushes which dislodge nymphs onto a revolving glass plate, creating a composite orchard sample that will be assessed under a stereomicroscope. Pear psylla counted from leaves samples will include 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 may also be counted on glass plates from leaf samples.

Pear psylla honeydew on leaves in commercial sites will be measured weekly to understand the correlation with infestation and injury levels. Ten leaves will be collected from each of 10 randomly selected trees distributed throughout each plot. Leaves will be selected in the same way as leaf brush samples and mixed into a single plastic zip top bag for each plot. In the laboratory, deionized water (100 ml) will be added to each container and shaken for 60 seconds, left to soak for 5 minutes, and then shaken again for 60 seconds to wash honeydew from leaves. The elute from each container will be poured into labeled 100ml plastic solo cups. Three 50 µl subsamples of wash will be pipetted onto a RX-5000α-Bev digital refractometer (Atago Co. Ltd.) to measure BRIX. The mean BRIX value from the three subsamples will be reported for each wash sample. In addition, we will measure BRIX of the wash sample using a handheld BRIX meter (i.e., Aetomce 0-90% BRIX Meter Handheld Refractometer $22.99 @ Walmart on 7 Jan 2023) to compare the BRIX measurement given by an affordable option that a grower may use.

Fruit honeydew levels in plots will be measured by visual inspection monthly. Twenty fruit per tree will be evaluated on 10 randomly selected trees in each plot. Honeydew on fruit will be graded by evaluating for honeydew (present, absent), black damage, and russet.

Additionally, we will monitor individual fruit at the unsprayed WSU-TFREC orchard through the entire season to understand the pattern of damage caused by honeydew. Flower clusters will be marked with flagging tape early in the season and those fruits will be visually inspected and evaluated for honeydew damage weekly. Photographs of individual fruits will be taken when they are visually inspected. Honeydew-caused damage will be thus followed from deposition of honeydew through fruit marking and will further inform when washes should be applied.

Fruit grading will be evaluated in commercial orchard sites at mid- and end-of-season one week prior to harvest. Ten pear fruits from low on the tree and 10 from high on the tree will be inspected on 20 randomly selected trees at each site. Care will be taken to look at fruits both near the canopy center and on the periphery. Fruits will be categorized based on USDA pear packing grades for pear psylla marking (USDA, 2007) by the U.S. #1, Washington Fancy, or Cull designation.

Data Analysis

Insect population, honeydew, and fruit damage data will be analyzed using repeated measures analysis of variance (ANOVA). Treatment differences will be discerned using Tukey's honest significant difference test ($\alpha = 0.05$). To estimate which factors best predict wash timings, multiple regression analysis will be used.

Objective 2: Compare honeydew washing efficacy

Honeydew washing methods will be compared at the unsprayed WSU-TFREC and Sunrise pear orchards. In a randomized block designed experiment we will compare efficacy of overhead washing systems, tractor with airblast sprayer, tractor with handgun, and unwashed control at managing honeydew.

In 2022 we installed an individually controlled overhead washing system at the WSU-TFREC orchard, and Sunrise orchard was designed with a similar system. Using these systems, in 2023-24 we will create small plots that will receive overhead washing treatments, while other plots will receive the airblast, handgun and control treatments (Table 1). Honeydew will be assessed by the above BRIX method before treatment and then again weekly for six to eight weeks after treatment to determine treatment impact and how long a single wash will provide protection from fruit damage. Wash timings will occur based on the recommendations in the pear psylla degree day model on the WSU decision aid system. In addition to honeydew BRIX assessments, fruit will be monitored by visual inspection for damage before and after 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, we will compare seasonal wash timings based on reaction to weekly population and honeydew monitoring, or calendar applications (i.e., weekly, biweekly, once/generation, degree day model) to determine if targeted washing at different times of the season is an effective strategy for fruit protection. For population reacting treatments, when psylla populations exceed the thresholds established by Burts (1988), or BRIX exceeds 2%, we will wash "reactive plots."

Data will be analyzed using ANOVA, and treatment differences discerned by Tukey's HSD to separate means.

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

In 2023-2024 we will compare water alone with soaps' ability to remove honeydew from pear trees. Using the airblast sprayer to administer treatment, we will establish plots in a randomized block design that will receive water washes alone or water plus surfactant washes. Washes will occur when there is a high amount of honeydew present on trees as determined by weekly sampling. Treated tree honeydew will be measured using the above BRIX method before and weekly for up to eight weeks after treatment to determine if surfactants or soaps augment water's ability to wash honeydew, and if the impact continues to protect fruit longer than water alone. Surfactants and soaps that may be tested include nonionic spreader-activators, nonionic organosilicone wetting agents, and insecticidal soaps. Data will be analyzed using ANOVA, and treatment differences discerned by Tukey's HSD to separate means.

Objective 4: Provide Extension

Project findings will be submitted for peer reviewed publication. In addition, we will provide information directly to the industry in the form of updated fact sheets on the Tree Fruit Website, extension publications, and in person at field days and other extension events. If our findings are likely to directly impact current management practices, we will work with the DAS administrators and WSU Crop Protection Guide editors in a timely manner to modify those resources accordingly.

From these experiments we expect to generate information on precise wash timings, optimized systems, and if the inclusion of other materials aid in honeydew removal and fruit protection. Our overall goal is to help farmers produce clean fruit through sustainable pest management programs with reduced inputs that conserve natural enemies.

Potential pitfalls: Rain events during the post-treatment period will reduce the honeydew levels across all plots being treated and may reduce the measurable impact of the treatments. However, since measurements will be taken immediately before and after treatment, important treatment impacts will be measured and discernable. In addition, because this is a two-year study, single weather events will have minimal impact on data collected.

RESULTS AND DISCUSSION

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

In 2023 we monitored honeydew deposition in 17 commercial pear orchards that were also monitored by Dr. Orpet's project "Assessing and supporting effective areawide pear pest management." 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).

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 is ongoing. However, at the otherwise unmanaged WSU-TFREC orchard, damage is high early in the season when psylla pressure is high, but due to high rates of predation by yellowjackets, damage reduces through the season as pears grow in size and damage is diluted across the increased surface area.

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 is clearest in conventionally managed orchards, where most damage occurs 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.

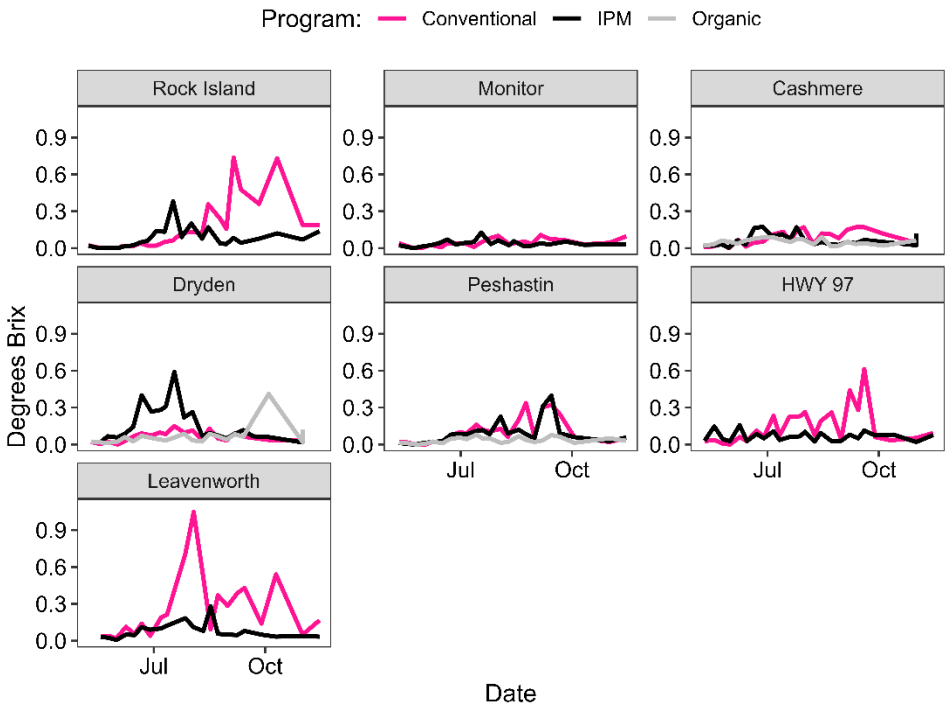


Figure 1. BRIX measurements in Washington commercial pear orchards (n=17) by region.

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

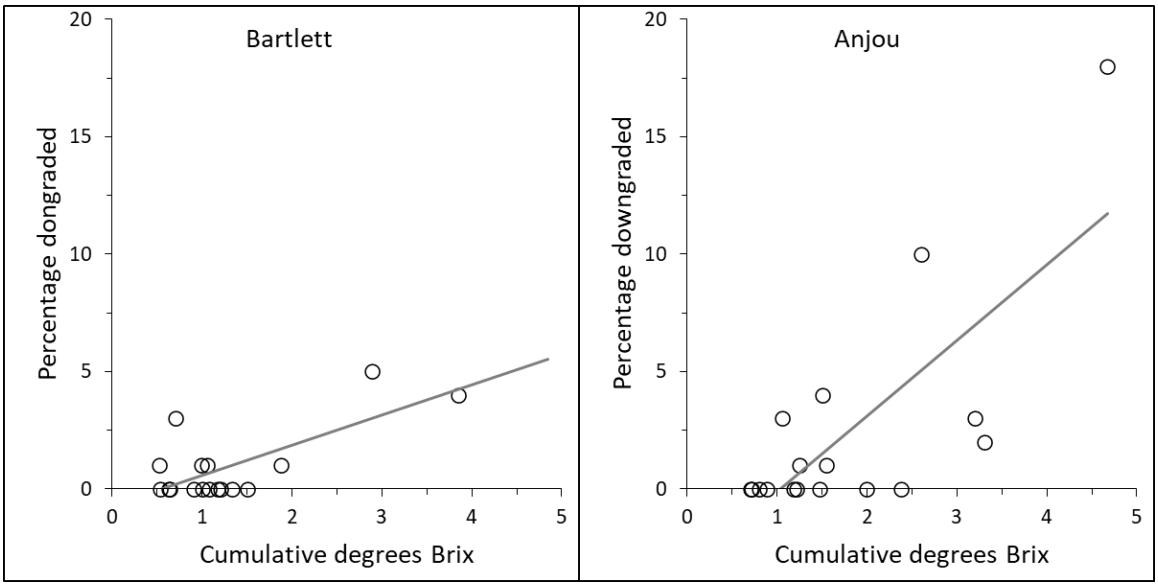


Figure 3. Relationship between the brix measurements and percentage fruit downgraded on Washington commercial pear orchards (n=17).

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

Figures 3-5 show that washing has an impact on honeydew levels, but not psylla eggs, nymphs, or adults. However, the key observation from 2023 is that more water is more effective at washing. 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. These observations are reflected in Fig. 3 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 believe better results can be achieved in 2024 with longer wash times and higher volume nozzles.

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

Figures 3-5 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. Based on these results we may either 1) test another surfactant in 2024, or 2) eliminate this objective and recommend against adding surfactants to washes.

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.

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.

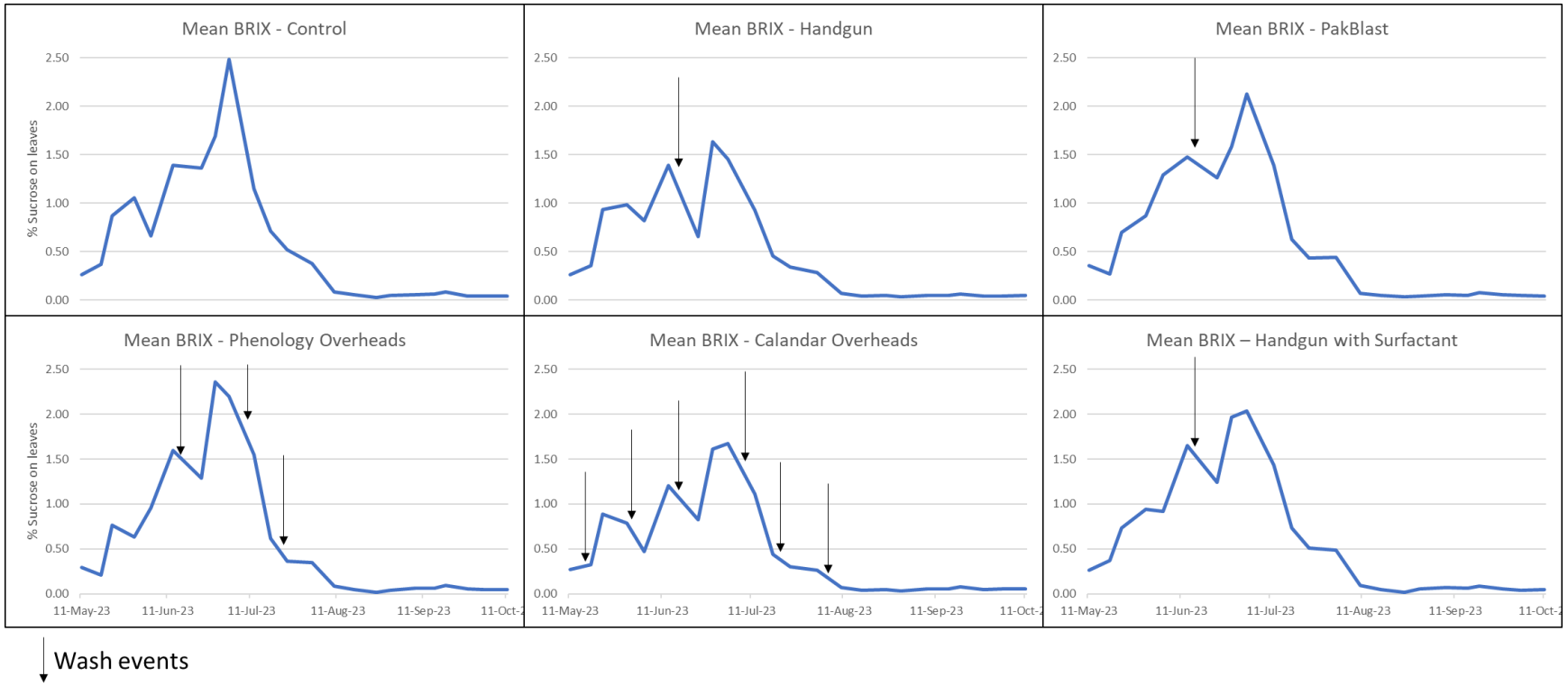


Figure 3. Season-long BRIX measures (% soluble solids) in plots receiving six washing treatments.

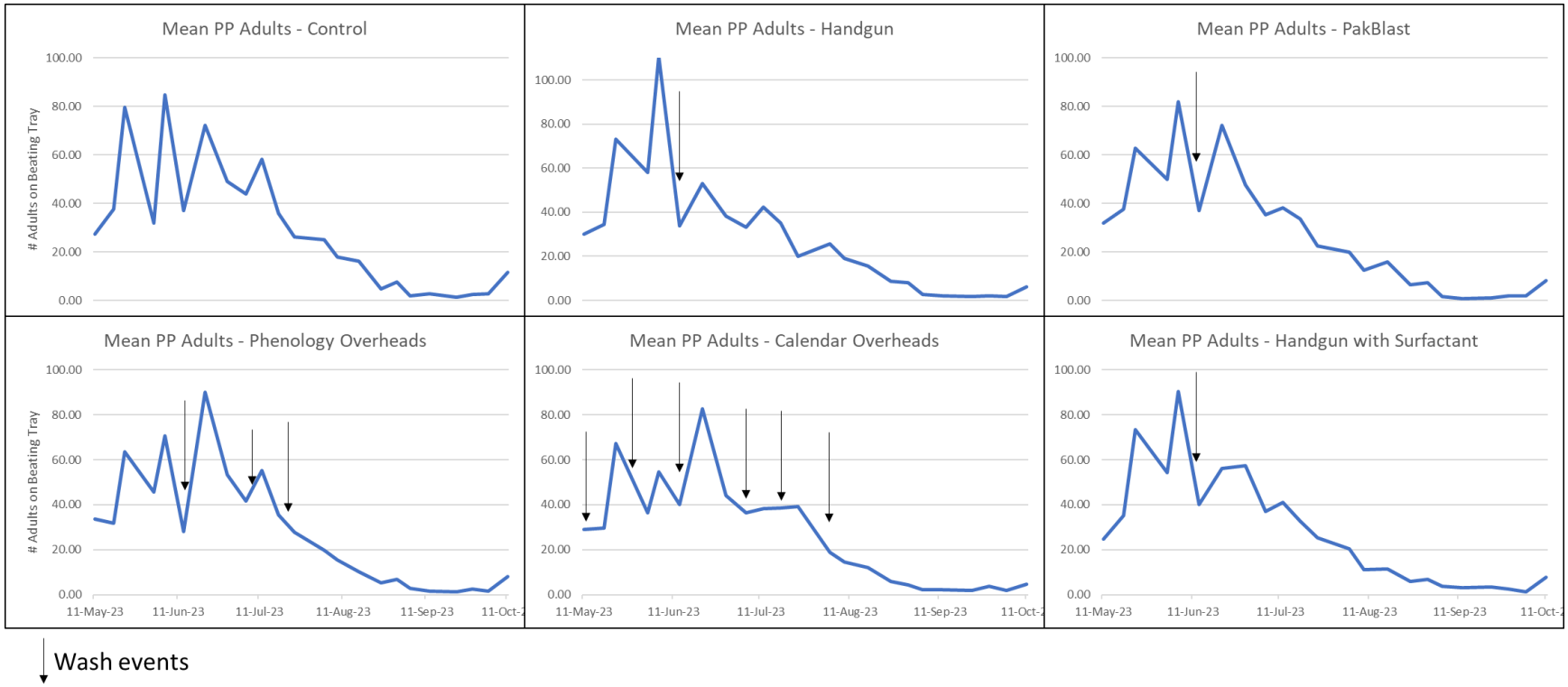


Figure 4. Beating tray samples (number per tray) for adult pear psylla in plots receiving six washing treatments.

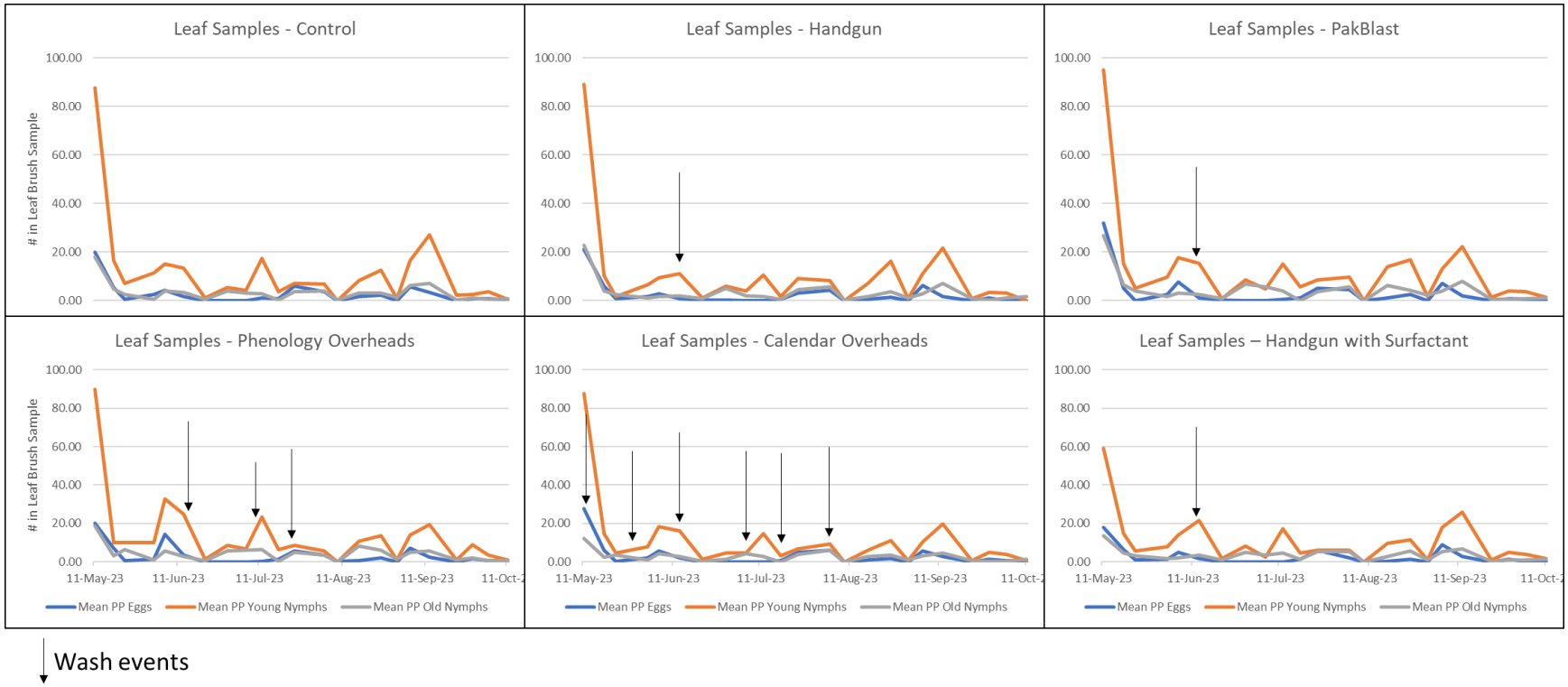


Figure 5. Leaf brush samples for pear psylla eggs and nymphs (number per sample) in plots receiving six washing treatments.

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

Report Type: Continuing Project Report

Primary PI: Srdjan G. Acimovic
Organization: Virginia Tech
Telephone: 540-232-6037
Email: acimovic@vt.edu
Address: 595 Laurel Grove Rd
Address 2: Alson H. Smith Jr. Agricultural Research and Extension Center
City/State/Zip: Winchester, VA 22602

Co-PI 2: Achala N. KC
Organization: Oregon State University,
Telephone: 541-772-5165 ext. 222
Email: achala.kc@oregonstate.edu
Address: 569 Hanley Rd
Address 2: Southern Oregon Research and Extension Center
City/State/Zip: Central Point, OR 97502

Cooperator: Kenneth Johnson
Organization: Oregon State University
Telephone: 541-737-5249
Address: Cordley Hall 4105, 2701 SW Campus Way
Address 2: Department of Botany and Plant pathology
Email: Kenneth.Johnson@oregonstate.edu
City/State/Zip: Corvallis, OR 97331

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

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 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
Contract administrator email address: Jessilp2@vt.edu
Station Manager/Supervisor: Lesley Mitchell
Station manager/supervisor email address: lesleyg@vt.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

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

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.

Significant Findings

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.

Methods

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

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 equipment. 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.

Maintenance sprays. Thiram 4/19/2023 and 5/5/2023 as deer repellent.

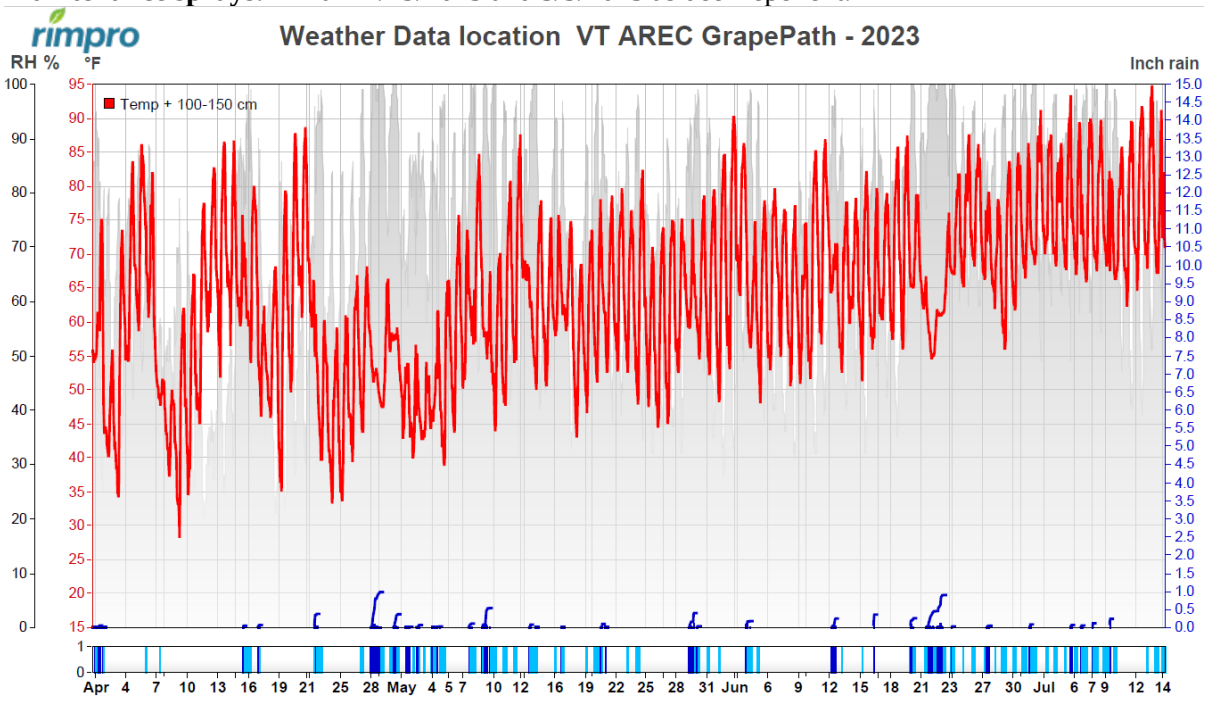


Figure 1. Weather conditions in 2023 during the fire blight experiment at Winchester, VA. Source: RIMpro B.V., Netherlands via NEWA, Cornell University, NY, U.S.A.

Inoculation. 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 2 – 4) 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 2-4).

Problems or limitations that were encountered. 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.

Results and Discussion

Summary graphics.

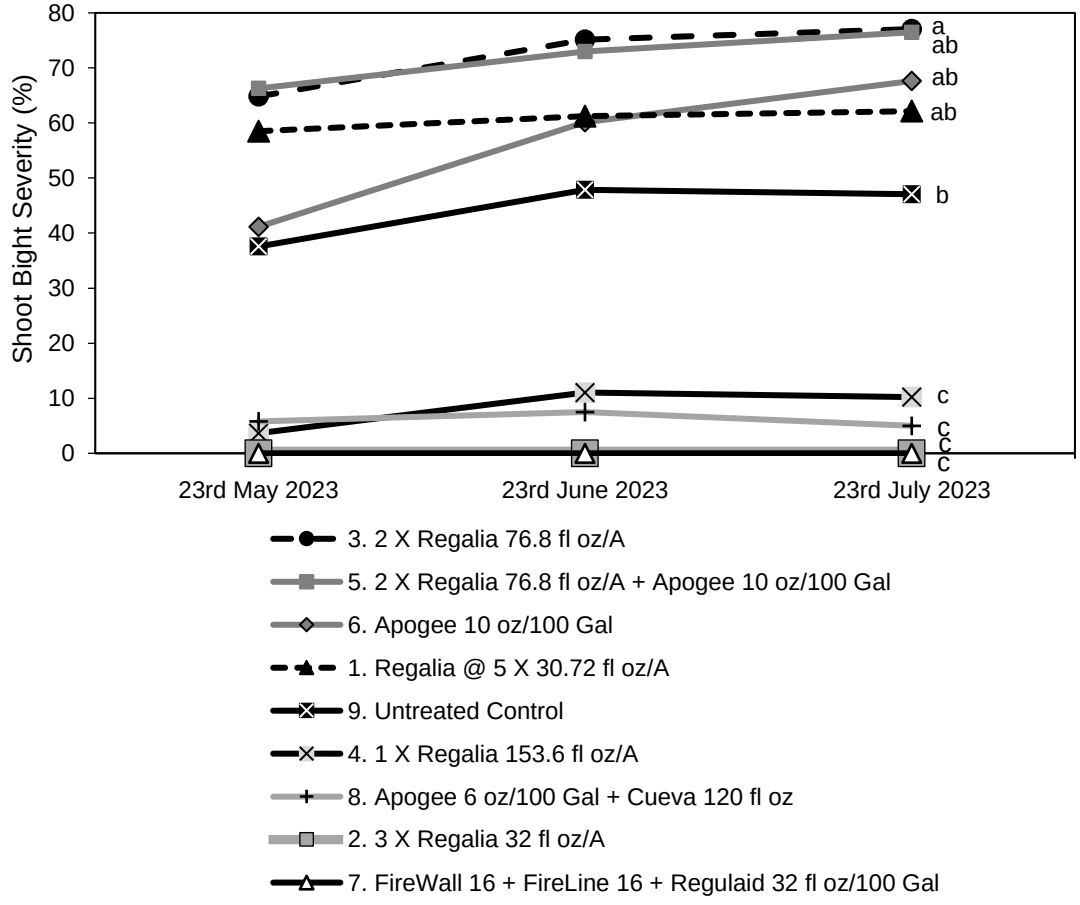


Figure 2. Shoot blight severity on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Shoots were inoculated on 25 April at 2.5- to 5-cm shoot size with *Erwinia amylovora* (2 × 10^8 CFU/ml). Treatment lines followed by different letters are significantly different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

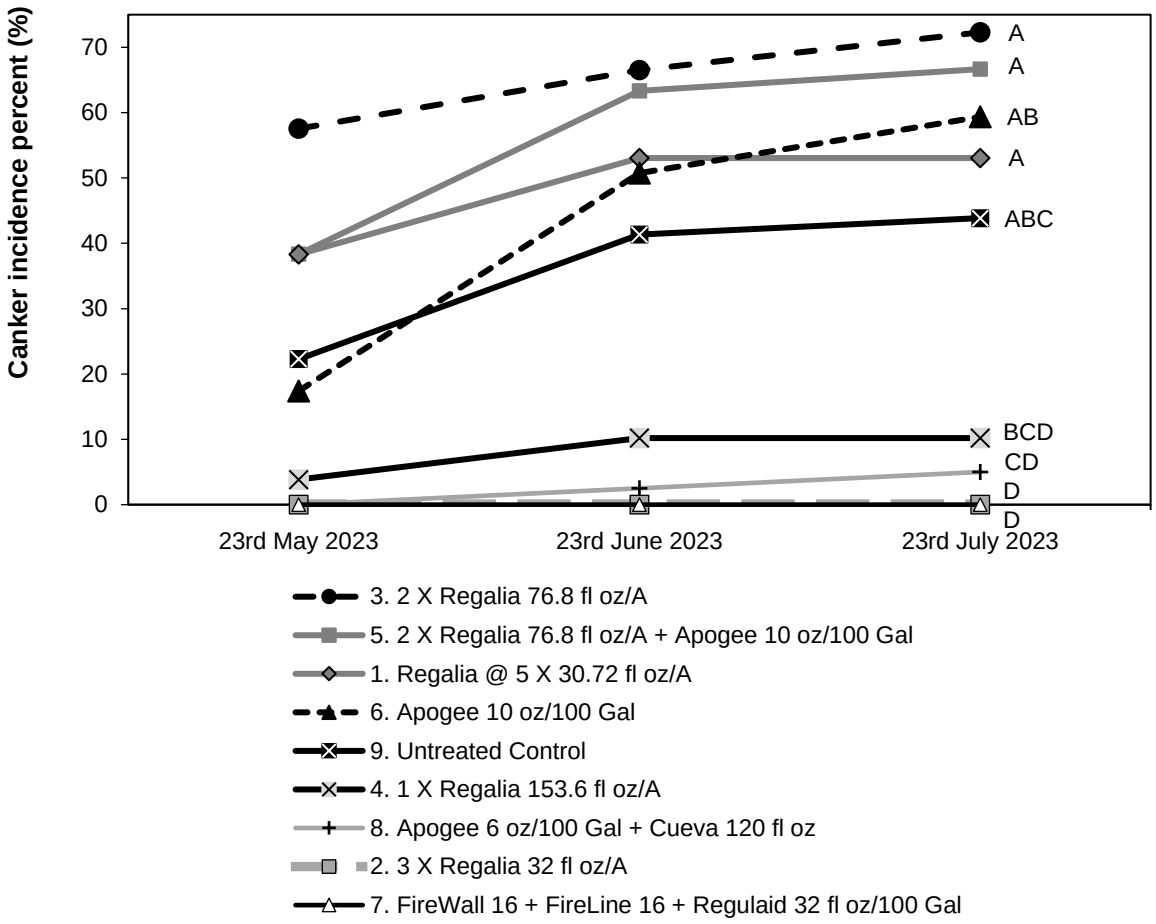


Figure 3. Canker incidence on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

Based on the data from Virginia and when compared to Oregon (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.

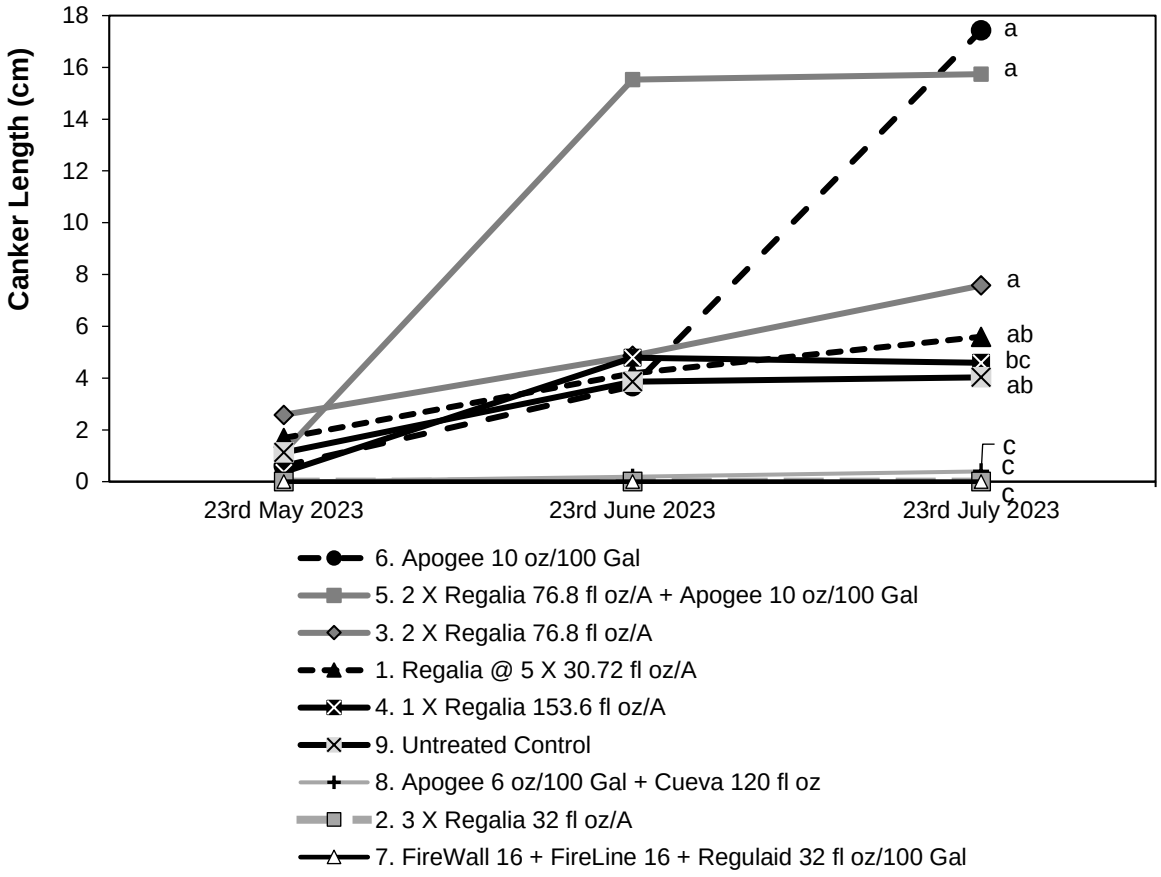


Figure 4. Length of fire blight cankers on perennial wood of pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees,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

2. OREGON

Significant Findings

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

Methods

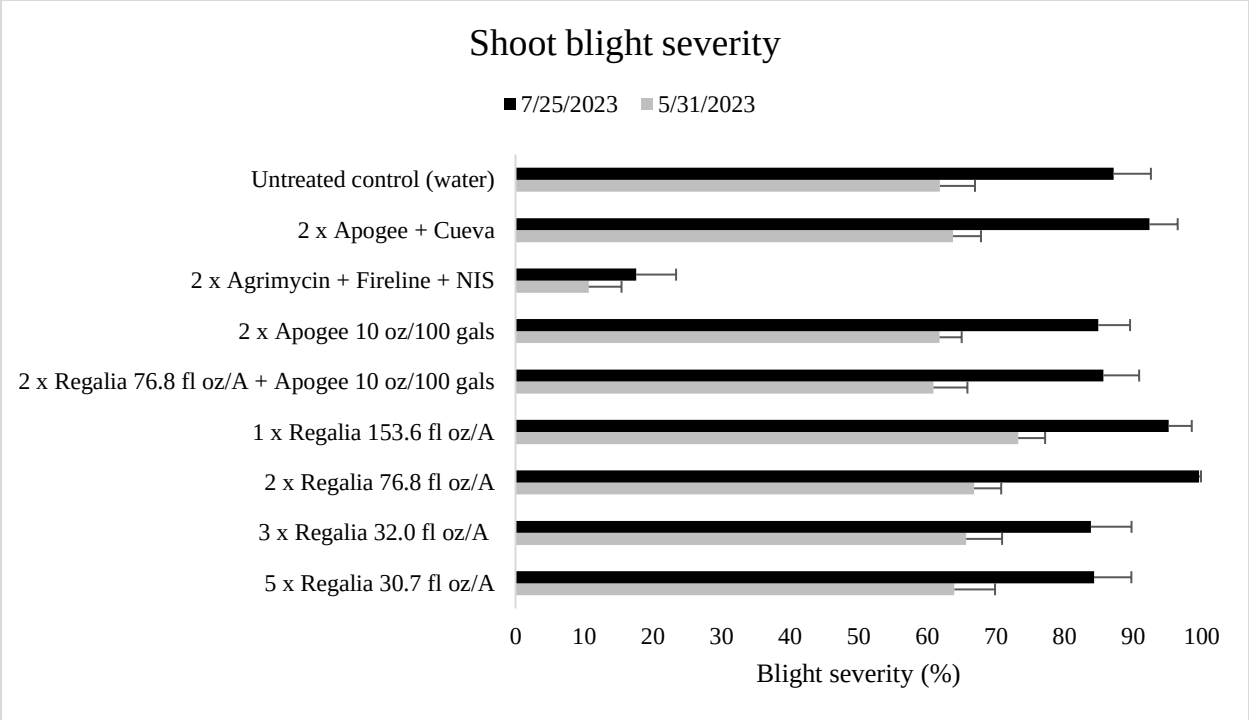
The 'Bartlett' trees at Southern Oregon Research and Extension Center, in Central Point, OR, are planted at 14.8 ft. between rows and 9 ft between trees within rows. Each spray program (treatment) listed in Table 1 were applied to three replicate trees in Oregon. Trees were assigned in a completely randomized 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

Treatment #	Number of spray applications, materials, and amount	Applied at pear growth stage (spray timing)	Dates of treatment application (2023)
1	5 X Regalia 30.72 fl oz/A	BB, GC, WB, PF, FS	04/05; 04/18; 04/24; 05/09; 05/18
2	3 X Regalia 32 fl oz/A (lower label rate)	WB, PF, FS	04/24; 05/09; 05/18
3	2 X Regalia 76.8 fl oz/A	PF, FS	05/09; 05/18
4	1 X Regalia 153.6 fl oz/A	FS	05/18
5	2 X Regalia 76.8 fl oz/A + Apogee 10 oz/100 gals	PF, FS	05/09; 05/18
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
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
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
9	Untreated control	-	-

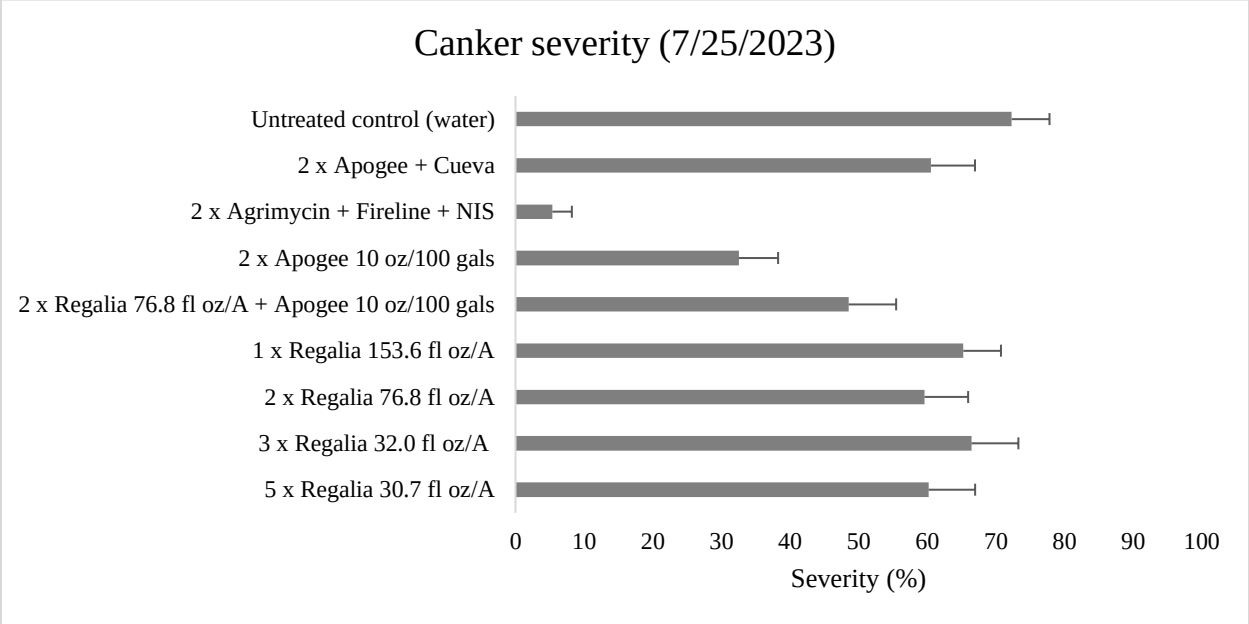
Data on total shoot length and lesion length were measured on May 31, and July 25, 2023. Cankers were noticed on secondary and tertiary branches during July 25 data collection and the total branch length and canker length were measured. For shoot blight analysis, severity was calculated as ratio of lesion 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. 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. 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 perent severity data was analyzed using ANOVA and the treatment means were compared using Fisher's protected LSD ($P<0.05$).

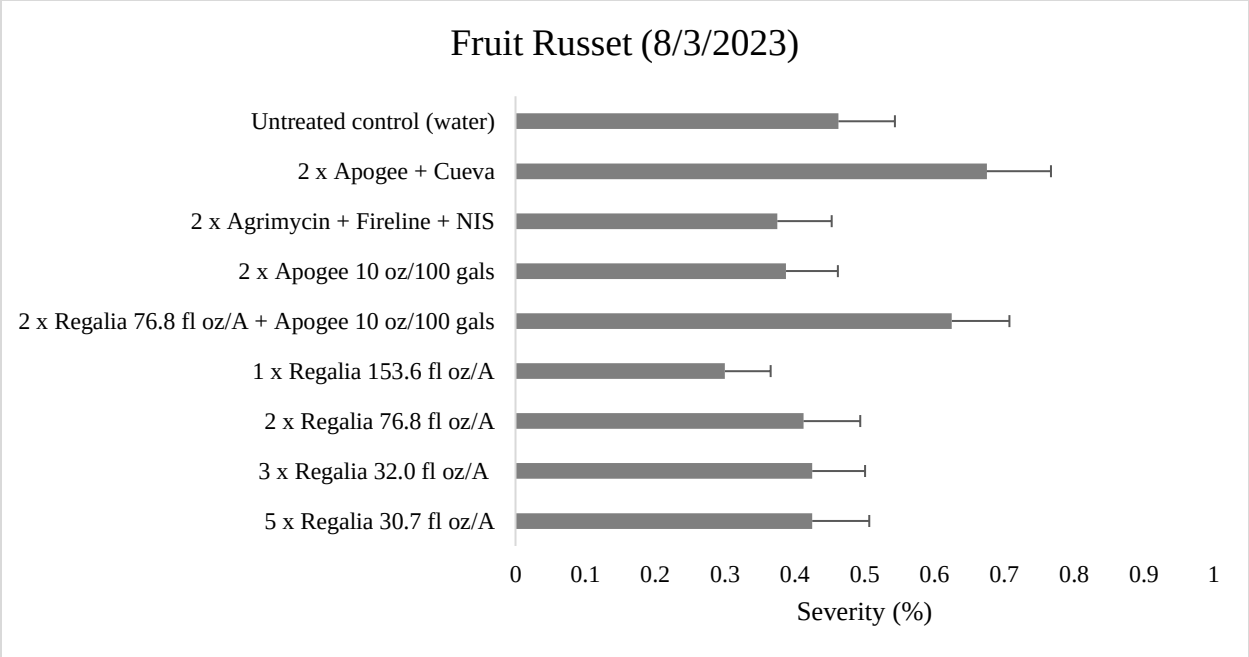
Results and Discussion



Only the commercial standard, where antibiotics were used during full bloom and one day before shoot inoculation significantly reduced the shoot blight severity. 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.

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.***

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

Report Type: Continuing – project start delay / No cost extension

Primary PI: Rachel Leisso
Organization: USDA-ARS Tree Fruit Research Lab – Hood River Worksite
Telephone: (541) 561-1420
Email: Rachel.Leisso@usda.gov
Address: 3005 Experiment Station Drive
City/State/Zip: Hood River, OR 97031

Co-PI 2: David Rudell
Organization: USDA-ARS Tree Fruit Research Lab
Telephone: (509) 664-2280
Email: David.Rudell@usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

CO-PI 3: Loren Honaas
Organization: USDA-ARS Tree Fruit Research Lab
Telephone: (509) 664-2280
Email: Loren.Honaas@usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Co-PI 4: Achala KC
Organization: Oregon State University - Southern Oregon Research and Extension Center
Telephone: (541) 776-7371
Email: Achala.KC@oregonstate.edu
Address: 569 Hanley Rd.
City/State/Zip: Central Point, OR 97502

Co-PI 5: Jim Mattheis
Organization: USDA-ARS Tree Fruit Research Lab
Telephone: (509) 664-2280
Email: James.Mattheis@usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Mt. Adams Fruit; Duckwall Fruit; Peshastin Hi-UP; Dr. Yu Dong

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.
Organization Name: USDA-ARS TFRL
Contract Administrator: Mara Guttman
Telephone: 510-559-5619
Contract administrator email address: Mara.Guttman@usda.gov
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
Miscellaneaous: sequencing (Honaas)

Budget 2
Co PI 2: Achala KC
Organization Name: OSU Ag. Res. Foundation
Contract Administrator: Josh Kvidt
Telephone: 541-737-4066
Contract administrator email address: josh.kvidt@oregonstate.edu
Station Manager/Supervisor: Richard Roseberg
Station manager/supervisor email address: richard.roseberg@oregonstate.edu

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. Research activities will compare these specific CA regimes:

   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)

   Long-term vision: Contingent upon research results, future projects could involve scaled-up evaluation of CA regimes of interest on bins and boxes in larger CA research rooms.

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

   Research activities in this objective will systematically evaluate the optimum firmness 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 lb), and late (17 lb) 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 + <0.1% CO_2. Fruit quality and samples collected in this objective will

contribute to research for molecular maturity indicators (led by Dr. Honaas) and the utility of California 'Bartlett' maturity indices (Mitcham et al., 1996) evaluated.

Long-term vision: Utilizing results from this objective, further studies use an adjusted firmness value or other indicator for optimal harvest maturity.

3. **Evaluate the influence of modified 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, 36 and 42 °F in boxes with MAP liners post-long-term CA storage. Although research indicates utility for certain types of MAP for 'Bartlett' when used immediately after harvest, whether MAP continues to provide significant benefit and retains aroma and quality in late-term storage post-CA has not been examined.

   Long-term vision: Depending on success of this approach, a future proposal could validate research outcomes by evaluating MAP for 'Bartlett' fruit post-CA from multiple packinghouses in collaboration with interested industry partners.

Specific deliverables include:

1) **determination of optimal CA conditions for long-term (>6 months) storage of 'Bartlett' fruit**
2) **revisiting optimal at harvest firmness for 'Bartlett' fruit destined for long-term storage**
3) **progression towards non-firmness-based maturity indicators**
4) **delineation of the interaction of firmness and size near harvest (e.g. what is lost in terms of fruit size when fruit are harvested at higher firmness)**
5) **determination of whether MAP packaging contributes to post-CA-storage fruit quality extension**

Significant findings

We do not have any findings to report in Year 1. In early August 2023, we submitted a request to delay the start of this project, which was approved by Dr. Hanrahan. There were two reasons for this request. One of our young summer employees passed away the week before the Hood River 'Bartlett' harvest began, and our small research group was grieving as well as missing one of our incredibly capable and hard-working team members. Additionally, our controlled atmosphere controller was not installed in June 2023 as scheduled; installation has been delayed until winter 2023-24. In-house CA is critical for both **Objectives 1 and 2**, as both involve CA conditions not typically used by commercial storage entities. We have decommissioned the on-station non-functioning nitrogen generator, and the new nitrogen generator is installed; however, there have been some delays with start-up for this equipment as well. We anticipate all these CA issues will be resolved by fall 2024. Due to this delay, we are modifying **Objective 3** to include fruit obtained post-CA from industry partners in order that we can begin work on this objective in winter 2023-2024.

Since we are delaying the start of this project, the project years will effectively be harvest years 2024 and 2025, with the second year formally being a no cost extension (NCE). We will have a continuing report in February 2025, a NCE report in February 2026, and an additional NCE report in February 2027, since fruit harvested in fall 2025 will still be in storage at the time of the Research Review for winter 2025-2026.

Methods

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

Proposed CA regimes

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)

The purpose of this objective is to determine whether ultra-low oxygen (<0.5% O_2 + <0.5% CO_2) significantly increases storage longevity and fruit quality in comparison to more typical low-oxygen CA programs.

In year 1, we will obtain fruit from one orchard at 19.5 lb firmness. Fruit will be rapidly cooled to 30 °F and stored in CA conditions as above. Fruit at harvest will be assessed according to standard fruit quality measures and evaluated 1 day after removal from storage (held at 68 °F after storage removal) for color, ethylene, respiration, external abiotic disorders and rot, and 7 days after removal from storage for color, firmness, soluble solids content (SSC), ethylene, respiration, disorders, rot incidence and type, and titratable acidity at 3-, 6-, and 7-months storage. Fruit samples at-harvest and post-storage will be further evaluated for treatment influence on fruit aroma.

Results from year 1 will inform CA conditions evaluated in year 2, with adjustments to or removal of CA conditions accordingly, and, in year 2, selected CA regimes will be evaluated on fruit from 3 orchards, representing both Hood River and Central Washington production regions.

The specific deliverable from this objective is determination of optimal CA conditions for long-term (>6 months) storage of 'Bartlett' fruit.

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

We aim to determine whether a) 19.5 lb firmness remains optimum for harvest maturity for long-term low oxygen CA (1.5% O_2, <0.5% CO_2) and b) California indicators of maturity/storage potential have utility for Oregon and Washington. Plus, this project will contribute valuable samples to research for molecular markers for 'Bartlett' maturity.

Each year, we will harvest all fruit on separate selected trees from an orchard block at either early (22.5 lb), mid (19.5 lb), or late (17 lb) flesh firmness and store fruit at 30 °F and 1.5% O_2 + <0.5% CO_2 to evaluate the influence of harvest maturity on stored fruit quality outcomes. We will record fruit size distribution based on diameter and weights approximating industry sizes (e.g. sizes 60 to 180 or smaller), dry matter, soluble solids content (SSC, also called °Brix), diameter, weight, and color, as well as flash freeze peel and cortex tissue for further molecular analyses, or biochemical and molecular analyses. After storage, color, ethylene production, and respiration will be evaluated upon removal of fruit from storage daily until final evaluation of color, firmness, SSC, titratable acidity, rot incidence and type 7 days after removal from CA.

The specific deliverables from this objective are:

a. **revisiting optimal at harvest firmness for 'Bartlett' fruit destined for long-term storage**
b. **progression towards non-firmness-based maturity indicators**
c. **delineation of the interaction of firmness and size near harvest (e.g. what is lost in terms of fruit size when fruit are harvested at higher firmness)**

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

In year 2, fruit will be harvested at 19.5 lb firmness, and stored at the optimum CA as determined in objective 1, for ~6 months, and then be packed into MAP liners (LifeSpan) in boxes while a separate portion will be stored in standard commercial liners. Boxes will be stored at 30, 36 and 42 °F to evaluate the influence of potential temperature changes during shipping and distribution, and a subset of fruit evaluated for quality every two weeks until 2 months post packing. Frozen tissue samples will be evaluated packaging effects on aroma.

We are modifying this objective to include fruit post-commercial CA storage in the 2023-2024 storage season.

The specific deliverable addressed by this objective is determination if MAP packaging contributes post-storage fruit quality extension.

Literature review

Ultra-low oxygen

Based on chlorophyll fluorescence, the lower oxygen limit (LOL) for 'Bartlett' pears is approximately 0.2% O_2 (2004-2005 storage season), but varies with lot and storage duration (Mattheis, PR-04-433, 2006). Fruit stored at 0.4% O_2 were slightly greener than those stored at 1.5% O_2 when removed from storage and had lower incidence of core browning (4% versus 29%) and decay at 6 months (Mattheis, PR-04-433, 2006). However, CA-stress related disorders can vary by orchard and are influenced by maturity. Overall, published information on the influence of ultra-low oxygen versus low oxygen CA on 'Bartlett' fruit quality is sparse.

Importantly, unlike some pear cultivars, 'Bartlett' aroma is apparently not extremely affected by ultra-low oxygen storage (Zlatić et al., 2016). As with other quality parameters, little information regarding the comparison of low oxygen versus ultra-low oxygen on 'Bartlett' aroma is readily available.

Maturity and storage potential

For 'Bartlett', harvest decisions balance rapidly increasing fruit size with decreasing fruit firmness, yet little published information exists to delineate the relationship between fruit size increase and firmness decrease. In the Hood River area, Bartlett harvest typically begins around 19.5 lb firmness (Wang and Sugar, 2013) and ends around 17 lb firmness (Wang et al., 2016). An extension publication suggests harvesting Washington 'Bartlett' pears from 17-15 lb firmness (Tvergyak, 1985), although discussions with warehouse managers concluded 19.5 lb was the recommended firmness for Bartlett for long-term CA storage in Washington (Meheriuk et al., 1988). However, in the Medford district, harvest begins around 22 lb and ends around 19 lb (Sugar and Powers, 1994). Few studies have systematically evaluated harvest maturity in relation to long-term storage for 'Bartlett', although research suggest fruit harvested at lower firmness, e.g. ~18 lb, can have high incidence of internal breakdown by 6 months storage (Bai et al., 2006).

Other pear studies indicate regional and local influences on fruit quality (Whitaker et al., 2009; Wang and Sugar, 2015), which suggests recommended firmness is somewhat district dependent. Regarding maturity biosignatures that can be deployed across districts *and* European pear cultivars, recent work by Honaas has shown that relatively fine contrasts of pear fruit maturity can be distinguished using gene activity data (Honaas et al., 2021). This suggests that new higher performance maturity indices based on molecular signatures may be possible. Honaas' most recent work in this area has shown that pome fruit samples can be ordered in time based solely on gene activity data (see Final Report AP-19-103: Honaas et al., 2022) – but importantly, while the performance of biosignature models generally increases with more data, adding more experimental variables (like CA) to the models has the inverse effect. This project will contribute valuable samples (derived from fruit stored in CA) that will help the team improve prototype maturity biosignature models by adding data, but also by adding breadth to fruit storage conditions so models can be applied to the widest possible range of pear storage regimes.

In California, 'Bartlett' maturity indices incorporate firmness, soluble solids content (SSC, or Brix), along with fruit diameter and color, and fruit with up to 22 lb firmness may be harvested, provided threshold values for all parameters are sufficient (Mitcham et al., 1996). Information on how widely these maturity indices are used in California is not readily available. Dry matter at harvest can indicate stored fruit quality (Goke et al., 2020), although models for dry matter are developed individually for cultivars (Escribano et al., 2016; Goke et al., 2018), and whether there is a location-dependent influence is unclear. Dry matter models have been developed for Wenatchee area 'Bartlett' (Goke et al., 2018) and California 'Bartlett' (Escribano et al., 2016).

MAP packaging after long-term CA

Wang and Sugar (2013) evaluated the influence of several types of modified atmosphere packaging (MAP) on 'Bartlett' storage outcomes. After harvest, fruit were packed immediately into boxes with MAP liners (LifeSpan or an unspecified experimental type) and stored for either 1 or 3 months, followed by storage at a range of temperatures (from 35.6 to 50.0 °F) to simulate transit. In this study, the MAP liner that equilibrated at an average of 12.3% O_2 + 5.6% CO_2 (LifeSpan) was suitable for 4 months of storage (at 30 °F) or 3 months of storage (at 30 °F) plus transport duration of up to 3 weeks at 40 °F, depending on maturity.

An important unknown regarding MAP packaging following longer-term CA for 'Bartlett' pears is the production and influence of ethylene by produced fruit post-storage. Ethylene in storage environments can increase the incidence of 'Bartlett' disorders (Bower et al., 2003), although temperature management is more important in preserving fruit quality overall. Ethylene production in MAP packaging was not reported by Wang and Sugar (2013) nor by Drake (2004).

References

Bai, J., Mattheis, J.P., Reed, N., 2006. Re-initiating softening ability of 1-methylcyclopropene-treated 'Bartlett' and 'd'Anjou' pears after regular air or controlled atmosphere storage. The Journal of Horticultural Science and Biotechnology 81, 959-964.

Bower, J.H., Biasi, W.V., Mitcham, E.J., 2003. Effect of ethylene in the storage environment on quality of 'Bartlett pears'. Postharvest Biology and Technology 28, 371-379.

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

Drake, S.R., 2004. Quality and condition of winter pears as influenced by harvesting, handling, packing and storage, Washington Tree Fruit Research Commission. Project PH-02-227 (2002-2004): pp. 1-4. Available online: https://treefruitresearch.org/report/quality-and-condition-of-winter-pears-as-influenced-by-harvesting-handling-packing-and-storage/

Escribano, S., Biasi, B., Elkins, R., Slaughter, D., Mitcham, E., 2016. Predicting Ripening and Postharvest Quality of 'Bartlett' Pears. California Pear Advisory Board, Sacramento, CA, pp. 1-19. Available online: https://www.calpear.com/_pdf/research-reports/2015/Postharvest/Predicting%20ripening%20and%20postharvest%20quality%20of%20Bartlett%20pears.pdf

Goke, A., Serra, S., Musacchi, S., 2018. Postharvest dry matter and soluble solids content prediction in d'Anjou and Bartlett pear using near-infrared sSpectroscopy. HortScience 53, 669-680.

Goke, A., Serra, S., Musacchi, S., 2020. Manipulation of fruit dry matter via seasonal pruning and its relationship to d'Anjou pear yield and fruit quality. Agronomy 10, 897.

Hansen, E., Mellenthin, W.M., 1979. Commercial Handling and Storage Practices for Winter Pears. Oregon State University Mid-Columbia Research and Extension Center, Hood River, OR, pp. 1-11. Available online: ir.library.oregonstate.edu/downloads/sx61dn23q

Honaas, L., Ficklin, S., Mattheis, J., 2022. Apple genomes for postharvest fruit quality biomarkers. Washington Tree Fruit Research Comission. Final Project Report AP-19-103, 1-13.

Honaas, L., Hargarten, H., Hadish, J., Ficklin, S.P., Serra, S., Musacchi, S., Wafula, E., Mattheis, J., dePamphilis, C.W., Rudell, D., 2021. Transcriptomics of Differential Ripening in 'd'Anjou' Pear (Pyrus communis L.) 12: Article 609684, 1-18.

Mattheis, J., 2006. Harvest and postharvest practices for optimum quality. Washington Tree Fruit Research Commission. Final Project Report: PR-04-433, 1-10. Availble online: https://treefruitresearch.org/report/harvest-and-postharvest-practices-for-optimum-quality/

Meheriuk, M., Evans, C., Talley, E., Kupferman, E., 1988. Harvest maturity and storage regime for pears. Post Harvest Pomology Newsletter 6, 1-7.

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 online: https://postharvest.ucdavis.edu/files/259434.pdf

Sugar, D., Powers, K.A., 1994. Maturity and Storage Performance of `Bartlett' and `Sensation Red Bartlett' Pears. HortScience HortSci 29, 18-19.

Tvergyak, P.J., 1985. Pear maturity indexes used to time harvest date. Post Harvest Pomology Newsletter 3, 1-6.

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., Sugar, D., 2015. 1-MCP efficacy in extending storage life of 'Bartlett' pears is affected by harvest maturity, production elevation, and holding temperature during treatment delay. Postharvest Biology and Technology 103, 1-8.

Wang, Y., Xie, X., Song, J., 2016. Preharvest aminoethoxyvinylglycine spray efficacy in improving storability of 'Bartlett' pears is affected by application rate, timing, and fruit harvest maturity. Postharvest Biology and Technology 119, 69-76.

Whitaker, B.D., Villalobos-Acuña, M., Mitcham, E.J., Mattheis, J.P., 2009. Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. Postharvest Biology and Technology 53, 43-50.

Zlatić, E., Zadnik, V., Fellman, J., Demšar, L., Hribar, J., Čejić, Ž., Vidrih, R., 2016. Comparative analysis of aroma compounds in 'Bartlett' pear in relation to harvest date, storage conditions, and shelf-life. Postharvest Biology and Technology 117, 71-80.

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

Report Type: Continuing Project Report

Primary PI: Dr. Christopher Gottschalk
Organization: USDA-ARS
Telephone: 304-725-3451 x264
Email: Christopher.gottschalk@usda.gov
Address: Appalachian Fruit Research Station
Address 2: 2217 Wiltshire Rd.
City/State/Zip: Kearneysville, WV 25430

Co-PI 2: **Dr. Tami Collum**
Organization: USDA-ARS
Telephone: 304-725-3451 x358
Email: tami.collum@usda.gov
Address: Appalachian Fruit Research Station
Address 2: 2217 Wiltshire Rd.
City/State/Zip: Kearneysville, WV 25430

CO-PI 3: **Dr. Lauri Reinhold**
Organization: USDA-ARS
Telephone: 541-738-4200
Email: lauri.reinhold@usda.gov
Address: National Clonal Germplasm Repository
Address 2: 33447 Peoria Rd.
City/State/Zip: Corvallis, OR 97333

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: 2024 - 2028
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.

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
Primary PI: Dr. Christopher Gottschalk
Organization Name: USDA ARS
Contract Administrator: Stephanie Kreger
Telephone: 304-725-3451 x332
Contract administrator email address: stephanie.kreger@usda.gov
Station Manager/Supervisor: Dr. Tracy Leskey
Station manager/supervisor email address: tracy.leskey@usda.gov

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

Footnotes:

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

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

Budget 2
Co PI 2: Dr. Lauri Reinhold
Organization Name: USDA ARS
Contract Administrator: Stefani Morgan
Telephone: (541) 738-4023
Contract administrator email address: stefani.morgan@usda.gov
Station Manager/Supervisor: Carolyn Scagel
Station manager/supervisor email address: carolyn.scagel@usda.gov

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

Footnotes:

Objectives
Our 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 50 genotypes (50+ from AFRS and 29 from NCGR), 20 of which have two consecutive years of data (AFRS sourced) for harvest and cold conditioning requirements.
- 20 pear genotypes were evaluated for susceptibility to *Penicillium expansum* and *Colletotrichum fioriniae*.
- Identified four genotypes that were significantly less susceptible to *P. expansum* and *C. fioriniae* compared to Gem.
- The biocontrol agent isolated form pear has been identified as a *Streptomyces sp.* and inhibited the growth of multiple pathogens on in vitro plate assays including *P. expansum* (43% inhibition), *C. fioriniae* (58% inhibition) and *Diaporthe eres* (63% inhibition).

Objective 2:

- Identified genotypes associated with large fruit size, high sugar, and high acidity.

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 in mature. 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 we began to collect and phenotype during the 2023 season.

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. First-year results were obtained in the Fall and Winter of 2022 and second-year results were obtained in the Fall of 2023 and are on-going.

Twenty pear genotypes that were evaluated for natural disease incidence in year 1 were selected to be directly challenged with *Penicillium expansum* or *Colletotrichum fioriniae* using a wound inoculation method. 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 1x104 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.

An additional 19 pear genotypes that were not evaluated in year 1, were evaluated for natural disease incidence in year 2. A total of 24 fruits harvested from each genotype were divided into three replicates of eight fruits and were evaluated weekly for the presence or absence of disease during cold storage. After twelve weeks in cold storage 8/19 of the new genotypes evaluated had low natural disease incidence (<15%). These included US 446, 672451-015, 79423-023, 71643-047, ‘Talsarskara Krasavitza’, ‘Pai Li’, NJ 12, and NJ Rock R27 T65. When a disease was identified, pathogens were sampled and plated for identification of pathogen species and/or complex based on morphology and DNA sequence using universal fungal primers ITS1 and ITS4. Data collection and analysis are ongoing from fruit collected in 2023. Additionally, fruit wound inoculation experiments will be conducted using these genotypes with low natural disease incidence in year 3.

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

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 will continue 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. All measurement for the 2022 fruit has been completed except the polyphenols. We had a delay in acquisition of a new high-throughput plate reader but intended to complete those measurements at the same time as the 2023 fruit. The 2023 fruit evaluations are on-going and are anticipated to be completed in the early spring of 2024.

Objective 3: We will evaluate each genotype for resilience to stress associated with the supply chain including bruising, scuffing, and puncturing. This objective will begin during the 2024 season due to the limited fruit available during the 2022 season 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 qualitatively note damage to fruit received from NCGR. As for fruit obtained from AFRS, when a genotype has acquired two consecutive years of storage data, it will be selected for evaluation. For each of the three injury tests, five replicate pear fruits – at optimal fruit maturity – will be removed from storage and subjected to stress tests. For evaluation of resistance to bruising, we will utilize a penetrometer to apply pressure to the fruit at a marked location on the fruit's surface. The penetrometer will apply an even pressure of 7 lbf to the fruit (the peel is not removed during this test). The fruit will then be rested at room temperature for 5 days. Following the rest period, the fruit will be dissected across the marked bruising site. The injury, if present, will then be documented for color (oxidation) and depth of bruising. Qualitative data from NCGR fruit began collection during the 2023 season. 20 genotypes of AFRS fruit will undergo evaluations starting in 2024.

An alternate approach will utilize a robot arm to simulate container loading and unloading which would cause bruising. However, the robot arm is currently still unavailable due to equipment failure and COVID-19 disruptions to the supply chain for replacement parts. We hope to fix and make this machine available for use during the upcoming years of the project. The robotic stress will be applied by having the robot's arm traverse the lower ¼ quadrant of a circle at a speed setting that mimics truck movement on the roadway and a drop treatment that covers a distance of 600 mm in < 1 sec. The robotic-associated testing will occur at AFRS under the guidance of Dr. Amy Tabb who has performed similar simulations (Nixon et al., 2019). To evaluate scuffing, a simulated conveyor belt will be constructed that consists of a rectangular box outfitted with fruit conveyor belt material. The box containing five replicate fruits will then be placed onto a shaker table that will operate at 100 RPMs for five mins. Following the stress, the fruit will be rested for 5 days at room temperature and then evaluated for presence/absence of scuffing and scuffing severity. The final evaluation test will be a puncture test where five replicate fruits will be subjected to a penetrometer outfitted with a 4 mm plug. The pressure it takes for the plug on the penetrometer to puncture the peel of the fruit will then be recorded.

Objective 4: The results gained from Objectives 1-3 will be presented and distributed to the research community and stakeholders through various channels such as presentations at the WTFRC Pear Research Review, published in a horticultural-focused journal(s), and data indexed into the USDA GRIN database for public accessibility.

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. 44 of the 50 genotypes were below the threshold of fruit required and as a result attention was focused on the germplasm available at AFRS. The AFRS germplasm had 38 lines with enough fruit to evaluate and determine harvest date and cold conditioning requirements, natural disease presence/absence, and measurements for Obj. 2 fruit

quality traits. In year two, we were able to source 29 varieties from the NCGR and an additional 59 genotypes from AFRS. 20 of the AFRS genotypes had data collected on them during year one of the project.

For the past two seasons, we have documented harvest date and cold conditioning requirements for >50 unique genotypes. We have documented a strong peak in harvesting dates for pears August 15th and August 30th followed by a short plateau of a high number of varieties harvested through September 15th and taper off in early October (Fig 1). However, a few varieties were found to be harvest after October 15th and represent extremely late ripening genotypes. The cold conditioning requirements for the selected pear germplasm was far more variable than harvest date with a trimodal distribution. The first peak in conditioning requirements being met around September 14th, a second on November 7th, and a final third around December 10th (Fig 1). Although moderately correlated (Kendall's $\tau = 0.49$), harvest date is not an accurate predictor for cold conditioning requirement. We found many genotypes that were 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 such as US 79423-023 and US 82728-016. 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.

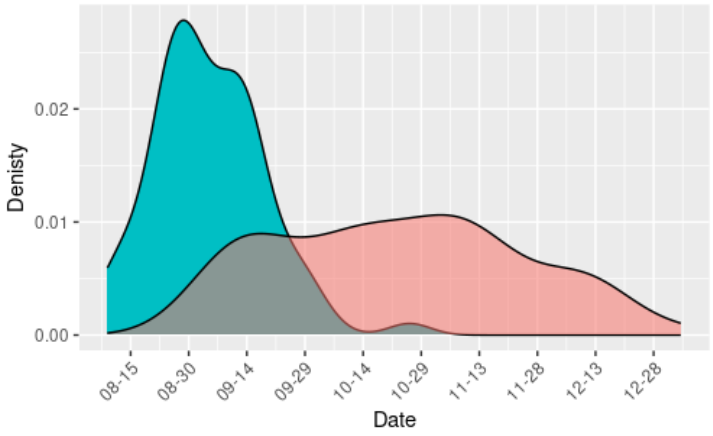


Figure 1. Variation in harvest date and cold conditioning requirements in pear germplasm.

Twenty genotypes that were evaluated for natural disease incidence in year 1 were directly challenged *P. expansum* or *C. fioriniae* using a wound inoculation method. 'Gem', 'Bell', and 'Shenandoah' were included as controls that are all highly susceptible to both *P. expansum* and *C. fioriniae*. We found five genotypes had significantly reduced lesion sizes when challenge with *P. expansum* compared to 'Gem'. These included US 68309-106, US 82728-016, US 83825-223, US 84907-166, and US 83825-261 (Fig 2A). Four of these genotypes (US 82728-016, US 83825-223, US 84907-166, and US 83825-261) also had significantly reduced lesion sizes when wound inoculated with *C. fioriniae* (Fig 2B). Interestingly, US 83825-223, US 84907-166, and US 83825-261 were identified in year 1 as having low natural disease incidence (<15%) while US 82728-016 had moderate natural disease incidence (33%). Wound inoculation experiments will be repeated in year 3.

The bacterium isolated from a pear surface that displayed antagonistic activity against *Diaporthe sp.* has been identified as a *Streptomyces sp.* We evaluated the ability of the *Streptomyces sp.* to reduce the growth of several pome and stone fruit pathogens using *in vitro* plate assays. We found that *Streptomyces sp.* had > 40% inhibition of several pathogens including *P. expansum* (43% inhibition), *C. fioriniae* (58% inhibition), and *D. eres* (63% inhibition) (Fig 3). Experiments applying *Streptomyces sp.* to fruit surfaces to evaluate disease control are underway as well as efforts to determine the bioactive compound responsible for reducing pathogen growth.

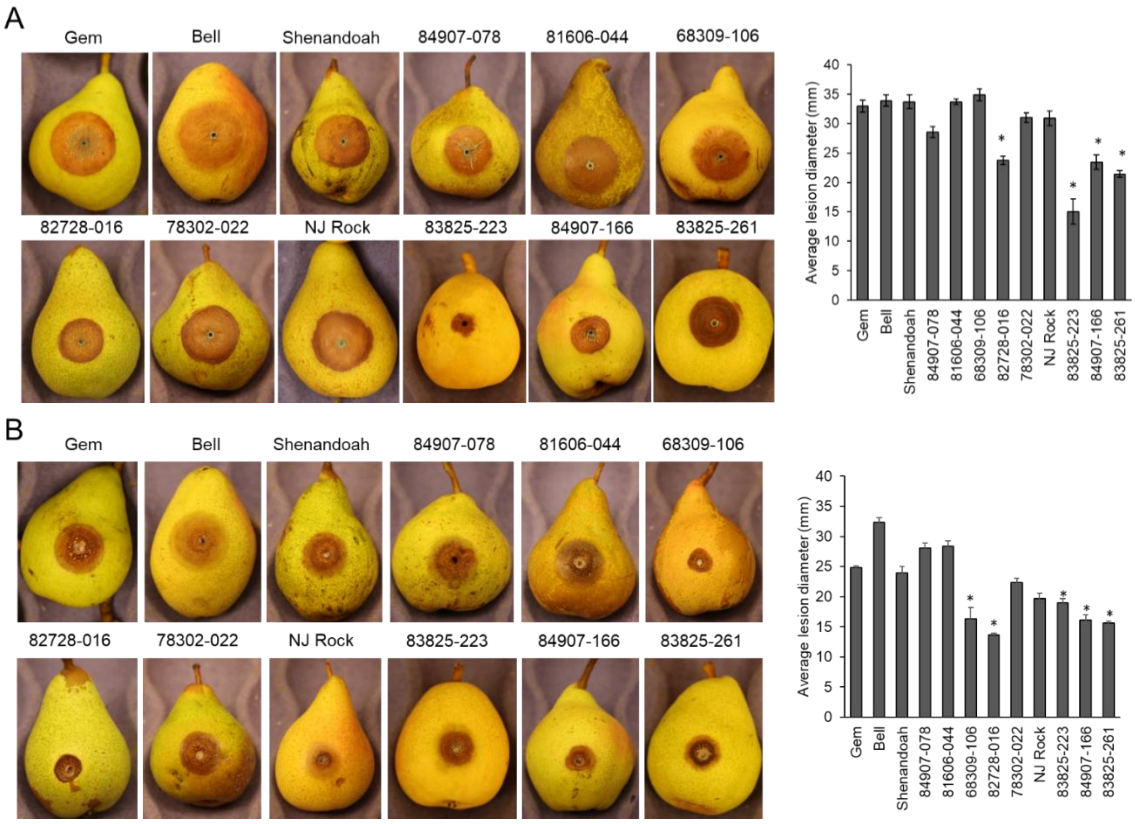


Figure 2. Wound inoculation of pear genotypes with *Penicillium expansum* (A) or *Colletotrichum fioriniae* (B). A representative image of lesion development 7 days post inoculation is shown for selected pear genotypes. Bars represent the mean lesion diameter (mm) ± standard error, N = 20 fruit. A * indicates a significant difference of $p < 0.01$ compared to Gem using a one-way ANOVA with post-hoc Tukey HSD test.

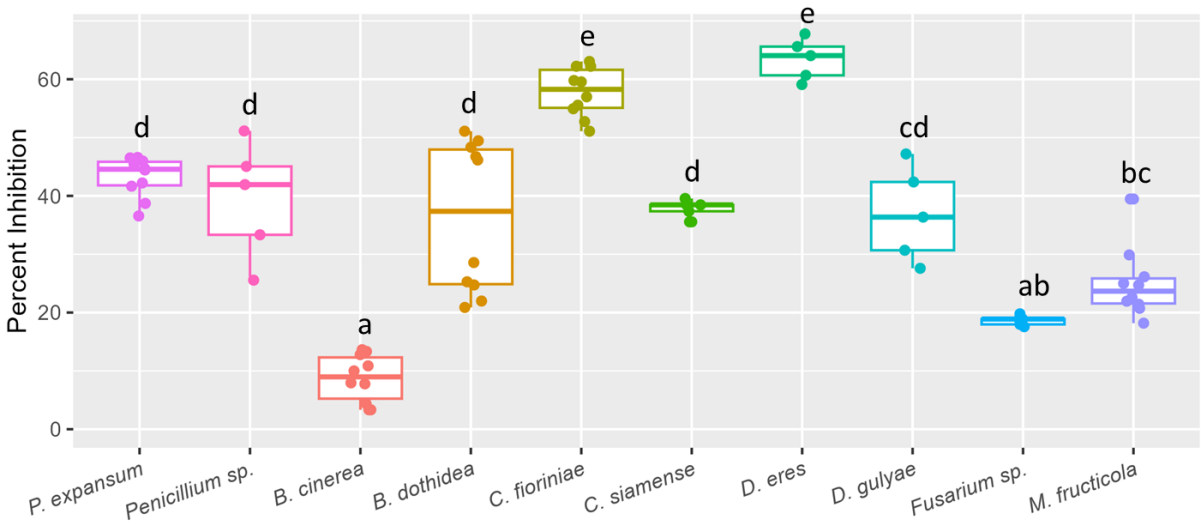


Figure 3. Percent inhibition of fungal pathogen growth by *Streptomyces* sp. under *in vitro* conditions on potato dextrose agar media. Box and whisker plots represent average percent reduction in growth by the pathogen towards the *Streptomyces* colonies after seven days grown at 26 °C.

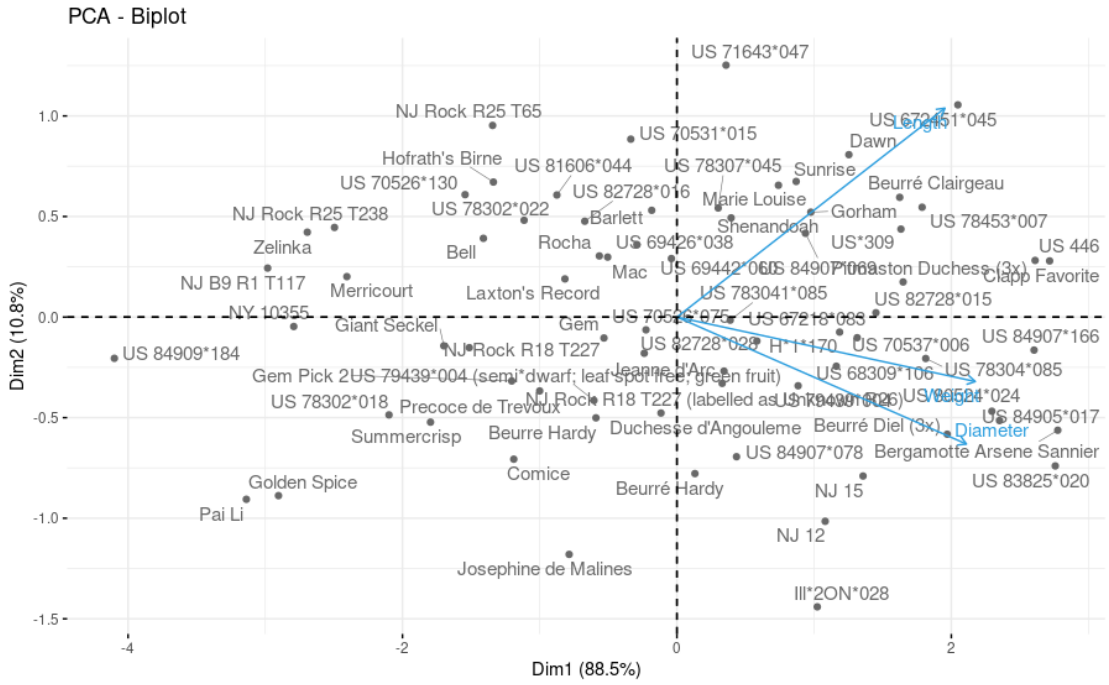


Figure 4. PCA of pear fruit size.

Objective 2: We are currently analyzing the fruit quality traits from the 2023 season. Thus far, we have documented fruit size measurements for the 2022 and 2023 season. 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). We have also begun to analyzed fruit quality metrics for 31 varieties of pears collected in 2022. We have obtained replicated measurements for soluble sugar content (Brix), pH, titratable acidity (TA). As anticipated, pH and TA have inverse relationships and are relatively independent of soluble sugar content (Fig 5). The results thus far have illuminated a lack of germplasm with high sugar content. Of what we tested, breeding lines NJ Rock R18 T227, US 79439-004, and US 84905-017 are high sugar with a range of 14 to 17 Brix. We have also identified lines with high acidity, a component of flavor that is traditionally lacking in pear, such as US 78453-007 and NJ Rock R25 T65. This information is invaluable to aiding in the improvement of pear flavor (Fig 5).

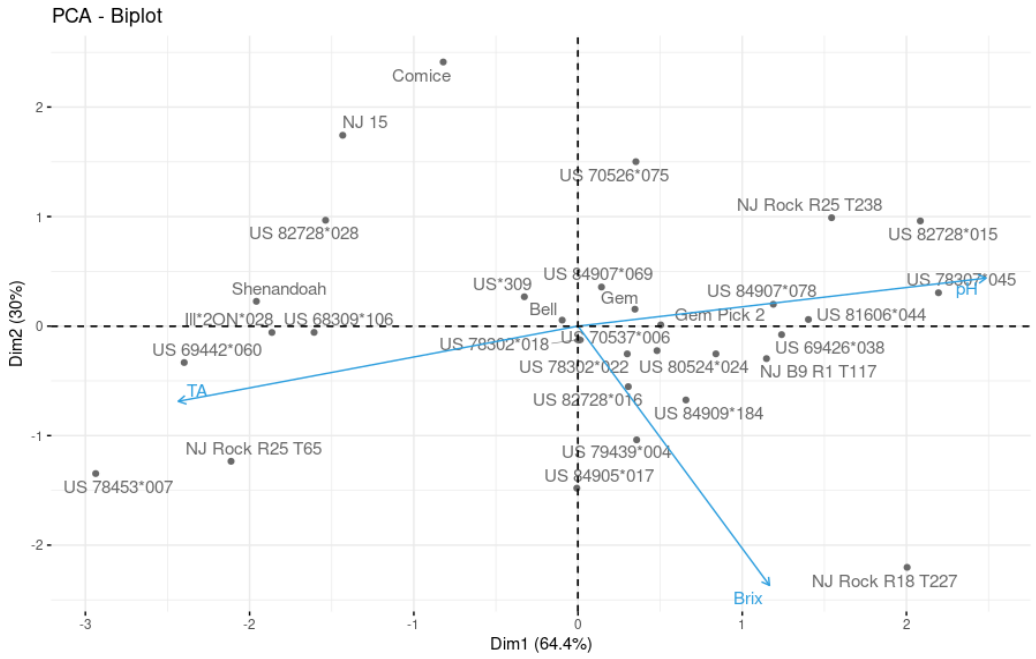


Figure 5. PCA of pear fruit quality metrics Brix, pH, and titratable acidity (TA).

Objective 3 and 4: Results have not been obtained yet for the final two objectives. We anticipate collecting results for Objective 3 in Fall of 2024. Objective 4 results are anticipated to begin in spring of 2025 when this project finishes and final reports are made.

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

Continuing Project Report Year 2 of 3

Primary PI: David Rudell
Organization: USDA-ARS
Telephone: 509-664-2280 x245
Email: David.Rudell@usda.gov
Address: Tree Fruit Research Laboratory
Address 2: 1104 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Co-PI 2: James Mattheis
Organization: USDA-ARS
Telephone: 509-664-2280 x247
Email: James.Mattheis@usda.gov
Address: Tree Fruit Research Laboratory
Address 2: 1104 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

CO-PI 3: Carolina Torres
Organization: WSU
Telephone: 509-293-8808
Email: ctorres@wsu.edu
Address: Tree Fruit Research and Extension Center
Address 2: 1100 N. Western Ave
City/State/Zip: Wenatchee, WA 98801

Co-PI 4: Rachel Leisso
Organization: USDA-ARS
Telephone: 541-561-1420
Email: Rachel.Leisso@usda.gov
Address: Tree Fruit Research Laboratory (Hood River)
Address 2: 3005 Experiment Station Dr.
City/State/Zip: Hood River, OR 97037

Collaborator: Dr. DoSu Park

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)

Budget 1
Primary PI: David Rudell
Organization Name: USDA-ARS
Contract Administrator: Sharon Blanchard
Telephone: 509-664-2280
Contract administrator email address: Sharon.Blanchard@usda.gov

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

Item	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 ULO CA.
3. Evaluate tests that indicate disorder control effectiveness during ULO CA.

Significant Findings:

1. Ultra-low O_2 (ULO) CA storage (0.5% O_2/0.5% CO_2) resulted in pears with better integrity for distribution while retaining ripening capacity.
2. Superficial scald was nearly eliminated in susceptible orchards by storing in 0.5% O_2.
3. Higher storage temperatures resulted in elevated levels of pithy brown core and internal browning.
4. 'D'Anjou will not ripen following 1-MCP treatment within 4 weeks of harvest combined with ULO-CA storage.

METHODS

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

For Year 1, d'Anjou pears were harvested from 3 orchards (Cashmere, WA; Dryden, WA; Hood River, OR) at commercial maturity. Pears were transported to the Tree Fruit Research Laboratory, 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, 33 °F, or 37 °F. Each combination was initially represented by 72 pears. Pear quality and ripeness (imaged, firmness, soluble solids, 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 weeks, assessing quality/ripeness 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.

In year 2, pears were harvested from the Cashmere orchard 1 week prior to, at commercial maturity, and 1 week following commercial maturity. The same storage conditions and evaluation is underway.

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

Pears were harvested from an orchard near Cashmere at commercial maturity, transported to the Tree Fruit Research Laboratory, sorted, and harvest maturity/fruit quality analyzed. To test the impact of delayed 1-MCP treatment on ripening capacity, pears were treated at harvest with 150 ppb 1-MCP for 12 h in air, then placed in ULO CA (0.5% O_2: 0.5% CO_2), or treated with 1-MCP in the same fashion after 0.5 or 1 month ULO CA storage. Pears were stored under these conditions for 8 months. At 8 months, a subset of these were treated with 150 ppb 1-MCP as indicated. Pear fruit quality/ripeness (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 weeks, sampling quality/ripeness immediately upon removal as well as following 7 days at 68 °F.

In Year 2, 1 ppm, rather than 150 ppb, will be applied for post-CA storage 1-MCP treatment on pears from the Cashmere orchard.

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

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, 3, 6, and 8 months to track changes in levels of natural chemicals associated with disorder risk in apples and pears. Tissue was processed, stored, and analyzed using 3 in-house analyses for natural chemicals, including those associated with superficial scald (apples and pears), CO_2 sensitivity (apples), and soft scald/soggy breakdown (apples). These analyses also include those directed towards confirming links between pithy brown core and natural peel chemicals in an earlier study.

This experiment was repeated in Year 2.

RESULTS AND DISCUSSION

Ultra-low O_2 (ULO) storage (0.5% O_2/0.5% CO_2) resulted in pears with better integrity for distribution while retaining ripening capacity.

Harvest maturity was varied among orchards with pears picked in Dryden (12.2 lbs) and Cashmere (12.3 lbs) less firm than Hood River (14.0 lbs). More mature fruit would be expected to perform worse when stored in warmer storage temperatures and higher O_2 levels. However, this was not evident in the current study.

Table 1. Firmness of d'Anjou pears from 3 orchards after 8 months controlled atmosphere storage at different O_2 and temperature condition combinations. Evaluations were made immediately upon removal at 8 months and following simulated cold chain (4 weeks)/retail shelf (7 days). Pears were in good condition to ship regardless of O_2 percentage if stored at 31 or 33 °F. While pears stored in 0.5% O_2 were firmer at every temperature, storage at 37 °F was not sufficient for slowing ripening to 8 months. Different lowercase letters indicate a difference among storage conditions according to a z-test ($p \leq 0.05$).

Temperature	Treatments	Firmness (lbs)					
		Hood River		Cashmere		Dryden	
		8M	8M+4w+d7	8M	8M+4w+d7	8M	8M+4w+d7
31 °F	0.5 %	13.6 a	2.4 a	15.4 a	3.8 ab	15.4 a	3.9 ab
	1.0 %	13.7 a	2.9 a	15.4 a	4.3 a	16.3 a	4.1 a
	1.5 %	13.8 a	2.5 a	14.6 a	3.9 ab	16.4 a	3.6 ab
33 °F	0.5 %	13.7 a	1.9 b	15.6 a	3.6 ab	16.7 a	3.4 ab
	1.0 %	13.2 a	2.1 b	15.1 a	3.2 b	16.0 a	2.9 ab
	1.5 %	13.2 a	1.8 b	15.8 a	3.3 b	16.4 a	3.9 ab
37 °F	0.5 %	5.1 b	1.5 b	13.5 a	2.8 c	14.4 b	2.8 b
	1.0 %	7.8 b	2.0 b	5.5 b	2.2 c	4.9 b	-
	1.5 %	5.3 b	-	3.8 b	-	4.2 b	-

The warmest temperature (37 °F) is not recommended, even when coupled with ULO atmosphere. This high temperature was tested as a potential means of storing pears under ULO conditions while minimizing risk of developing internal disorders. Little softening had occurred and pears appeared

acceptable for distribution at 8 months for every storage regime except those stored at 37 °F (Table 1; Note that firmness values are higher at 8 months than at harvest as firmness was analyzed on cold fruit at 8 months). At 37 °F, pears from Cashmere and Dryden that were stored under 0.5% O_2 were still unripe yet had softened in comparison with those stored in colder temperatures indicating they had begun to ripen and were unfit for distribution. Pears from Hood River had obviously ripened under these conditions in all cases at 37 °F. Pears were ripe after a 4-week post-storage cold chain followed by 7 days at 68 °F (Table 1). Spoilage was most prominent in fruit stored under 37 °F.

Superficial scald was nearly eliminated in susceptible orchards by storing in 0.5% O_2

Peel and cortex disorders were also impacted by storage conditions. This included superficial scald of the peel and pithy brown core (Figure 1). Pears harvested from the Cashmere and Dryden orchards developed severe superficial scald after the 4-week post-storage cold chain and 7 days at 68 °F (Figure 2). Superficial scald symptoms were not present before the final evaluation after any storage condition (not shown). Pears from the Hood River orchard did not develop superficial scald. Storage temperatures within the 30-33 °F range had no consistent influence on scald development. Due to loss from mucor infection and/or spoilage, the impact of 37 °F could not be determined. However, storage O_2 percentage had a profound impact, especially when comparing incidence on fruit stored at 0.5 % with the higher O_2 levels, which was different only on fruit stored at 31 °F. While this clearly indicates ULO conditions are adequate for controlling superficial scald even beyond 8 months storage and distribution, questions still arise regarding the safety of these conditions when considering internal disorders.

Figure 1. Disorder symptoms developing d'Anjou pears in year 1. These included pithy brown core (left) and superficial scald (right).

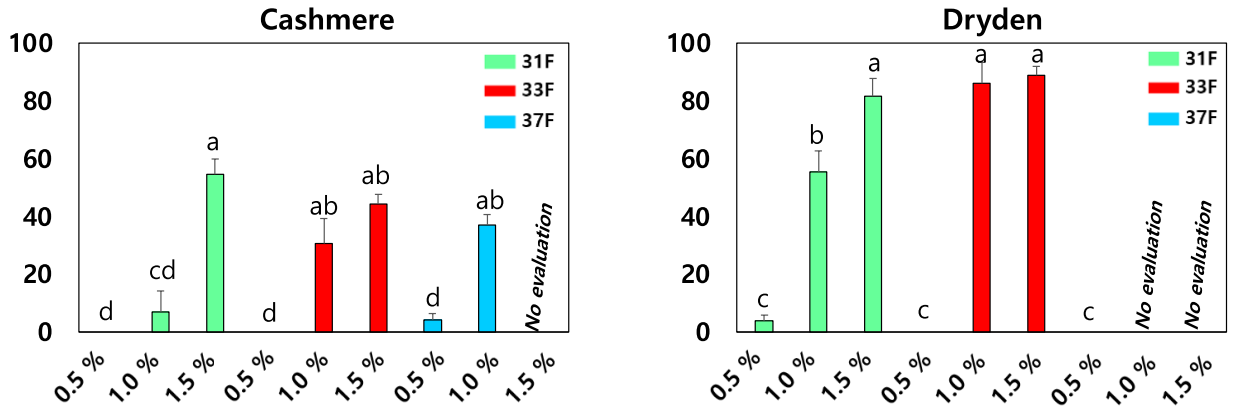


Figure 2. Superficial scald incidence on d'Anjou pears from 2 orchards following 8 months of storage under variable O_2 and temperature conditions followed by 4 weeks at 33 °F and 7 days at 68 °F to simulate distribution and retail shelf time. Scald was nearly eliminated when pears were stored in 0.5% 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 ($p \leq 0.05$).

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 lead to elevated rates 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 (Figure 3). Only insignificant levels of the disorder occurred earlier than this final evaluation (not shown). This pattern seems to indicate the symptoms observed here were associated in some way with ripeness or related factor rather than chilling damage. Other results reveal that pithy brown core is also reduced when 150 ppb 1-MCP is applied at harvest or during ULO storage at 2 weeks in pears from the Cashmere orchard supporting this possibility (not shown).

Disorder risk appears to be orchard or, potentially, ripeness-specific. **However, <u>caution</u> is required in this interpretation here as similar symptoms can have very different causes or etiologies.** This could very well be the case here where other chilling stress related symptoms failed to present themselves in this season. Another possibility would be CO_2 accumulation and resulting sensitivity in particularly dense regions of pear cortex. We plan to add CO_2 concentration as a variable in Year 3.

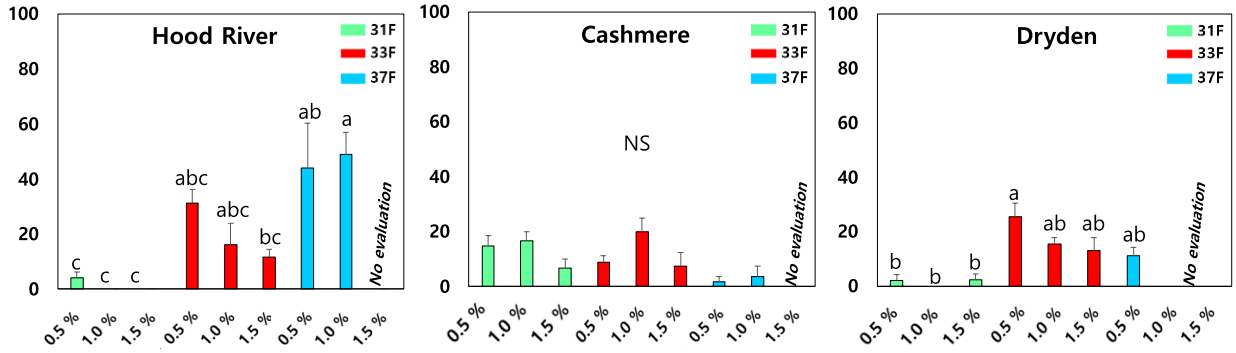


Figure 3. Pithy brown core incidence in d'Anjou pears from 3 orchards following 8 months storage under variable O_2 and temperature conditions followed by 4 weeks at 33 °F and 7 days at 68 °F to simulate distribution and retail shelf time. Pithy brown core was reduced by lower temperature or remained 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 ($p \leq 0.05$).

'D'Anjou will not ripen following 1-MCP treatment within 4 weeks of harvest combined with ULO-CA storage.

Based on present results, combining even a low rate of 1-MCP (150 ppb) at harvest followed by 0.5% O_2 :0.5% CO_2 CA (31 °F) will likely result in pears that will not ripen, even after long term storage. Pears treated with 150 ppb MCP at harvest and after 2 or 4 weeks ULO CA storage and then stored 8 months in the same conditions followed by 4 weeks in air (33 °F) and, finally, 7 days at 68 °F still did not ripen as indicated here by relative softening (Table 2). However, if applied at the same rate after 8 months ULO CA storage, 1-MCP did not have any obvious impact on ripening or quality (Figure 4). As our objective was to determine approaches using 1-MCP that have a limited impact on ripening during the post-CA storage cold chain, Year 2 is focusing on applying higher rates (up to 1 ppm) after 8 months storage. Results from Year 1 indicate that further mitigation of ripening and superficial scald may be unneeded if ULO conditions are used, at least for cold chains up to 4 weeks. We also intend to extend our cold chain trial out to 2 months for this experiment in Year 2.

Table 2. Firmness and titratable acidity of d'Anjou treated with 150 ppb 1-MCP at harvest and following different delays during storage under ultra-low oxygen (ULO; 0.5% O_2:0.5% CO_2, 33 °F) for 8 months. Evaluations were made immediately after removal from storage at 8 months or 8 months plus 4 weeks in air at 33 °F and 7 days at 68 °F to simulate distribution and retail. Results indicate that this rate of 1-MCP alongside ULO CA storage rendered pears incapable of ripening. Treatment at this low rate after 8 months of storage had no impact. Different lowercase letters indicate a difference among treatment according to a z-test ($p \leq 0.05$).

Temperature	1-MCP application	Firmness (lbs)		Titratable acidity (%)	
		8M	8M+4w+d14	8M	8M+4w+d14
33 °F	None	15.1 a	1.7 b	0.24 b	0.31 a
	At harvest	14.9 a	13.4 a	0.24 b	0.31 a
	After 2W	14.9 a	15.1 a	0.26 ab	0.32 a
	After 4W	14.3 a	15.1 a	0.29 a	0.32 a
	After 8M	15.0 a	2.0 b	0.27 ab	0.34 a

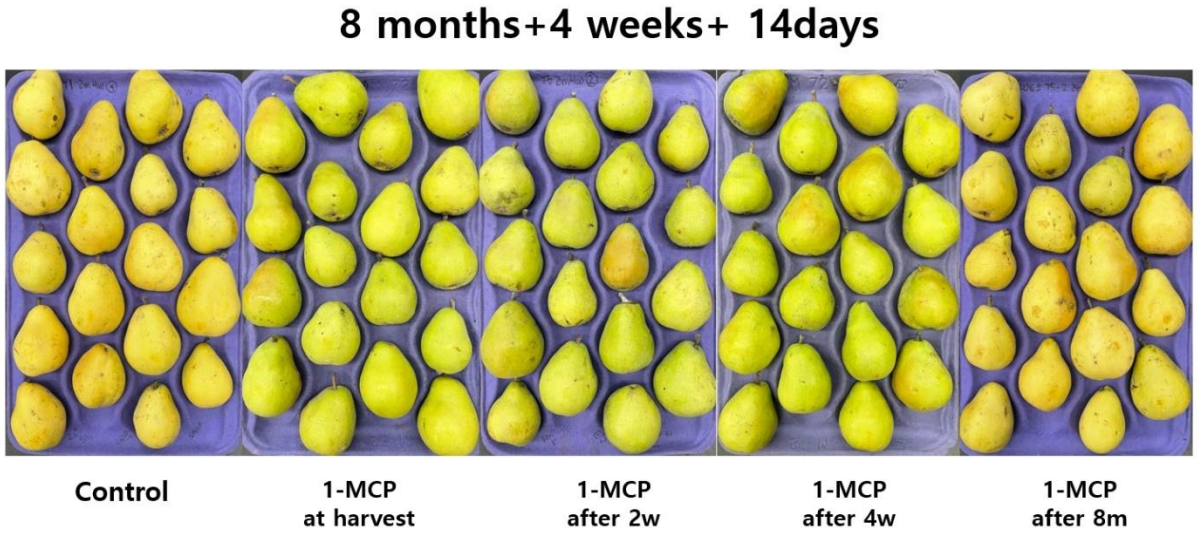


Figure 4. Appearance of d'Anjou treated with 150 ppb 1-MCP at harvest and following different delays during storage under ultra-low oxygen (ULO; 0.5% O_2:0.5% CO_2, 33 °F) for 8 months. Evaluations were made immediately after removal from storage at 8 months or 8 months plus 4 weeks in air at 33 °F and 7 days at 68 °F to simulate distribution and retail. Pears treated with 1-MCP at harvest or during storage at 2 or 4 weeks did not ripen by the end of the study. Ripening of pears treated upon removal from CA storage at 8 months was not influenced by 1-MCP treatment at this rate. Pears were stored in 0.5% O_2 for this experiment and, consequently, did not develop superficial scald.

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

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 (Figure 5). 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, the phytosterols, associated with changes in plant cellular membranes (the envelopes holding cell components in the correct place that must remain fluid at all temperatures) were identified earlier in apple as associated 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 these (ASG/SE) increased in peel with O_2 percentage, as did scald incidence, although the link was not as clear with regard to temperature with pears stored at 37 °F having low a low ratio compared with those stored at lower temperatures yet similar scald incidence (Figure 6).

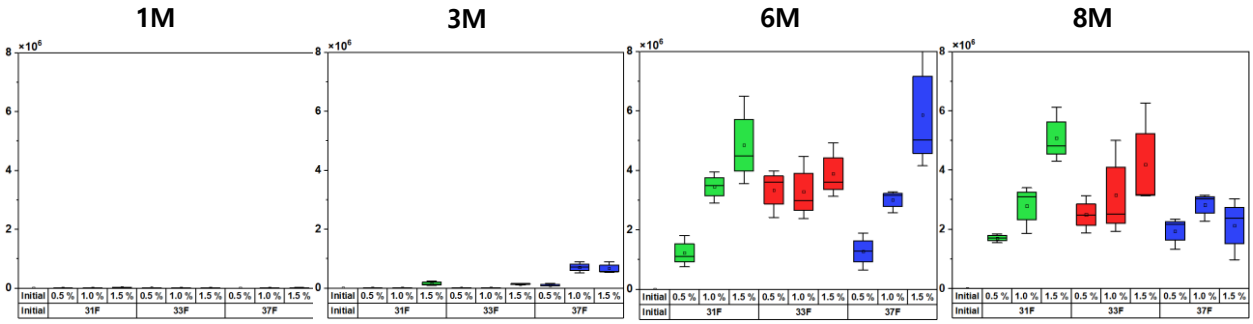


Figure 5. Accumulation of conjugated trienol (CTOL) in peel of d'Anjou pears harvested from Cashmere, WA and stored in 0.5% CO_2 plus 0.5%, 1%, or 1.5% O_2 at 31, 33, or 37 °F. CTOL accumulation is associated with the environmental conditions that cause superficial scald. Results here reflect the final scald incidence presented for this orchard in Figure 1. Error bars represent standard deviation.

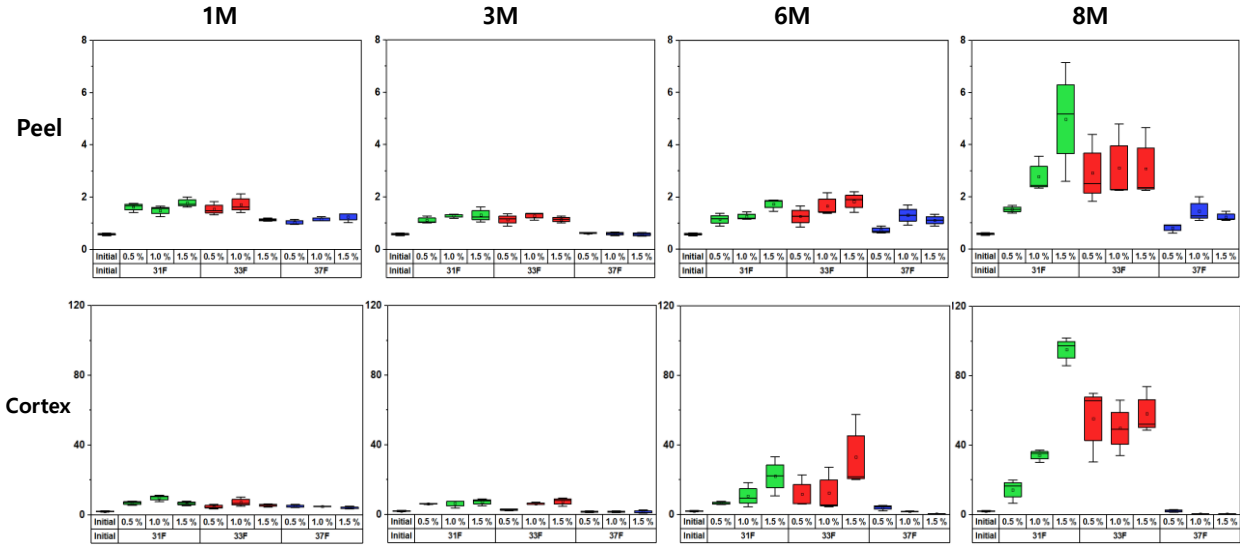


Figure 6. Changes of the ASG to SE ratio in peel and cortex of d'Anjou pears harvested from Cashmere, WA and stored in 0.5% CO_2 plus 0.5%, 1%, or 1.5% O_2 at 31, 33, or 37 °F. Elevated ASG/SE in peel is associated with superficial scald in apple and, in cortex, with soggy breakdown and CO_2-related browning also in apple. Results here reflect the final scald incidence presented for this orchard in Figure 1. However, no association with pithy brown core incidence (Figure 2) is apparent. Error bars represent standard deviation.

Summary (Year 1)

D'Anjou stored in 0.5% O_2:0.5% CO_2 at 31 °F fully ripened after 8M only developing insignificant incidence of superficial scald and little impact on pithy brown core incidence after a simulated 4-week distribution period and 7 day of room temperature simulated retail presentation. More conventional O_2 percentages did not control scald as well. O_2 percentage had little consistent impact on pithy brown core. Furthermore, colder storage temperature appeared to both reduce superficial scald while not impacting or even reducing pithy brown core. 1-MCP treatment, even at 150 ppb, at harvest or within the first month

following storage establishment, in combination with ULO CA at 33 °F stopped pears from ever ripening. This combination is not recommended. 1-MCP treatment at 150 ppm after 8 months ULO CA did not influence ripening, and our subsequent work will test higher rates alongside ULO CA. CTOL analysis continues to be a useful approach for assessing superficial scald risk as relative levels represented the final scald incidence as influenced by O_2 percentage. Altogether, Year 1 results contradict conventional understanding by indicating that a combination of ULO CA and conventional pear storage temperatures is an effective means of maintaining peak quality for distribution and ripening even after long-term storage. However, these results must be evaluated cautiously as there are other factors unaccounted for in this study, including seasonality and maturity, which we will be looking at in years 2 and 3. Furthermore, as d'Anjou can be sensitive to CO_2, causing internal browning, we will include this as a factor in year 3.

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

Report Type: Continuing Project Report – No Cost Extension

Primary PI: Jessica Waite
Organization: USDA-ARS Wenatchee
Telephone: 509-209-7970
Email: jessica.waite@usda.gov
Address: 1104 N. Western Ave.
Address 2:
City/State/Zip: Wenatchee, WA 98801

Cooperators: Sean Cutler, UC Riverside; Kate Evans, WSU; Amit Dhingra, WSU; Chris Dardick, USDA-ARS Kearneysville

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
Primary PI: Jessica Waite
Organization Name: USDA-ARS Wenatchee
Contract Administrator: Mara Guttman & Sharon Blanchard
Telephone: 510-559-5619 (MG), 509.664.2280 (SB)
Contract administrator email address: mara.guttman@usda.gov, sharon.blanchard@usda.gov
Station Manager/Supervisor: Dave Rudell
Station manager/supervisor email address: david.rudell@usda.gov

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.

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

- Continued successful transformation of callus tissue, as indicated by the red fluorescent marker gene included in the RCB construct. However, shoot regeneration from transformed callus has not yet been achieved.
- Obtained similar levels of callus transformation in 'OHxF 97' and 'OHxF 87' as 'Bartlett' when similar protocols were applied.
- Determined that using different micropropagation medias for plant growth prior to transformation improved callus transformation with the RCB construct.

Methods

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

1a. Selection of germplasm to be transformed

In Year 1, we were able to obtain 'OHxF 87', '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. Additionally in year 3, we recently 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). We plan to use 'Conference' in the future as a potential 'proof of principle' for transformation of our inducible RCB construct.

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). This year we sequenced this version of the construct to confirm it is correct, and plan to use it in transformation experiments in the coming year.

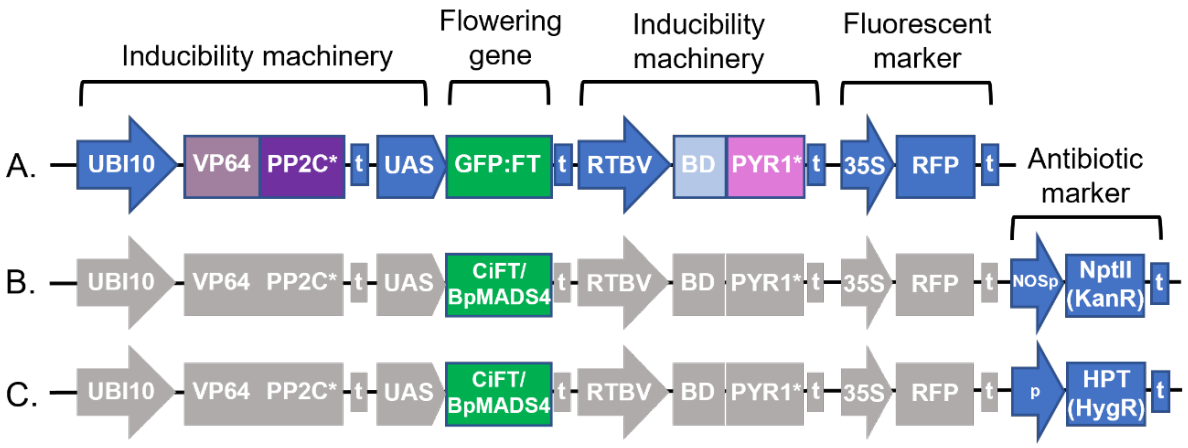


Figure 1. Construct development. A. Original construct received from Cutler lab. B. Construct developed in Year 1, containing flowering genes for pear and a Kanamycin-resistance gene (NptII). C. Construct developed in Year 2 containing a Hygromycin-resistance gene (HPT), replacing KanR.

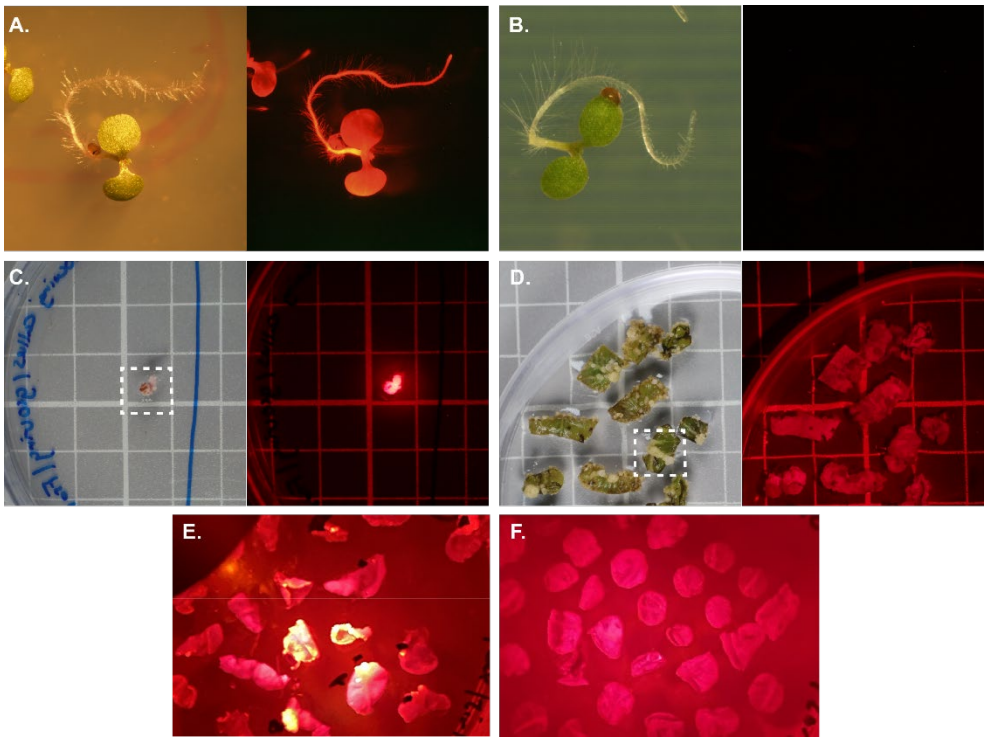


Figure 2. Red fluorescent marker indicates transformation of Arabidopsis and pear callus tissue. A. Arabidopsis seedlings that have been successfully transformed with the RCB construct and selected on Kanamycin, in white light (left) and green light to excite the red fluorescence (right). B. Arabidopsis 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.

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 right conditions.

Throughout year 3 we have 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.

A report from research on ‘OHxF 333’ regeneration used sorbitol as a carbon source, as opposed to sucrose, which led us to compare carbon sources in transformation trials (9). We compared 3 treatments: full replacement of sucrose with 30g/L sorbitol in the solid regeneration media, 1:1 ratio of sucrose to sorbitol (15g sucrose and 15g sorbitol/L), and sucrose. Sorbitol appeared to make no improvement on regeneration and thus we continued to use sucrose as a carbon source (RCB_230315, Table 1).

After identifying improved hormone combinations for adventitious shoot regeneration in our gene-editing project, we applied these hormone combinations to our transformation trials. From this point forward, we used 9uM TDZ as cytokinin and 4.9uM IBA as auxin in our solid regeneration media (post co-cultivation) (RCB_230622 and later, Table 1). In most recent experiments, we have also included an additional step of transferring leaf discs to a second shoot expression media after the first 30 days, which contains 4.4uM TDZ and no auxin.

Through personal communication, our colleagues at UC Davis informed us that transformation systems in some other plant species include a “pre-culture” step prior to inoculation, which allows callus to begin forming before agrobacterium is introduced. Upon literature review, we were able to find a couple of reports in poplar and one in apple that included and explained this step (10-12). To test this in pears, we excised leaves, soaked them, and cut leaf discs as usual, then allowed leaf discs

to grow on solid regeneration media (we used NN69 with 22uM TDZ and 10uM NAA) for 5 days prior to inoculation with agrobacterium, then continued the rest of the protocol as usual. A total lack of red fluorescent tissue in this trial led us to not include this step in future attempts.
The media on which pears are grown on during micropropagation can influence adventitious shoots regeneration (13). Communication with cooperators on this project suggested that this may be applied to increase the competency of cells to regenerate after transformation as well. Thus, we conducted a transformation trial with leaves grown on three different basal medias (PM2, QL, DKW (13, 14)), with or without different cytokinin treatments (no cytokinin, 4.4uM BA, or 5uM meta-Topolin (mT)). Plantlets were stripped of leaves and transferred to the different medias, and allowed to grow for 8 weeks (two 4-week rounds of transfers to fresh media). Leaves were then excised and the protocol was followed as usual, including the modifications of using 9uM TDZ and 4.9uM IBA in regeneration media and transfer of leaf discs to 4.4uM TDZ, auxin free shoot expression media after 30 days.

In the coming year, we will be 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 containing the construct of interest) to enhance transformation rates of very difficult-to-transform cultivars of eucalyptus and poplar (16). In initial trials, we will inoculate pear discs in a combination of S82 with Agrobacterium containing our RCB construct, mixed at three different ratios: 1:1, 1:10, and 1:25. Further, we will test three different conditions for co-cultivation: liquid NN69 media (as we have used previously, and helps with avoiding oxidation of leaf disc tissue), semi-solid NN69 media with a low concentration of Gelzan as gelling agent, and fully solid NN69 with a higher concentration of Gelzan. We are including this test, as S82 may overgrow in the liquid media. After co-cultivation and removal of both agrobacterium strains using antibiotics, leaf discs will be transferred to media containing no hormones, as the S82 strain naturally produces hormones that encourage plant regeneration. Leaf discs will be grown in darkness for 2 weeks and moved to unlit shelves, as with our standard protocol. Similarly, callus transformation will be recorded at 4 weeks and regeneration at 8 weeks.

We will also test transformation of our RCB construct into ‘Conference’, using published protocols designed to work for this cultivar specifically (2). While ‘Conference’ is not our target germplasm, this will help us understand more about our RCB construct, different transformation requirements for different cultivars, whether published protocols contain all necessary information needed to repeat experiments.

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 have 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. We have extract DNA from these plants, using a Qiagen DNeasy Plant Pro kit, and will use PCR to see if we can detect the transgene in these plants. Transgene DNA from agrobacterium will be used as a positive control, and non-transformed pear tissue will be used as a negative control. 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. 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
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. 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 (17-19), 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.

Experiment ID	Experimental parameters tested	Cultivar	Callus transf.	Shoot trans.	Control regen.	Notes
RCB_221205	From Year 2, for reference: Testing leaf punches and liquid co-cultivation media	Bartlett	65/150	0	ND	
RCB_221215	From Year 2, for reference: Second round of RCB_221205	Bartlett	35/173	0	ND	
RCB_230315	Testing carbon source for growth of callus and potential transformants: Sucrose, Sorbitol, 1:1	Bartlett	22/50 (sucrose) 14/49 (sorbitol) 15/49 (1:1)	0	1/20 (sucrose) 0/20 (sorbitol) 0/22 (1:1)	Prior to finding improved hormone combination or second "expression" phase.
RCB_230316	Testing Bartlett parameters with OHxF 97	OHxF 97	62/101	0	10/26	Prior to finding improved hormone combination or second "expression" phase.
RCB_230622	Testing initial hormone combination found from regeneration studies:	Bartlett, OHxF 87	56/90 (Bartlett) 53/85 (87)	0	5/10 (Bartlett) 3/11 (87)	Prior to adding shoot expression phase.
RCB_230715	Testing pre-culture method: Allowing callus formation prior to transform. with agrobacterium	Bartlett, OHxF 87	0/75	0	ND	No red spots found - did not continue with these plants.
RCB_230928	Testing microprop. media used to grow leaves prior to transform.: PM2, PM2 + BA, PM2 + mT, DKW, DKW + BA, DKW + mT	Bartlett	ND	ND	ND	Majority of boxes in experiment had fungal contamination - unable to determine results.
RCB_231016	Testing micropropagation media used to grow leaves prior to transform.: QL + BA, QL + mT, DKW + BA	Bartlett	64/55 (DKW+BA) 54/69 (QL+mT) 22/56 (QL+BA)	0	0	Control results dhowed auxin used for shoot expression phase, IBA, had degraded.

Table 1. Callus transformation and adventitious shoot regeneration results from trials in Year 3. Callus transformation is reported as (# of glowing red cell clusters/ total # leaf discs in experiment). Transformed shoots are reported as (# transformed shoots / total # leaf discs). Regeneration in the control leaf discs is reported as (# regeneration shoots / total # leaf discs in control). ND - not determined.

Results and Discussion

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. Here we report continued successful transformation of pear callus tissue (Fig. 2), but 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. 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.

This year, we found that when our base protocol was applied to 'OHxF 97', we saw similar, if not higher, amounts of callus transformation, indicated by the number of red fluorescent cell cluster in the callus tissue (RCB_230316, Table 1). With the modified protocol, which included optimized hormones in the initial regeneration media, we saw similar numbers of red fluorescent clusters between 'Bartlett' and 'OHxF 87' (RCB_230622, Table 1). 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.
Early results from growing plants on different micropropagation medias prior to transformation were promising, even with losses of some groups due to fungal contamination. When plants were grown on DKW media supplemented with 4.4uM BA or QL media supplemented with 5uM meta-Topolin, the number of fluorescent red cell clusters within callus tissue, per total number of leaf discs transformed, was much higher (RCB_231016, Table 1). This suggest that growth on these medias improve the competency of callus tissue to be transformed with Agrobacterium. Moving forward, we will use these media for growing 'Bartlett' leaves prior to transformation, as well as test different media types for 'OHxF 87' and 'OHxF 97'.

Several parameters tested this year had little effect or a negative effect on callus transformation: replacement of sorbitol as a carbon source, and pre-culturing leaf discs prior to transformation. The number of fluorescent red cell clusters in the callus tissue was similar or slightly lower after transformations in which sorbitol was used instead of sucrose, or when they were used in a 1:1 ratio. This test used 'Bartlett' as a cultivar, and we cannot rule out sorbitol as an optimized carbon source for other cultivars. Pre-culturing by growing leaf discs on regeneration media prior to inoculation results in a total lack of callus transformation. This may be due to the amount of time that wounded tissue was allowed to heal prior to inoculating. Pre-culturing for a shorter time, or pre-culturing leaves prior to wounding may useful tests to try in the coming year.

The altruistic transformation method that we aim to apply in the coming year has proved successful for difficult-to-transform plants in the Strauss lab at Oregon State University (16). This method relies on using a strain of Agrobacterium that still contains some of its natural genes, which sense the plant cell environment and stimulate production of tissue growth through auxin and cytokinin production. These genes have been removed in most agrobacterium strains we use in laboratory settings.

Researchers in the Strauss lab found that when S82 was co-transformed with a standard agrobacterium (containing a construct-of-interest, initially a marker gene), some callus tissue is transformed with S82, and some with the construct-of-interest. The S82 callus tissue generates a large amount of callus tissue but doesn't regenerate adventitious shoots. Instead it sends signals to the callus tissue transformed with the construct-of-interest to regenerate adventitious shoots (16). The S82 construct contains a different fluorescent marker from the construct-of-interest, and thus callus tissue can be easily identified from one another. Upon deeper literature review, we found a similar approach applied previously in an Asian pear species (*P. betulaefolia*), which allowed for adventitious shoot regeneration after transformation in a very difficult-to-transform species (20). We are excited about applying this work to European pears in the coming year, particularly with more difficult-to-regenerate cultivars.

An unexpected reduction in staff down to one technician for most of this year, as well as recent challenges with fungal contamination in the lab, led to some delays and losses in our experiments. However, we have recently been able to hire new staff and find professional cleaning service to help clean and mitigate future contamination issues. We are very hopeful to make even more progress in the coming year.

References

1. S. Montanari, J. Postman, N. V. Bassil, D. B. Neale, Reconstruction of the Largest Pedigree Network for Pear Cultivars and Evaluation of the Genetic Diversity of the USDA-ARS National Pyrus Collection. *G3 (Bethesda)* **10**, 3285-3297 (2020).
2. F. Mourgues, E. Chevreau, C. Lambert, A. de Bondt, Efficient Agrobacterium-mediated transformation and recovery of transgenic plants from pear (Pyrus communis L.). *Plant Cell Rep* **16**, 5 (1996).
3. N. Matsuda *et al.*, Early Flowering Phenotype in Transgenic Pears (Pyrus communis L.) Expressing the CiFT Gene. *Journal of the Japanese Society for Horticultural Science* **78**, 410-416 (2009).
4. H. Flachowsky, A. Peil, T. Sopanen, A. Elo, V. Hanke, Overexpression of BpMADS4 from silver birch (Betula pendula Roth.) induces early-flowering in apple (Malus×domestica Borkh.). *Plant Breeding* **126**, 137-145 (2007).
5. Q. T. Fu *et al.*, An efficient protocol for Agrobacterium-mediated transformation of the biofuel plant Jatropha curcas by optimizing kanamycin concentration and duration of delayed selection. *Plant Biotechnol Rep* **9**, 405-416 (2015).
6. N. Matsuda, M. Gao, K. Isuzugawa, T. Takashina, K. Nishimura, Development of an Agrobacterium-mediated transformation method for pear (Pyrus communis L.) with leaf-section and axillary shoot-meristem explants. *Plant Cell Rep* **24**, 45-51 (2005).
7. M. L. Ming *et al.*, Highly efficient CRISPR systems for loss-of-function and gain-of-function research in pear calli. *Hortic Res-England* **9** (2022).
8. J. P. Nitsch, C. Nitsch, Haploid plants from pollen grains. *Science* **163**, 85-87 (1969).
9. N. Dimitrova, L. Nacheva, An Optimized Micropropagation Protocol by Ex Vitro Rooting of Pear Rootstock OHF 333 (Pyrus communis L.). *Acta Agrobotanica* **74**, 1-9 (2021).
10. R. Sharma, M. Modgil, P. Sharma, U. Saini, Agrobacterium-mediated transfer of chitinase gene in apple (Malus x domestica Borkh.) rootstock MM106. *Indian J Hortic* **69**, 1-6 (2012).
11. P. Maheshwari, I. Kovalchuk, Agrobacterium-Mediated Stable Genetic Transformation of Populus angustifolia and Populus balsamifera. *Frontiers in Plant Science* **7** (2016).
12. M.-S. Kim, N. B. Klopfenstein, Y. W. Chun, "Agrobacterium-mediated Transformation of Populus Species" in Micropropagation, genetic engineering, and molecular biology of Populus*,* N. B. Klopfenstein, Y. W. Chun, M.-S. Kim, M. R. Ahuja, Eds. (USDA Forest

Service, Fort Collins, CO, 1997), vol. General Technical Report RM-GTR-297, chap. 8, pp. 51-59.
13. R. L. Bell, C. Srinivasan, D. Lomberk, Effect of nutrient media on axillary shoot proliferation and preconditioning for adventitious shoot regeneration of pears. *In Vitro Cell Dev-Pl* **45**, 708-714 (2009).
14. B. M. Reed, J. Denoma, S. Wada, J. Postman, Micropropagation of pear (Pyrus sp.). *Methods Mol Biol* **11013**, 3-18 (2013).
15. F. B. Laboratory, Presentations. *Forestry Biotechnology Laboratory, https://biotechlab.forestry.oregonstate.edu/presentations*, Accessed 12 Jan 2024 (2023).
16. S. Strauss, G. S. Goralogia, 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 (2023).
17. S. Montanari *et al.*, A QTL detected in an interspecific pear population confers stable fire blight resistance across different environments and genetic backgrounds. *Molecular Breeding* **36** (2016).
18. A. Peil *et al.*, Improvement of Fire Blight Resistance in Apple and Pear. *International Journal of Plant Breeding* **3**, 1-27 (2009).
19. J. D. Zurn, J. L. Norelli, S. Montanari, R. Bell, N. V. Bassil, Dissecting Genetic Resistance to Fire Blight in Three Pear Populations. *Phytopathology* **110**, 1305-1311 (2020).
20. J. Kaneyoshi, H. Wabiko, S. Kobayashi, T. Tsuchiya, *Agrobacterium tumefaciens* AKE10-mediated transformation of an Asian pea pear, *Pyrus betulaefolia* Bunge: host specificity of bacterial strains. *Plant Cell Reports* **20**, 622-628 (2001).

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

Report Type: Final Project Report

Primary PI: **Jessica Waite**
Organization: USDA-ARS Tree Fruit Research Lab
Telephone: 509-209-7970
Email: jessica.waite@usda.gov
Address: 1104 N. Western Ave.
Address 2:
City/State/Zip: Wenatchee, WA 98801

Co-PI 2: Pat Brown
Organization: UC Davis
Telephone: 530-752-4288
Email: pjbrown@ucdavis.edu
Address: Dept. of Plant Sciences, One Shields Ave., 1057 Wickson Hall
Address 2:
City/State/Zip: Davis, CA 95616

CO-PI 3: Charles Leslie
Organization: UC Davis
Telephone:
Email: caleslie@ucdavis.edu
Address: Dept. of Plant Sciences, One Shields Ave., 1055 Wickson Hall
Address 2:
City/State/Zip: Davis, CA 95616

Co-PI 4: Franklin Lewis
Organization: UC Davis
Telephone:
Email: flewis@ucdavis.edu
Address: Dept. of Plant Sciences, One Shields Ave., 1092 Wickson Hall
Address 2:
City/State/Zip: Davis, CA 95616

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: Requested and Awarded
Funding Duration: Awarded 2022-23 and 2023-24, Requested 2024-25
Amount: Awarded $8994 (2022-23), $8289 (2023-24), Requested $8234
Agency Name: California Pear Advisory Board
Notes: We were awarded funding for this project for the previous two years, and this year have requested another, final year of funding from CPAB

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Jessica Waite
Organization Name: USDA-ARS Tree Fruit Research Lab
Contract Administrator: Mara Guttman & Sharon Blanchard
Telephone: 510-559-5619 (MG), 509.664.2280 (SB)
Contract administrator email address: mara.guttman@usda.gov, sharon.blanchard@usda.gov
Station Manager/Supervisor: Dave Rudell
Station manager/supervisor email address: david.rudell@usda.gov

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

Objective 1: Optimize methods for tissue generation needed for protoplast isolation and plant recovery.
Objective 2: Optimize methods for generating pear protoplasts from *in vitro* tissues.
Objective 3: Design and generate gene-editing machinery and introduce into plant cells.

Significant Findings

- Characterized different callus tissue types in 'Bartlett' and 'OHxF 87' resulting from using different hormone inputs after wounding leaves.
- Optimized adventitious shoot regeneration in 'Bartlett' and partially in 'OHxF 87' and 'OHxF 97'.
- Waite Lab learned protoplast isolation methods from Brown Lab and began applying these techniques in both locations.

Methods

This project was co-funded by both the Fresh and Processed Pear Committees and the California Pear Advisory Board. Work took place in both the Waite Lab in Wenatchee and the Brown Lab at UC Davis. Each lab designed experiments to tackle distinct and overlapping parts to the objectives. The methods and results specify the different experiments done in each lab.

Plant Materials:

For micropropagation in the Waite Lab, shoots were sub-cultured in Magenta GA-7 boxes (Magenta Corp., Chicago, IL, USA) with 50 ml medium per container. For Bartlett, the base medium used was PM2 (Pear Medium 2) which is similar to (MS) (Murashige and Skoog, 1962) but contains 2x of all mesos (Ca, Mg, P minerals), as well as 2.5 mg/L thiamine, 250 mg/L myo-inositol, 3% w/v sucrose, 4.4 µM 6-benzylaminopurine (BAP), 0.6% agar (A111, PhytoTechnology Labs, Shawnee Mission, KS, USA) adjusted to pH 5.7 and autoclaved. For OHxF97, the basal medium used was Pear Rootstock (PRS-propagation) medium, which is similar to PM2 but contains 2.5x MS level of mesos (Ca, Mg, P minerals). OHxF87 was also grown on PRS-propagation medium, but with 1.2x of MgSO4 (instead of 2.5x). Shoots were transferred into fresh medium every four weeks and multiplied. Pear shoot cultures were grown at 20°C under a 16-h photoperiod with an average of 50 µmol/m2s irradiance.
In vitro shoots of Bartlett pear obtained from the Waite Lab were maintained in the Brown Lab on Murashige and Skoog (MS) media modified with 5 µM BAP, 0.5 µM indole-3-butyric acid potassium salt (K-IBA), 3% w/v sucrose, and 0.6% w/v A111 agar with pH adjusted to 5.7 before autoclaving. Cultures were kept under a 16-hr photoperiod with transfer every 3 weeks.

Tissue regeneration (Obj. 1):

All tissue regeneration experiments in the Waite lab used NN69 with 2% sucrose and 0.8% gellan gum (Gelzan™ G3251, Phytotech Labs) as a base media, unless otherwise noted (Nitsch and Nitsch, 1969). For Experiment 1, Phase 1, recently unfurled leaves from 'Bartlett' and 'OHxF 87' were removed from micropropagated plants and soaked for 1 hour in liquid NN69 media containing 2% sucrose, 10µM NAA, and 22.7µM TDZ. 20-30 leaf discs per treatment were removed from the leaves using a 4mm biopsy punch, placed back in the liquid soaking media until all discs were made and placed on solid media containing 1 of 6 treatments (see Table 1). Leaf discs were punched from the petiole-end of the leaves (2 discs per leaf) and contained midrib tissue, both of which contain tissue

that is more competent to regenerate adventitious shoots. Three replicate experiments were performed. Leaf discs were left in the dark on these treatments at 20C for 30 days and callus quality and shoot regeneration was recorded. For Phase 2, leaf discs were transferred to media containing 4.9µM IBA and 9µM TDZ for a subsequent 30 days and shoot regeneration was recorded, and for Phase 3, leaf discs were transferred to media containing only 9µM TDZ as the cytokinin, without auxin. Regeneration rates were recorded after 30 days. For Experiment 2, leaf discs were harvest and soaked in the same way as for Experiment 1. 16 leaf discs were used per treatment. Leaf discs were placed on 1 of 4 treatments (see Table 2) and placed at 20C in the dark. Leaf discs were examined at 3, 5, 7, 10, 15, 21, and 30 days for callus formation and shoot regeneration. Three replicate experiments were performed, and a fourth replicate was kept in the dark for the full 30 days and examined at the end. After 30 days, leaf discs were transferred to plates containing a lower level of TDZ (4.5µM) only, and regeneration was recorded after an addition 30 days (60 days total). Following these experiments, regeneration has been carried out with the following protocol for Bartlett: leaves removed and soaked 1 hour in liquid NN69 media containing 2% sucrose, 10µM NAA, and 22.7µM TDZ, transferred to solid NN69 media with 2% sucrose, 0.8% gellan gum, 4.9µM IBA, and 9µM TDZ and grown in the dark for 30 days, then transferred to solid NN69 media with 2% sucrose, 0.8% Gelzan, and 4.5µM TDZ and grown in the dark for an additional 30 days.

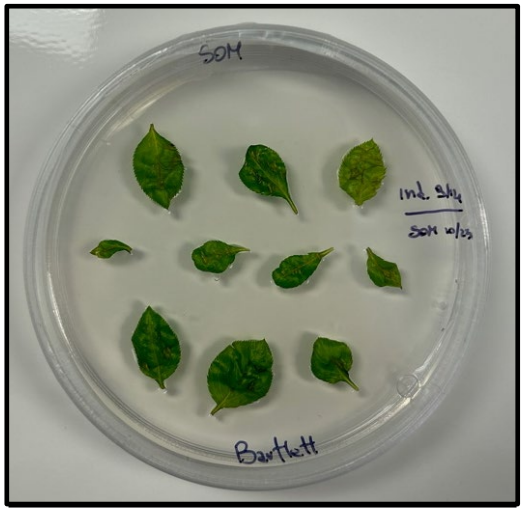
Table 1. Callus Induction Treatments for Exp. 1

		Cytokinin	
		13.6µM TDZ	22.7µM TDZ
Auxin	1µM NAA	T1	T2
	10µM NAA	T3	T4
	4.5µM 2,4-D	T5	T6

Table 2. Regeneration Treatments for Exp. 2

	Auxin	Cytokinin	Base media
Treatment 1	10µM NAA	22.7µM TDZ	NN69
Treatment 2	10µM NAA	22.7µM TDZ	MS
Treatment 3	4.9µM IBA	9µM TDZ	NN69
Treatment 4	4.9µM IBA	9µM TDZ	MS

Shoot organogenesis experiments in the Brown lab were performed by Giuseppe Vaia, a visiting scholar from the University of Tuscia. The first 5-6 apical leaves excised from 3-week-old shoot were used as starting explants for adventitious shoot induction experiments. The adaxial surface of each leaf was randomly wounded with forceps and placed (10 per plate), adaxial side up, on shoot organogenesis medium (SOM) (Figure 1), consisting of MS modified basal medium with Gamborg vitamins (PhytoTech, M404) supplemented with an additional 100 mg/L of myo-inositol, sucrose 3% (w/v), 15 µM of thidiazuron (TDZ) and 1µM 1-napthaleneacetic acid (NAA) (pH 5.7, gelled with 0.6% agar – PhytoTech A111).



Three additional different compounds were tested by adding to the SOM, at the concentration commonly reported in literature: silver nitrate ($AgNO_3$) 10 mg/L, salicylic acid 10 mg/L, and cefotaxime 200 mg/L. Previous works have reported improved plant regeneration using inhibitors of ethylene such as silver

nitrate and salicylic acid (Plus et al., 1993; Chae and Park, 2012; Park et al., 2012). Cefotaxime, is also known to enhance callus growth and plant regeneration. It is assumed that plant enzymes called esterases can break down cefotaxime to new compounds that might have growth-regulating properties. Moreover, it was proposed that cefotaxime might inhibit ethylene production in cultures, which is positively correlated with plantlet differentiation from the callus mass.

Plant protoplast isolation and digestion (Obj. 2):

Protoplast isolations in the Waite Lab were performed using 0.5 g of recently unfurled fully expanded leaves obtained from 'Bartlett' tissue culture plants transferred to and grown for 3 weeks on QL media containing 5µM *meta*-Topolin (*m*T) as cytokinin. Harvested leaves were cut into thin 1-2 cm ribbons and submerged in 5 mL enzymatic digestion buffer. Enzymatic digestion was performed using 1% Cellulase RS, 0.2% Pectinase and 0.2% Macroenzyme R10 dissolved in buffer containing 5mM MES, 10mM CaCl, 11% mannitol as the osmoregulator, 0.1% BSA, and 0.3% glycine (pH 6.0). All enzymatic digestions were carried out at either 22C for 12 or 16 hours or at 25C for 6 hours on a rotary shaker at 40 rpm. The digest solution was filtered through a 45µm nylon mesh filter (Sigma) into 50 mL conical tubes. Protoplasts were separated from the digestion solution by centrifugation at 100xg for 10 mins at 22C. Purification of protoplasts was performed as previously described elsewhere (Ochatt and Power, 1988) using a modified CPW buffer containing 5 mM HEPES, 100mM CaCl, 40uM glycine and 600uM mannitol containing 200 uM BSA. Protoplast were visualized using Evans blue staining on an Olympus BX53 microscope and Olympus DP74 attached camera, using the 40x objective and 10x ocular (for a total of 400x).

Protoplast isolations in the Brown lab were also performed by Giuseppe Vaia. The first attempt followed the grape protoplast isolation protocol described by Tricoli (2019), however, there were incomplete digestions and the tissues browned quite badly. To fight the enzymatic browning, we tested plasmolysis, which involves using a high-solute-containing solution to allow the cell membrane to pull away from the cell wall. Tissue was soaked for 1 h in 3 ml of osmotically adjusted washing solution (WS) containing 0.6 M mannitol, 3 g/L Glycine, 2mM $CaCl_2$, 0.1% bovine serum albumin (BSA) and 0.12% HEPES, and the addition of a modified Tricoli (2019) antioxidant mix (AOx) to the enzyme solution. The antioxidant mix consisting of 0.1% ascorbic acid, 0.15% sodium citrate dihydrate, 0.1% N-Acetyl-L-cystein and 0.03% L-Glutathione reduced.
Protoplasts of 'Bartlett' were isolated from tissue derived from shoot organogenesis pre-conditioning, obtained as described above. These tissues were primarily callus but contained some leaf tissue. Approximately 0.5 g of material was collected and then sliced with a scalpel blade and immediately transferred to a 3 ml of a cell-wall digestion enzymatic solution composed by 0.5% Cellulase Onozuka RS, 0.25% Macerozyme R10, 0.25% Pectinase, 1% BSA, 5 mM $CaCl_2$, 5 mM 2-(N-morpholino) ethane sulfonic acid (MES), 3% Glycine and 0.6 M mannitol, pH adjusted to 6.0 (Tricoli, 2019), the solution was filter-sterilized with 0.2 µm nylon mesh.
The containers (Nalgene screw-top) were placed in a rotary shaker at room temperature in the dark at 50-60 rpm overnight. After approximately 16 hours incubation, the protoplast solution was filtered through a 40 µm screen and the protoplasts were collected by pelleting via centrifugation at 350 rpm (26 *g*) for 10 minutes. The supernatant was discarded and the pellet of protoplasts was slowly re-suspended in 3 ml of osmotically adjusted washing solution (WS), after they were centrifuged again at 350 rpm for 10 minutes.
Protoplasts were purified using a dextran gradient consisting of 2 ml of a 13% dextran solution, containing also 0.4 M sucrose, 2mM $CaCl_2$, 0.1% BSA and 0.12% HEPES, overlaid with 2 ml of 0.6 M WS. Protoplasts in dextran gradient were then centrifuged at 350 rpm for 8 minutes. The ring of viable protoplasts, visible in the layer interface, was aspirated by using a Pasteur pipette.

Gene editing machinery and methods for introduction (Obj. 3):

We have identified several companies that manufacture ribonucleoproteins (RNPs) for delivering CRISPR machinery into plant cells. We will be using a Phytoene Desaturase (*PDS*) gene as an initial target, as resulting plant material is identifiable by its white tissue, due to a lack of chlorophyll. The specific PDS gene in pear we will target is Pycom04g02050.

RESULTS AND DISCUSSION

Tissue Regeneration

In both labs, culture of leaf explants showed 100% callogenesis (growth of callus) after 4 weeks, regardless of regeneration media or leaf explant type (full leaves or discs), concentrated particularly in

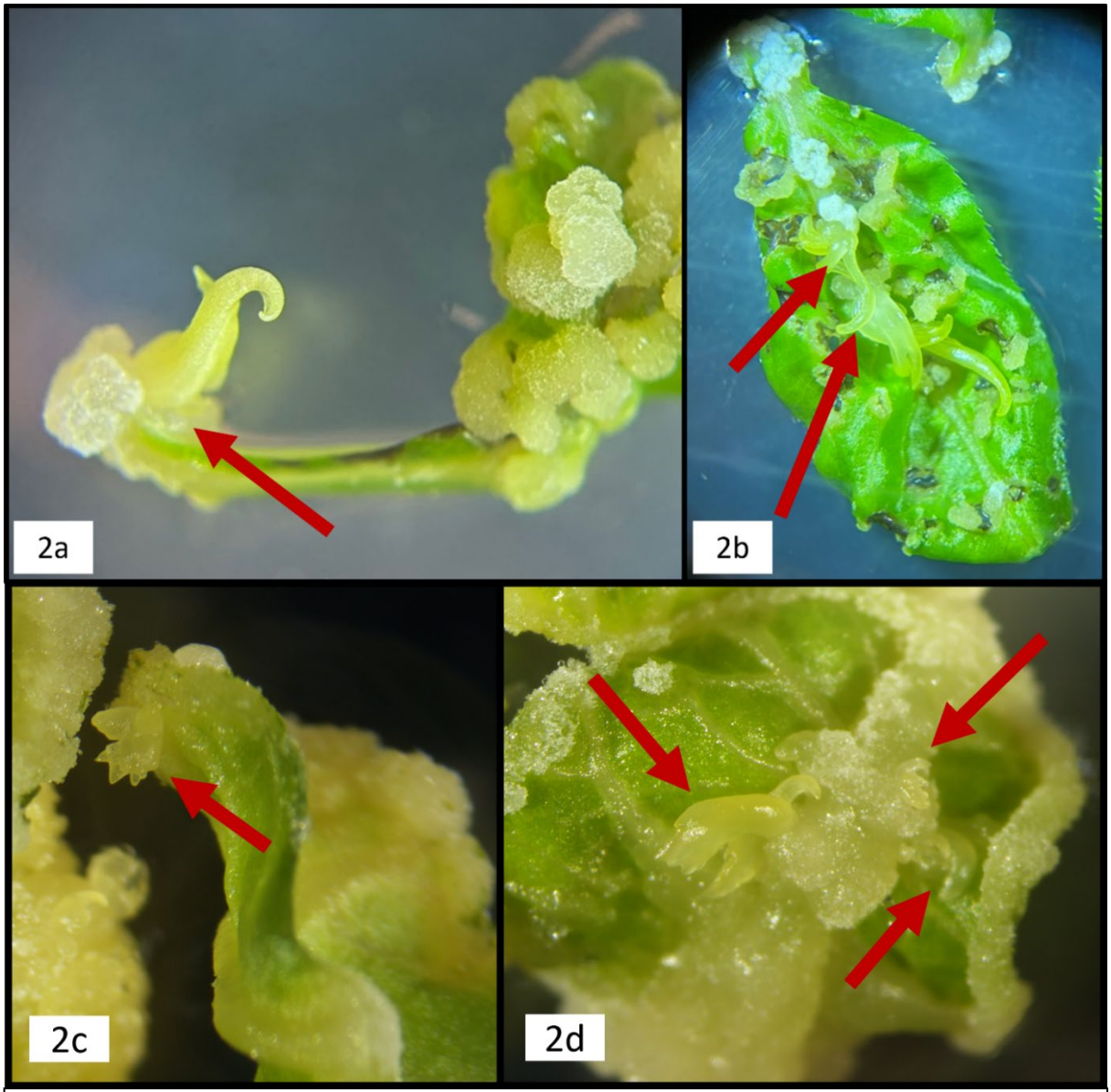


Figure 2. Adventitious shoots forming on full leaf explants (2a and b) and leaf discs (2c and d). Shoots are localized in the petiole (2a) and midrib (2b and d) areas, and occasionally at wound sites on leaf discs (2d).

the petiole, midrib and wounded areas. However, the regenerated shoots developed mainly in the petiole and midrib area (Figure 2), concentrated in the proximal area of the leaf, while adventitious buds were rarely or never observed in the wounded areas.

The Waite lab worked toward optimizing tissue regeneration from leaf discs, starting with 'Bartlett' and 'OHxF 87'. Previously, callus production on 'Bartlett' leaf discs occurred on all leaf discs, but the efficiency of regeneration of adventitious shoots from that callus was low – less than 10%. Note that for experiments in the Waite Lab, regeneration efficiency is reported as total number of adventitious shoots divided by total number of leaf discs, x100, which does not account for average number of shoots per leaf disc, which we will record for all future experiments. To better understand how different hormone types, levels, and combinations affected callus formation and quality, we performed an experiment subjecting 'Bartlett' and 'OHxF 87' leaf discs to six different hormone

		Phase 1		Phase 2		Phase 3	
		Average	SEM	Average	SEM	Average	SEM
Bartlett	T1	4.68	0.37	2.92	1.48	2.92	1.48
	T2	1.39	1.39	11.46	4.89	1.75	1.75
	T3	3.03	3.03	12.24	3.06	4.78	2.64
	T4	4.35	4.35	12.97	2.32	2.78	1.39
	T5	0	0	1.67	1.67	0	0
	T6	0	0	0	0	0	0
OHxF 87	T1	1.33	1.33	0	0	5.22	0.78
	T2	4.33	2.33	1.33	1.33	9.11	2.73
	T3	4.70	2.63	1.52	1.52	7.73	5.42
	T4	0	0	0	0	11.21	5.90
	T5	0	0	0	0	8.02	3.14
	T6	0	0	0	0	2.98	1.50

Table 3. Regeneration Rates for Phases 1, 2, and 3 of Experiment 1. Regeneration rates were calculated as total number of shoot regenerants divided by total number of leaf discs, x100 for percent values. Average of three replicate are presented, with standard error of the mean reported. Yellow highlights represent the three highest rates for each cultivar.

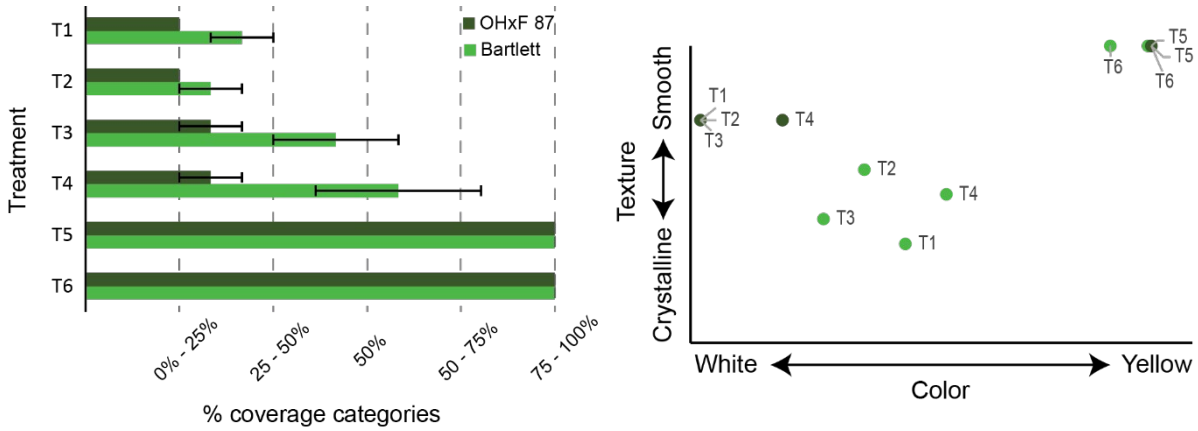


Figure 3. Callus growth/coverage and quality in response to hormone treatments. (Left) Average percent of leaf discs that were covered by callus tissue 30d after growth on the six different hormone treatments (Table 2). (Right) Texture and color of callus grown on different hormone treatments were observed, assigned a number category, and averaged across replicates. Dark green dots represent OHxF 87 on the six different treatments, light green dots represent Bartlett.

treatments (Table 1). Callus coverage and quality parameters were measured, as well as any shoot regeneration during the first 30 days (Figure 3 and Table 3). From this part of the experiment, we noted that treatments 1, 3, and 4 were highest for 'Bartlett' and had similar regeneration rates (4.7, 3.0, and 4.3%, respectively), and treatments 2 and 3 were highest for 'OHxF 87' (4.3 and 4.7%, respectively).

We performed a literature review covering regeneration from pear callus tissue, and identified a hormone treatment that had performed well for another group developing protocols for Pyrodwarf (4.9µM IBA and 9µM TDZ) (Vujovic et al., 2014). To test whether the callus types generated from Experiment 1, Phase 1 could regenerate equally in response this treatment, leaf discs were transferred to media containing these hormones and grown for an additional 30 days (Table 3). 'Bartlett' callus generated on Treatments 2, 3 and 4 responded well to this treatment, whereas any callus generated on the auxin 2,4-D (Treatments 5 and 6) showed almost no regeneration. 'OHxF 87' callus generated on all treatments showed low to no regeneration during Phase 2.

Our literature review also revealed that some groups have had regeneration success transferring callus tissue onto plates containing only cytokinin and no auxin (Leblay et al., 1991; Caboni et al., 2002; Bell et al., 2011). To test whether this would have a positive effect on our callus tissue, we performed one final transfer onto 1/2x MS media containing 9µM TDZ only. These plates contained either sucrose or sorbitol as a carbon source, but we saw little difference between these, and thus combined results are reported (Table 3). We saw some additional regeneration in 'Bartlett', for callus that was originally generated on Treatments 1-4. 'OHxF 87' rates increased, particularly for callus generated on Treatments 2-5. It is possible that this is a response to the treatment in Phase 3, or signifies delayed regeneration, as compared to 'Bartlett'. This question will require further exploration.

Based on these results, we decided to compare the best performing treatments from Experiment 1 (Treatment 4 from Phase 1 (10µM NAA and 22.7µM TDZ) and the hormone combination from Phase 2 (4.9µM IBA and 9µM TDZ)) with different base medias (NN69 and MS), and look at callus formation and shoot regeneration over time in each cultivar. Leaf discs were grown on these treatments (Table 2) for 30 days, observing callus formation at 3, 5, 7, 10, 15, and 30 days, and then

				Regen. @ 30d on treatment		Additional regen. after 30d on TDZ-only	Total regen. rates after 60 days
	Auxin	Cytokinin	Media	Avg	SEM	Avg	Avg
Bartlett	10µM NAA	22.7µM TDZ	NN69	0	0	18.75	18.75
			MS	0	0	6.25	6.25
	4.9µM IBA	9µM TDZ	NN69	15.63	4.03	71.88	87.50
			MS	7.81	2.99	42.19	50.00
OHxF 87	10µM NAA	22.7µM TDZ	NN69	0	0	0	0
			MS	0	0	0	0
	4.9µM IBA	9µM TDZ	NN69	9.38	1.80	26.52	35.90
			MS	7.81	1.56	23.89	31.71

Table 4. Regeneration rates for Experiment 2. Regeneration rates were calculated as total number of shoot regenerants divided by total number of leaf discs, x100 for percent values. Average of three replicate are presented, with standard error of the mean reported. At the 60 days timepoint, data from all 4 replicates (16 leaf discs each) was pooled, so standard error could not be calculated. TDZ-only plates contained 4.5uM TDZ and no auxin.

transferred to TDZ-only plates, this time with a lower concentration (4.5µM TDZ), and grown for an additional 30 days. For both 'Bartlett' and 'OHxF 87', growth on NN69 media containing 4.9µM IBA and 9µM TDZ, followed by transfer to 4.5µM TDZ, resulted in the best regeneration rates (Table 4). These same hormone combination with MS base media also performed well, but to a lesser extent. Since these experiments, we have continued use of the two-phase protocol, starting with 30 days on NN69 with 4.9µM IBA and 9µM TDZ, followed by transfer to 4.5µM TDZ for 30 more days, and have regularly seen 80-90% regeneration rates for 'Bartlett'. Again, this rate calculation represents total number of shoots per total number of leaf discs, not accounting for number of shoots per leaf disc, which we will record in future experiments. We noted that for this second TDZ-only phase, some leaf discs had multiple shoot per disc, while others had none.
These findings have helped us in regenerating and producing the tissue we need to isolate protoplasts, and knowledge of an optimized hormone combination for regeneration in 'Bartlett' will further be useful in regenerating tissue from the protoplasts themselves.

Results from the Brown Lab's experiments comparing regeneration capacity of the explants excised from rooting media and those excised from the multiplication media were significant, while the three media modifications (addition of silver nitrate, salicylic acid, or cefotaxime) showed no improvement over the standard organogenesis medium. Regeneration efficiency (calculated by the number of leaves with at least one shoot per total explants x 100) was more than 35% for leaves from the rooting media, while for leaves from the multiplication media it did not exceed 3%. Nevertheless, no difference has been observed about the average number of shoots per regenerating leaf that was around one/two, with some exceptions even up to three. Previous papers showed that regeneration capacity is strictly linked to pear genotype, and our results seem to be in line with those reported in the same cultivar (Yousefiara et al., 2014). In addition, the data from cited articles was measure 8 weeks after wounding (as well as other related articles), so we expect a continued increase in the number of shoots forming in the coming weeks.
Further studies in the Brown lab will focus on different hormones concentration and type of salts in the regeneration medium, since has been reported that ammonium/nitrate ratio were essential in shoot regeneration of pear (Leblay et al., 1991).

Plant protoplast isolation and digestion

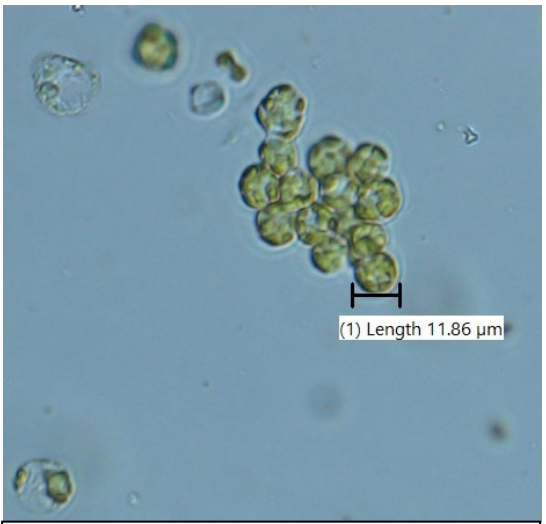


Fig 4. Bartlett protoplasts isolated in Waite Lab.

Members of the Waite lab were able to use CPAB funding this year to travel to the Brown lab at UC Davis and learn protoplast isolation protocols, resulting in both labs now being able to work towards this goal. Pear tissues have been difficult to fully digest and isolate protoplasts from. As a result, trials varying the digestion buffers and duration of digestion were performed. Digestions in the Waite lab carried out at 22C for 16 hours and 12 hours resulted in no visible protoplasts or non-viable protoplasts, respectively. Digestion at 25C for 6 hours yielded greater number viable protoplasts that were incompletely digested (Figure 4).
Results from the Brown Lab showed that addition of antioxidants improved protoplast isolations, resulting in a mixture that was clear and almost free of impurities and debris (Figure 5, right tube compared to left tube). This might be due to the production of phenolic compounds, which might substantially affect

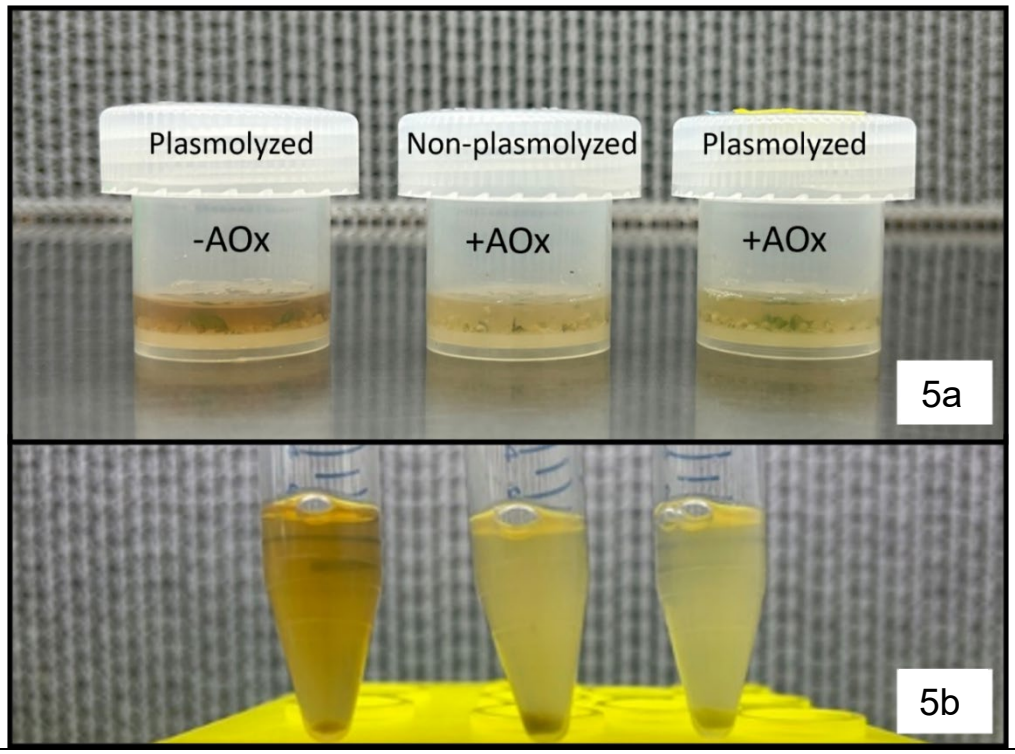


Figure 5. Cell-wall digestion enzymatic solution after 16h incubations (5a) and after the first centrifuge (5b). From left to right are the plasmolysed sample without antioxidant mix in the enzymatic solution, the non-plasmolysed sample with antioxidant mix in the enzymatic solution and the plasmolysed sample with antioxidant mix in the enzymatic solution.

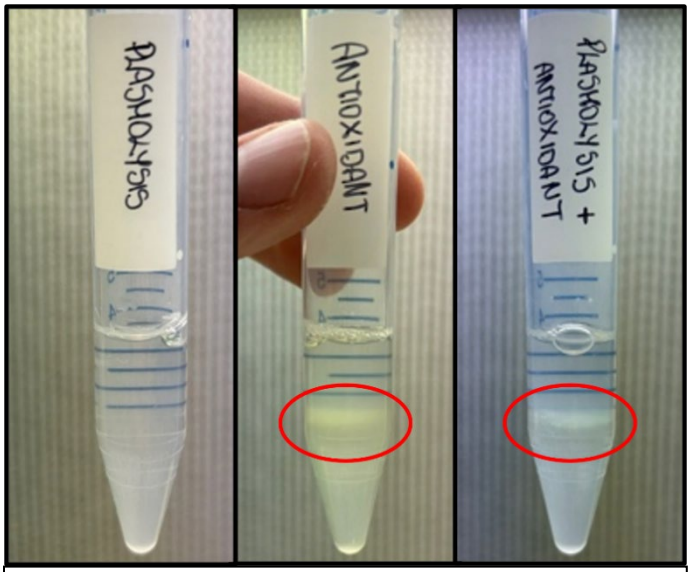


Figure 6. The ring of protoplasts, visible in the layer interface, after the centrifugation in dextran gradient.

the digestions of the cell walls. Indeed, after centrifugation, there were no visible protoplasts in the solution obtained without the antioxidant mix (Figure 6). When plasmolysis was tested (using high-solute solutions to separate the cell membrane from the cell wall), no differences were observed between plasmolysed and non-plasmolysed samples in terms of the solution color after 16 h incubations (Figure 5a) or the amount of protoplast visible in the layer interface after the dextran gradient (Figure 6). The protoplasts were harvested and counted using a counting chamber. The yield of the harvested protoplasts was around 1 x 10^6 cells per ml in the samples with the antioxidant mix in the enzymatic solution (Figure 6). These results showed the crucial importance of adding an antioxidant mix to the enzyme solution to prevent browning due to phenol production and achieve an improved protoplast yield.

Gene editing machinery

This year, we researched the specific *PDS* gene we will use as a gene-editing target. Starting with *PDS* as a target allows for quicker assessment of whether the gene-editing system is functional, as knocking out this gene results in white tissue as soon as plants are regenerated. The *PDS* gene in the Bartlett genome we will target is Pycom04g02050, which has been recently targeted in pear gene-editing, using a DNA-integrated system (Malabarba et al., 2021). Further, we have researched and found several biotechnology companies that manufacture CRISPR RNPs for gene editing that we can use for delivering the gene editing machinery, once we have generated protoplasts in the quantities needed for transformation.

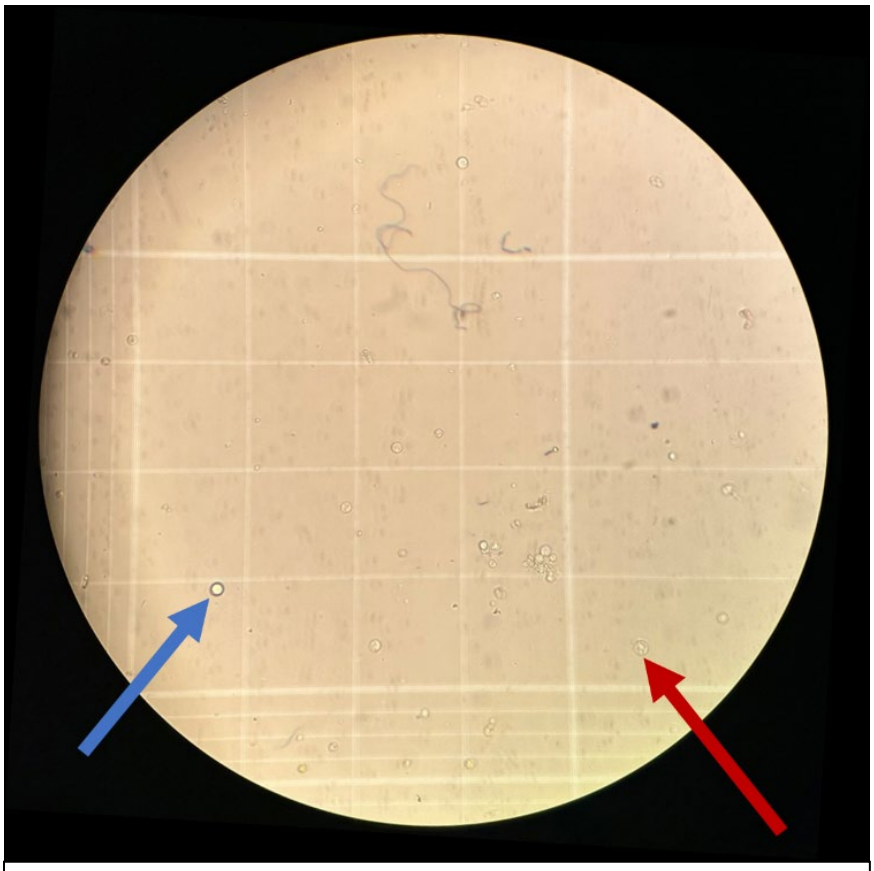


Figure 7. Counting of viable protoplasts (red arrow) with the help of counting chamber, observed with 100X magnification. 20 µL of protoplast solution have been diluted in 180 µL of washing solution before being counted. The spherical object indicated by the blue arrow is an air bubble and is not counted.

REFERENCES

Bell RL, Scorza R, Lomberk D (2011) Adventitious shoot regeneration of pear (Pyrus spp.) genotypes. Plant Cell, Tissue and Organ Culture (PCTOC) **108:** 229-236

Caboni E, D'Angeli S, Chiappetta A, Innocenti AM, Van Onckelen H, Damiano C (2002) Adventitious shoot regeneration from vegetative shoot apices in pear and putative role of

cytokinin accumulation in the morphogenetic process. Plant Cell Tissue and Organ Culture **70:** 199-206

Chae SC, Park SU (2012) Improved shoot organogenesis of DC treated with ethylene inhibitors. Life Science Journal-Acta Zhengzhou University Overseas Edition **9:** 1725-1728

Leblay C, Chevreau E, Raboin LM (1991) Adventitious Shoot Regeneration from Invitro Leaves of Several Pear Cultivars (Pyrus-Communis L). Plant Cell Tissue and Organ Culture **25:** 99-105

Malabarba J, Chevreau E, Dousset N, Veillet F, Moizan J, Vergne E (2021) New Strategies to Overcome Present CRISPR/Cas9 Limitations in Apple and Pear: Efficient Dechimerization and Base Editing. International Journal of Molecular Sciences **22**

Nitsch JP, Nitsch C (1969) Haploid plants from pollen grains. Science **163:** 85-87

Ochatt SJ, Power JB (1988) Plant-Regeneration from Mesophyll Protoplasts of Williams Bon Chretien (Syn Bartlett) Pear (Pyrus-Communis L). Plant Cell Reports **7:** 587-589

Park EH, Bae H, Park WT, Kim YB, Chae SC, Park SU (2012) Improved shoot organogenesis of gloxinia ('Sinningia speciosa') using silver nitrate and putrescine treatment. Plant Omics **5:** 6-9

Plus J, George L, Eapen S, Rao PS (1993) Enhanced plant regeneration in pearl millet (Pennisetum americanum) by ethylene inhibitors and cefotaxime. Plant Cell, Tissue and Organ Culture **32:** 91-96

Tricoli D (2019) Renewal Progress Report for CDFA Agreement Number# 18-0397. *In*,

Vujovic T, Ruzic D, Cerovic R (2014) Adventitious organogenesis via intermediate callus formation in representatives of Prunus, Pyrus and Rubus genera. Romanian Biotechnological Letters **19:** 9297-9309

Yousefiara M, Kermani MJ, Bagheri A, Habashi AA, Abdollahi H (2014) Induction of Direct Adventitious Shoot Regeneration in Pear (Pyrus communis L.). Plant Tissue Culture and Biotechnology **24:** 87-92

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. This year, we proposed to lay the groundwork for developing a transgene-free gene editing system in pears. To do this, we focused on optimizing adventitious shoot regeneration from pear callus tissue, began optimization of protoplast isolation from pear tissues, and researched gene targets and synthesis of gene editing machinery. Adventitious shoot regeneration from pear callus tissue was important for two reasons: allowing us to define a protocol for generating callus tissue that is competent to regenerate, and understanding the ideal hormone combinations each cultivar responds to for efficient regeneration. These will help us both to generate tissue for protoplast isolation and to regenerate plants from protoplasts. This year, we were able to screen different hormone combinations and identify an efficient protocol for 'Bartlett' callus formation and adventitious shoot regeneration. While we were able to increase efficiency slightly for OHxF 87 and 97, our future work will focus on improving efficiency for these cultivars. The two collaborating labs were able to meet this year and share methods, such that both groups have now begun the work of optimizing protoplast isolation. Our attempts thus far have narrowed the cell-wall digestion lengths but have struggled with partial digestion or oxidation issues. Future work will focus on testing different cell-wall digestion enzymes and concentrations, solution characteristics, and tissue sources. Finally, we identified the specific pear *PDS* gene and genetic sites to be targeted, as well as researched companies that can synthesize the RNPs we will use to introduce the gene-editing machinery into plant cells. All together, we took significant steps towards developing a transgene-free gene-editing system for pears and will continue working towards building this tool.

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

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

PI: Todd Einhorn
Organization: MSU
Telephone: 517-353-0430
Email: einhornt@msu.edu
Address:1066 Bogue St
Address 2:Soil Science Building
City/State/Zip: East Lansing/MI/48824

Co-PI (2): Stefano Musacchi
Organization: WSU-Wenatchee
Telephone: 509-663-8181 ext. 236
Email: stefano.musacchi@wsu.edu
Address: TFREC
Address 2: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI(3): Yongjian Chang
Organization: North American Plants, Inc.
Telephone: 503-474-1852
Email: ychang@naplants.com
Address: 9375 SE Warmington Rd.
Address 2:
City/State/Zip: McMinnville/OR/97128

Co-PI (4): Kelsey Galimba
Organization: OSU
Telephone: 541-386-2030 Ext. 38218
Email: kelsey.galimba@oregonstate.edu
Address: MCAREC
Address 2: 3005 Experiment Station Drive
City/State/Zip: Hood River/OR/97031

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

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

Other funding sources: **None**

WTFRC Budget: ***None***

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

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

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

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

Budget 2

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

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

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

Budget 3

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

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

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

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), the vast proportion of these rootstocks continued to perform very well. Genetic testing of all accessions indicated that four 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 somewhat surprisingly 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 supplied to Helios nursery. Helios nursery planted these in an OR field and grafted them with Comice interstems and, 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 22.001, 23.001, 57.001, and 65.001 were reported to have a high level of genetic similarity. These were in fact the accessions that had appeared to perform equivalently in the field. To make matters more interesting, these four accessions all grouped with Quince A, but not the Quince A developed in Europe and commercialized in Europe. The similarity was with Quince A found in the US, which differs markedly from the 'actual' Quince A. Further testing is being conducted from tissues sampled in 2023 to confirm these results. We will communicate the findings in a follow-up report when received. To be clear, there were no mixups in the plantings 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).

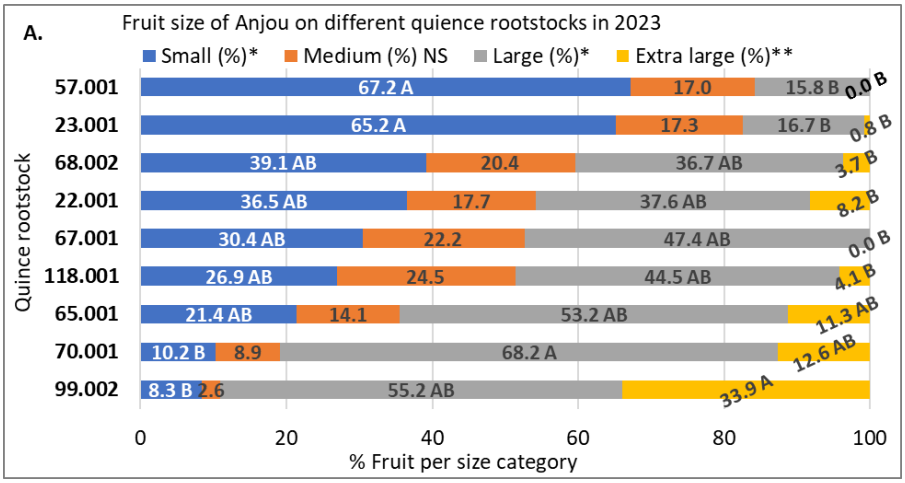
Table 4. Yield parameters for Bartlett and Anjou, with a Comice interstem grafted on quince accessions, on August 24th and August 29th, 2023 (harvest dates for Bartlett and Anjou, respectively). The means are averages of 3 trees per combination (N= 3). Significance, *p=<0.05, **p=<0.01, ***p=<0.001. 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



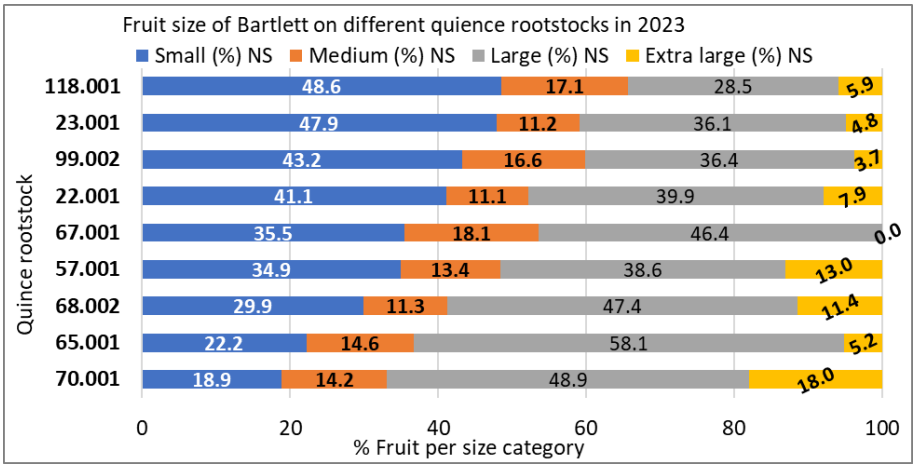


Figure 1. Fruit size distribution for 2023 harvest: **A.** Anjou with Comice interstem grafted on quince accessions, **B.** Bartlett with Comice interstem grafted on quince accessions. Fruit was harvested on Aug 24th, 2023 for Bartlett and Aug 29th, 2023 for Anjou and sorted on September 7th by the use 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.

Project Title: Pear Rootstock Breeding PR-22-102

Report Type: Continuing Project Report

Primary PI: Kate Evans
Organization: WSU TFREC
Telephone: 509-293-8760
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

Co-PI 2: Soon Li Teh (moved to new position at UMN 10.2.23)
Organization: WSU TFREC
Telephone: 509-293-8813
Email: soonli.teh@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

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

Project Duration: 3 Year

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
Primary PI: Kate Evans
Organization Name: WSU-TFREC
Contract Administrator: Anastasia (Stacy) Mondy
Telephone: 916-897-1960
Contract administrator email address: arcgrants@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) who is (was) the point person for pear rootstock (plans for re-hiring will be discussed); Wages for time-slip labor for orchard management and trait phenotyping; In-state travel between TFREC and orchards for orchard management and trait phenotyping.

OBJECTIVES

1. Develop seedling populations to produce new rootstocks
2. Conduct marker-trait association for rootstock-conferred traits in seedling populations
3. Validate stability/repeatability of preliminary dwarfing locus
4. Maintain a relevant pear rootstock parent germplasm
5. Evaluate B × A and B × C selections

SIGNIFICANT FINDINGS

- Approximately 2,000 *Pyrus* seedlings were evaluated for scion and rootstock vigor traits in winter 2023/2024.
- Ten precocious seedlings that were previously micropropagated (10 replicates per seedlings) are being maintained in the WSU TFREC hoop house.
- All 37 B × A and B × C trees produced flowers in spring 2023; 25 trees produced fruit which is helping further differentiate the selections.
- Further analysis of the two dwarfing loci identified (on chromosomes 5 and 15) was completed to characterize their haploblocks and their relative contributions to vigor reduction.

METHODS

Objective 1: Develop seedling populations to produce new rootstocks

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

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

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

Table 1: Existing data collected prior to the start of this project of various rootstock seedling and scion (d'Anjou) traits for breeding and selection.

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

In addition, these seedling populations are being leveraged through collaborations with Dr. Sindhuja Sankaran (WSU Department of Biological Systems Engineering) and Dr. Lee Kalcsits (WSU

Department of Horticulture) to develop more efficient, reliable and accurate phenotyping of vigor/dwarfing traits.

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

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

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

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

Objective 3: Validate stability/repeatability of preliminary dwarfing locus

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

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

Objective 4: Maintain a relevant pear rootstock parent germplasm

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

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

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

Previously, seedlings from crosses of 'Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice' that exhibited dwarf seedling stature in the greenhouse were selected and replicated (PI: Dhingra; "Establishing NW-acclimated *Pyrus* Rootstock Breeding Material; PR-14-107). In 2017, a total of 14

selections in triplicate (approximately 45 trees) were planted at the WSU Columbia View orchard. (PI: Evans; "Pear Rootstock Breeding"; PR-15-105). These trees were budded with d'Anjou. Evaluation for dwarfing potential and precocity is ongoing. Trees are just starting to fruit with six accessions bearing fruit in fall 2021. Ten of the 14 accessions did not bloom in spring 2021.

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

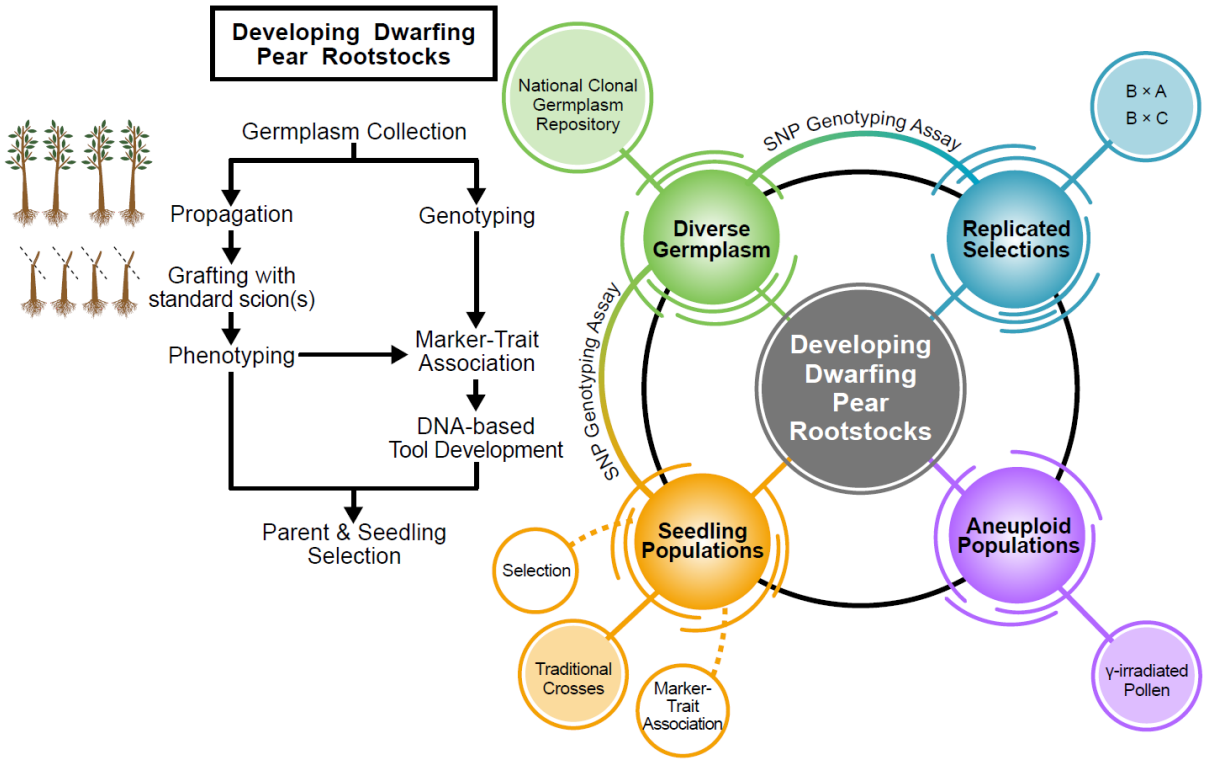


Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. Proposed endeavors include (a) expansion of existing seedling populations, (b) propagation of rootstock seedlings with 'd'Anjou', (c) collection of scion and rootstock phenotypic data, (d) DNA genotyping/sequencing,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

RESULTS AND DISCUSSION

Objective 1: Develop seedling populations to produce new rootstocks

Pear seedlings from cross years 2016, 2017 and 2019 (all budded with d'Anjou') were evaluated for scion and rootstock vigor traits in winter 2023/2024 (**Table 2**).

We currently have 4 years of robust vigor data for the ~600 seedlings (oldest seedlings in the ground) to make selections by the end of 2024 for replicated evaluation in Phase Two, which is beyond the timeframe of this project.

Cross year	Number of seedlings	Phenotypic data collected in winter 2023/2024
2016	~600	Scion trunk diameter
2017	~320	Scion trunk diameter Tree height Canopy volume
2019	~1,000	Internode length Scion trunk diameter Tree height Canopy volume

Table 2: Phenotypic data of rootstock and scion (i.e., d'Anjou) traits collected for Pyrus seedling populations in winter 2023/2024.

Bloom data was collected for all populations in the spring, however due to risk of fire blight infection, once bloom is recorded, it is removed.

Approximately 1400 new pear seeds were produced following crossing in 2023.

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

Further analysis of the two dwarfing loci identified (on chromosomes 5 and 15) 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 collaborating 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.

Objective 4: Maintain a relevant pear rootstock parent germplasm

In our previous project (PI: Evans; PR-19-108), the ten precocious seedling candidates that were identified, selected and micropropagated (10 replicates per seedling), are still being maintained in the WSU TFREC hoop house as they are too small to be established in the parent germplasm set at Sunrise Orchard.

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

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

In spring 2023, all 37 B × A and B × C trees produced flowers. Fruit was harvested from 25 trees, with number of fruit and individual fruit weights recorded. Fruit is currently in cold storage and will be evaluated to determine if there are differences in fruit quality. By the end of 2024, rootstocks of low dwarfing potential and non-precocious bearing will be discarded.

OUTREACH

Presentations

- Soon Li Teh presented "Introduction to WSU pear rootstock breeding" at the Wenatchee Sunrise Rotary Club. January 3, 2023.
- Soon Li Teh presented "Pear rootstock breeding in the U.S. Pacific Northwest" at the XIV International Pear Symposium, Stellenbosch, South Africa. January 26, 2023.
- Soon Li Teh presented "Updates and progress of WSU pear rootstock breeding" at the Northwest Wholesale Cashmere grower meeting. January 31, 2023.
- Kate Evans presented "Plant breeding from a pome fruit perspective" at the Texas A & M Plant Breeding Symposium. February 16, 2023.
- Soon Li Teh presented "QTL mapping and haplotype characterization of two major-effect dwarfing loci in reciprocal *Pyrus* rootstock seedling families" at the 11th Rosaceae Genomics Conference, Nelson, New Zealand. March 15, 2023.
- Soon Li Teh presented "WSU pear rootstock breeding program" for the U.S. Pear Crop Germplasm committee meeting (virtual). March 24, 2023.
- Kate Evans presented "WSU pome fruit breeding program" for WSU Research & Extension Experience Undergraduate Introductory Symposium, on-line on June 2, 2023.
- Kate Evans hosted WSU plant breeding graduate students visit to CV orchard on October 18, 2023.

Publications

- York Z, Teh SL, Evans K. (2023) Fire blight susceptibility of 20 diverse pear (Pyrus spp.) rootstock breeding parents. Journal of the American Pomological Society. 77(2): 66-74
- Teh SL, York Z, Evans K. (2023) QTL mapping and haploblock characterization of two major dwarfing loci in reciprocal Pyrus rootstock seedling families. Fruit Research. 3:20 DOI: 10.48130/FruRes-2023-0020
- Raman MG, Marzougui A, Teh SL, York ZB, Evans KM, Sankaran S. (2023) Rapid assessment of architectural traits in pear rootstock breeding program. Remote Sensing. 15(6), 1483.
- Teh SL, Evans K. (accepted) Pear rootstock breeding in the U.S. Pacific Northwest. XIV International Pear Symposium. Acta Horticulturae

Project Title: The Next Fruit 4.0

PI:	Peter Frans de Jong	Co-PI (2):
Organization:	Wageningen University & Research	Organization:
Telephone:	+31 4884 73744 (voicemail) +31 (0)6 30475029 (SMS/Whatsapp)	Telephone:
Email:	peterfrans.dejong@wur.nl	Email:
Address:	Lingewal 1,	Address:
Address 2:	Droevendaalsesteeg 4,	Address 2:
City/State/Zip:	Randwijk, Gelderland, 6668 LA	City/State/Zip:
City/State/Zip2:	Wageningen, Gelderland, 6708 PB	

Cooperators: Manoj Karkee and Lav Khot (Washington State University), Joseph Davidson (Oregon University)

Project size
Amount: 3,156k€ for 4 years
Agency Name: Dutch ministry of Ministry of Agriculture, Nature and Food Quality
Notes: Total project size is 3,156k€ for 4 years, the other half (1,578k€) is financed by Dutch growers and companies (in cash/in kind) and the Washington Tree Fruit Research Commission. The part that is financed by WTFRC is stated below.

Item	2021	2022	2023
Salaries	$54,000	$54,000	$54,000
Benefits			
Wages			
Benefits			
Equipment	$5,000	$5,000	$5,000
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total	$59,000	$59,000	$59,000

Executive Summary The Next Fruit 4.0

The object is to make fruit cultivation more efficient, intelligent, sustainable, and future-proof. This requires us to be able to monitor, manage, and make decisions at the level of individual trees with the help of smart technology. The first example is the development of a precision sprayer that can spray at a nozzle level with sensors that detect the volume of the trees. Two prototypes were build and one needs further development and the other is ready for field trials. A later add on are camera's that can detect pests and diseases. Precision spraying for fruit thinning showed that aiming on the trees with a high amount of flowers gave the best results on effects on return bloom. The second example is the development of sensor platforms that detects blossom in the orchard or a platform that can examine the fruit quality of a storage bin. Specially for pear an algorithm was developed to measure the size. Colour measurements will follow. The third example is the use of a non-destructive sensor to measure fruit quality like firmness and brix. The sensor Fresco showed reliable outcomes on a set of more than 20 samples. And finally the fourth example is the build of end effectors for picking and pruning to make robots multifunctional. The first end effector to pick pears was made and tested with success in the field. This winter red currant plants will be pruned with the pruning end effector.

Objectives overall project
Making fruit cultivation more efficient, intelligent, sustainable, and future-proof requires us to be able to monitor, manage, and make decisions at the level of individual trees. **Smart Technology** will enable getting the most out of an orchard through the targeted, efficient use of crop protection agents, plant hormones and fertilizers, while saving on labour and minimizing food waste. This all contributes to the creation of a sustainable fruit cultivation system.

The project has therefore three key objectives in relation to technology development:

1. Improving the sustainability of cultivation and the supply chain by:
    a) developing ways of applying crop protection agents, plant hormones or fertilisers to individual trees (or parts of trees) based on new ways of detecting stress, pests, and diseases (using sensors and new algorithms) and
    b) by combining data to develop new decision support models using AI. This will, for example, give decision support in storage duration and conditions to prevent loss and waste of the fruit, or help to determine the optimal dose of crop protection agents, growth regulators and fertilisers.
2. Maximising yields by optimising cultivation and storage through the optimisation of individual tree growth.
3. Minimising costs by developing multifunctional robots to replace human labour and ensure the efficient use of inputs.

The need to achieve these objectives has led to the project being organised in four case studies. A brief description of the four case studies is provided below, including an explanation of how they mutually reinforce each other.

Case study 1: Further development of precision sprayer
The former project Fruit 4.0 demonstrated that precision spraying at the level of individual trees is possible. In The Next Fruit 4.0 we want to further develop and broaden the application of precision spraying by controlling it down to individual nozzles and by using sensors to detect pests and diseases and apply sprays in response. Being able to control sprays at the level of individual nozzles also optimises the use of regulators for growth and fruit setting, resulting in a more uniform orchard. Hot spots of insect infestation can also be controlled without spraying the whole orchard.

Case study 2: Advanced crop management and yield registration
This case study is based on the use of sensors to collect data and translate it into decision support models visualised as clear dashboards. This will involve making the sensor platform from the Fruit 4.0 project applicable to more than just apples. The wide range of data and information gathered will also be distilled into clear insights around cultivation management. With help from experts and the use of modern AI algorithms, decision models will be created that can contribute to optimising and improving the sustainability of fruit cultivation.

Case study 3: Cool data
Apples and pears are often stored for a long time, even up to the following harvest. Storing the fruit for any length of time often leads to substantial losses due to a lack of clear, objective information on how long a particular batch can be stored. This case study will focus on maximising the use of data derived from the cultivation phase (climate, crop, and soil) and the focused application of new technology (sensors), leading to decision models that deliver better risk assessments and storage strategies. This will help reduce loss and waste during storage.

Case study 4: Multifunctional robot
Finally, The Next Fruit 4.0 will also work on expanding the functionality of existing robots which are already in development (e.g. by adding a gripper for picking pears, or for pruning and removing suckers) and which could perform more efficiently through technological improvements

and better orchard design. All of this will help solve the problem of increasingly limited availability of seasonal labour.

The results presented are from the last 12 months. Results are presented per case study.

Case study: Precision sprayer

Objectives
A validated prototype precision sprayer for several fruit crops, which is directed at nozzle level on the basis of smart algorithms and decision models and combined with stress, disease and pest detection.

Significant Findings

- Laser scanner data can be translated into spray actions
- 2 prototype sprayers were build
- 1 prototype has been tested, finding is that the system was functioning well but a constant driving speed was needed. In the field the results therefor were not satisfying. The other prototype can handle difference in speed and is now far enough in development for testing in November and December 2023.

Methods
The third year of the project concentrated on:

- Building an improved sensor platform for a sprayer with LIDAR and GPS and (later in the fourth year of the project with RGB sensors).
- Processing data into usable data for spray decisions at nozzle level
- Build 2 sprayers with laser scanner that can spray at nozzle level and that can adapt dose on tree volume

Results and Discussion
In practice, the most important benefit is that in the future fewer spray products will be needed to achieve the same result and that emissions to the environment will be further limited. The LIDAR scanners that make this possible are placed at the front of the sprayer. They determine the tree volume and gaps while driving. Both spray systems use PWM (Pulse Width Modulation) technology to vary the amount of spray liquid. This is done by changing the length of those pulses. Based of the tree volume an algorithm determines the amount of spraying liquid for each nozzle.

Within this work package, two types of sprayers have being build. The first is from Munckhof, the second from KWH. In the past period, the focus has been on getting both systems working. In collaboration with Munckhof, a first so-called timing measurement has been made, not yet in the field, but on asphalt with art objects and water-sensitive paper. This showed that the system already functions well, but that it is still very sensitive to driving speed. The results of the first measurements taken in the orchard showed that the deposition was lower than the standard sprayer that was used. In order to do further testing, the system needs to be improved.

With the KWH sprayer, work was mainly done to get communication between the different parts of the system going. The Lidar sensors are read by a separate computer. This computer also decides whether and how much to spray. These instructions are then communicated to the sprayer system (from the company BBLEAP). The entire system is now basically working and the first measurements will be taken in the coming weeks.

Below 2 pictures of the sprayers, one in the field during tests and one during installation of the components.

Case study: Advanced crop management and yield registration

Objectives

- Validated sensors and algorithms to collect physiological and phytopathological characteristics of apple and pear.
- Validated decision models developed on the basis of collected data and expert knowledge; targeted on production optimization.

Significant Findings

- Blossom detection method did not work sufficient enough, a higher resolution camera is needed in combination with flash lights.
- Detection method to detect fruit tree canker and apple blossom weevil
- Trunk detection to get the GPS locations for individual trees.
- Field trial on blossom and fruit thinning showed for third year in row that precision spraying on trees with a high amount of flowers is the most effective strategy to make the orchard more uniform.
- Experiments were done to develop a thinning decision support system for Conference pear.
- Proof of principle was demonstrated for automated detection of apples and also pear in top layer of storage bins.

Methods
The third year of the project concentrate on:

- Testing systems for automated detection of pear in top layer of storage bins.
- Building data and decision support models and dash boards for growers for presentation and management at tree level
- Setting up trails on thinning based on sensor input

Results and Discussion

At harvest, growers and sales organisations like to know what the fruit quality is in the storage bins. For apple the size can easily be determined by making a picture from the top of a bin. For pear it is in development now. For that reason an algorithm was developed for the Conference pear.

Image processing photos storage bin

Within the project, WUR is developing image processing in which the size distribution of the pear is initially determined from photos of the storage bin. In subsequent steps, other quality aspects can also be analysed, such as fruit shape, colour and certain damages.
For the size measurement specific points in the shape are now detected. This concerns the stem and nose position and the widest point of the fruit to determine the diameter. Several steps are required to validate the data. First, it must be determined how reliable the size measurement for the detected pears is and then it must be determined how well this size distribution corresponds to the entire storage bin or the entire batch.

The image processing model is running on a trial basis at the project partner Bodata. The goal is to bundle the collected information into a quality report. We are currently discussing with the

consortium partners involved how the analyses can be incorporated into daily practice. Preparations are also being made for market introduction.

Drive through automatic photo portal for picking trains
Because there is little time during the harvest to photograph each storage bin by hand, it was thought that it would be practical to drive a picking train under a gate where the photos could be taken automatically. By then linking the photo to this storage bin via an RFID chip, it will be possible to quickly gain insight of a complete batch.
A test setup was tested at the experimental orchard Randwijk during the past harvest period. As soon as a storage bin passes the camera, a photo is automatically taken and the RFID chip is scanned. To ensure consistent photo quality, it was decided to shield the portal from daylight and artificially illuminate it with construction lights. To minimize motion blur in the photo, the picking train had to pass in the lowest gear. Integration with RFID stickers turned out to work fine. There are still some points that require attention, such as fruit brilliance and colour correction.

Develop crop growth model
Within this work package, Delphy is working on developing a crop growth model. The aim is to predict the June drop and the final fruit numbers for Conference pear.

Many counts and measurements were again carried out in various tests in 2023. In addition to validating the model, work has been done to collect information about the course of the June drop and the factors that influence it. The results of all tests have now been worked out.
It is clear what causes this difference. As is known, there are many factors that influence moulting, such as planting system, planting year, number of flower clusters, soil, crop health, etc.

This year, time was also spent on developing the dashboard for fruit growers together with the project partner Agromanager. An important point of attention here is the easy exchange of data.

Precision thinning
Last season, WUR and Delphy carried out an extensive thinning test on a task map at the Experimental orchard in Randwijk on Elstar apple. A total of 7 treatments in 4 flowering classes, i.e. 28 combinations, were carried out.
Counting was carried out at three times, namely at the end of June (end of June drop), in July (hand thinning) and in August (just before harvest). Just before harvest, a random fruit size measurement was also carried out in all treatments. The results will be analysed in the near future
It is clear that it was a difficult thinning year. As with many growers, it was difficult to spray under ideal conditions. As a result, the thinning result was often disappointing in the trial.

Unfortunately, the Apple Blossom Beetle also caused noise in the data because counted flower clusters did not yield any fruits due to damage. The June drop may also have been less strong than we expected. It is clear that a strong thinning treatment on trees with many flower clusters does not quickly lead to over-thinning.

In Conference pear, ongoing fruit thinning research based on The Next Fruit 4.0 has been expanded with additional treatments to clarify the opportunities for precision thinning and precision fruit setting. Trees with many flower clusters have been thinned more often with Brevis to reduce the manual thinning. Trees with few flower clusters were stimulated to fruit set to increase yield. The number and weight per tree were determined during the harvest in September. These results are also currently being analysed.

Case study: Cool data

Objectives

The focus for this year was to select and evaluate tools for non-destructive quality assessment of fruit both preharvest and postharvest. Observed differences between batches of fruit should be related to relevant quality characteristics of the fruit. Not only aiming at quality assessment of freshly harvested fruits but also related to storage behavior of the respective batches.

Results and Discussion

First the tools to evaluate the fruit have been selected. Non-destructive measurements using new tools are being related to common (destructive) quality assessment methods.

Common quality assessment

- Firmness, Brix, Weight
- Photographic analysis (color, shape, percentage russeting)

Non-destructive assessment

- Near Infrared – both a hand held sensor from the project partner Kubota and hyperspectral imaging from our in-house facility
- Microwave based –a hand held sensor from the project partner Vertigo

The project partner Kubota decided to pause the further development of the NIR hand held sensor. Therefor the focus was on Fresco sensor from Vertigo.

During the past harvest period, photographic recordings were made of 20 storage boxes per sample at 19 locations, of a total of 23 samples of Conference pear.

Vertigo was also present at a number of locations to validate the Fresco in practical situations in order to look at the effects of variation in light, temperature and moisture.

The preliminary results are shown in the figure below. Companies were visited in the most important Conference growing regions (Limburg, Zeeland, the Betuwe, Utrecht, Flevoland, North Holland and the Belgian fruit region). In some cases, the storage boxes were labelled so that they can be reanalysed as soon as they leave storage. Fruits from each batch were collected and stored in parallel at WUR Randwijk. Photo material and data about hardness and sugar content are added to the Agromanager database as much as possible. Agromanager is data platform for fruit growers where all data can be collected and analysed by the grower.

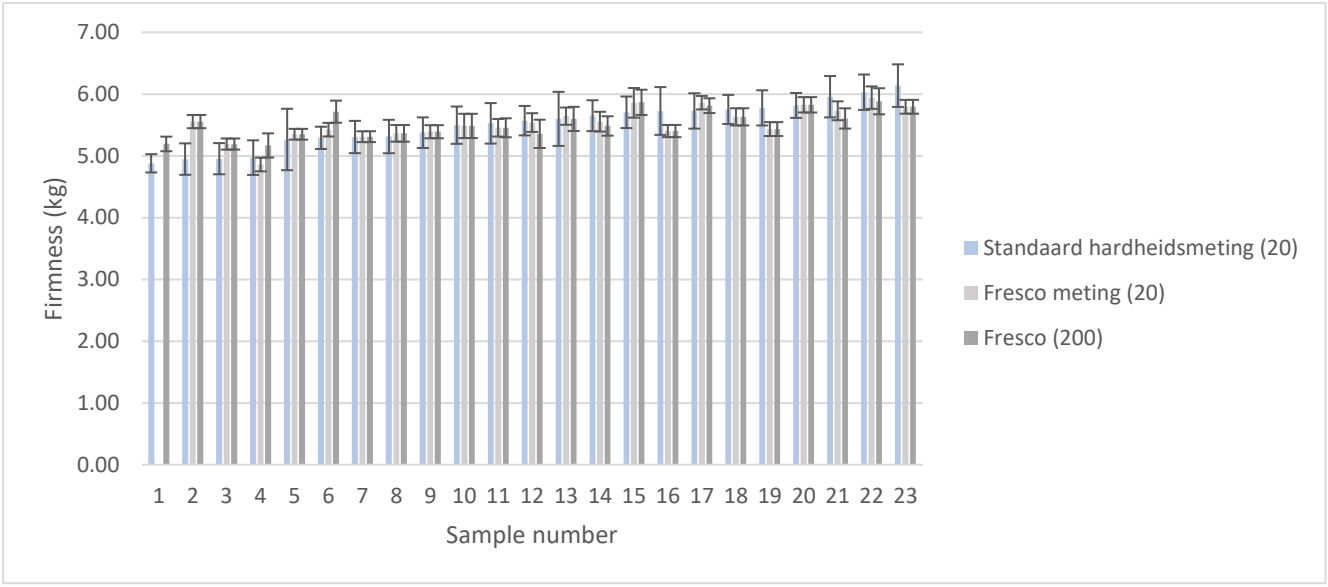


Figure: results of measurement of 23 different samples of pear fruit by hand (standard in blue), with the Fresco sensor (light grey sample of 20 fruits and dark grey with 200 samples)

Case Multifunctional robot

Objectives
The main objective of the multifunctional robot case is to expand the functionality of existing orchard robots and of orchard robots currently under development in parallel research projects. The focus of the work is on two topics, namely the development of a sensing system and a gripper for picking pears and on a sensing system, robot control and end-effector(s) for robotic pruning of fruit trees and red currant bushes. On the longer term additional tasks such as automatic thinning, removing weeds and precision spraying will be targeted.

Significant Findings

- Detection system developed for robotic harvesting pear to detect the position but also the orientation and some other key points of the fruit.
- Prototype gripper that can do the required motion to detach a pear from a tree which is significantly different from that to detach an apple.
- Extensive knowledge and expertise on automatic pruning and fruit harvesting is exchanged with Washington State University and Oregon State University. Close cooperation and knowledge exchange between Dutch and US researchers is of mutual benefit.
- A prototype gripper for pruning is developed and tested on red currant.

Methods
The third year of the project concentrated on:

- Designing first prototypes for pruning and picking end effectors.
- Designing an algorithm to detect pears and pose estimation.
- Testing different camera’s for making 3D models of dormant red currant plants.

Results and Discussion
Gripper testing pear picking
Within the project the prototype to pick pears was tested in the field. The most important innovation lies in the moving gripper system (photos 2 and 3) with suction cup. Unlike conventional methods that use the robot's arm movement to loosen the fruit from the tree, the new

concept allows the gripper mechanism itself to perform the crucial picking motion. The gripper also has an integrated colour and 3D (RGB-D) camera.
WUR researchers wrote software to integrate the deep-learning peer detection algorithm developed earlier in the project into an operating system for the robot using ROS2 (Robot Operating System 2).
After the first tests on the indoor test setup, a two-day test was carried out with the harvest of Conference pears at Experimental orchard of Randwijk during the harvest period in September 2023. The results are convincing: the robot can detect and harvest pears without damaging the pear. But we're not there yet. The tests in the orchard have provided valuable insights into what works well and what can be improved. The data collected during these picking experiments will be analysed to further refine the robot's capabilities and make necessary improvements.

Pruning red currant bushes
The past summer months have been used to explore better options for the sensor system. The research team is looking for high-quality sensors that can map plant architecture in 3D.
Two way are being followed for this.
On the one hand, (combinations of) various 3D sensors (cameras, LIDARs) and associated classification algorithms are investigated in collaboration with the sensor experts from the company IMEC. On the other hand, the collaboration between WUR (Jochen Hemming) and Oregon State University (Alex You, Joe Davidson and Cindy Grimm) contributed to a study investigating how a branch can be mapped in 3D with a simple 2D camera. The results of this research will be presented at a leading scientific robotics conference in Japan (ICRA 2024) in May next year

Photo 1 Robot setup in orchard

Photo 2 Gripper with suction cap

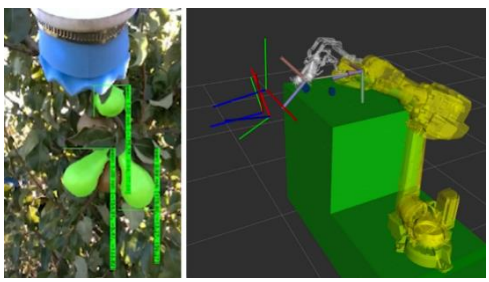


Photo 3

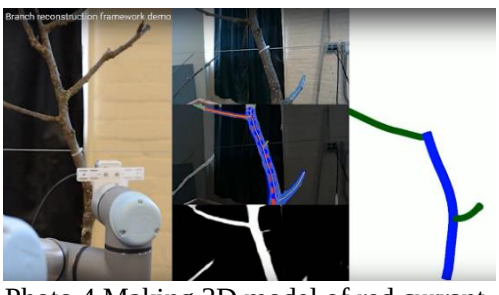


Photo 4 Making 3D model of red currant