Northwest Pear Research Review

Columbia Gorge Hotel, Hood River, OR

Thursday, 2/21/2019

Time	Page	Presenter	Title	Yrs
			Final reports	
8:45	1	Amiri	Enhancement of postharvest decay management in pear	17-19
9:00	7	Dhingra	Assessment of organoleptic traits in sliced pears	18-19
9:15	15	Rendon	Ecology of *Trechnites,* an important parasitoid wasp of pear psylla	19
9:30	28	Dong	Delivering quality pear fruit to consumers	16-18
9:45	40	Serra	Dry matter assessment in pear and consumer perception	16-17
10:15			***Break***	
			Continuing Projects 10:15 - 11:45	
1	50	DuPont	Fire blight management: new products and effective rates	18-20
1	58	Nottingham	Integrated fruit production for pears: ***No-cost extension***	17-19
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1	84	Cooper	Using transcriptomics to target key behaviors of pear psylla	19-21
1		Willett	Technology projects (See tech reports)	19
2	90	Serra	Field evaluation of pear cultivars on cold hardy quince rootstocks	19-21
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2	104	Dhingra	Greenhouse screening of 49 dwarf rootstock candidates	18-20
2	109	Dong	Mechanisms and practical solutions to control scald of pears	18-20
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FINAL PROJECT REPORT
WTFRC Project Number: PR-16-103

Project Title: Enhancement of postharvest decay management in pear

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Cooperators: Kelly Wallis (Oregon), multiple packers in WA and OR, Craig Christensen (Cashmere, WA).

Total Project Request: **Year 1:** $32,284 **Year 2:** $33,284 **Year 3:** $34,323

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization name: WSU-TFREC **Contact Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 x221 **Email:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	2017	2018
Salaries[1]	17,550	18,252	18,982
Benefits[1]	7,434	7,732	8,041
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[2]	4,100	4,100	4,100
Travel[3]	2,000	2,000	2,000
Miscellaneous	0	0	0
Plot Fees[4]	1,200	1,200	1,200
Total	32,284	33,284	34,323

Footnotes:
[1] Salaries for a research intern (Laxmi Pandit, 0.65 FTE) at 42.4% benefit rate.
[2] Supplies include Petri dishes, multi-well plates, microbiological media for fungi growth and fungicide sensitivity tests.
[3] Travel to multiple packinghouses in WA and OR for fruit collection.
[4] Plot fees for an experimental orchard to be used for field studies.

ORIGINAL OBJECTIVES

1- Conduct a general disease survey to identify and quantify major postharvest rots.

2- Conduct a general resistance monitoring program across multiple pear orchards and packinghouses in WA and OR to TBZ, pyraclostrobin, boscalid, fludioxonil and pyrimethanil.

3- Evaluate the efficacy of fungicides applied by thermofogging and investigate the possibility of reducing fungicide input.

4- Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.

SIGNIFICANT FINDINGS

Objective 1: *Conduct a general disease survey to identify and quantify major postharvest rots*

- 243 grower lots including 124 and 119 lots in 2017 and 2018, respectively, from 9 packinghouses, including 4 packinghouses in WA and 5 packinghouses in Hood River OR, were surveyed from February to May of 2017 and 2018. Overall, 166 and 77 lots were surveyed from OR and WA, respectively in the two years.
- In 2018, gay mold followed by *Nectria* rot and Cladosporium rot were most predominant in Washington, whereas blue mold followed by gray mold and Mucor rot were most predominant in Oregon.
- The quarantine pathogen *Phacidiopycnis pyri* was found at about 8 and 4% of total decay in OR and WA, respectively. Its frequency was slightly higher in 2018 compared to the previous season.
- Gray and blue molds make for up to 50% of total decay in both regions.
- *Nectria* rot seems to be emerging as a potential problem. Geographical variabilities in its distribution have been observed. Further research is needed.

Objective 2: *Conduct a general resistance monitoring program across multiple pear orchards and packinghouses in WA and OR*

- A total of 1,140 isolates of *Penicillium expansum* (blue mold) and 2,000 isolates of *Botrytis spp.* (gray mold) were collected from the different packinghouses surveyed in 2017 and 2018 (Objective 1). These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), pyrimethanil (Penbotec) and fludioxonil (Scholar) for both *P. expansum* and *Botrytis* and to pyraclostrobin + boscalid (Pristine) and fluxapyroxad (Merivon) for *Botrytis* only.
- Overall, resistance frequencies of *P. expansum* for TBZ and Penbotec decreased significantly in 2018 after most packers rotated with Scholar.
- Resistance in *Botrytis* stayed steady in 2018 compared to 2017 except to TBZ for which it decreased by almost 3 times in 2018.
- 243 decay and resistance profiles were created and sent to the participating packers and growers before the beginning of the new season to allow them change strategies and spray regimes based on decays and resistance found at their lots.

Objectives 3: Because it was not possible to identify a packer who drenches, fogs or aerosols at the same time, this objective was not conducted. The information collected from the 243 surveyed lots indicate that:

- About 64% of packers have been applying the fungicides through thermo-nebulization (Fog or aerosol), 21% on the packing line and 3% only through drench at harvest.
- Similar studies conducted on apple revealed an uneven distribution of fungicide residues when applied through fog or aerosol inside the storage room and also within bins.
- Trials on apples also revealed that decay on wounded fruit may show up earlier on fruit treated dry than those drenched at harvest.
- Dry application of fungicides will likely reduce incidence of blue mold and Mucor rot in storage but may have a lower efficacy against most field pathogens which initiate infections months or weeks before harvest.
- Rotation of Pristine and Merivon with Topsin-M seemed more effective than solo applications

Objective 4: *Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.*

- Delaying harvest, a week to 10 days after commercial maturity increased decay significantly on fruit stored for 8 months. The grower at the commercial orchard where the trial was conducted harvest at the later date.
- Adding Ziram to Pristine or Merivon preharvest, reduced postharvest disease losses by 15 to 50% compared to Pristine or Merivon solo.

RESULTS AND DISCUSSION

Objective 1. *Prevalence of postharvest diseases*

As in 2017, gray mold (*Botrytis*) was predominant in both states with average frequencies of 43 and 24% in WA and OR, respectively (Figure 1). Incidence of blue mold (*Penicillium*) in OR decreased significantly from 25% in 2017 to 8% in 2018, whereas blue mold frequency in WA remained the same as in 2017 around 10%. We have noticed a sharp increase in the incidence of *Phacidiopycnis* rot in both states in 2018 but specially in OR where it jumped from 7% in 2017 to 22% in 2018. The incidence of *Nectria* rot increased in OR in 2018 compared to the previous season and remained steady in WA around 4% of total decay (Figure 1).

Besides the group of other diseases which is made of some minor diseases and few unknown pathogens, about 60% of the total decays is caused by pathogens infecting fruit in the orchards versus 25 to 28% of total decays caused by typical postharvest pathogens such as *Penicillium*, Mucor and *Cladosporium*. This strongly indicates that while postharvest disease management efforts are needed, preharvest management should be enhanced and started earlier than the 2 weeks to few days preceding the harvest.

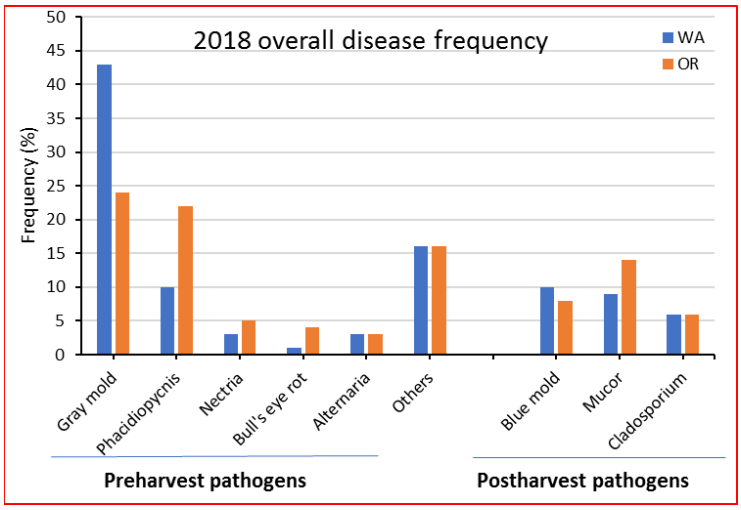

Figure 1. Overall incidence of major postharvest diseases found in in 2018 in Washington State (blue bars) and Hood River, OR (orange bars). Results are average from 119 growers lots.

Objective 2. *Fungicide resistance occurrence and frequencies*

A total of 480 isolates of *Penicillium expansum* (blue mold) and 1030 isolates of *Botrytis* (gray mold) were collected from the different packinghouses surveyed in 2018 (Objective 1). These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), pyrimethanil (Penbotec) and fludioxonil (Scholar) for both *P. expansum* and *Botrytis* and to pyraclostrobin + boscalid (Pristine) and fluxapyroxad (Merivon) for *Botrytis* only. Overall, resistance frequencies of in *P. expansum* (blue mold) decreased sharply to TBZ and Penbotec compared to 2017 because most packers switched to Scholar application at harvest in 2017-18 season. The frequency of *P. expansum* isolates with reduced sensitivity to Scholar increased a bit in 2018 as a result of the switch to this fungicide but the resistance frequency remains relatively low around 10%.

On the other hand, resistance frequencies to *Botrytis* (gray mold) in 2018 decreased for TBZ by about 55% than in 2017 whereas frequencies remained steady for the other fungicides. The most worrisome finding is that resistance to Penbotec remained high around 60% of the total population in 2018 but variations were observed between lots. Overall, resistance frequencies were similar in WA and OR for *Penicillium* but were slightly higher in OR for *Botrytis*.

More details and specific numbers will be shared at the Pear Review meeting in February 2019.

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- Similar studies conducted on apple revealed an uneven distribution of fungicide residues when applied through fog or aerosol inside the storage room and also within bins.
- Trials on apples also revealed that decay on wounded fruit may show up earlier on fruit treated dry than those drenched at harvest.

❖ Dry application of fungicides will likely reduce incidence of blue mold and Mucor rot in storage but may have a lower efficacy against most field pathogens which initiate infections months or weeks before harvest.

Objectives 4. *Evaluate the impact of fungicide rotations and mixtures in orchards on postharvest decay and resistance development*

In 2016, two new pre-harvest fungicides Pristine (new to pear but commonly used on apple) and Merivon were tested as solo or tank-mixed with the multi-site Ziram. Two harvest dates were tested, one at the end of August and the second one in early September.

Except for the untreated control, all treatments resulted in disease incidence lower than 10% on fruit harvested late August, whereas disease incidences ranged from 15 to 33% when fruit were harvested 10 days later in September (Figure 2).

Interestingly, Ziram' s efficacy was equal to that of Pristine or Merivon tank-mixed with Ziram. The inconveniency of irritation caused by Ziram to pickers should be avoided by wearing proper clothing during harvest. Moreover, economically it should be more beneficial to growers to include Ziram in their management programs. We have not tested for fungicide resistance in plots where Ziram was used, but previous studies on mixing single-sites with multi-sites fungicides such as Ziram, thiram or captan has delayed selection for resistance to single-sites such as TBZ and boscalid (Pristine).

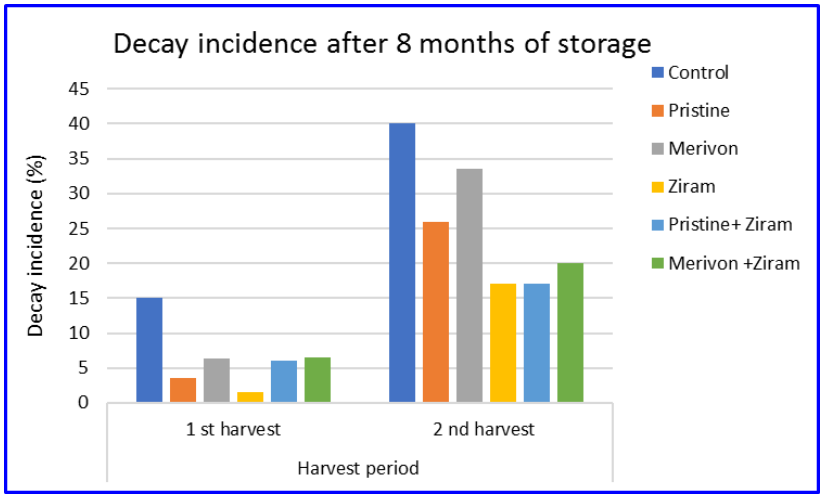

Figure 2. Overall decay incidence on d 'Anjou pear treated with Pristine, Merivon, or Ziram preharvest after 8 months of storage at 33F in a regular atmosphere. 1st harvest was done in 1st week of August 2016 followed by a 2nd harvest 10 days slater.

Additional trials were conducted in the 2017-18 season including Topsin-M, Merivon, Pristine and Ziram applied as solo products, rotated or tank-mixed. Results indicated that Pristine alone or rotated with Topsin-M was the most effective, whereas the rotation of Merivon with Topsin-M or tank-mixture of Merivon + Ziram improved the efficacy of Merivon. The full results will be presented and discussed at the Pear Review meeting in February 2019

EXECUTIVE SUMMARY

Summary of findings:

The traditionally-known disease threats such as blue and gray mold, in addition to the emerging new pathogens, mostly reported from Washington, have put a tremendous pressure on growers and packers in recent years in term of improving management and reducing losses. We first worked to accurately assess the real threats and their extent to develop sustainable solutions. We conducted two years of regional (WA and north OR) decay surveys combined with a regional fungicide resistance monitoring to assess any possible impact in recent shifts in cultural and management practices and climate change on emergence or exacerbation of pathogen populations or increased fungicide resistance frequencies. Such information was highly needed to improve fruit production sustainability. Fungicide resistance monitoring programs will be crucial to pinpoint location-specific problems, evaluate the potential impact of environmental conditions between regions and different spray regimes on resistance development. Moreover, the impact of target and no-target sprays in orchards as well as storage conditions on decay control efficacy and potential resistance development problems need to be assessed to improve disease management.

Summary of findings: We have a better understanding of the risks caused by pear pathogens in term of occurrence, distribution and importance. More specifically, we acquired new knowledge about the emerging quarantine pathogens (*Phacidiopycnis, Nectria*) in term of importance and distribution and control of *Phacidiopycnis*. One the major outcomes from the project is a better assessment of existing risks of fungicide resistance in pear fruit systems in Washington and North Oregon. Although, resistance has emerged to most fungicides, levels of resistance can be considered lower compared to other crop systems. However, caution is needed to avoid catastrophic scenarios due to control failure if resistance continues to increase because rationale solution and practices are not implemented. We have documented a positive effect on control level of adding multi-site fungicides such as Ziram to pre-harvest spray programs either solo, in rotation or tank-mixed with other single-site fungicides.

Project Outcomes:

- Peer reviewed publications: 2 (3 more pending)
- Extension publications: 5
- Professional presentations: 8
- Extension presentations: 25

Future directions:

- Develop specific management programs for major diseases: Gray mold and Blue mold
- Better understand the epidemiology of preharvest pathogens to enhance management in storage.
- Acquire more knowledge about the emerging (quarantine) pathogens such as *Phacidiopycnis* and *Nectria* in term of epidemiology and best management practices.
- Continue research efforts in assessing the effect of storage conditions on decay and fungicide resistance development and develop adequate solutions.

FINAL PROJECT REPORT

Project Title: Assessment of organoleptic traits in sliced pears

PI: Amit Dhingra
Organization: Washington State University
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City/State/Zip: Pullman, WA 99164

Cooperators: Blue Bird Growers – Ron Gonzales; Blue Star Growers – Smart fresh treated fruit; Crunch Pak: Ozgur Koc, WSU: Seanna Hewitt and Scott Mattinson

Other funding sources

Agency Name: WSDA
Amt. requested/awarded: $249,926 **(requested)**
Notes: Production of high quality fresh sliced pears

Agency Name: Crunch Pak
Amt. requested/awarded: $30,000 **(Requested – matching funds for the WSDA proposal)**
Notes: Support for pear slicing, packaging, purchase of fruit, labor and fruit quality analysis

Agency Name: NIH Protein Biotech Training Program
Amt. requested/awarded: $52,234 **(awarded)**
Notes: Support for Seanna Hewitt, Ph.D. student includes stipend, travel, medical, tuition and fees

Total Project Funding: $67,808

Budget History:

Item	2017	2018
Salaries[1]	16,800	17,472
Benefits	8,106	8,430
Supplies[2]	7500	7500
Travel[3]	1000	1000
Total	33,406	34,402

Footnotes:
1. Technical support for evaluation and analysis of fruit
2. Support for purchasing fruit, chemical compounds, and modified atmosphere bags
3. Travel to warehouses for fruit procurement

OBJECTIVES

The objectives of this project aimed to quantify the profile and content of volatile compounds critical for a positive consumer experience. In order to produce market ready fresh sliced pears, quantitative information regarding the volatile profile in modified atmosphere bags with different OTRs is needed to complement the promising results obtained from the consumer trials and willingness to pay study that was published recently.

The two objectives of this study were:

1. Assess commercially valuable traits of sliced pears
2. Evaluate flavor profile of the sliced fruit using HPLC and GCMS in a time course experiment

SIGNIFICANT FINDINGS

- Under regular atmosphere, the respiration rate of 1-MCP pears after slicing is lower than non 1-MCP pears (Figure 1).
- The respiration rate as measured by percentage carbon dioxide generated from 1-MCP sliced pears treated with the ripening compound increases steadily as a function of number of days in modified atmosphere bag with an OTR of 140. OTR 140 represents the thinner MA bag used for production of fresh sliced apples. However, the average rate of respiration in 1-MCP treated sliced pears is lower than non-1-MCP treated pear controls (Figure 2), which is somewhat expected.
- In the thicker MA bag with a lower OTR of 100, carbon dioxide percentage was nearly 1.5x – 2x more than the MA bag with OTR of 140 (Figure 3).
- The Ripening compound results in an increased accumulation of esters, which are critical component of pear aroma.
- The thicker MA bag with a lower OTR of 100 produced more ethyl acetate, which may not be desirable for consumers.
- Shelf life of 21 days was easily achieved with 1-MCP treated pears sliced and ripened with the Ripening Compound.
- Overall, a need to test a MA bags with OTRs ranging from 110 to 130 for pears has been identified.

METHODS

Objective 1: Assess commercially valuable traits of sliced pears

Smart fresh (1-MCP)-treated and untreated Anjou pears were sliced. The ripening compound treatment was applied in conjunction with anti-browning solution provided by Crunch Pak. Typically, 1 gallon of the solution was prepared with varying concentrations of the ripening compound. The sliced fruit was dipped in the solution for 1 minute prior to being packaged in two different types of modified atmosphere bags along with a regular atmosphere control.

> Shelf life and brix of the sliced product with smart fresh treated fruit packed in November and March and packed in 3 different types of modified atmosphere bags was assessed. Five, 2 oz. bags of sliced fruit were sampled every 3 days till day 21 for these analyses.

At each sampling time point duration, all samples were frozen at 0 deg C. Within 1 month the samples were removed and by using frozen tissue from 2 MA bag replicates an extract was prepared. The sample was thawed and blended with a Waring blender by adding an additional 20% of ddH2O.

Samples were filtered using cheese cloth and once again frozen at 0 deg C in 3 x 20 ml aliquots. Samples were stored in plastic cone capped scintillation vials.

Objective 2: Evaluate flavor profile of the sliced fruit using HPLC and GCMS in a time course experiment

Volatile compounds from dynamic headspace were collected and analyzed by GC/MS. The volatile profiles included esters, alcohols, hydrocarbons, aldehydes, and ketones. Five, 2 oz. bags of sliced fruit were used for HPLC analysis. Fruit were sampled every 3 days till day 21 for these analyses.

Respiration rates for regular atmosphere were analyzed on treated cut slices by randomly assigning up to 500 g of slices into individual jars. Two jars per treatment were used. Lids on the jars were left open until the day of sampling. Lids were shut for 1 hour, then a 0.5 ml gas sample was removed and injected into a HP-5890 GC, containing a 10 m X 1.0 cm CRC column. The injector was packed while it was held at a temperature of 100 deg C and the TCD detector was also held at 100 deg C. The GC oven was held at 30 deg C during sampling. The quantification of oxygen and carbon dioxide was performed by injecting a gas standard, 10% oxygen in helium, and a 10% carbon dioxide standard in nitrogen. Both standards were purchased from Alltech Scientific.

Volatile Analysis:
The headspace solid phase microextration technique was performed using 2.0 ml of pear extract in a 4.0 ml vial (Supelco, Bellefonte, PA). The pear sample was mixed with 30% NaCl and was stirred at room temperature for one hour. An SPME device with a fused silica fiber coated with 65 µm polydimethylsiloxane/ divinylbenzene was exposed to the sample headspace. The samples were injected using splitless injection for 2 min. at 200 deg C into an Agilent 6890/5973 GC/MSD(Agilent,Wilmington, Del.) equipped with Chemstation C1024 A02. The column consisted of a DB-1MS 60 m column (Phenomenex,Torrance,CA.) with a bore of 0.32 mm i.d. and 1.0 µm film thickness. Chromatographic conditions used were as per Mattheis et al. (1991) with the exception that the transfer line and ion source temperature were held at 250 deg C and 150 deg C respectively. The GC inlet contained a 0.75 mm SPME injection sleeve, which assured peak sharpness especially for early eluting peaks (Yang and Peppard, 1994). Initial compound identification was made by matching the results with the Wiley/NIST library (Wiley 125K) and later confirming the match with commercial standards. Volatile quantification followed by analyzing mixtures of compounds per functional group. Thereafter, the standard curves were used for final quantification.

RESULTS AND DISCUSSION

Objective 1: Assess commercially valuable traits of sliced pears

The commercially valuable traits included – shelf life, sugars and brix.

The 1-MCP treated ‘D’Anjou’ sliced and treated with three concentrations of ripening compound consistently achieved a shelf life of 21 days. As the head space was sampled from the bags of different OTRs, the fruit tissue was juiced and stored. The analysis of %Brix and sugars is currently underway.

Objective 2: Evaluate flavor profile of the sliced fruit using HPLC and GCMS in a time course experiment. All fruit packaged in different OTR bags was stored at 39 deg F.

1. Baseline respiration: First step was to understand the difference in the basal respiration rates. As is evident in Figure 1, fruit stored at regular atmosphere does not show substantial difference in respiration rates between the type of fruit (control or 1-MCP treated). Overall the rate is lower in case of 1-MCP treated fruit.

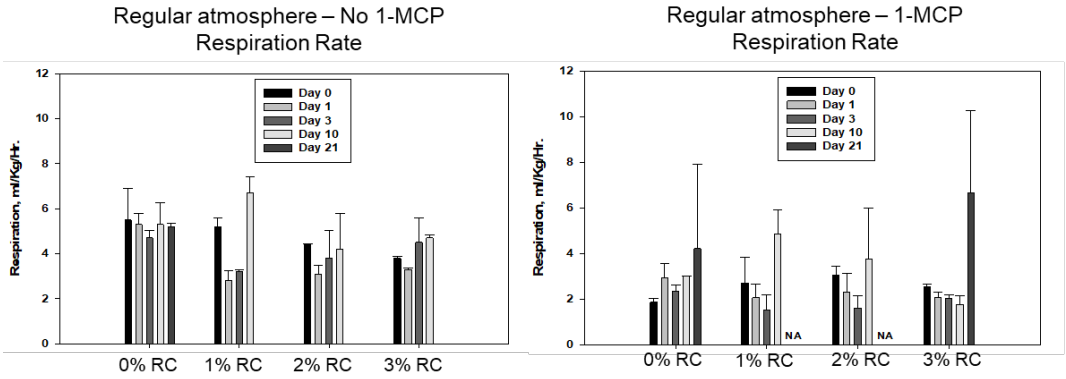

Figure 1: Respiration rate of non 1-MCP and 1-MCP treated sliced pears under regular atmosphere. The fruit was stored at 4 deg C or 39 deg F.

2. Respiration rates under modified atmosphere – non 1-MCP and1- MCP fruit packaged in two different OTR bags. The MA bag used for producing fresh sliced apples has an OTR of 140. In addition, a MA bag with an OTR of 100 was also used.

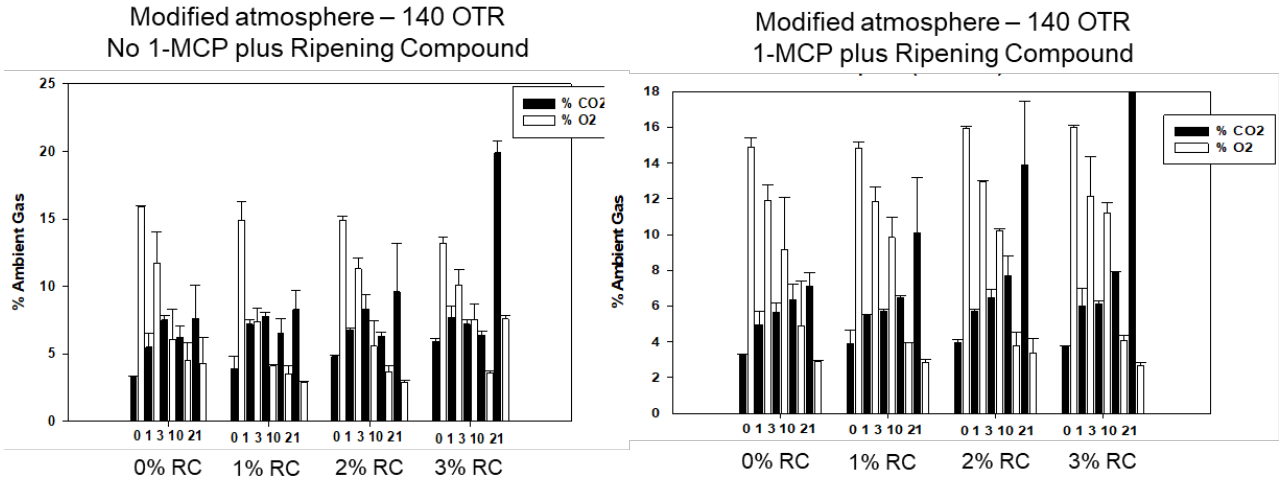

Figure 2: Percentage ambient gas generated by non 1-MCP and 1-MCP treated sliced pears under modified atmosphere bag OTR = 140. The fruit was treated with 0, 1, 2, or 3% of Ripening Compound (RC) and bags were stored at 4 deg C or 39 deg F after slicing. X-axis is number of days and Y-axis represents percentage ambient gas. Open bars – oxygen and dark bars – carbon dioxide.

Figure 2 shows percentage ambient gas in the MA with an OTR of 140. As is clear, addition of the Ripening Compound results in a consistent increase of carbon dioxide. The increase is consistent with an increase in the percentage of the Ripening Compound.

When the fruit was packaged in MA bags with a lower OTR of 100, the rates of respiration were much higher (Figure 3). This data is very important in determining the correct OTR for pears. The data on 'D'Anjou' can also help guide production of fresh sliced pears from other varietals as well.

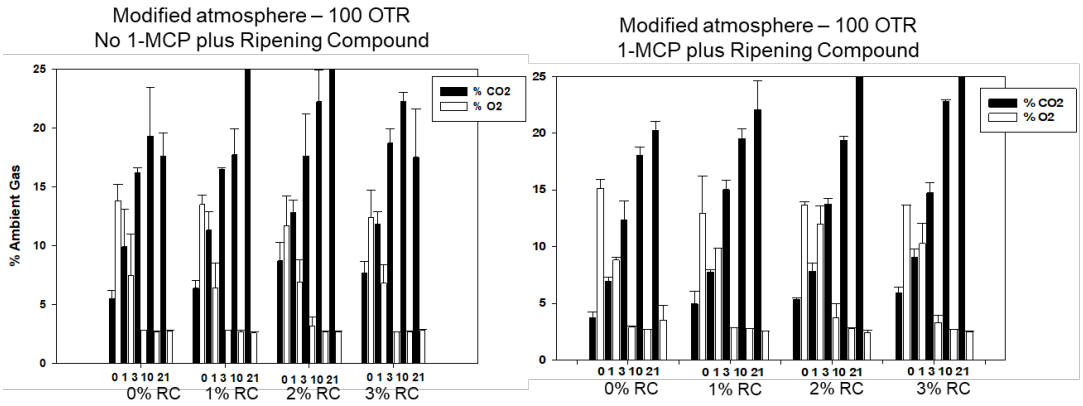

Figure 3: Percentage ambient gas generated by non 1-MCP and 1-MCP treated sliced pears under modified atmosphere bag OTR = 100 – low permeability – thicker bag. The fruit was treated with 0, 1, 2, or 3% of Ripening Compound (RC) and bags were stored at 4 deg C or 39 deg F after slicing. X- axis is number of days and Y-axis represents percentage ambient gas. Open bars – oxygen and dark bars – carbon dioxide.

3. Estimation of aldehydes and esters, and alcohols

The 1-MCP treated fruit when sliced and treated with ripening compound resulted in increased production of esters. The concentration of aldehydes remained low in both types of MA bags. Esters are essential for pear aroma. While the MA bag with an OTR of 100 produced far more esters (Figure 4), it is important to consider this result in light of amount of alcohols produced. There has to be a good balance between all these components.

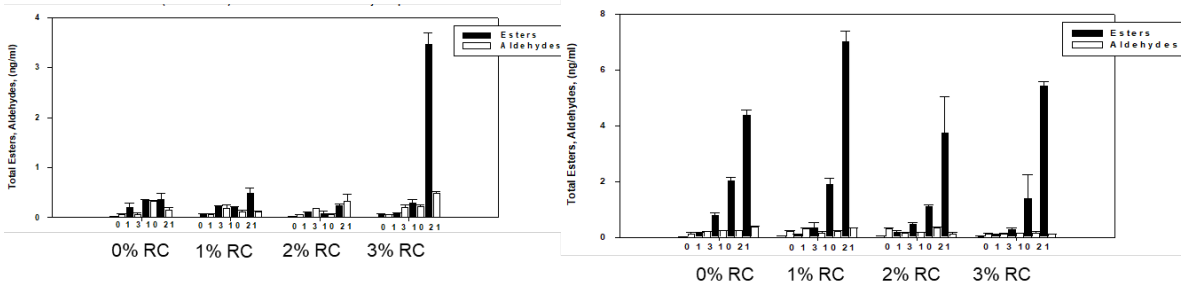

Figure 4: Total esters and aldehydes. Open bars – aldehydes and dark bars – esters. Represented as a factor of number of days on the shelf. This data is for 1-MCP treated sliced pears under modified atmosphere bag OTR = 140 (left panel) and OTR – 100 (right panel). The fruit was treated with 0, 1, 2, or 3% of Ripening Compound (RC) and bags were stored at 4 deg C or 39 deg F after slicing. X- axis is number of days. A.

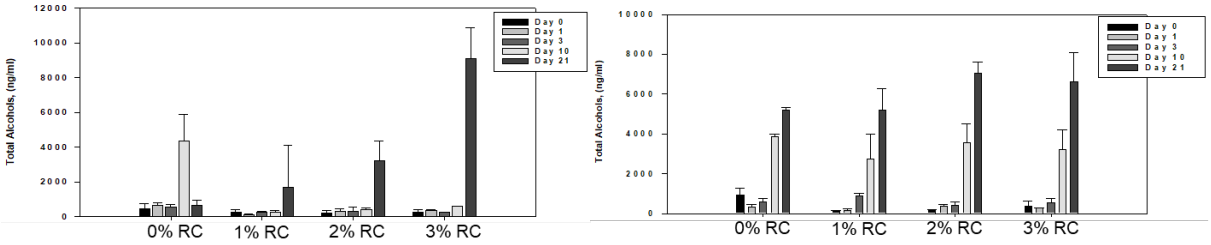

Figure 5: Total alcohols as a function of number of days on the shelf. This data is for 1-MCP treated sliced pears under modified atmosphere bag OTR = 140 (left panel) and OTR – 100 (right panel). The fruit was treated with 0, 1, 2, or 3% of Ripening Compound (RC) and bags were stored at 4 deg C or 39 deg F after slicing. X- axis is number of days.

A similar pattern was also observed for total alcohols. The thicker (OT – 100) MA bag resulted in production of higher amounts of total alcohols. While these alcohols did not produce offensive smell or flavor, a proper consumer trial is essential to quantify the impact on consumer decision.

In summary, the data presented here shows that a MA bag with an OTR around 120 might be best for production of fresh sliced pears. However, the pears need to be treated with 1-MCP and subsequently treated with the Ripening Compound to obtain desirable shelf life and flavor profile. As the data regarding sugars is finalized, the results from this work will be compiled into a manuscript for publication.

As the decision to utilize a MA bag of different OTR is being considered, the fact that the modified atmosphere bags have a range of oxygen transmission rates (OTRs) and carbon transmission rates (CO2TR) is important to keep in mind. It is known that the transmission of gases across packaging structures is governed by factors described by the Fick's law:

Jgas = *A x ACgas/R*

Jgas is the total flux of gas (cm3/s)
A is the surface area of the film (cm2)
Cgas is the concentration gradient across the film
R is the resistance of the film to gas diffusion (s/cm)

For any given fresh – cut produce, the choice of optimal OTR and CO2TR is dependent upon its respiration rate, weight, the internal package dimensions, the targeted atmosphere composition, and product handling temperature. Therefore, it is necessary to select a modified atmosphere bag where oxygen and carbon dioxide transmission rates match the needs of the product. Carbon dioxide diffusion rates are two to five times faster than oxygen and the ratio of carbon dioxide transmission rate to oxygen transmission rate of a polymer in the bag. It seems that the OTR of 140 is somewhat optimal and OTR of 100 can be detrimental to quality.

NOTE: I plan to provide an update to the final report once the data on %Brix and total sugars is collected and analyzed.

Outreach

Peer-reviewed publication - Ikiz D, Gallardo RK, Dhingra A, Hewitt S (2017) Assessing consumers' preferences and willingness to pay for novel sliced packed fresh pears: A latent class approach. Agribusiness

Good Fruit Grower article - Dhingra A, Gallardo K (2017) Customers are willing to pay a premium only on high quality, fresh sliced pears. In: Good Fruit Grower. Washington State Fruit Commission, Wenatchee, WA, pp 36-39

Presentation - Sliced Pears—How to add $1 million to the pear market's bottom line in the PNW. Seanna Hewitt and Amit Dhingra. Annual Washington State Tree Fruit Association Meeting, December 2017.

Presentation to WA and OR packing houses regarding the possibility of producing fresh sliced pears.

Manuscript under preparation – Impact of different OTRs on production of fresh sliced pears.

EXECUTIVE SUMMARY

Executive summaries are required for ending projects. This one-page report should include significant progress or outcomes and summary of finding and future directions (if applicable). Formatting requirements are the same as other project reports. The executive summary should be attached to the end of the final report and sent as one file. This page does not count towards the 10-page final report maximum.

Increasing the per capita consumption of pears has been the most urgent and important priority of the pear industry since the publication of a seminal paper by George Ing (late commissioner, WTFRC) in 1994. Consumers continue to be dissatisfied due to inconsistent quality of pear fruit available on the grocery store shelf.

Respiration (CO2 production) is one of the primary physiological processes involved in fruit maturation and ripening. The rate of respiration of sliced fruit is greater than that of whole fruit. Since no prior data on respiration rates of sliced pears grown in the PNW exists, there is a critical need to determine the respiration rate for sliced pears. This knowledge will be necessary in order to test and develop modified atmosphere (MA) bags with optimal oxygen/carbon dioxide transmission rates, which will maintain high quality of the slices while reducing overall oxidation (browning), retain pear aroma, and extend the shelf life of the sliced fruit for up to 3 weeks.

The aim of our research is to develop a market ready fresh sliced pear product by identifying the optimal modified atmosphere (MA) packaging that will produce highest quality sliced fruit with reduced browning, retention of pear aroma, and extend the shelf life to 21 days. This study has demonstrated that MA bag with an OTR of 140 is somewhat optimal but not ideal and MA bag with an OTR of 100, while producing higher concentration of esters that are critical for pear aroma, may produce less than ideal product due to higher accumulation of alcohols and acetates.

Future research should evaluate MA bags of OTR 110, 120 and 130 combined with consumer trials. There are new types of packages with specialized vials that can regulate OTR tightly and such incorporate of innovations in packaging along with the Ripening Compound can enable the production of market ready fresh sliced pears.

FINAL PROJECT REPORT

Project Title: Ecology of *Trechnites,* an important parasitoid wasp of pear psylla

PI: Dalila Rendon
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Email: vaughn.walton@oregonstate.edu

Cooperators: Tianna DuPont, Louis Nottingham, Elizabeth Beers, Christopher Strohm (Washington State University); Steve Castagnoli (Oregon State University), Chris Nickelsen (Nickelsen orchards, Hood River, OR).

Budget: $ 30,623 **Year 1 (2018):** $ 30,623

Other funding sources: None

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: (541)7374866, (541)772-5165 **Email address:** l.j.koong@oregonstate.edu

Item	2018
Salaries[1]	$14,440
Benefits[2]	$1,444
Supplies	$4,000
Travel[4]	$1,000
Plot Fees[5]	$775
Total	$21,659

Footnotes:
[1]Salaries: 800hr for a Biological Science Tech. at $18.00/hr (20 hrs per week for 40 weeks, 0.5 FTE).
[2]Benefits: 10% of the wage.
[3]Supplies: Trapping material, containers, insect cages for colonies, insecticides for bioassays, plant enclosures
[4]Travel: Weekly travel to orchards for 40 weeks at $0.535 per mile
[5] Plot fees at MCAREC (0.25 acre) for field enclosure experiments

Budget 2
Organization Name: OSU- SOREC **Contract Administrator:** L.J. Koong
Telephone: **Email address:**

Item	2018
Wages	$2,240
Benefits	$224
Plot Fees	
Total	$2,464

Budget 3
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: 510/559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2018
Salaries[1]	$2,500
Supplies[2]	$4,000
Total	$6,500

Footnotes:
[1]Time-slip labor for summer collecting of psyllids and parasitoids
[2]Funds to purchase PCR reagents and other PCR supplies

ORIGINAL OBJECTIVES:

1) To determine the seasonal phenology of *Trechnites insidiosus* in pear orchards and extra-orchard habitats using different monitoring techniques.
2) To determine which floral resources are used by *Trechnites insidiosus* both inside and outside of orchards, and to document use of other psyllid species as alternative hosts.
3) To determine the parasitism capacity of *Trechnites insidiosus* in field enclosures
4) To determine the lethal and sub-lethal effects of common insecticides on larvae and adults of *Trechnites insidiosus*.

SIGNIFICANT FINDINGS

1) *Trechnites* overwinters as larvae in mummified psylla nymphs, and emerge as adults in a single peak during spring (Mid May – Mid June, depending on location). Their population drops during summer, presumably due to low host availability.
2) It was not possible to extract plant DNA fron *Trechnites* to determine floral hosts. Preliminary reports show that *Trechnites* does not parasitize other psyllid species, such as willow psylla.
3) Laboratory experiments showed that, when exposed to *Trechnites,* survival to adulthood of psylla nymphs is reduced by 51%. Furthermore, exposure to *Trechnites* causes psylla nymphs to disperse, potentially contributing to additional mortality without parasitism.
4) Mortality rates in adult *Trechnites* sprayed with Abamectin, Esteem, and Ultor were 82%, 53%, and 26% respectively. Esteem, an insect growth regulator, was surprisingly toxic to adult wasps, while Ultor was the most benign. In larvae (mummified nymphs), mortality with Abamectin, Esteem, and Ultor, was no different than the water control. In all cases the percent unemerged wasps was high, ranging from 87% to 92%.
5) We found a hyperparasitoid complex associated with *Trechnites* and pear psylla, which can potentially affect populations of *Trechnites* in pear orchards. Two different species of parasitoid wasps (and possibly more) also emerged from parasitized nymphs: *Dilyta rathmanae* and *Pachyneuron* sp.

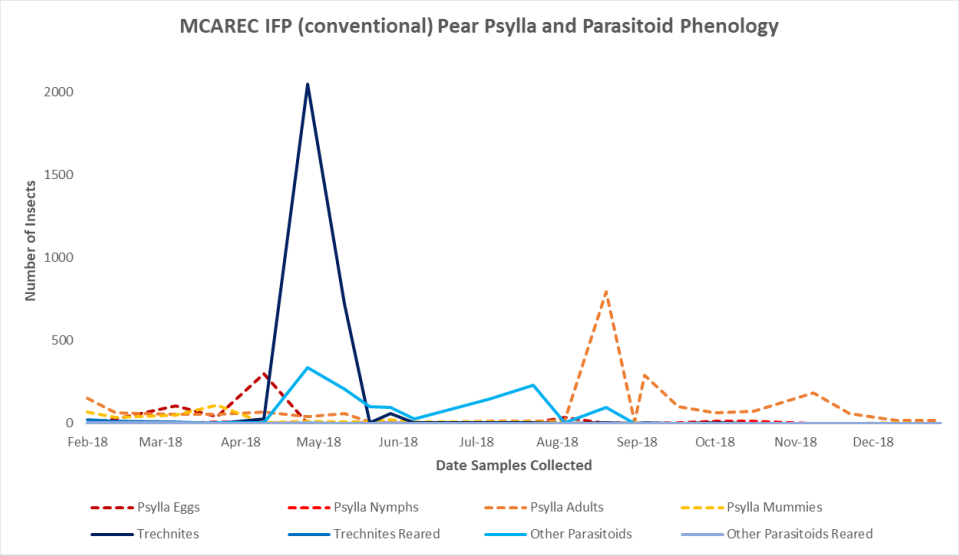
RESULTS AND DISCUSSION

Objective 1: Seasonal phenology of *Trechnites insidiosus*

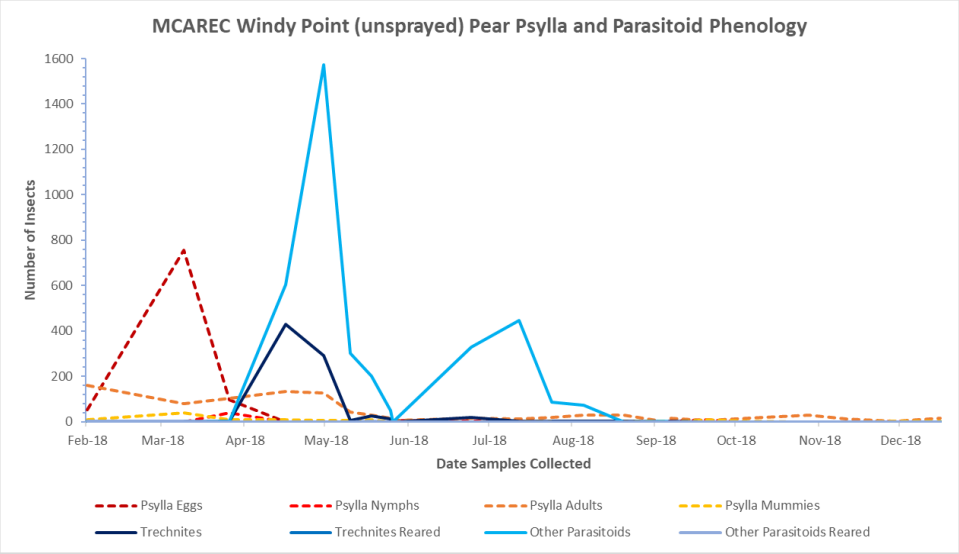
Trechnites overwinters as larvae in psylla mummies in orchards, and adult wasps appear in pear orchards in a single "explosive" peak. In Hood River, most adult *Trechnites* emerged in early May (around 650DD; Fig. 1a, 1b), while in Southern Oregon, *Trechnites* emergence occurred much later (mid-June, around 1700DD; Fig 1c, 1d). In Wapato WA, *Trechnites* emerged at the end of May (around 1500 DD; Fig. 1e). There was very low psylla pressure during the summer, and it is possible that this early abundance of *Trechnites* contributed to keep psylla populations low. The population of *Trechnites* also remained low throughout the season, suggesting that they might be dispersing to other environments, potentially seeking other hosts, or that their population crashed as it might be tightly associated with pear psylla abundance. Number of overwintering mummies collected was low relative to *Trechnites* wasp emergence. This suggests two things, either that overwintering mummies hide on parts of the tree bark where they are difficult to collect, or that additional *Trechnites* adults are migrating into the orchards from other nearby hosts. Overwintering and early spring ecology of *Trechnites* should be further investigated.

Figure 1. Phenology of *Trechnites insidiosus* and *Cacopsylla pyricola* in pear orchards in Hood River OR (a) conventional, (b) unsprayed), Medford OR (c) conventional, d) unsprayed), and Wapato WA (e) unsprayed).

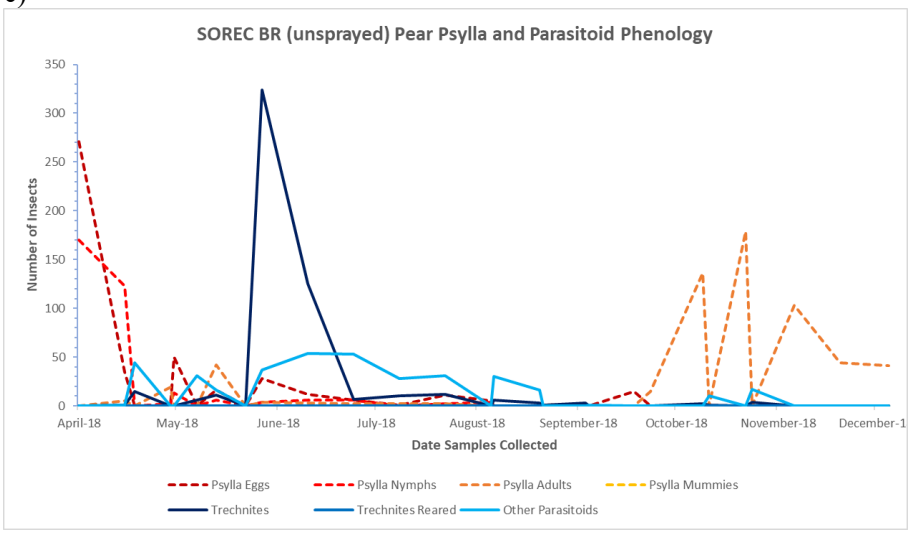
a)



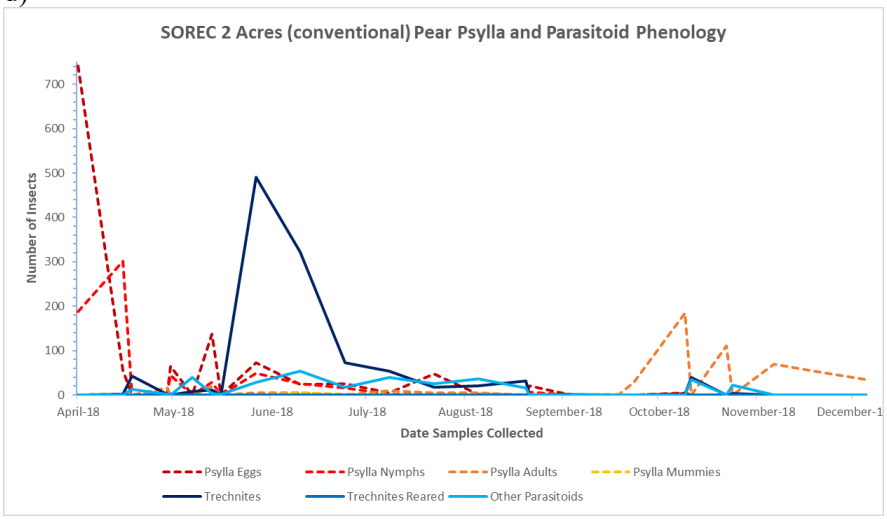
b)



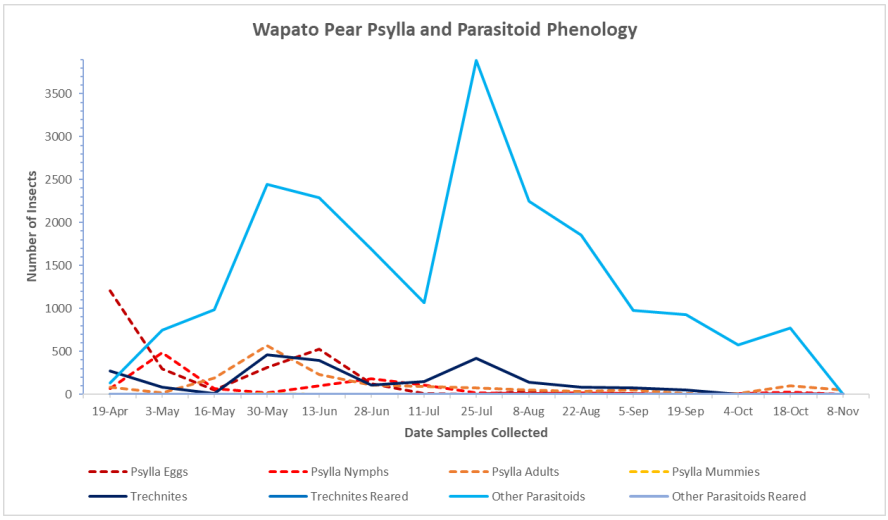
c)


SOREC BR (unsprayed) Pear Psylla and Parasitoid Phenology
Number of Insects
350
300
250
200
150
100
50
0
April-18
May-18
June-18
July-18
August-18
September-18
October-18
November-18
December-1
Date Samples Collected
Psylla Eggs
Psylla Nymphs
Psylla Adults
Psylla Mummies
Trechnites
Trechnites Reared
Other Parasitoids

d)


SOREC 2 Acres (conventional) Pear Psylla and Parasitoid Phenology
Number of Insects
700
600
500
400
300
200
100
0
April-18
May-18
June-18
July-18
August-18
September-18
October-18
November-18
December-1
Date Samples Collected
Psylla Eggs
Psylla Nymphs
Psylla Adults
Psylla Mummies
Trechnites
Trechnites Reared
Other Parasitoids

e)


Wapato Pear Psylla and Parasitoid Phenology
Number of Insects
3500
3000
2500
2000
1500
1000
500
0
19-Apr
3-May
16-May
30-May
13-Jun
28-Jun
11-Jul
25-Jul
8-Aug
22-Aug
5-Sep
19-Sep
4-Oct
18-Oct
8-Nov
Date Samples Collected
Psylla Eggs
Psylla Nymphs
Psylla Adults
Psylla Mummies
Trechnites
Trechnites Reared
Other Parasitoids
Other Parasitoids Reared

Objective 2: Floral resources and psyllid hosts.

We attempted to extract pollen from *Trechnites* wasps to determine which floral resources they use, as it has been done in other insects (i.e., psylla, bees). Due to the diminute size of *Trechnites,* it was not possible to wash pollen from their bodies, therefore we could not amplify plant DNA. Future studies should investigate alternative methods to determine which in-bloom plant species does *Trechnites* visit and feed from.

Other species of psyllid hosts, such as willow psyllid, were examined for *Trechnites* parasitism. Preliminary results show that there is no overlap among the wasp species that parasitize pear psylla and willow psylla, but this work is still in progress.

Objective 3: Parasitism capacity of *Trechnites*

Maintaining *Trechnites* wasps in the laboratory was very challenging, as they are very fragile and experience high mortality when manipulated. We were not able to rear a colony of *Trechnites* or psylla, therefore all our parasitism trials were done with field-collected wasps and psylla nymphs. It was not possible to assess whether field-collected nymphs were already parasitized, so we ran a baseline assay without wasp exposure to find out roughly what proportion of nymphs were already parasitized. To reduce unsuitable conditions, we performed this experiment in the laboratory rather than the field.

Five nymphs were placed in a clear cylindrical container with a fresh pear leaf (Fig. 2), and allowed to develop until adulthood. We tested two treatments, one with no wasp (n = 64), and one where one *Trechnites* wasp was introduced into the container for 72 h (n = 37). Without a wasp, 35% of the nymphs reached adulthood, while only 18% reached adulthood when exposed to one *Trechnites* wasp ($X^2 = 8.12$, df =1, p =< 0.01; Fig. 3). We also observed that nymphs sometimes managed to crawl out of the container through the bottom. In treatments without wasps, 4% of the nymphs were recorded as missing, while 14% of the nymphs went missing in treatments with a *Trechnites* wasp ($X^2 = 4.04$, df =1, p =< 0.04; Fig. 3). This suggests that *Trechnites* exerts non-consumptive effects on psylla nymphs. This phenomenon happens when predators modify the behavior of a prey without consuming it; in this case, psylla nymphs were more likely to disperse when a wasp was present. This shows that the presence of *Trechnites* can affect psylla nymphs even without parasitism, as dispersing nymphs can likely die when moving to different environments. Another option is behavior modification through parasitism, and it is possible that parasitized nymphs seek refuge away from the leaves to protect the developing wasp larva.

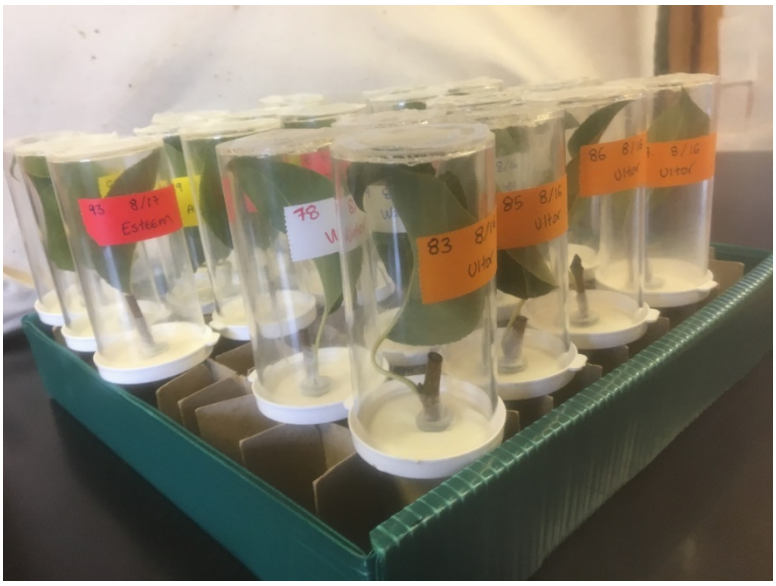


Figure 2. Wasp enclosures to assess parasitism and mortality.

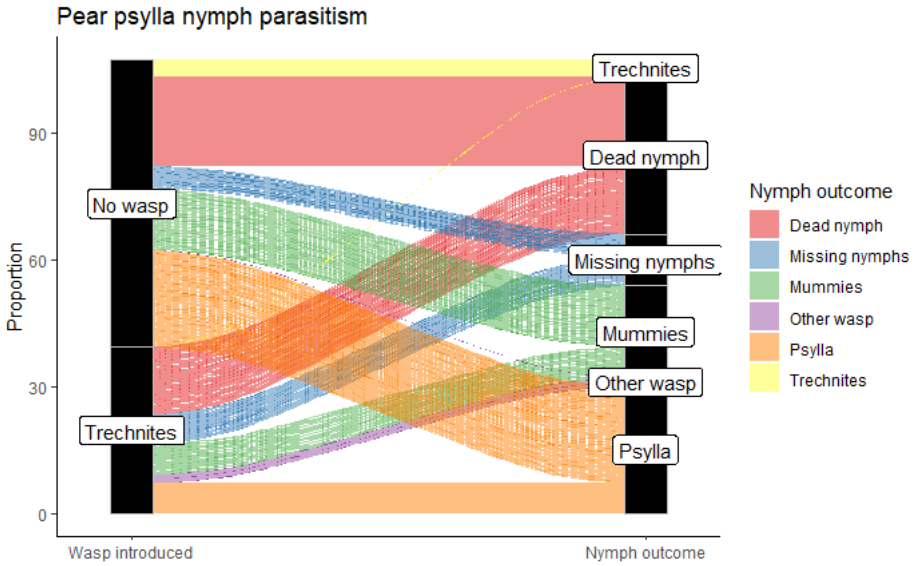


Figure 3. Survival outcome proportions from five psylla nymphs in a leaf container with or without one *Trechnites* wasp.

Objective 4: Insecticide effects on *Trechnites* adults and larvae

Using a potter spray tower at 6 PSI, we sprayed adult *Trechnites* wasps and larvae (developing inside psylla mummies) with 2 mL (0.06 fl oz) of three insecticides at the maximum rate registered for pear psylla: Abamectin 0.15LV (20 fl oz / acre), Esteem 0.86 EC (pyriproxyfen, 16 oz / acre), and Ultor (spirotetramat, 14 fl oz / acre).

A single adult wasp was sprayed in an arena (Fig. 4). After exposure, we kept the wasp for 1 h in the spray arena, and then we transferred the wasp to a leaf container with water and honey. Mortality was recorded after 48h. When sprayed with Abamectin (n = 34), Esteem (n = 32), and Ultor (n = 30), mortality rates in adult wasps were of 82%, 53%, and 26% respectively (Fig. 5). Wasps sprayed with a water control (n = 32) resulted in 15% mortality, with was similar to mortality in Ultor ($X^2 = 0.57$, df =1, $p = 0.45$), but lower than mortality with Esteem ($X^2 = 8.38$, df =1, $p < 0.01$), and Abamectin ($X^2 = 15.12$, df =1, $p < 0.01$). The results with Esteem were surprising, as this is an insect growth regulator targeting juvenile stages and is not supposed to affect adult insects, but it caused greater than expected mortality in adult wasps. Overall, Ultor was the most benign insecticide for adult wasps, and Abamectin was the most detrimental.

We also recorded the number of wasps emerging from a group of ten mummies attached to a filter paper. After being sprayed with Abamectin (n = 28), Esteem (n = 29), and Ultor (n = 28), the percentage of unemerged (dead) wasps from psylla mummies was 87%, 92%, and 88%, respectively (Fig. 6). This mortality was high, but not different from unemerged wasps after being sprayed with a water control (87%, n = 30; $X^2 = 1.65$, df =3, $p = 0.64$). This could be interpreted as a positive result, indicating that wasp larvae developing inside psylla mummies are protected from detrimental effects of insecticide applications. Unfortunately, wasp emergence from mummies was very low, reflecting either a natural trend in the field or higher mortality due to manipulation in the laboratory.

Tying phenology data with insecticide assay results, it is possible to give some recommendations for spray applications to try to minimize negative impact on *Trechnites.* While Esteem might cause some mortality on *Trechnites* larvae developing in mummies in early spring, this impact is minimal. It is not recommended to apply Abamectin during bloom, but it is essential to time the first application such that it is before peak adult *Trechnites* emergence. Abamectin may not significantly impact *Trechnites* larvae in mummies, but it causes high mortality in adults. If Abamectin is applied during or shortly after adult *Trechnites* emergence, the population will be significantly affected. More research on degree days models is thus necessary to more accurately predict when *Trechnites* wasps emerge. Ultor is usually applied following bloom, after *Trechnites* adult emergence, and this is the most benign product unlikely to significantly affect *Trechnites* populations. Mortality data from other commonly used insecticides is needed.

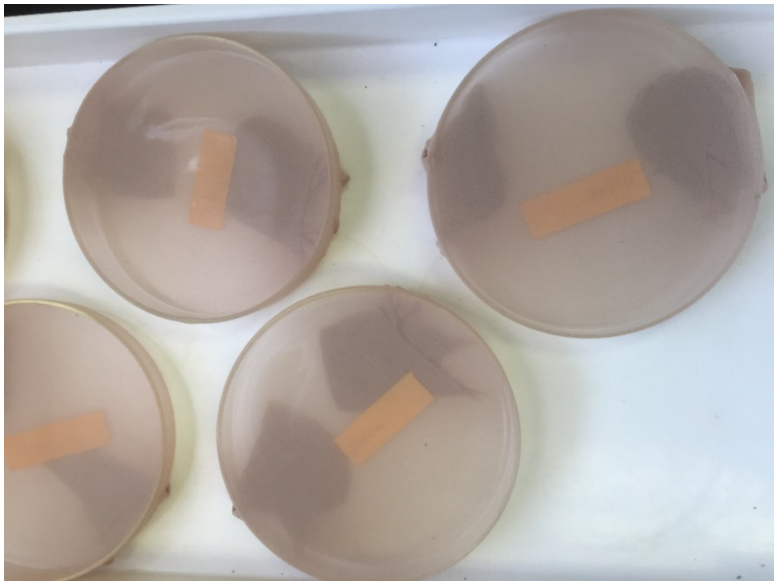


Figure 4. Insecticide assay arenas for spraying adult wasps

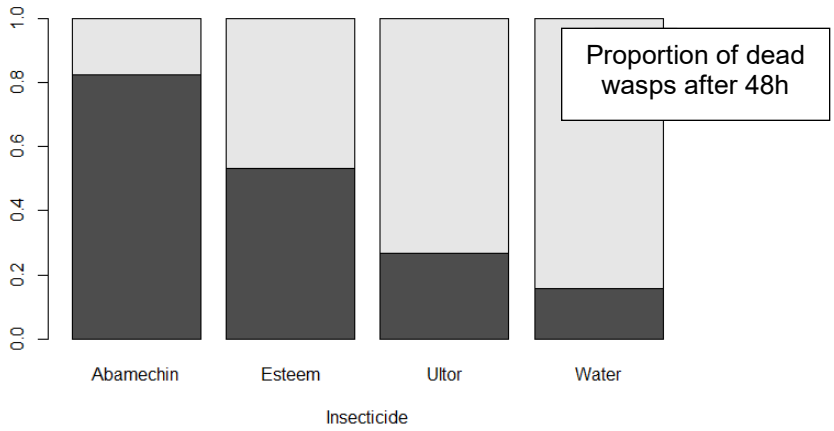


Figure 5. Adult wasp mortality 48 h after insecticide exposure.

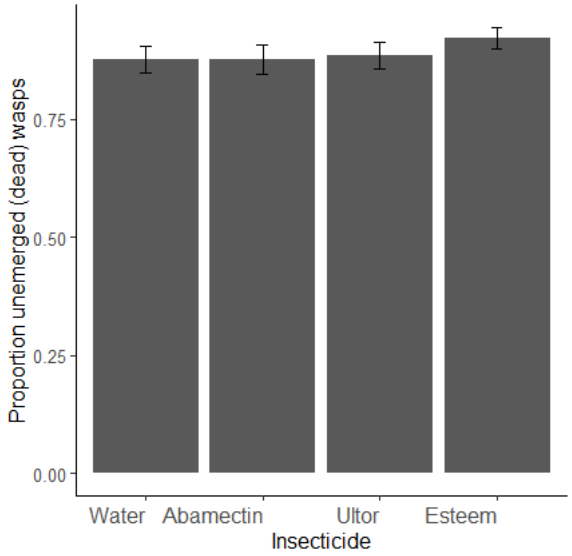


Figure 6. Proportion of unemerged (dead) wasp larvae from mummies after insecticide exposure

Additional results: hyperparasitoid complex

From the insecticide assays, we found that besides *Trechnites* (Fig. 8a), there are at least two more species of wasps that emerge from pear psylla mummies (Fig. 7). These wasps were sent to taxonomists and identified as *Pachyneuron sp.* (Hymenoptera: Pteromalidae; Fig 8b), and *Dilyta rathmanae* (Hymenoptera: Figitidae; Fig. 8c). From previous records (Menke and Evenhuis 1991, Schick 1994), these wasps are reported as being hyperparasitoids; that is, they lay eggs inside *Trechnites* larvae developing inside psylla nymphs. The presence of these hyperparasitoids can affect *Trechnites* populations, and the interactions among parasitoids in pear warrant further research.

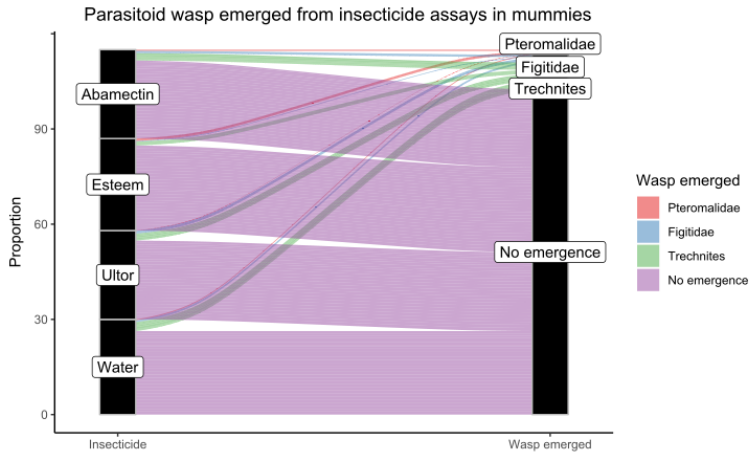


Figure 7. Parasitoid wasp emergence proportions from insecticide assays in psylla mummies.

a)

b)

c)

Figure 8. parasitoids reared from *Cacospsylla pyricola* nymphs. a) *Trechnites insidiosus* (Hymenoptera: Encyrtidae), b) *Pachyneuron* sp. (Hymenoptera: Pteromalidae), c) *Dilyta rathmanae* (Hymenoptera: Figitidae).

Additional results: *Trechnites* DNA detection in psylla mummies

Given that there are at least three species of parasitoid wasps in pear orchards, and that there is a very low rate of wasp emergence from mummies, it is not possible to know which wasp has parasitized a psylla mummy found in the field. We are currently testing DNA identification from mummies. In preliminary assays, we found that using a primer designed for *Trechnites,* it was possible to identify *Trechnites* DNA from parasitized psylla mummies, including empty mummies (the exoskeleton left behind after the wasp emerges; Fig. 9). Future work should focus on designing primers to identify DNA from the other parasitoid wasps found in pear orchards, which will help answer many questions regarding interactions among the parasitoid wasps and pear psylla.

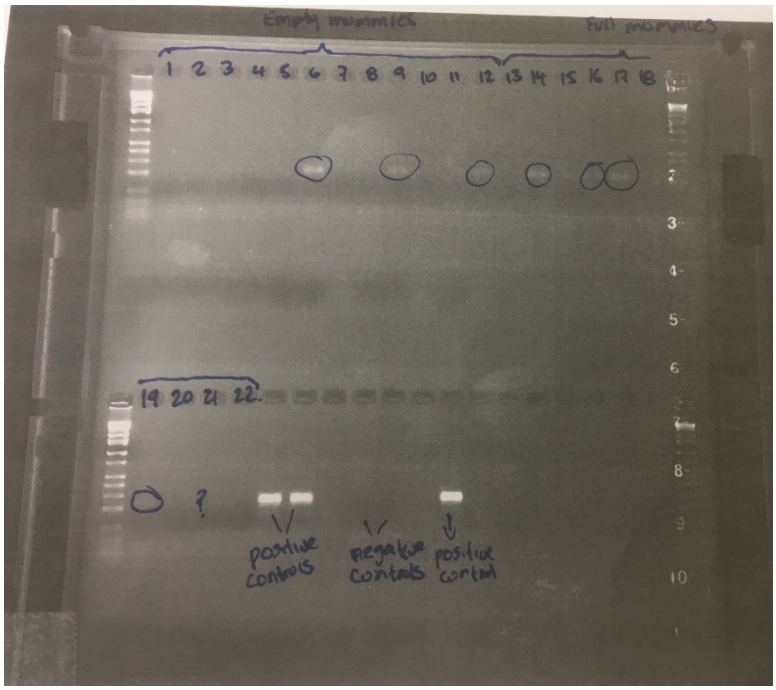


Figure 9. Results from a polymerase chain reaction (PCR) aimed to detect *Trechnites* DNA in parasitized psylla mummies. Circled marks represent samples where *Trechnites* DNA was detected in "empty" mummies (exoskeleton left behind after the wasp emerged), and "full" mummies (with the wasp larva still developing inside).

EXECUTIVE SUMMARY

Trechnites overwintered as larvae in psylla mummies in orchards, and adult wasps appeared in pear orchards in a single "explosive" peak. In Hood River, most adult *Trechnites* emerged in early May (around 650DD), while in Southern Oregon, *Trechnites* emergence occurred much later. In Wapato WA, *Trechnites* emerged at the end of May. There was very low psylla pressure during the summer, and it is possible that this early abundance of *Trechnites* contributed to keep psylla populations low. The population of *Trechnites* also remained low throughout the season, suggesting that they might be dispersing to other environments, potentially seeking other hosts, or that their population crashed as it might be tightly associated with pear psylla abundance. Number of overwintering mummies collected was low relative to *Trechnites* wasp emergence. This suggests that overwintering mummies hide on tree bark where they are difficult to collect, or that additional *Trechnites* adults are migrating into the orchards from other nearby hosts.

When pear psylla nymphs were not exposed to *Trecnhites,* 35% reached adulthood, while only 18% reached adulthood when exposed to one *Trechnites* wasp. We also observed that psylla nymphs were more likely to disperse (presumably to safer environments) when a wasp was present. This shows that the presence of *Trechnites* can affect psylla nymphs even without parasitism, as nymphs can likely die when moving to different environments.

When sprayed with Abamectin, Esteem, and Ultor, mortality rates in adult wasps were of 82%, 53%, and 26% respectively. Wasps sprayed with a water control had 15% mortality, with was similar to mortality in Ultor, but lower than mortality with Esteem, and Abamectin. The results with Esteem were surprising, as this is an insect growth regulator targeting juvenile stages and is not supposed to affect adult insects, but it caused greater than expected mortality in adult wasps. Overall, Ultor was the most benign insecticide for adult wasps, and Abamectin was the most detrimental.

After being sprayed with Abamectin, Esteem, and Ultor, the percentage of unemerged (dead) wasp larvae from psylla mummies was 87%, 92%, and 88%, respectively. This mortality was high, but not different from unemerged wasps after being sprayed with a water control (87%). This could be a positive result, indicating that wasp larvae developing inside psylla mummies are protected from detrimental effects of insecticide applications. However, wasp emergence from mummies was very low, reflecting either a natural trend in the field or higher mortality due to manipulation in the laboratory.

Tying phenology data with insecticide assay results, it is possible to give some recommendations for spray applications to try to minimize negative impact on *Trechnites.* While Esteem might cause some mortality on *Trechnites* larvae developing in mummies in early spring, this impact is minimal. It is not recommended to apply Abamectin during bloom, but it is essential to time the first application such that it is before peak adult *Trechnites* emergence. Abamectin does not significantly impact *Trechnites* larvae in mummies, but it causes high mortality in adults. If Abamectin is applied during or shortly after adult *Trechnites* emergence, the population will be significantly affected. More research on degree days models is thus necessary to more accurately predict when *Trechnites* wasps emerge. Ultor is usually applied after *Trechnites* adult emergence, and this is the most benign product, unlikely to significantly affect *Trechnites* populations. Mortality data from other commonly used insecticides is needed.

FINAL PROJECT REPORT

Project Title: Delivering quality pear fruit to consumers

PI: Yu Dong
Organization: Oregon State University MCAREC
Telephone: 541-386-2030 (ext. 38229)
Email: dongyu@oregonstate.edu
Address: 3005 Experiment Station Drive
City/State/Zip: Hood River/OR/97031

Cooperators: Steve Castagnoli, Paul Chen, Drs. Shunchang Cheng, Yingli Li, Shaoying Zhang

Other funding sources: none

Total Project Funding: **Year 1:** $25,725 **Year 2:** $26,390 **Year 3:** $27,073 **Year 4:** $0

Budget History:

Item	2015:	2016:	2017:	2018 (No-cost extension)
Salaries	13,088[1]	13,481	13,885	0
Benefits	1,250[2]	1,300	1,352	0
Wages	6,715[3]	6,917	7,124	0
Benefits	672[4]	692	712	0
Equipment				
Supplies	3,500[5]	3,500	3,500	0
Travel	500[6]	500	500	0
Plot Fees				
Miscellaneous				
Total	25,725	26,390	27,073	0

Footnotes:
[1]Postdoctoral Research Associate: 1/3 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 500hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 10% of the wage, with a 3% annual increase.
[5]Supplies: maintaining cold rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), and gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

OBJECTIVES:

1. Elucidate the cell metabolic mechanisms and pre/postharvest factors affecting the development of buttery-juicy melting texture (BJMT) during ripening of pears.
2. Study pre/postharvest factors influencing the chilling requirement for ripening capacity (CRRC) of pears.
3. Develop conditioning protocols for 1-MCP treated Anjou pear.

SIGNIFICANT FINDINGS

1. Elucidate the cell metabolic mechanisms and pre/postharvest factors affecting the development of BJMT during ripening of pears.

a. ***Cell wall pectin metabolism.*** Water-soluble pectins (WSP), CDTA-soluble pectins (CSP), and pectin methylesterase (PME) were positively correlated with BJMT. WSP are hygroscopic and give consumers the BJMT feeling when eating pear.

b. ***Ethylene.*** The expression of *PcACO1* gene triggers ethylene synthesis and is associated with pectin metabolism.

c. ***BJMT index.*** BJMT index was developed to identify BJMT of pears based on extractable juice (EJ, mL $100g^{-1}$). BJMT index = (100 - EJ) / 10.

d. ***Factors affecting the development of BJMT***

1) ***Harvest maturity***. Anjou pears harvested at 15-14 lbs have an excellent BJMT and flavor after 4-7 months of regular-air (RA) storage at 30 °F plus 7 d at 68 °F, while pears harvested at 13-12 lbs have inferior BJMT and inferior flavor.

2) ***1-MCP + ethylene***. 150 ppb 1-MCP-treated Anjou pears (15-14 lbs) fail to develop BJMT following 8 months of RA storage plus 7 d at 68 °F. However, 1-MCP (150 ppb) + ethylene (150 ppb)-treated pears develop BJMT after 8 months of RA storage followed by 7 d at 68 °F.

3) ***Storage temperature***. Anjou pears stored at 30-32 °F develop BJMT in 5-7 months. Higher storage temperature, such as 34 °F, accelerate the development of BJMT; fruit stored at this temperature show increased development of superficial scald.

4) ***Controlled atmosphere (CA) storage***. Anjou pears develop an excellent BJMT after 3-8 months of regular CA (~1.5% O_2 + < 0.05% CO_2) or 3-10 months of low-O_2 CA (~0.8% O_2 + < 0.05% CO_2) storage at 30 °F plus 7 d at 68 °F.

2. Study pre/postharvest factors influencing the CRRC of pears.

a. ***Accumulated cold units (ACU*** *= hours < 50 °F during 42 d prior to harvest****) and Harvest maturity.*** On an orchard specific basis, ACU (0-300) affected CRRC when Anjou pears were harvested at 13-15 lbs. Higher ACU reduced CRRC. Harvest maturity also affected CRRC with more mature fruit generally having lower CRRC. Neither of these trends held across growing districts.

b. ***Ca content.*** Orchard elevation does not affect uptake of Ca in peel or pulp tissue. Six applications of 0.45% (w/v) $CaCl_2$ from 30 days after full bloom (DAFB) to 130 DAFB increased Ca absorption, and extended CRRC for ~10 d.

c. ***Temperature and ethylene conditioning.*** High ethylene exposure (100 ppm) with high storage temperature reduced CRRC and caused BJMT in the early ripening test. Fruit held at 68 °F and treated with 100 ppm ethylene for 72 h experienced elimination of CRRC, and

significant softening. These pears failed to develop a dessert quality comparable to chilled fruit.

3. Develop conditioning protocols for 1-MCP treated Anjou pear.

a. Late-harvested Anjou pears treated with 1-MCP-treated were able to ripen with minimal scald.
b. 300 ppb 1-MCP combined with 300 ppb ethylene improved ripening capacity (RC) of Anjou pears after long-term CA storage (i.e. > 7-8 months).

METHODS

Objective 1. Lab procedures were developed to quantify cell wall total pectin substances (TPS), WSP, CDTA-soluble pectin (CSP), and sodium carbonate-soluble pectin (SSP). Polygalacturonase (PG) and pectin methylesterase (PME), key enzymes regulating the pectin degradation process, were monitored. Anjou pear fruit harvested at commercial maturity was ripened for 7 d at 68 °F after storing at 30 °F for 0, 1, 2, 3, 4, 5, 6, 7, or 8 months in RA. Tissue samples were frozen in liquid N_2 and stored at -80 °C until analysis. The effects of harvest maturity (FF = 15-12 lbs), 1-MCP+ethylene, storage temperatures (30, 32, and 34 °F), and CA storage on cell wall pectin metabolism and buttery-juicy texture development were studied. An industry standard methodology was developed to objectively quantify the buttery-juicy texture.

Objective 2. Pears were sampled from 10 orchards in the North Central WA, Mid-Columbia, and Southern OR growing districts on several dates over two seasons. The orchards ranged in elevation from 540 ft to 1890 ft. A total of 44 samples were collected for this study.

Objective 3. To facilitate early marketing of 1-MCP treated Anjou pears, commercially feasible conditioning protocols were developed to ensure that conditioned fruit with ripening capacity had optimal shipping firmness and post-conditioning storage life. Conditioning parameters included ethylene conditioning, intermediate temperature conditioning, and ethylene + intermediate temperature conditioning.

RESULTS

Elucidate the cell metabolic mechanisms and pre/postharvest factors affecting the development of BJMT during ripening of pears.

a. Cell wall pectin metabolism. Anjou pears harvested at 15-14 lbs from MCAREC showed no RC expressed by fruit firmness following 1-3 months of RA storage at 30 °F plus 7 d at 68 °F. These pears began to soften below 5 lbs after 4 months of storage and developed excellent BJMT. However, coarse and dry texture was observed after 8 months of RA storage during the ripening test (Fig. 1). The development of BJMT was negatively correlated with RC, but was positively correlated with WSP, CSP, or PME (Table 1). No relationship between BJMT and TPS, SSP, or PG was observed at any length of storage period.

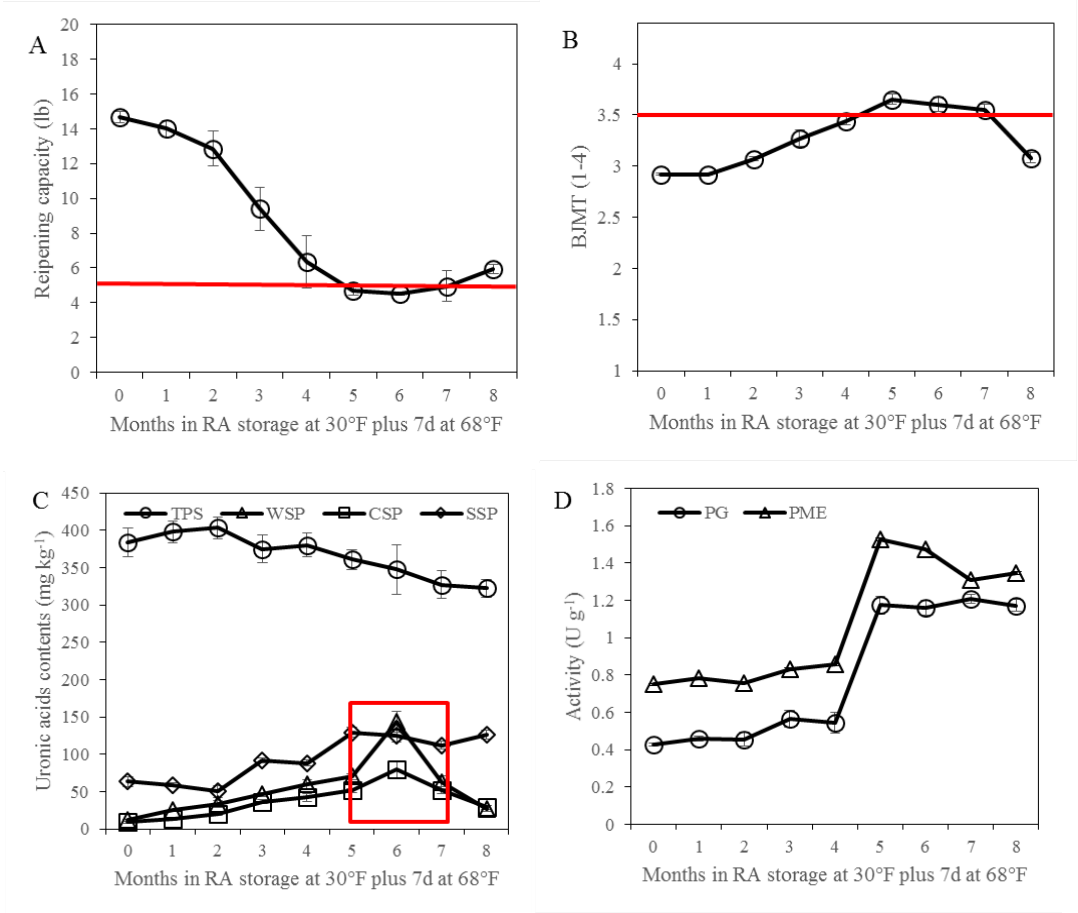


Fig. 1. Ripening capacity (RC) expressed by fruit firmness (A); BJMT (B); total pectin substances (TPS), water-soluble pectin (WSP), CDTA-soluble pectin (CSP), sodium carbonate-soluble pectin (SSP) (C); polygalacturonase (PG), and pectin methylesterase (PME) (D) of Anjou pears following 8 months of RA storage at 30 °F plus 7 d at 68 °F.

Table 1. Correlation analysis among RC, BJMT, TPS, WSP, CSP, SSP, PG, and PME.

	RC	BJMT	TPS	WSP	CSP	SSP	PG	PME
RC								
BJMT	**-0.909****							
TPS	0.781*	-0.488						
WSP	-0.691*	**0.919****	-0.366					
CSP	-0.863**	**0.818****	-0.562	0.953**				
SSP	-0.396*	0.299	-0.866**	0.599	0.368			
PG	-0.001	0.001	0.727*	0.074	-0.130	-0.348		
PME	-0.859**	**0.668***	-0.501	0.870**	0.724*	0.427	-0.486	

*, ** significant difference at $P = 0.05$ and 0.01 levels, respectively.

b. Ethylene. The *PcACO1* gene was a critical factor correlated with ethylene production. Ethylene synthesis triggered the development of RC and participated in pectin metabolism (Fig. 2).

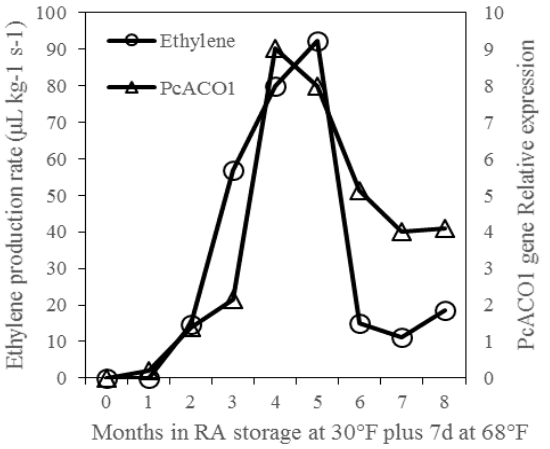


Fig. 2. Ethylene production rate and expression of PcACO1 gene of Anjou pears following 8 months of RA storage at 30 °F plus 1 d at 68 °F.

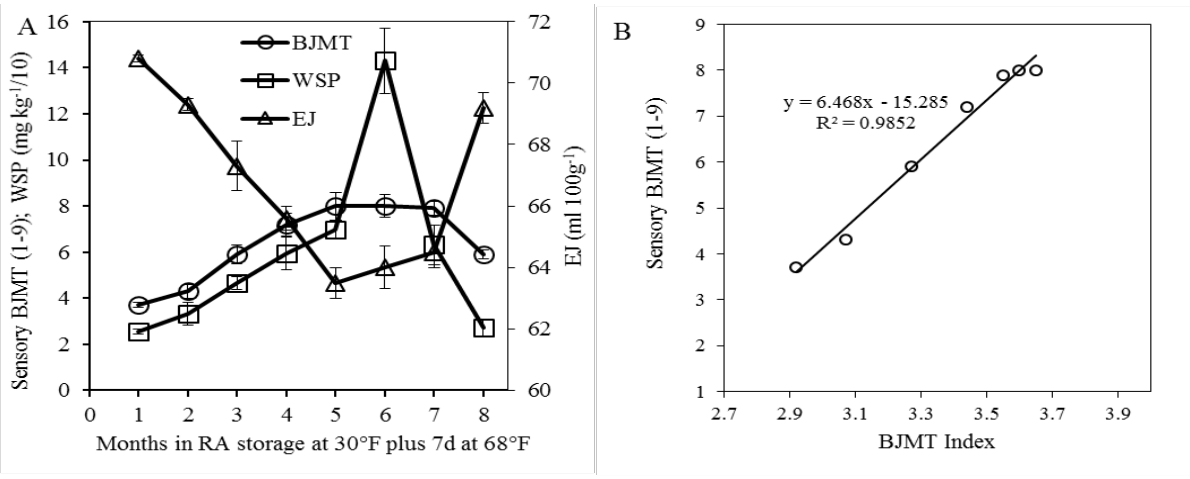


Fig. 3. BJMT, WSP, and extractable juice (EJ) (A) and the correlation of BJMT index with sensory BJMT scores (B) of Anjou pears following 8 months of RA storage at 30 °F plus 7 d at 68 °F.

c. Factors affecting the development of BJMT.

1) ***Harvest maturity***. In 2016, Anjou pears were harvested at 15-11 lbs from MCAREC. Flesh firmness for H1 was 14.7 lbs; for H2, 12.8 lbs; for H3, 11.2 lb. H2 or H3 pears developed a BJMT after 2-4 months of RA storage at 30 °F followed by 7 d at 68 °F. By contrast, H1 pears developed BJMT after 4-6 months under the same storage conditions. Flesh firmness in H2 and H3 was below 5 lbs following 4-6 months of RA at 30 °F plus 7 d at 68 °F, resulting in a coarse and dry texture in both H2 and H3 pears, as well as a dramatic reduction of WSP (Fig. 4).

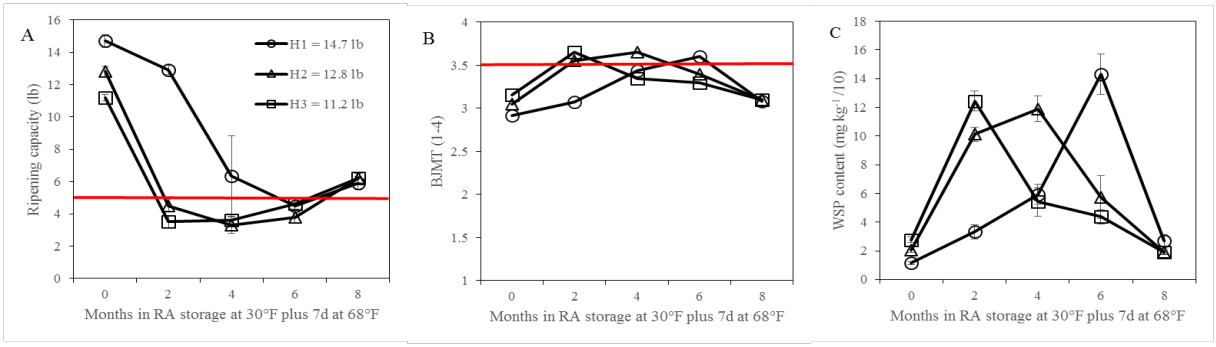


Fig. 4. RC (A), BJMT (B), and WSP (C) affected by harvest maturity of Anjou pears at MCAREC following 8 months of RA storage at 30 °F plus 7 d at 68 °F.

2) Assessments of RC and BJMT indicated that Anjou pears harvested from MCAREC at 15-14 lbs developed BJMT after 5-7 months of RA storage at 30 °F plus 7 d at 68 °F. 1-MCP-treated pears failed to develop BJMT over the whole of the storage period, while 1-MCP combined with ethylene recovered RC and developed BJMT at higher WSP levels after 8 months of RA storage at 30 °F plus 7 d at 68 °F (Fig. 5).

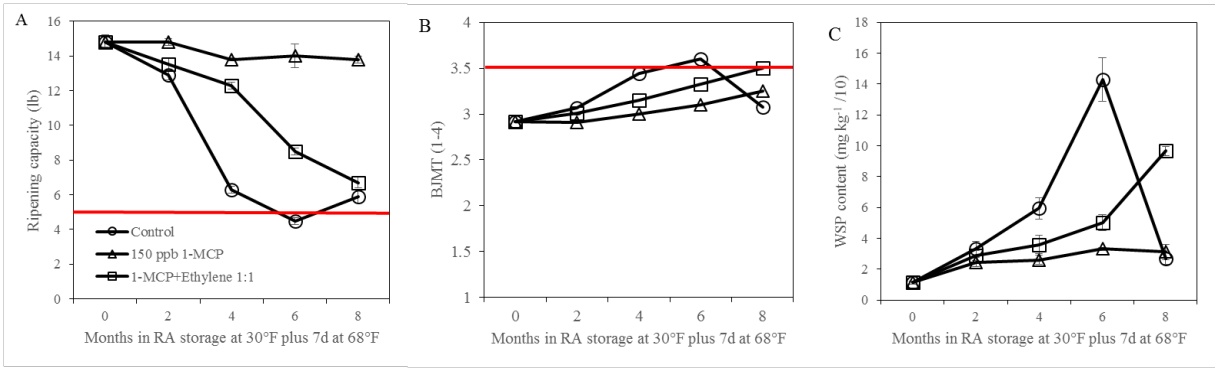


Fig. 5. RC (A), BJMT (B), and WSP (C) in Anjou pear treated with 150 ppb 1-MCP alone or with a combination of 150 ppb 1-MCP plus 150 ppb ethylene of Anjou pears following 8 months of RA storage at 30 °F plus 7 d at 68 °F.

3) Although Anjou pears harvested at 15-14 lbs developed BJMT after 5-7 months of storage at 32 °F, these fruit had a higher incidence of superficial scald during ripening. After 4 months of 34 °F, fruit had developed BJMT, indicating that the higher storage temperature can promote ability to ripen. The most effective storage temperature for Anjou pears appeared to be 30 °F (Fig. 6).

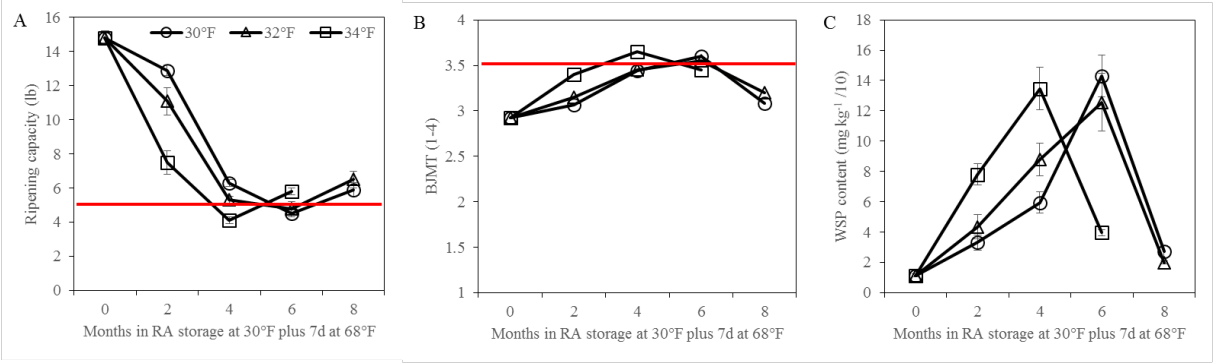


Fig. 6. RC (A), BJMT (B), and WSP (C) affected by storage temperature of Anjou pears following 8 months of RA storage at 30 °F plus 7 d at 68 °F.

4) ***CA storage.*** Anjou pears were harvested from MCAREC at 15-14 lbs and subjected to regular CA; these pears developed BJMT during 3-8 months of storage at 30 °F plus 7 d at 68 °F. Further, pears subjected to low-O_2 CA developed longer-lasting BJMT and maintained higher WSP than regular CA- or RA-treated pears. Decreasing O_2 to 0.8% extended the life of the BJMT quality. (Fig. 7).

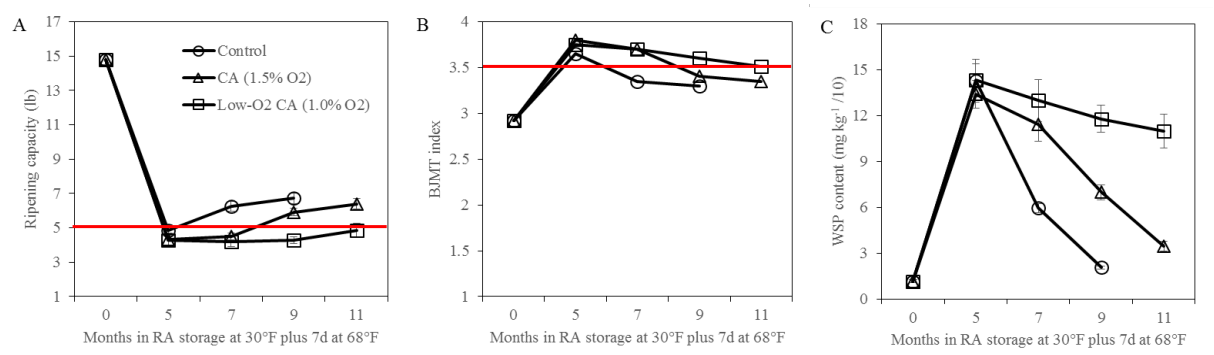


Fig. 7. RC (A), BJMT (B), and WSP (C) as affected by regular CA (~1.5% O_2 + < 0.05% CO_2) or low-O_2 CA (~0.8% O_2 + < 0.05% CO_2) of Anjou pears following 5, 7, 9, and 11 months of storage at 30 °F plus 7 d at 68 °F.

<u>Study pre/postharvest factors influencing the CRRC of pears.</u>

a. ACU and harvest maturity. In preliminary studies with fruit from the Hood River Valley harvested at ~15 lbs from altitudes of ~500 - 2,000 ft ACU ranged from 0 to 269 units. Fruit with ACU >200 units had significantly greater WSP and a significantly higher BJMT index (Fig. 8A and B). Production elevation and harvest maturity significantly influenced CRRC (Fig. 8C). Fruit from low elevation had higher CRRC than pears harvested from higher elevation at the same harvest maturity.

For the 44 fruit samples obtained over two seasons from 10 orchards in North Central WA, Mid-Columbia, and Southern OR, there was a slight trend toward decreased CRRC with higher ACU, however, this relationship was very weak (R^2=0.05). There was a slightly stronger trend toward decreased CRRC with more advanced maturity (R^2=0.14). These results indicate that, both ACU and harvest maturity were only weakly correlated CRRC, so

neither seems useful in modeling and predicting CRRC at harvest time across regions. For any single orchard in a given season, the relationship between ACU and CRRC was generally much stronger (R^2= 0.34 to 0.95), so ACU may be a useful indicator of CRRC on a site specific basis. As indicated above, orchard elevation can have an effect on ACU and consequently on CRRC. In the Hood River Valley, for example, a negative temperature gradient follows the north-south elevation gradient and generally results in higher ACU at higher elevation (R^2=0.81). The corresponding trend in CRRC with increased elevation, is however, much weaker (R^2=0.12). Together, these results indicate that additional factors must be considered in developing a predictive model for CRRC.

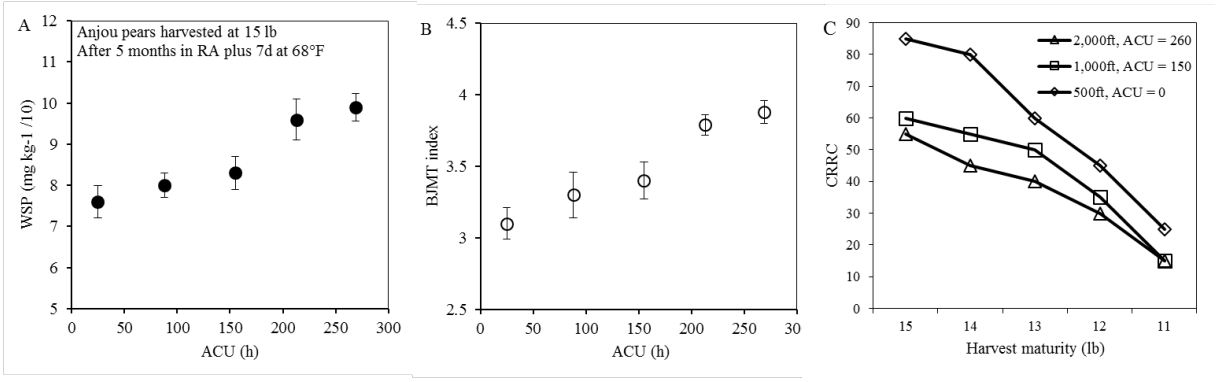


Fig. 8. WSP (A) and BJMT index (B) of Anjou pears from the Hood River Valley as affected by accumulated cold units (ACU) after 4 months of RA storage at 30 °F plus 7 d at 68 °F. Relationship between CRRC and harvest maturity (C) in Anjou pears.

b. ***Ca content.*** Anjou pears were harvested at ~13 lbs from five orchards with elevations ranging from ~500 - 2000 ft, and peel and pulp Ca content was measured. There were no significant differences in peel Ca content among the five orchards (Fig. 9A), although pulp tissues differed. There was no relationship between CRRC and Ca content in peel or pulp tissue (Fig. 9A and B). At MCAREC, we sprayed 0.45% (w/v) $CaCl_2$ 6 times at 20-day intervals from 30 DABF to 130 DAFB. These applications significantly increased Ca content in peel and pulp tissue at harvest and required longer CRRC (about 10 days) than untreated fruit (Fig. 9C).

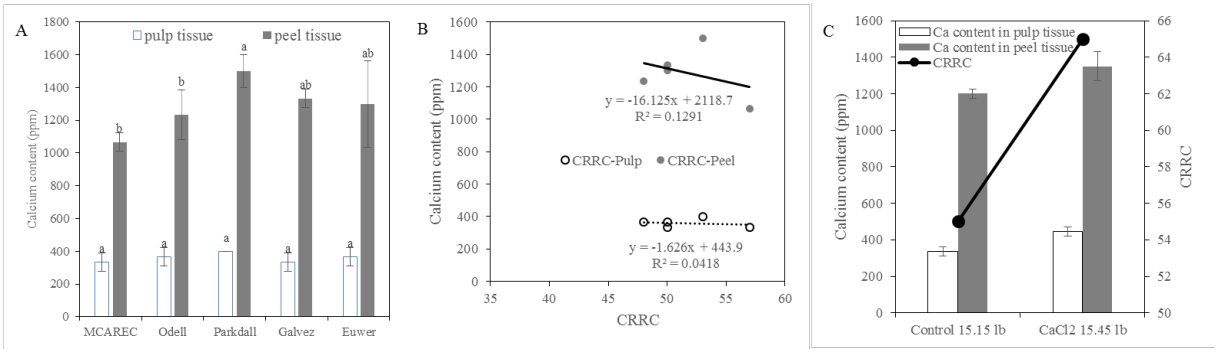


Fig. 9. Calcium content in pulp and peel tissue (A) of Anjou pears from five orchards in the Hood River Valley, OR. Relationship between calcium content in pulp or peel tissue and CRRC (B) in Anjou pears. Calcium content and CRRC (C) affected by $CaCl_2$ sprays to Anjou pears at MCAREC.

c. ***Temperature and ethylene conditioning.*** Anjou pears were harvested at 14 lbs and treated with 100 ppm ethylene at 68 °F for 24, 48, and 72 hr. After each 24 h period, fruit were removed and separated into two groups. One group was held at 30 °F, the other at 50 °F. As shown in Table 2, the longer the ethylene exposure duration, the shorter the CRRC required. In addition, the higher storage temperature reduced CRRC requirement. Ethylene at 100 ppm for 72 hrs can eliminate CRRC, but the ethylene-treated fruit did not develop dessert quality.

Table 2. CRRC in Anjou pears condition in 100 ppm ethylene for 0, 24, 48, 72 h at 68 °F followed by temperature condition at 30 °F or 50 °F.

Ethylene conditioning duration (h)	Condition temperature (°F)	
	30	50
0	65	15
24	30	5
48	10	5
72	0	0

<u>Develop conditioning protocols for 1-MCP treated Anjou pears.</u>

a. ***Late-harvest pears treated with 1-MCP.*** Late-harvested fruit are prone to loss of firmness and green color during storage. These pears are more susceptible to superficial scald after removal from RA storage and develop a coarse and dry texture at ripening. Fruit mature faster on the tree in hot seasons; labor shortages in recent years have resulted in fruit being harvested at over-mature stages with reduced storability. In this study, late-harvest pears treated with 150 ppb 1-MCP at LM1 and LM2 developed BJMT and less superficial scald compared to untreated fruit after 6 months of RA storage at 30 °F plus 7 d at 68 °F (Table 3).

Production elevation influenced the effect of 1-MCP on later-harvested fruit (Table 4). Pears produced at 688 ft, harvested at FF ~12.5 lbs, and treated with 1-MCP developed BJMT after 7 months of RA storage at 30 °F plus 7 d at 68 °F. Pears produced at 1752 ft and treated with 1-MCP failed to develop BJMT after 7 months, but RC decreased dramatically and these pears tended to achieve RC after long-term storage.

Table 3. Changes in RC, soluble solids content (SSC), titratable acidity (TA), BJMT, and superficial scald (SS) of commercial maturity (CM) and late-harvest maturity (LM1 and LM2) Anjou pears on day 7 at 20 °C as affected by 150 ppb 1-MCP following storage at –1.1 °C for 4 and 6 months.

Harvest periods	Treatment	Storage period (months)	RC (lbs)	SSC (%)	TA (meq. L^{-1})	BJMT (1-4)	SS (%)
CM – (14.8 lbs)	Control	4	4.9 ± 0.6 b	13.0± 0.2 a	33.88 ± 1.77 b	3.6 ± 0.2 b	5.7 ± 1.2 b
	Control	6	3.9 ± 0.4 c	13.0± 0.3 a	22.33 ± 1.17 d	3.8 ± 0.1 a	25.34 ± 3.7 a
	1-MCP	4	11.9 ± 0.6 a	13.1± 0.3 a	37.77 ± 1.39 a	3.2 ± 0.2 c	0 c
	1-MCP	6	11.6 ± 0.3 a	13.2± 0.5 a	25.03 ± 0.64 c	3.1 ± 0.2 c	0 c

LM1 – (12.8 lbs)	Control	4	3.3 ± 0.5 c	13.0 ± 0.2 a	28.97 ± 0.31 b	3.6 ± 0.2 a	11.9 ± 2.1 b
	Control	6	3.6 ± 0.4 c	13.2 ± 0.3 a	24.07 ± 0.31 c	3.5 ± 0.2 ab	40.3 ± 5.9 a
	1-MCP	4	8.3 ± 0.6 a	12.9 ± 0.3 a	31.34 ± 0.12 a	3.1 ± 0.2 c	0 d
	1-MCP	6	6.6 ± 0.2 b	13.3 ± 0.4 a	28.04 ± 0.25 b	3.4 ± 0.2 b	3.7 ± 1.0 c
LM2 – (11.2 lbs)	Control	4	3.6 ± 0.4 c	13.2 ± 0.3 a	25.69 ± 0.03 c	3.6 ± 0.2 a	32.5 ± 5.6 b
	Control	6	4.6 ± 0.3 b	13.3 ± 0.5 a	22.87 ± 0.11 d	3.5 ± 0.2 a	58.7 ± 7.9 a
	1-MCP	4	6.6 ± 0.3 a	13.1 ± 0.4 a	28.97 ± 0.51 a	3.4 ± 0.1 a	3.6 ± 0.6 d
	1-MCP	6	6.1 ± 0.5 a	13.4 ± 0.5 a	26.49 ± 0.34 b	3.5 ± 0.1 a	5.9 ± 1.3 c

Different letters indicate significant differences between treatments at each harvest period according to Fisher's protected LSD test at $P < 0.05$.

Table 4. Changes in RC, SSC, TA, BJMT, and SS of late-harvest Anjou pears on day 7 at 20 °C affected by 150 ppb 1-MCP and production elevation (Orchard 1 at 688 ft and Orchard 2 at 1752 ft) following storage at –1.1 °C for 5 and 7 months.

Production elevation	Treatment	Storage period (months)	RC (lbs)	SSC (%)	TA (meq. L^{-1})	BJMT (1-4)	SS (%)
Orchad1 – 688 ft (12.5 lbs)	Control	5	3.3 ± 0.2 c	13.1 ± 0.3 b	24.29 ± 0.83 b	3.7 ± 0.1 a	51.3 ± 4.5 b
	Control	7	3.7 ± 0.2 b	12.5 ± 0.2 c	22.15 ± 0.47 c	3.5 ± 0.2 ab	63.3 ± 7.2 a
	1-MCP	5	6.7 ± 0.4 a	13.5 ± 0.1 a	26.15 ± 0.99 a	3.3 ± 0.1 b	2.6 ± 0.6 c
	1-MCP	7	3.7 ± 0.3 b	13.1 ± 0.4 b	24.34 ± 0.02 b	3.6 ± 0.2 a	5.1 ± 0.5 c
Orchard2 – 1752 ft (12.6 lbs)	Control	5	3.0 ± 0.1 d	13.2 ± 0.4 a	23.67 ± 0.02 c	3.8 ± 0.2 a	55.0 ± 4.7 b
	Control	7	4.2 ± 0.1 c	12.3 ± 0.2 c	21.74 ± 0.51 d	3.6 ± 0.2 a	66.0 ± 5.5 a
	1-MCP	5	10.3 ± 0.2 a	13.1 ± 0.4 a	29.67 ± 0.78 a	2.8 ± 0.1 c	1.1 ± 0.2 d
	1-MCP	7	8.3 ± 0.3 b	12.7 ± 0.2 b	28.11 ± 0.34 b	3.2 ± 0.2 b	5.3 ± 0.3 c

Different letters indicate significant differences between treatments at each harvest period according to Fisher's protected LSD test at $P < 0.05$.

b. **Combination treatment of 1-MCP and ethylene in CA storage.** The 1-MCP+ethylene (1-MCP (300 ppb) + ethylene (300 ppb) treatment recovered the RC of Anjou pears after 8 months of CA storage. Untreated and ethylene-treated fruit developed RC following 4-8 months storage in CA storage at 30 °F plus 7 d at 68 °F (Fig. 10A). However, 1-MCP inhibited RC for 8 months. The combination treatment slightly increased SSC and inhibited the decline of TA during 8 months of CA storage (Fig. 10B and C). BJMT of untreated fruit decreased from 3.8 to 3.6 during 8 months

of storage. 1-MCP treated fruit had a BJMT lower than 2.7 during the same period of storage. The 1-MCP + ethylene treated fruit had excellent BJMT after 8 months of storage.

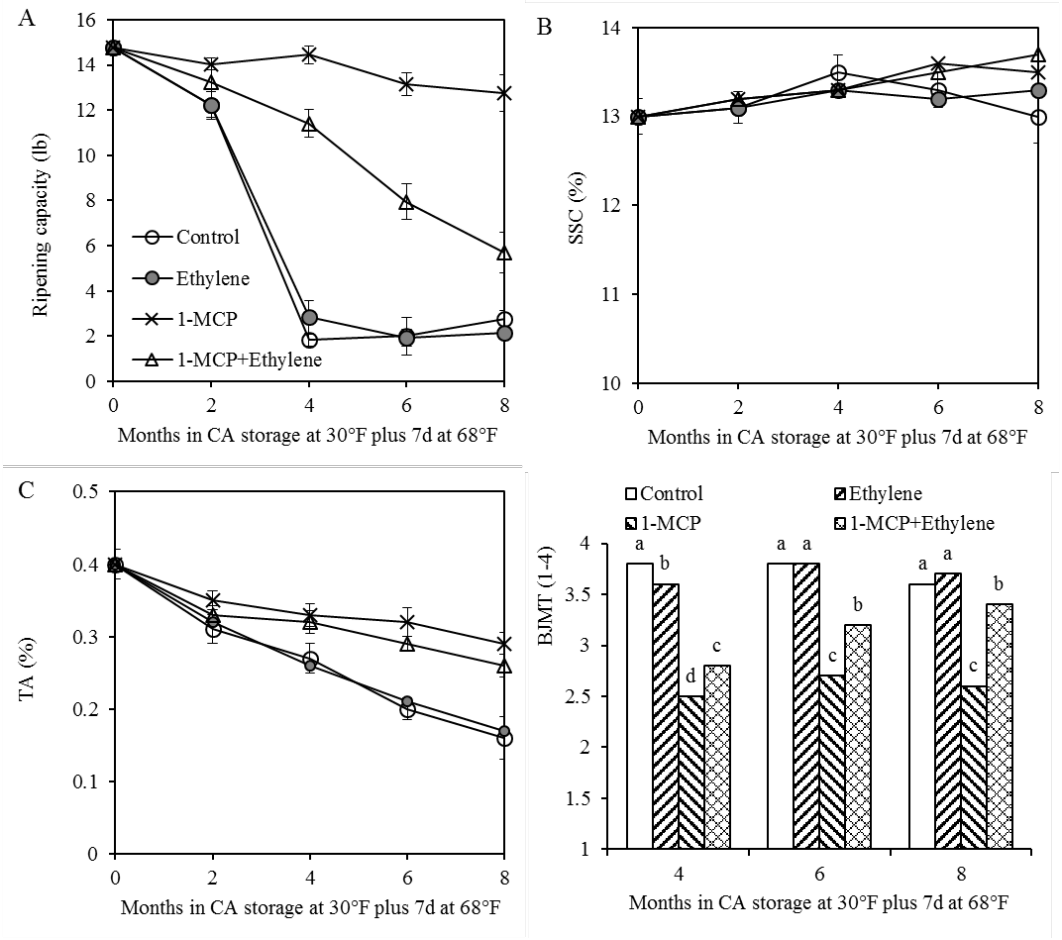


*Fig. 10. RC (A), SSC (B), TA (C), and BJMT (D) affected by 300 ppb 1-MCP and 300 ppb ethylene, alone or in combination with, in Anjou pears following 8 months of CA storage (*1.5% O_2 + < 0.05% CO_2*) at 30 °F plus 7 d at 68 °F.*

EXECUTIVE SUMMARY

Project title: Delivering quality pear fruit to consumers

European pears (*Pyrus communis* L.) are enjoyed by consumers when fruit have ripened to a buttery-juicy (melting) texture with full flavor development. However, after receiving the chilling requirement and/or ethylene conditioning, pear fruit can soften but may develop a dry-coarse (mealy) texture after ripening, especially those fruit harvested at later maturity stages or improperly stored. The lack of knowledge about the metabolic mechanisms resulting in buttery-juicy texture has precluded development of practical approaches to delivering fruit with this preferred texture, nor has any chemical or physical analysis been available for the industry to define the textural properties of ripened pears.

The current research indicated that water-soluble ponyuronides (WSP), CDTA-soluble pectins (CSP), and pectin methylesterase (PME) are positively correlated with the development of buttery-juicy melting texture (BJMT). WSP are hygroscopic and impart the BJMT feeling to the consumer. Measuring WSP is tedious, although extractable juice (EJ, mL $100g^{-1}$) is relatively simple to measure; EJ is negatively correlated with BJMT. An EJ-based index to score BJMT was developed: BJMT index = (100 - EJ) / 10. We used the new BJMT index to investigate how pre- and post-harvest factors affect the development of BJMT and concluded:

- Pears harvested at 15-14 lbs maturity had longer BJMT life for up to 8 months.
- Anjou pear harvested at 15-14 lbs and treated with 150 ppb 1-MCP failed to develop BJMT after 8 months of RA storage. Anjou pear harvested at 15-14 lbs and treated with 1-MCP + ethylene did recover ripening capacity and developed BJMT after 8 months.
- Higher storage temperature (i.e. 34 °F) accelerated development of BJMT, but this treatment was associated with higher incidence of superficial scald.
- Compared to regular CA conditions, decreasing the O_2 level to 0.8-1% at 30 °F extended BJMT life for an additional two months to as long as 11 months.

After pears achieve a minimum number of chilling days, they can develop acceptable ripening capacity. In the case of Anjou pears, the chilling requirement to reach reaching full ripening capacity varied significantly (50-90 d), and is influenced by pre- and post-harvest factors. From this, we conclude that:

- Accumulated cold units (ACU) affected the chilling requirement for ripening capacity (CRRC); higher ACU reduced CRRC.
- Harvest maturity also affected CRRC, with more mature fruit generally having lower CRRC. Neither of these trends held across growing districts.
- Ca levels affected the CRRC period. Pre-harvest Ca sprays increased CRRC by ~10 days.
- Storage temperature and ethylene conditioning affected CRRC periods, with higher storage temperatures and longer ethylene exposure reducing the time to reach CRRC.

Late-harvest at FF = 13-12 lbs enables complete ripening of the 1-MCP treated Anjou pears, while controlling superficial scald. A combination treatment of 300 ppb 1-MCP and 300 ppb ethylene improved the ripening capacity of Anjou pears after long-term CA storage (i.e. > 7-8 months).

FINAL PROJECT REPORT **YEAR:** 3 of 2 (No-cost extension)
WTFRC PROJECT NUMBER: PR16-105

PROJECT TITLE: Dry matter assessment in pear and consumer perception

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Cooperators: Alex Goke (WSU –TFREC)

Total Project Request: Year 1: $ 51,655 **Year 2**: $ 56,172 **Year 3:** $0

Other funding sources: None
WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU **Contract Administrator:** Kim Rains
Telephone: 509 663 8181 (221) **Email:** kim.rains@wsu.edu

Item	2016	2017	2018 (NCE)
Salaries[1]	24,000	24,960	0
Benefits[2]	8,414	8,750	0
Wages[3]	2,880	2,995	0
Benefits[4]	289	300	0
Equipment	0	0	0
Goods/Services[5]	14,572	17,667	0
Travel[6]	1,500	1,500	0
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	**51,655**	**56,172**	**0**

Footnotes:
[1] Salary for a new hire 50% Research Intern (Serra-Musacchi) paid the other 50% on other grant.
[2] Benefit on salary at 31.5%
[3] One non-Student temporary for 12 wks: 20hrs/wk at $12/hr (Serra-Musacchi).
[4] Benefits on temporary at 10% (Serra-Musacchi).
[5] Labware/consumable, fruit sample reimbursement (Serra-Musacchi), sensory panel costs (consumable and incentive advertising), electronic tongue: sensors, chemicals and glassware (Ross).
[6] 2778 miles/year for domestic travel ($0.54/mile) to go to the orchard and to Pullman to meet co-pi and deliver fruit.

RECAP ORIGINAL OBJECTIVES

1. ***Determine the reliability of the Felix F-750 Produce Quality Meter and therefore if this non-destructive dry matter assessment tool can be used as at harvest sorting step for more consistent fruit quality categories.***
2. ***Assess if higher dry matter in pear translates into greater consumer liking and acceptability through consumer preference and sensory analysis studies.***

SIGNIFICANT FINDINGS

1. ***Determine the reliability of the Felix F-750 Produce Quality Meter and therefore if this non-destructive dry matter assessment tool can be used as at harvest sorting step for more consistent fruit quality categories.***
    - A d'Anjou specific model for dry matter was developed and sequentially improved from 0.79 to 0.92 coefficient of determination (R^2) and 0.45 to 0.34 root mean square error (RMSE) over the course of this project.
    - A Bartlett specific model was also developed for dry matter with an R^2 of 0.86 and RMSE of 0.34.
    - Models generally performed better when used on fruit similar to those used to build the model in terms of maturity and post-harvest stage, suggesting models may be limited in their utility depending on the composition of fruit in the calibration set.
    - Predictive models can be improved in terms of lower RMSE and higher R^2 by including larger amounts of fruit with broader maturity levels during calibration.
    - Models for use on-tree can be built up to two months prior to harvest with fair accuracy (0.20 to 0.95 % dry matter RMSE; 0.36 to 0.48 °Brix RMSE), though model accuracy suffers when model is applied to growth stages other than what it was calibrated for.
    - Using a combined model developed over several timepoints is a fair compromise for quality prediction on-tree in the field.
    - Distribution of fruit dry matter predicted at-harvest varied between orchards and years, presumably leading to down-stream differences in fruit quality and consumer liking.
    - When instrumentally evaluated for quality at < 1 and after 5 months of post-harvest, higher dry matter d'Anjou pears were significantly lower in I_{AD} index (more ripe) and greater in soluble solids content (SSC, °Brix) and actual destructive dry matter (DM %).

2. ***Assess if higher dry matter in pear translates into greater consumer liking and acceptability through consumer preference and sensory analysis studies.***
    - From two orchards and two harvest years, d'Anjou fruits were sorted at harvest into low (< 13 %), moderate (13 - 16 %), and high (> 16 %) predicted dry matter classifications using the Felix F-750 Produce Quality Meter.
    - Consumers significantly ($p < 0.05$) favored high dry matter fruits over moderate and/or low matter fruits in terms of perceived appearance, aroma, firmness, crunchiness, juiciness, sweetness, and pear flavor.
    - In terms of overall liking, high dry matter fruits were significantly ($p < 0.05$) and uniquely favored over moderate and low dry matter fruits.
    - Overall liking was driven primarily by liking of flavor, followed by sweetness, firmness, and then juiciness.
    - Consumers were willing to pay premium prices for higher dry matter fruits at an estimated $0.20/lb above average retail prices.

METHODS

1. ***Determine the reliability of the Felix F-750 Produce Quality Meter and therefore if this non-destructive dry matter assessment tool can be used as at harvest sorting step for more consistent fruit quality categories.***

D'Anjou pears used in this project were grown in three commercial orchards in Cashmere, Washington, USA and Monitor, WA, USA. The first orchard ("Orchard 1") consisted of central leader d'Anjou/OHF87 trees planted in 1998 and spaced 14 ft x 8 ft (389 trees/A, equal to 4.3 m x 2.45 m and 950 trees/ha). The second orchard ("Orchard 2") consisted of open vase d'Anjou/Bartlett seedlings planted in the 1970's and spaced 20 ft x 20 ft (109 trees/A, equal to 6 m x 6 m and 278 trees/ha). The third orchard, ("Orchard 3"), was a Bartlett/OH87 planted in 2012 at 12 ft x 5 ft (726 trees/A equal to 3.7 m x 1.5 m spacing and 1,800 trees/ha) trained to either a spindle or bi-axis system. Harvest of Orchard 1 occurred 18-19th of August in 2016 and 11-12th in September for 2017, and the 29th of August in 2016 and 6th of September in 2017 for Orchard 2. Orchard 3 was harvested in 2016 only on August 5th. Immediately following harvest, fruit were washed and placed in regular atmosphere cold storage (1 °C = 33.8 °F) for sorting and experimental purposes.

To enable the sorting procedure, several non-destructive dry matter models were built over the course of 2016 and 2017 – two for d'Anjou cultivar and one for Bartlett cultivar. For each model, spectral profiles of the opposing faces of 100 fruit were collected across three internal fruit temperatures (approximately 34, 68, and 90 °F) to reduce temperature-associated noise in the model, as temperature strongly influences absorption and emission by water in critical spectral areas. Two sections of each fruit were then destructively evaluated for dry matter and used to calibrate the model resulting in 200 fruit samples per model. A preliminary model was also built in 2015 for d'Anjou with only two temperatures and 50 fruit. The purpose of the successive d'Anjou models was to determine if the model could be improved by incorporating a larger variety of fruit maturity levels in to the calibration set. Accuracy was measured by calculating the coefficient of determination (R^2) and root mean square error (RMSE) between predicted and actual destructive values. Accuracy in this way was evaluated internally at calibration by comparing predicted values generated from the model to known values of the fruits used in model calibration and later destroyed, as well by applying models to external validation datasets from the instrumental evaluation of Orchards 1, 2, and 3 from 2016 harvest. Models were also compared across d'Anjou and Bartlett cultivars to evaluate model specificity.

In addition to models developed for sorting purposes, three on-tree models were developed for the purpose of monitoring fruit quality on the tree during development. For this, dry matter and soluble solids content prediction models were developed using growing fruit from Orchard 1 at 84, 112, and 140 days after full bloom (DAFB; 24 April 2017 as date of full bloom, alternatively 2, 1, and 0 months prior to harvest) on 24, 24, and 64 fruit samples for 84, 112, and 140 DAFB, respectively. Models developed at each time point were then applied to the respective data captured on-tree in order to estimate dry matter and soluble solids content through time as the fruit matured. Models were also compared across growing stage to evaluate model specificity.

For at-harvest sorting, fruit were non-destructively measured on opposing faces (two readings per fruit) by a Felix F-750 Produce Quality Meter to acquire average predicted fruit dry matter (%). From this average value, fruits were classified in to categories of dry matter (e.g. 13-13.99%, 14-14.99%, etc.) and randomly divided in to three evaluation periods; (1) instrumental quality evaluation < 1 month post-harvest, (2) instrumental quality evaluation ~5 months post-harvest, and (3) consumer sensory evaluation ~5 months post-harvest. For instrumental quality assessment, weight, I_{AD} index, firmness, soluble solids content (°Brix), dry matter (%), titratable acidity (% malic acid), and pH were

evaluated after seven days of room-temperature ripening. Predicted dry matter classes were evaluated for differences in these parameters using ANOVA and SNK means separation.

2. ***Assess if higher dry matter in pear translates into greater consumer liking and acceptability through consumer preference and sensory analysis studies.***
Following dry matter estimates as described above at harvest, stratified random samples of predicted dry matter were selected for consumer sensory testing. Due to variation in dry matter production between years, for the purpose of consumer evaluation dry matter classes were defined as low (< 13 %), moderate (13 – 16 %), and high (> 16 %) predicted dry matter. Fruit were held in regular atmosphere cold storage (≈ 1 °C) for over five months then ripened at ambient temperature for seven days prior to sensory evaluation. Sensory evaluations were conducted across four panel days both in February of 2017 (2016 harvest) and in February/March 2018 (2017 harvest) at the Washington State University Sensory Evaluation Facility (Ross's lab, Pullman, Washington, USA). For consumer evaluation, fruit were first washed then cut stem-to-calyx in one-eight slices with the seed core removed and randomly presented to consumers. Consumers used a nine-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely) to rate the slices for appearance, aroma, firmness, crunchiness, juiciness, sweetness, bitterness, pear flavor, and overall liking. Consumers were then asked a series of yes/no willingness to pay (WTP) questions, first presented with a premium bid of $1.73/lb, followed by a base rate of $1.36/lb if unwilling to purchase at the premium bid, and a discount bid of $0.99/lb if unwilling to purchase at the base bid. Rates were established based on Northwest USA market prices of fresh pears in February 2017. Responses were evaluated with ANOVA and post hoc Tukey HSD. Drivers of overall liking was investigated using multiple linear regression with sensory liking attributes as predictors. Mean WTP was estimated utilizing a contingent valuation model based on a utility difference approach.

RESULTS & DISCUSSION

1. ***Determine the reliability of the Felix F-750 Produce Quality Meter and therefore if this non-destructive dry matter assessment tool can be used as at harvest sorting step for more consistent fruit quality categories.***
Table 1 shows a summary of model accuracy at the time of calibration among all sorting models developed in terms of coefficient of determination (R^2) and root mean square error (RMSE). **As shown, the d'Anjou specific model for dry matter were sequentially improved from 0.79 and 0.45 to 0.92 and 0.34 R^2 and RMSE**, respectively, over the course of this project. This is likely due to intentional stratified selection among various fruit maturities for inclusion in the model – we hypothesized that broader ranges of fruit in the calibration would increase model performance overall. The Bartlett model also performed well for dry matter with an R^2 of 0.86 and RMSE of 0.34. Soluble solids models performed poorer than dry matter counterpart for all developments. This is likely due to the non-specificity of dry matter (which by definition also includes soluble solids aka sugars), making it less sensitive to spectral interference by other compounds, whereas soluble solids would be more sensitive to such interference due to its narrower chemical definition.

Table 1: Calibration performance statistics for predictive models developed for d'Anjou and Bartlett variety pear harvested from Orchards 1, 2, and 3 in year 2016-2018.

Model	Parameter	RMSE	R^2
d'Anjou Preliminary Model	Dry Matter	0.45	0.79
d'Anjou First Generation	Dry Matter	0.29	0.92
	Soluble Solids	0.31	0.90
d'Anjou Second Generation	Dry Matter	0.36	0.94
	Soluble Solids	0.42	0.91
Bartlett	Dry Matter	0.39	0.86
	Soluble Solids	0.43	0.84

Beyond calibration, model performance varied greatly depending on the cultivar the model was developed on and the age of fruit being measured. Table 2 details accuracy of the second generation d'Anjou and Bartlett models used to predict dry matter in fruit from the full harvest of Orchards 1, 2, and 3 in 2016. Both models were used to predict dry matter in fruit that were destructively evaluated < 1 month and after 5 months of regular atmosphere cold storage. Predictions were then compared to actual dry matter values for each model, orchard, and evaluation period combination. Overall, all models largely performed acceptably in each application, though decreases in performance were apparent when a model developed on one variety was applied to a group of fruit of another variety. For instance, d'Anjou model performed between 0.799-0.957 R^2 and 0.434-0.709 RMSE on d'Anjou fruit from Orchards 1 and 2, but performance was reduced to 0.777-0.845 R^2 and 0.581-0.779 RMSE when used on Bartlett fruit from Orchard 3 (Table 2). There also appears to be an influence of storage stage on model performance as well. For instance, d'Anjou model when applied to d'Anjou fruit from Orchard 2 was more accurate when applied to fruit that were stored for less than 1 month (0.957 R^2 and 0.462 RMSE) relative to when fruit were measured following 5 months of cold storage (0.903 R^2 and 0.570 RMSE, Table 2). These errors are likely due to differences in fruit characteristics between those selected for use in building the models (at a uniform storage stage) and those used in this validation exercise (e.g. fruit of both varieties held in storage for either < 1 or ~ 5 months). Model performance for use on both varieties could likely be improved by incorporating both varieties in the calibration, as well as different maturity stages and storage stages.

Table 2: External validation performance statistics of predictive models developed for d'Anjou and Bartlett variety pear applied to fruit from Orchard 1 and 3 evaluated at harvest (less than one month of cold storage) or post-storage (after 5 months of cold storage).

Validation Set	Model	Evaluation	n	RMSEP	R^2
Orchard 1 (Bartlett)	Bartlett	Pooled	120	0.781	0.777
		At-Harvest	60	0.732	0.733
		Post-Storage	60	0.415	0.921
	d'Anjou	Pooled	120	0.779	0.777
		At-Harvest	60	0.719	0.743
		Post-Storage	60	0.581	0.845
Orchard 1 (d'Anjou)	Bartlett	Pooled	203	0.836	0.722
		At-Harvest	108	0.692	0.813
		Post-Storage	95	0.405	0.914
	d'Anjou	Pooled	203	0.709	0.799
		At-Harvest	108	0.663	0.821
		Post-Storage	95	0.434	0.901
Orchard 2 (d'Anjou)	Bartlett	Pooled	75	0.742	0.874
		At-Harvest	45	0.444	0.960
		Post-Storage	30	0.482	0.931
	d'Anjou	Pooled	75	0.659	0.900
		At-Harvest	45	0.462	0.957
		Post-Storage	30	0.570	0.903

Similar trends were found for models developed for purpose of on-tree monitoring during fruit growth (Fig. 1). For both dry matter and soluble solids models, the lowest RMSE was obtained when applied the model to fruit of the same age (e.g. the 112 DAFB applied to 112 DAFB fruit). When applying a model to a fruit of a different age, RMSE would drastically increase (e.g. the dry matter model developed at 84 DAFB and applied at 140 DAFB suffers an 0.53 increase in RMSE). This effect was more pronounced for dry matter than soluble solid predictions. One solution for this problem would be to combine all models together, resulting in better performance through time by accommodating different fruit in the calibration. However, the combined model for dry matter did not

substantially improve the longitudinal accuracy of the model. Only the combined soluble solids model was stable (flat line) over time (Fig. 1). This would indicate that changes in fruit tissue ultrastructure and water content throughout development strongly impact model performance, much more so for dry matter than soluble solids content. Further work could address this limitation by evaluating different combinations of NIR spectral data to account for changes in fruit ultrastructure with the goal of making the models less sensitive to fruit changes over time.

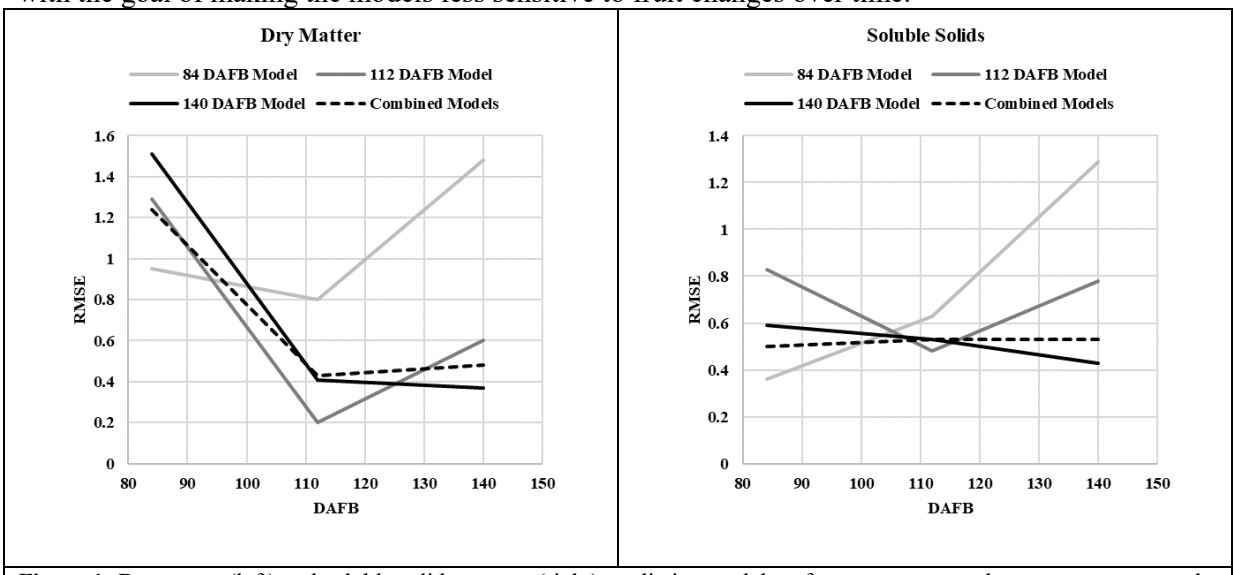


Figure 1: Dry matter (left) and soluble solids content (right) predictive model performance measured as root mean squared error (RMSE) as a function of time of calibration (DAFB) and application of the model.

As determined from first d'Anjou cultivar specific dry matter models in 2016 and second generation model in 2017, Figure 2 depicts distributions of predicted dry matter of representative subsamples of fruits harvested among years and orchards (≈ 2400 in 2016 and 1480 in 2017 picked from 20 or more trees sampled from harvest bins across varying degrees of coloring, size, and canopy position as proxies for maturity). Most of the harvest fruit exhibited moderate levels of predicted dry matter (e.g. 13-13.99, 14-14.99, and 15-15.9%), while comparatively fewer fruits were produced above and below this range (respectively high and low predicted dry matter categories). Distribution of predicted dry matter for Orchard 2 was notably skewed higher than that of Orchard 1 for both harvests. These orchards varied in terms of age, tree architecture, rootstock, and the cultural practices that have been applied to them – any of which could cause the observed differences. Additionally, the 2016 harvest tended to produce higher predicted dry matter fruits compared to 2017 harvest. As a result, very few higher predicted dry matter fruits were harvested from Orchard 1 in 2017. This may be due to the anomalous 2017 growing season characterized by a late bloom and shorter season. These observations highlight the need for non-destructive determinations of quality-related parameters at harvest such as dry matter prediction, as fruit can deviate substantially between orchards and years in maturity and quality and harvest which substantially impacts consumer-side sensory experiences.

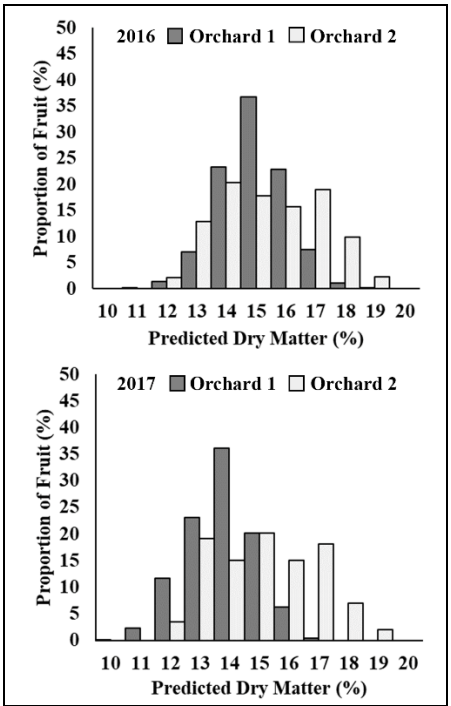


Figure 2: Distribution of predicted dry matter (%) of d'Anjou fruit harvested in Cashmere, WA, from Orchard 1 (dark gray) and Orchard 2 (light gray) in 2016 (upper) and 2017 (lower) among predicted dry matter ranges.

Table 3: Instrumental fruit quality parameters among predicted dry matter classes < 1 and after 5 months post-harvest from 2016 and 2017 harvests of Orchard 1. Different letters long columns indicate significant difference in means ($p < 0.05$, SNK). Model significance is not reported for simplicity.

Evaluation	Predicted Dry Matter Range (%)	Weight (g)		I_{AD} Index		Firmness (kg)		SSC (°Brix)		Destructive Dry Matter (%)		pH		Titratable Acidity (% Malic Acid)	
Harvest 2016 < 1 mo. Post-Harvest	**11-12.99**	156	C	2.04	A	7.49	AB	11.6	F	13.9	F	4.04	B	0.33	B
	13-13.99	195	B	1.98	A	7.64	A	12.7	E	15.2	E	3.97	BC	0.35	AB
	14-14.99	207	AB	1.89	B	7.43	AB	13.6	D	15.9	D	3.86	D	0.36	A
	15-15.99	221	A	1.80	C	7.31	AB	14.2	C	16.7	C	3.86	D	0.35	AB
	16-16.99	217	A	1.67	D	7.28	AB	14.9	B	17.5	B	3.95	C	0.32	B
	17-17.99	201	AB	1.51	E	7.08	B	15.9	A	18.6	A	4.10	A	0.29	C
Harvest 2016 ~5 mo. Post-Harvest	**11-12.99**	169	CD	1.36	A	0.89	CD	12.2	F	13.5	F	4.14		0.25	
	13-13.99	187	BC	1.03	B	0.78	D	13.3	E	14.1	E	4.07		0.25	
	14-14.99	205	AB	0.89	C	0.85	CD	14.0	D	15.0	D	4.07		0.24	
	15-15.99	217	A	0.82	CD	0.95	BC	14.6	C	15.7	C	4.07		0.24	
	16-16.99	205	AB	0.74	DE	1.07	B	15.4	B	16.7	B	4.07		0.24	
	17-17.99	165	D	0.63	E	1.31	A	16.6	A	18.3	A	4.03		0.23	
Harvest 2017 < 1 mo. Post-Harvest	**10-11.99**	163	D	1.89	A	5.79	AB	10.5	E	12.5	E	4.20	A	0.28	B
	12-12.99	188	C	1.84	AB	6.20	A	11.6	D	13.7	D	4.06	B	0.31	AB
	13-13.99	208	B	1.79	B	6.05	A	12.5	C	14.3	C	3.98	C	0.33	A
	14-14.99	231	A	1.69	C	5.38	B	13.4	B	15.5	B	3.92	C	0.33	A
	15-15.99	242	A	1.68	C	5.32	B	14.5	A	16.2	A	3.95	C	0.33	A
Harvest 2017 ~ 5 mo. Post-Harvest	**10-11.99**	158	C	1.54	A	1.93	A	10.7	E	12.2	E	4.44	A	0.22	AB
	12-12.99	191	B	1.42	A	1.85	A	12.0	D	13.2	D	4.39	AB	0.23	AB
	13-13.99	209	AB	1.09	B	1.14	B	12.8	C	13.8	C	4.26	BC	0.24	A
	14-14.99	226	A	1.00	B	1.05	B	13.6	B	14.7	B	4.17	C	0.24	A
	15-15.99	204	AB	0.98	B	1.32	B	14.6	A	16.0	A	4.36	AB	0.20	B

From the classes determined at harvest, Table 3 details the results of instrumental quality evaluation < 1 and after 5 months post-harvest of the 2016 and 2017 harvests of Orchard 1. For I_{AD} index, soluble solids, and actual destructive dry matter, trends were consistent over years and evaluation periods – higher dry matter fruits were significantly lower in I_{AD} index (more ripe) with greater soluble solids and dry matter. Firmness was also negatively related to dry matter with the exception of 2016 ~5 months post-harvest fruit, where the greatest firmness was interestingly found in higher dry matter fruits, though the magnitude between greatest and lowest firmness was only ~ 0.5 kg. Higher dry matter fruit also appeared generally larger (greater weight), though this was not entirely consistent between dry matter classes. Titratable acidity and pH did not demonstrate any clear relationship to dry matter class, though differences were often significantly different with the exception of 2016 ~5 months post-harvest fruit.

2. ***Assess if higher dry matter in pear translates into greater consumer liking and acceptability through consumer preference and sensory analysis studies.***

Table 4: Mean consumer liking scores (1 = dislike extremely, 9 = like extremely) of sliced fruit samples between low (< 13 %), moderate (13 – 16%), and high (> 16 %) predicted dry matter categories of pears harvested in 2016 and 2017 from Orchard 1 and Orchard 2 in Cashmere WA. Different letters in a column indicate statistically significant difference in mean liking among predicted dry matter categories at $p < 0.05$ (Tukey HSD).

Predicted Dry Matter	Sliced Fruit Sensory Attributes							
	Appearance	Aroma	Firmness	Crunchiness	Juiciness	Sweetness	Bitterness	Flavor
Low	6.47 c	6.23 c	6.08 c	5.80 c	5.64 b	5.36 c	5.32 b	5.51 c
Moderate	6.61 b	6.55 b	6.56 b	6.19 b	6.82 a	6.52 b	5.76 a	6.60 b
High	6.87 a	6.89 a	6.85 a	6.53 a	6.93 a	7.04 a	5.96 a	6.97 a

Table 4 depicts mean liking of consumer sensory parameters across low, moderate, and high predicted dry matter categories for fruit harvested in 2016 and 2017 from Orchards 1 and 2. As shown, high predicted dry matter fruits were favored by consumers for all attributes evaluated. For many attributes, high dry matter fruits were significantly ($p < 0.05$) and uniquely more favored – most notably in terms of liking of perceived firmness, sweetness, and flavor.

Figure 3 represents mean overall liking across low, moderate, and high predicted dry matter categories for fruit harvested in 2016 and 2017 from Orchards 1 and 2. As shown, high predicted dry matter fruits were rated significantly more favorable than both moderate and low predicted dry matter fruits. This would seem to indicate a strong positive relationship between dry matter and consumer preferences – as dry matter increases, so does overall consumer liking in addition to liking of perceived sensory attributes including appearance, aroma, firmness, crunchiness, juiciness, sweetness, bitterness, and flavor.

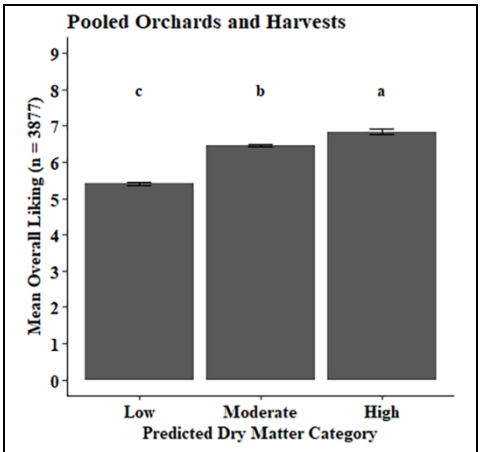


Figure 3: Mean consumer overall liking (1 = dislike extremely, 9 = like extremely) for both ("pooled") orchards and harvest years (2016 and 2017). Different letters indicate statistical difference in mean overall liking at significance $p < 0.05$ (Tukey HSD). Bars indicate standard error.

Liking attributes did not contribute equally to overall acceptance (Table 5). Overall liking was best associated with liking of flavor (β = 0.46***), followed by sweetness (β = 0.23***), firmness (β = 0.15***), then juiciness (β = 0.13***). Relative contribution of these terms to the overall model fit was 28.41, 22.31, 9.69, and 16.09 %, respectively, placing more emphasis on juiciness than firmness. Flavor, as a complex sensory outcome of numerous physical properties and chemical compounds, is immensely difficult to measure instrumentally. Dry matter may serve as a surrogate for flavor quantification in that it encompasses not only sugars, but other compounds (fibers, minerals, acids, etc.) that may contribute to flavor either directly or

Table 5: Multiple linear regression and relative contribution of sensory liking scores on overall liking of pears harvested in 2016 and 2017 from Orchards 1 and 2.

Parameters	Estimate	SE	Relative Contribution (%)
Sensory Scores			
Appearance	0.04***	0.01	2.48
Aroma	-0.01	0.01	4.12
Firmness	0.15***	0.01	9.69
Crunchiness	0.06***	0.01	7.35
Juiciness	0.13***	0.01	16.09
Sweetness	0.23***	0.01	22.31
Bitterness	0.07***	0.01	9.54
Flavor	0.46***	0.01	28.41
Model			
Intercept	-0.81***	0.07	
R^2	0.85		

indirectly through biochemical evolution during storage and ripening. This is supported by liking scores compared among predicted dry matter classes (Table 4) which shows high predicted dry matter fruits being significantly more favored in terms of flavor relative to moderate and low predicted dry matter fruits.

Table 6: Willingness to pay (WTP) estimated means and 95% confidence intervals in dollars per pound ($/lb) among predicted dry matter groups (low, < 13 %; moderate, 13 – 16 %; high, > 16 %) of fruit harvested in 2016 and 2017 from Orchard 1 and 2 in Cashmere, WA. Price premiums shown as difference between mean estimate and base retail price of $1.36/lb.

	WTP Estimate		
Predicted Dry Matter	**Mean** ($/lb)	**95 % Confidence Interval ($/lb)**	**Price Premium ($/lb)**
Low	1.25	1.22-1.28	-0.11
Moderate	1.49	1.47-1.51	0.13
High	1.56	1.52-1.62	0.20

As for consumers' willingness to pay for increases in dry matter, Table 6 describes estimated WTP among predicted dry matter categories for fruit harvested in 2016 and 2017 from Orchards 1 and 2. Mean WTP increased from low to moderate to high dry matter, with low being valued below average retail price ($1.36/lb) and moderate and high predicated dry matter valued above market price at a magnitude of $0.13/lb and $0.20/lb, respectively. This finding may have particularly strong implications for an industry evolving towards targeted consumer-oriented strategies such as quality threshold-based trademarking and value-added products, where there may exist an opportunity to segregate fruit at harvest in to various tiers of quality that are then marketed accordingly at a higher purchasing price.

EXECUTIVE SUMMARY

Fruit dry matter is increasingly recognized as a reliable indicator of fruit quality &d consumer acceptance for numerous commodities, though this relationship has yet to be thoroughly explored in European pears. The use of NIR spectroscopy, recently popularized for quality control in many horticultural products, may be used as a non-invasive tool for the determination of internal quality parameters but prior to this work has suffered from a lack of proof of concept demonstrations in soft-ripening pears. This project gave us the possibility to address these limitations through investigation of the importance of dry matter in summer and fall/winter pears (Bartlett and d'Anjou) and its impact on consumer preference in Washington State. The use of a non-destructive tool as Felix F750 Quality Meter based on NIR spectroscopy allowed us to develop cultivar specific models to predict with fairly good accuracy the fruit dry matter and soluble solids. Particular effort was made to evaluate the accuracy of models applied to fruit of varying maturity levels and post-harvest storage stages as well on-tree. This approach gave us the opportunity to work on a meaningful larger number of fruit than would be possible using traditional destructive methods for dry matter evaluation. This technology was employed to sort pears at harvest with the goal to create more homogenous groups of fruit for increased consumer liking. These results have practical applications in the PNW Pear industry and be a good resource to increase pear consumption by delivering high quality product to the pear market.

PROJECT OUTCOMES

Publications:

Goke A., Serra S.*, Musacchi S. (2018). "Postharvest Dry Matter and Soluble Solids Content Prediction in d'Anjou and Bartlett Pear Utilizing NIR Spectroscopy". *HortScience* 53(5), pp.669-680.

Serra S.*¥, Goke A. ¥, Diako C., Vixie B., Ross C., Musacchi S. (xxx) "Consumer perception of dry matter in d'Anjou pear determined at harvest using near-infrared spectroscopy". *International Journal of Food Science and Technology* (submitted).

Serra S., Goke A., Musacchi S. (xxx) "Manipulation of Fruit Dry Matter via Seasonal Pruning and its Relationship to Growth, Yield, and Quality of d'Anjou Pear" (in preparation).

Presentations:

Serra S., Musacchi S., Ross C., Goke A.: "Dry matter assessment in pear and consumer perception (continuing report)," WTFRC-NWPB, Hoodriver, OR February 16, 2017.

Serra S.: "DA Meter and Dry Matter". Invited oral presentation in IFTA session IV: New Instrument Panel discussion. 2017 IFTA Annual Conference, from bud to bin, Wenatchee WA (23 February 2017).

Serra S., Goke A., Knerl A., Sheick R., Musacchi S.: "Non-destructive dry matter prediction in d'Anjou pears: a new sorting tool?" (Oral presentation by Serra S.) ASHS annual meeting, Pomology 1 - Waikoloa, Hawaii, 20th September 2017.

Goke A., Knerl A., Serra S., Musacchi S.: "Utilizing handheld NIR for on-tree quality assessment in developing d' Anjou pear". (Oral presentation by Goke A. during "Research News Flash 2017") at 113th Washington State Tree Fruit Association Annual Meeting. Kennewick, WA, December 2017.

Serra S., Musacchi S., Ross C., Goke A.: "Dry matter assessment in pear and consumer perception (continuing report)," WTFRC-NWPB, Wenatchee, WA, February 15, 2018.

Serra S., Goke A., Knerl A., Sheick R., Ross C., Vixie B., Musacchi S.: "d'Anjou pear sorting by predicted dry matter and its effect on consumer preference" (Oral presentation by Serra S.). XIII International Pear Symposium, Montevideo, Uruguay, December 4th-7th 2018.

Serra S., Goke A., Knerl A., Sheick R., Ross C., Vixie B., Musacchi S.: "d'Anjou pear sorting by predicted dry matter and its effect on consumer preference" (Oral presentation by Serra S.). 73rd Lake Chelan Horticulture Day – January 21, 2019, Chelan, WA.

FUTURE DIRECTIONS

- Further explore the influence of maturity, storage period, and ripeness on NIR model accuracy.
- Match consumer responses to dry matter on a per-fruit basis to determine exact thresholds for fruit quality groupings at harvest.
- Expand dry matter and soluble solids prediction models to production scale with an NIR-equipped packing line to sort fruit aimed towards maximizing consumer satisfaction.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-17-100

Project Title: Fire blight management: new products and effective rates

PI: S. 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

Cooperators: None

Total Project Request: Year 1: 14,134 Year 2: 13,812 **Year 3: 14,256**

Other funding sources

Agency Name: Industry Gift Grants
Amt. requested: $1,500 per product/rate screened.
Notes: For screening of individual new products. Does not include multiple rates or individual products proposed here.

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509-293-8803 **Email:** arcgrants@wsu.edu/kim.rains@wsu.edu

Item	2017	2018	**2019**
Salaries[1]	7,800	8,112	8,436
Benefits[2]	2,884	3,000	3,120
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[3]	950	200	200
Travel[4]	500	500	500
Miscellaneous	0	0	0
Plot Fees[5]	$2,000	$2,000	$2,000
Total	14,134	13,812	**14,256**

Footnotes:
[1]Salary for one technician at $3,900 per month for two months.
[2] Benefits at 37% for one technician.
[3]Supplies include a new power misting backpack sprayer in year one ($750), and safety and application materials in all years.
[4]925 miles per year for travel to research plots, to organize project and present results.
[5]Plot fees included here are for a pear block at Sunrise Research Orchard for russet trials.

OBJECTIVES

1. Test the efficacy of three commercially available copper and biological products (Cueva, Previsto, Blossom Protect) and one experimental product (Alum) at five rates in order to determine at which rates products are effective. Treatments will be assigned randomly to plots within a randomized complete block and compared to untreated inoculated and untreated non-inoculated controls.
2. Test new products for potential control.
3. Investigate russet potential in order to determine when products are effective with little or no russet risk. Four products will be applied at four rates in a randomized complete block and assessed for russet.
4. Provide research-based recommendations to pear producers on appropriate rates for new products.

SIGNIFICANT FINDINGS

- Metallic copper content between 0.2 and 0.25 lbs/A/100 gal provides the best control based on a regression of all WA copper treatments between 2013 and 2017.
- Russet levels were higher for 4 and 5 qrt Cueva and Previsto applications with a significant regression between rate and russet but all russet levels were low in 2018 with few russeted fruit rated above 3 on a 1 to 15 scale.

METHODS

Site: A 0.42 acre mature Bartlett & Anjou pear block at WSU Columbia View Orchard Orondo, WA was used for russet evaluations. A 1.4-acre research block of mature Red Delicious apples at WSU Columbia View Orchard 48 Longview Rd. East Wenatchee, WA 98802-8283 was used for the inoculated trial. Soils are a Cashmont Gravely Sandy Loam with a 3-8% slope. The site has good air drainage and some wind protection.

Plots: Three blocks of 24 trees were designated (3-4 tree rows each). Individual trees were marked as plots in a randomized complete block where suitable trees were selected based on sufficient bloom (100+ flowers on lower branches).

Inoculum: Ultrafreeze-preserved cultures (−80°C) of the *Erwinia amylovora* 153 (streptomycin sensitive fireblight strain) were grown for 72 hours 28°C in NYDA agar to propagate dormant colonies. Subsequent inoculations were made transferring cultures to fresh NYDA plates every 24 hours to ensure fresh (<48 hrs old) plates.

Cluster Inoculation: Fresh cultures were diluted to $1x10^7$ CFU ml^{-1} and verified using an optical density spectrometer. A 1:9 dilution of the $1x10^7$ CFU ml^{-1} solution was used to obtain $1x10^6$ CFU ml^{-1} solution used in field inoculation. A one-liter sprayer was used to lightly wet each cluster. 100+ clusters per tree (all clusters up to ~7 feet high) were inoculated when the blooms were at an average of 100% bloom on the branch. An untreated and un-inoculated check treatment was included.

Treatments: Products were applied by tree to the area of the tree to be inoculated according to manufacturer recommendations (see Table 1) using a Stihl SR420 blow mister backpack sprayer with a wetting agent (NuFilm, organic; Regulaid, conventional; or proprietary). Products were applied to wet, near dripping previously

calibrated to equal 100 gal/A (approx. 0.5 gal per tree).

Included in this trial as a comparison and as "treated checks" were FireLine (oxytetracycline 17%) at 1.5 lbs. / 100 gal. / A and FireWall (streptomycin sulfate 17%), at 1.5 lbs. / 100 gal. / A, both antibiotics from AgroSource, Inc., and critical for comparisons as long-term standards. An untreated and inoculated check treatment and an untreated non-inoculated check treatment were included.

Application dates for pear russet trials were April 14 (20% bloom); April 18 (50% bloom); April 20 (80% bloom); April 22 (full boom); April 24 (full bloom +2). Application dates for new products trial (apple) were: April 26 (20% bloom); April 27 (50% bloom); April 28 (80% bloom); April 29 (full bloom); April 30 (full bloom +1); May 4 (Petal fall/+3 days); May 9 (plus 10 days); May 16 (plus 17 days).

Table 1. Fire Blight Treatments New Product Trial WSU 2018

Treatment	*Rate per 100 gallons water*	*Timing**	*Spreader Sticker (rate per 100 gal)*	
Untreated, NOT Inoculated Check	water	100% bloom	N/A	N/A
Untreated, Inoculated Check	water	100% bloom	N/A	N/A
*Standard strep (Firewall 17)**	28.8 oz	50% bloom, 100% bloom, petal fall	Regulaid	32oz
*Standard oxytet (Fireline 17)**	24 oz	50% bloom, 100% bloom, petal fall	Regulaid	32oz
*5**	9 oz	50% bloom, 100% bloom, petal fall	Regulaid	32oz
*6**	9.6 oz	50% bloom, 100% bloom, petal fall	Regulaid	32oz
Blossom Protect + Buffer Pro.	1.25 lb + 8.75 lb	20% bloom, 80% bloom	none	
Blossom Protect + Buffer Protect, VP20	1.25 lb + 8.75 lb 9 lb	20% bloom, 80% bloom; 100% bloom, petal fall	none/ Nufilm	3oz
VP20	9 lb	100% bloom, petal fall	Nufilm	3oz
1BW165N	3 lbs	one day before 100% bloom, +7 day	Nufilm	3oz
Omnilytics Phage		70% bloom, 100% bloom, petal fall	Nufilm	3oz
13	24 oz +128 oz	antibiotic 50% bloom, 100% bloom, petal fall; JA at 3, 10 and 17 days after 100% bloom	Regulaid	32oz
21	3 qt	day before and day after 100% bloom	none	
30	1.25 lb + 5.9 lb	20% bloom, 80% bloom	none	
31	1:1 (10 ppt: 100%)	day before and day after 100% bloom	Nufilm	3oz
32	1 qt	day before and day after	none	
33	3 qt	100% bloom	none	
34	3 qt		none	
35	12 oz	full bloom and petal fall	Brandt 719	1 pint
36	3 oz			
37		20-40% bloom, 70% bloom	none	
38	5 mM	80% bloom, 100% bloom,	Regulaid	
39	5 mM	day after	none	
40	5 mM		Regulaid	

* Buffered to 5.6.

Table 2. Treatments Russet Rate Trial Pear

Treatment	*Rate per 100 gallons water*	*Timing**
Untreated, NOT Inoculated Check	water	100% bloom
Untreated, Inoculated Check	water	100% bloom
Firewall 17 standard strep w Tech Mg	28.8 oz	50% bloom, 100% bloom, PF
Fireline 17 (standard oxytet) w Tech Mg	24 oz	50% bloom, 100% bloom, PF
Cueva	1 qrt	day before and day after 100% bloom
Cueva	2 qrt	day before and day after 100% bloom
Cueva	3 qrt	day before and day after 100% bloom
Cueva	4 qrt	day before and day after 100% bloom
Cueva	5 qrt	day before and day after 100% bloom
Previsto	1 qrt	day before and day after 100% bloom
Previsto	2 qrt	day before and day after 100% bloom
Previsto	3 qrt	day before and day after 100% bloom
Previsto	4 qrt	day before and day after 100% bloom
Previsto	5 qrt	day before and day after 100% bloom
Alum (0.5%)	4 lb	100% bloom, petal fall
Alum (0.75%)	6 lb	100% bloom, petal fall
Alum 1%	8 lb	100% bloom, petal fall
Alum (1.25%)	10 lb	100% bloom, petal fall
Blossom Protect PLUS Buffer Protect	1.25 lb + 8.75 lb	20% bloom, 80% bloom
Blossom Protect + Buffer Pro. (1.5x)	1.25 lb 13 lb	20% bloom, 80% bloom
Blossom Protect + Buffer Pro. (0.5x)	1.25 lb 4.4 lb	20% bloom, 80% bloom

Evaluation: Trees were visually evaluated for flower cluster infection every week following treatment. Infections were noticeable starting 2 weeks after inoculation. Cluster infection counts were summed across all dates. Fruit were evaluated for russet fruit skin marking during the third week in July.

Analysis: Statistical analysis was performed using an analysis of variance ANOVA and multiple means comparison T test (LSD) (SAS). Regression analysis was performed using proc reg (SAS).

Environmental Conditions and Cultural Practices: Overall temperatures were warm to moderate during bloom but with high temperatures right after bloom (and during bloom of later varieties).
Multiple wetting events during the bloom period (about once per week) resulted in severe natural fire blight infections throughout the region. Treatment blocks were at the end of their bloom period as wetting events began to occur.

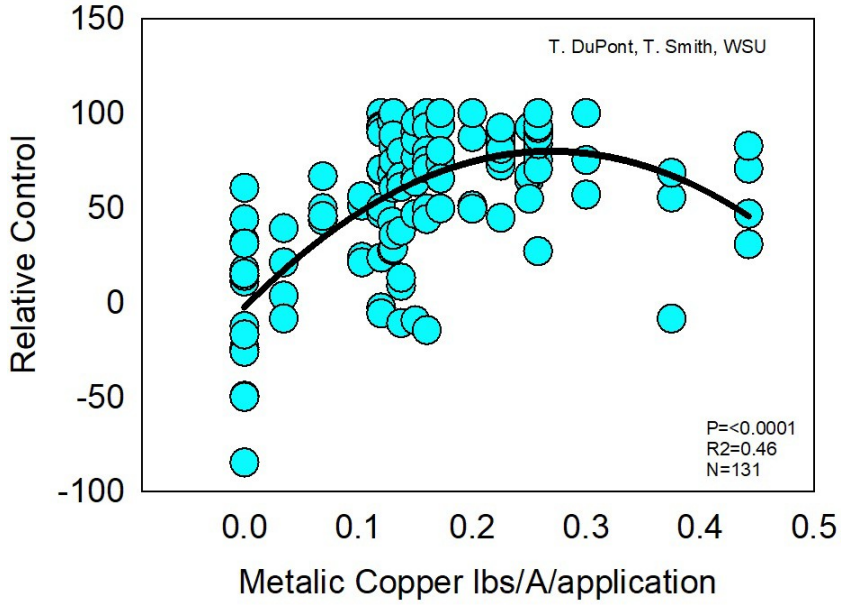
Table 3. Environmental Conditions During Bloom

DATE	MAX AIR TEMP	LEAF WETNESS	PRECIP	DATE	MAX AIR TEMP	LEAF WETNESS	PRECIP
14-APR	61.5	0.08	0	**4-May**	81.1	0	0
15-APR	56.2	0.24	0.44	**5-May**	71.2	0	0
16-APR	51.6	0.36	0.22	**6-May**	72.2	0.02	0
17-APR	56.5	0	0	**7-May**	81.9	0	0
18-APR	56.5	0	0	**8-May**	82.3	0.07	0.11
19-APR	66.8	0	0	**9-May**	73.2	0.21	0
20-APR	68.8	0	0	**10-May**	70.9	0	0
21-APR	60.1	0	0	**11-May**	79.7	0.01	0.01
22-APR	64.2	0	0	**12-May**	84	0.02	0
23-APR	68.6	0	0	**13-May**	87.9	0	0
24-APR	73.7	0	0	**14-May**	91.5	0	0
25-APR	80.8	0	0	**15-May**	93	0	0
26-APR	81	0	0	**16-May**	89.4	0	0
27-APR	81.5	0	0	**17-May**	86.3	0.09	0.66
28-APR	67	0.18	0.28	**18-May**	60.9	0.51	0.4
29-APR	68.5	0.02	0	**19-May**	74	0.25	0.01
30-APR	67.8	0	0	**20-May**	80.5	0.14	0.02
1-MAY	71.5	0	0	**21-May**	84.7	0	0
2-MAY	81	0.01	0	**22-May**	88.4	0.03	0
3-MAY	79.8	0.01	0				

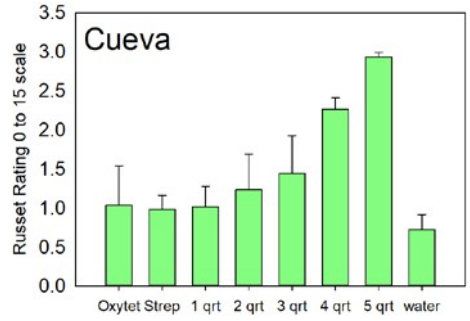
RESULTS RATE TRIALS

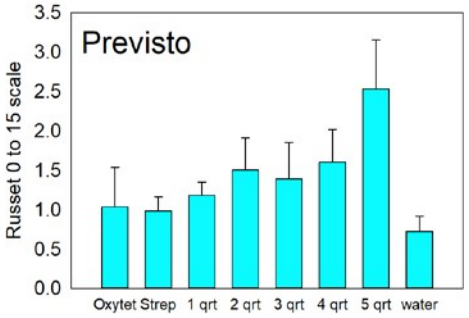
All copper treatment results from 2013 to 2017 were combined based on relative control (% control compared to infection in the inoculated untreated control). Each product was normalized by % metallic to pounds metallic copper per acre. The regression was significant (R^2=0.46). While a large variation in relative control existed, the highest control was obtained with metallic copper applications of 0.2 to 0.3 lbs per acre (Figure 1). This is interesting as at 3.3% metallic copper a 3 qrt rate of Previsto would equal 0.225 lbs of metallic copper but Cueva at 1.8% metallic copper would have only 0.16 lbs of metallic copper at a 4 qrt rate.

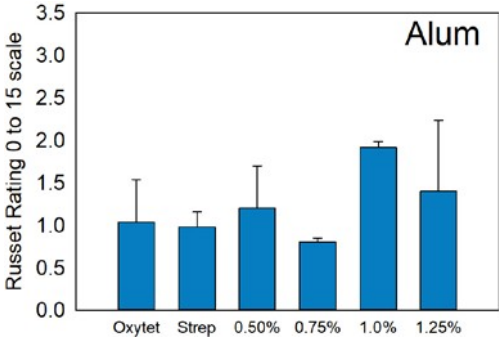
Figure 1 Relative Control Pounds of Metallic Copper Applied

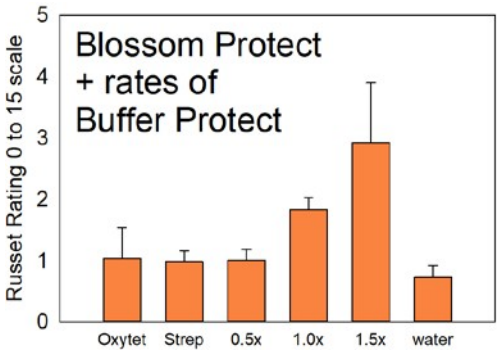


Russet evaluations for products applied at different rates resulted in relatively low levels of russet for all treatments on a 0 to 15 scale. No treatments exceeded a rating of 3. However, regressions for russet were positive/ significant for Cueva ($R^2=0.75$; $P<0.001$) and Previsto ($R^2=0.40$; $P=0.004$).









RESULTS NEW PRODUCT TRIALS

- Overall infection levels were unexpectedly low. There was a high level of variability within the block with significant differences between blocks. Differences between treatments found in this trial should be viewed conservatively as the trial did not have a sufficient level of pressure to provide confident separation between treatments.
- Under low pressure the biological BW175N provided control similar to organic standard Blossom Protect.
- Under low pressure the test product VP20 performed better than the untreated check.
- Under low pressure Blossom Protect followed by VP20 provided good control.
- Under low pressure the Omnilytics Phage provided control similar to Blossom Protect.
- No products had unacceptable levels of russet.

Table 4. 2018 Washington State Fire Blight New Products Trial Efficacy

	*Strikes per 100 clusters***				*Strikes per tree*			
Strep Standard (Firewall 17)	0	±	0	a	0	±	0.0	a
Untreated not inoculated check	0	±	0	ac	0	±	0.3	a
5	1	±	0	ac	2	±	0.9	a
Blossom Protect + Buffer Pro, VP20	1	±	0	ace	2	±	0.0	a
13	1	±	0	abce	1	±	0.3	a
32	1	±	1	abce	2	±	0.3	a
33	1	±	0	abce	2	±	0.5	a
6	2	±	1	abce	4	±	1.8	a
Omnilytics Phage	2	±	1	abce	4	±	1.7	a

Blossom Protect/ Buffer 1.25 lb	2	±	1	abce	4	±	1.5	a
BW165N	2	±	1	abce	3	±	1.2	a
34	3	±	1	abce	7	±	1.9	ab
40	3	±	1	abce	5	±	2.3	a
VP20	3	±	1	abce	6	±	1.7	ab
31	3	±	1	abce	5	±	1.7	a
36	3	±	1	abcde	11	±	5.6	ab
Fireline 17 oxytet standard	4	±	2	abcde	13	±	6.6	ab
38	4	±	2	abcde	16	±	11.1	ab
Untreated inoculated check	5	±	2	abcde	11	±	3.9	ab
39	5	±	2	abcde	6	±	2.1	ab
21	6	±	3	bcde	15	±	11.0	ab
37	7	±	5	bde	16	±	11.8	ab
30	7	±	5	bd	15	±	9.4	ab
35	9	±	6	d	22	±	17.2	b

Table 5. Russet Evaluation New Products

Treatment	*Russet Rating 1 to 15 scale*			
13	2.8	±	0.4	d
35	1.6	±	0.3	c
33	0.5	±	0.2	b
21	0.3	±	0.3	ab
6	0.2	±	0.1	ab
36	0.2	±	0.1	ab
Omnilytics Phage	0.1	±	0.1	a
Blossom Protect + Buffer, VP20	0.1	±	0.1	a
40	0.1	±	0.1	a
30	0.0	±	0.0	a
5	0.0	±	0.0	a
Blossom Protect + Buffer Protect	0.0	±	0.0	a
VP20	0.0	±	0.0	a
37	0.0	±	0.0	a
31	0.0	±	0.0	a
Untreated, NOT Inoculated Check	0.0	±	0.0	a
Untreated, Inoculated Check	0.0	±	0.0	a
38	0.0	±	0.0	a
39	0.0	±	0.0	a
*Standard oxytet (Fireline 17)**	0.0	±	0.0	a
32	0.0	±	0.0	a
34	0.0	±	0.0	a
BW165N	0.0	±	0.0	a
*Standard strep (Firewall 17)**	0.0	±	0.0	a

* Buffered to 5.6.

DISCUSSION

Low levels of infection in the block were unexpected as disease pressure in the region was very high. This block blooms toward the early end of the bloom period and this year was finishing bloom when the wet periods that initialize infections occurred. The block was inoculated to improve the likelihood of consistent disease pressure throughout the block. However, bloom progressed very quickly and as such inoculation may have been later than optimal. Additionally, several methods have been identified to potentially improve the quality and virulence of the inocula itself so as to hopefully provide more consistent pressure in future trials.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (No Cost Extension)
WTFRC Project Number:

Project Title: Integrated fruit production for pears

PI: Elizabeth H. Beers
Organization: WSU-TFREC
Telephone: 509-663-8181 x 234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: None

Total Project Request: **Year 1:** $105,424 **Year 2:** $121,474 **Year 3**: $125,811

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509-293-8803 **Email:** arcgrants@wsu.edu/kim.rains@wsu.edu

Item	2016	2017	2018	2019
Salaries[1]	63,597	75,054	78,056	
Benefits[2]	21,932	26,250	27,300	
Wages[3]	6,240	6,490	6,749	
Benefits[4]	626	651	677	
Equipment	0	0	0	
Supplies[5]	4,000	4,000	4,000	
Travel[6]	3,529	3,529	3,529	
Miscellaneous	0	0	0	
Plot Fees[7]	5,500	5,500	5,500	
Total	105,424	121,474	125,811	0

Footnotes: [1]Research Intern, 7 months (year 1), 12 months (years 2 and 3) 0.40 FTE. Post-Doc, 3 years [2]Benefits for Research Intern 38.6%, Post-Doc 33.5%. [3]Wages for time-slip help, 1.0 FTE, summer. [4]Benefits for time-slip 10%. [5]Supplies – office and lab supplies, electronics, statistical consulting. [6]Travel to plots – motor pool rental. [7]5.5 acres total: 2.7 acres (TF8,9), 2.8 acres (WSU Sunrise)/yr x $1,000/acre, 3 years.

Objectives:

1. *Evaluate selective pesticides and non-insecticidal tactics for supplementing broad-spectrum insecticides for pear pests.*
2. *Determine the potential for the use of insect growth regulators (IGRs) as pre-bloom and post-harvest sprays for reducing overwintering psylla populations.*
3. *Evaluate tree washing techniques for control of pear psylla and mites.*
4. *Evaluate non-target effects on the predatory mite Galendromus occidentalis.*
5. *Evaluate pesticide efficacy for specific pesticide and pest issues.*
6. *Communicate project results as they become available*

2018 Significant Accomplishments:

- Adult bioassays: Many conventional and organic materials were screened for overwintering adult mortality. Materials achieving good mortality (greater than 90%) were: Bexar 27 fl oz/acre[1], Malathion 5EC 32 floz/acre, Dimethoate 4EC 32 fl oz/acre, Dormant oil 4%, Assail 70WP 3.4 oz/acre, Cinnerate 40-60 fl oz/acre, and Lime sulfur 3 gal/acre. Variability in mortality occurs between slide dip and potter spray tower methods for certain products including Surround and Bexar; the ability to walk/fly may alter the effects of these products.
- Field spray trials: Pre-bloom adulticides were more effective when applied at delayed dormant than popcorn. Summer spray programs using only selective materials (Cinnerate at 30 fl oz/acre[2], AzaDirect 48 fl oz/acre, and Celite 40 lb/acre) successfully managed psylla following bloom and had negligible impacts on natural enemy densities.
- Soft vs. conventional management programs: *Prebloom*: No additional control of psylla was gained from malathion in addition to Surround, compared with Surround alone. Reflective mulch and Surround (tested separately) provided equal prevention of egg-lay by winterforms as the conventional spray program. *Postbloom*: Soft programs using only Surround, IGRs, AzaDirect, and a soft codling moth program (oil, Cyd-X, Intrepid) resulted in lower honeydew residues on both leaves and fruit compared with the conventional spray program.
- Plant Defense Elicitors: Single applications of Actigard and Employ at various timings on three-tree plots in commercial orchards had no effect on psylla densities. Methods for testing defense activation have been developed using qPCR to measure expression of defense genes.
- Fall Surround (kaolin) Sprays: Surround WP 100 lb/acre applied to 2-acre commercial plots in October of 2017 reduced psylla adult numbers the following spring. Residue analysis using Image J revealed that residues partially diminished over the winter, but the remaining film provided significant repellency of psylla adults and eggs.
- Communication of Project Results: Nine articles have been generated which can be found on the TFREC Pear IPM webpage http://treefruit.wsu.edu/crop-protection/pear-ipm/. Four open-access journal articles have been submitted for publications in the journal Arthropod Management Tests. Results of this project have been presented at 31 events (grower meetings, field days, or working groups) since the beginning of the project (19 in 2018).

[1] Pesticide solutions for lab bioassays reflect 100 gpa spray volume.
[2] Field sprays either used 100 or 200 gpa depending on tree size and timing.

Obj. 1.a. Soft vs. Conventional Plots.

Methods. Four psylla management programs were compared: two soft programs, one conventional program and one untreated check (Table 1). Each program was executed on four replicate plots. Each plot consisted of 40 trees in four rows (Fig. 1); two rows of Anjou and two Bartlett. The conventional program was developed with local fieldmen. The two soft programs only differed in pre-bloom management (one used Surround, the other reflective mulch). Measurements were taken on psylla life stages, spider mites, various natural enemies, fruit-set, honeydew residues on leaves, and fruit injury.

Fig. 1. Reflective mulch plot, Sunrise Orchard

Table 1. Products, rates and timings for pear psylla management programs, Sunrise Orchard, 2018.

	Conventional	**Surround**	**Reflective Mulch**	**Check**
Mar 16 (**Delayed-dormant)**	Malathion 5EC 32 fl oz Surround CF 50 lb 440 IAP oil 4%	Surround CF 50 lb 440 IAP oil 4%	(refl. mulch)	-
Apr 4 (Popcorn**)**	Assail 70WP 3.4 oz Rimon 0.83EC 32 fl oz	Surround CF 50 lb	(refl. mulch)	-
May 2 (Petal fall)	Actara 25WDG 5.5 oz Rimon 0.83EC 32 fl oz	-	(mulch removed)	-
May 21	Ultor 1.25L 14 fl oz Rimon 0.83EC 32 fl oz Exirel 0.83EC 20.5 fl oz	Surround WP 50lb	Surround WP 50 lb	-
May 30	Delegate 25WG 7 oz	Surround WP 50 lb Aza-Direct 1.2 L 48 fl oz Esteem 35WP 5 oz	Surround WP 50 lb Aza-Direct 1.2L 48 fl oz Esteem 35WP 5 oz	-
June 6	-	Aza-Direct 1.2L 48 fl oz Centaur 70WDG 46 oz	Aza-Direct 1.2L 48 fl oz Centaur 70WDG 46 oz	-
June 18	-	Aza-Direct 1.2L 48 fl oz Dimilin 2L 48 fl oz	Aza-Direct 1.2L 48 fl oz Dimilin 2L 48 fl oz	-
July 8	Delegate 25WG 7oz Assail 70WP 3.4 oz	Aza-Direct 1.2L 48 fl oz	Aza-Direct 1.2L 48 fl oz	-

*The entire block received baseline programs for fire blight, codling moth and mites. Codling moth suppression used mating disruption ties and sprays of oil, Intrepid, and Altacor. Mite suppression involved one application of Envidor following petal fall. All sprays following delayed-dormant received were mixed with 440 IAP Oil 0.25%.

Results and Discussion. *Pear psylla*. Psylla pressure was high throughout this experiment. All three management programs equally and effectively suppressed psylla colonization (adults and eggs) prior to bloom (Fig. 2). Summerform adult densities were lowest in the Surround program, followed by the reflective mulch program, the conventional program, and the check, respectively. Immediately following bloom, the there was a slight increase in psylla adults and nymphs in reflective mulch plots. Interestingly, a concurrent rise in natural enemies occurred in reflective mulch plots (Fig. 2). While in hindsight a petalfall spray in the reflective mulch plot should have been applied; this event provided evidence that reflective mulch is conducive for conservation biological control.

Summer soft programs involving more frequent spraying of soft materials resulted in better control of the first and second generation of summerform adults than less frequent but more potent conventional sprays. The second generation of nymphs (peaking late June) were in similar densities among the

three programs, and over 8x greater in the check. The third generation of nymphs (early August) was 2x greater in conventional program the two soft programs and the check.

Honeydew and Injury. The conventional program sustained greater fruit injury and honeydew residues than both soft programs; soft programs were not different for either of these variables. Psylla honeydew was extremely high in check plots, well beyond levels in the three programs.

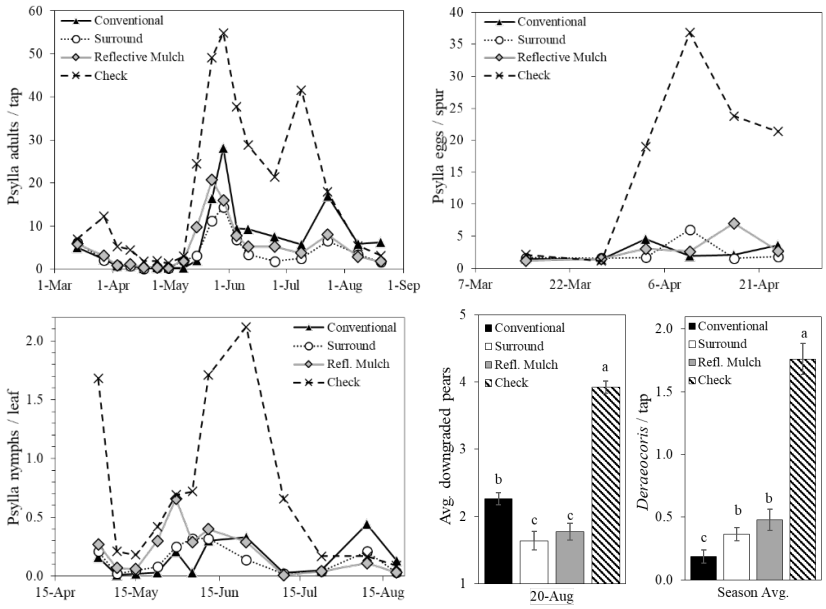


Fig. 2. Daily psylla counts, Deraeocoris season averages, and honeydew injury for two soft programs, one conventional program and a check.

Natural Enemies. Many natural enemies were present, but some groups like earwigs and *Campylomma* were very low. Most were spiders (39%), *Deraeocoris brevis* (25%), and *Trechnites insidiosus* (22%). Soft programs had more psylla specialists *Deraeocoris* and *Trechnites* than the conventional program.

Mites. Both soft programs experienced a noticeable, but sub-injurious, increase in twospotted mites in the final two samples of the season. This likely suggesting that mid-summer application of Surround flares secondary pests.

Discussion. Prebloom results suggest that broad spectrum sprays can be omitted from prebloom programs if Surround or reflective mulch are properly utilized. Most of the growers in Washington are already spraying Surround once or twice prior to bloom. Therefore, growers can save time and money, while conserving natural enemies, by simply focusing on pre-bloom Surround sprays.

Summer findings demonstrated that better control of psylla is achieved with more frequent sprays of soft materials, than less frequent sprays of broad spectrum materials. More frequent sprays of soft materials are more conducive with biological control. However, applying Surround after petalfall may disrupt natural enemies, and is probably unnecessary.

Obj. 2. Potential for pre-bloom and postharvest IGRs

IGRs for use pre-bloom and during the summer were tested in previous year bioassays. Post-harvest applications have not been perused further. Previous research has already addressed this topic and found it to have very low potential for control.

Obj. 3. Evaluate tree washing techniques for control of pear psylla and mites.

An overhead tree washing system was established at Sunrise Orchard in the spring of 2017. This system was used as part of a soft psylla program, and proved highly effective for increasing the threshold for psylla nymphs by removing honeydew. In 2018, we monitored the wetting time-line for achieving drip, and examined the addition of a non-ionic surfactant. It was determined that approximately 1 hour of 72 gal/min/acre washing from our overhead rotor sprinklers (R2000) was needed to completely wet all leaves and fruit; but larger trees could take longer. We think allowing drip to continue for another hour or more will result in greater honeydew removal. Injecting Regulaid

16 fl oz/acre half way into a 3-hour total cycle was performed to help further wet leaves and fruit, and remove psylla. Regulaid moved through the system in less than 1 minute. We continued to run the system for another 1.5 hours to remove all surfactant residues, but less time would probably suffice.

Obj. 4. Evaluate non-target effects on the predatory mite *Galendromus occidentalis*.

This objective was completed in 2016.

Obj. 5. A. Evaluate pesticide efficacy for specific pesticide and pest issues.

Table 2. Psylla adult mortality, lab bioassays

	% Mortality (winterforms)	
Product / rate	Slide Dip	Potter Tower
Bexar 15SC 27 floz/ac	66.0	100.0
Delegate 25WG 7 oz/ac	74.0	100.0
Malathion 5EC 32 floz/ac	100.0	95.0
Warrior II 2.08 21 floz/ac PBO 8 floz/ac	40.0	34.0
Dimilin 2L 48 floz/ac	18.0	80.0
Dimethoate 4EC 32 floz/ac	100.0	-
Assail 70WP 3.4 oz/ac	92.0	-
Wettable Sulfur 15 lb/ac	20.0	-
Lime Sulfur 3 gal/ac	78.0	100.0
Surround CF 50 lb/ac	25.0	60.0
Cinnerate 40 floz/ac	95.0	66.0
Cinnerate 60 floz/ac	100.0	100.0
Ecotec plus 64 floz/ac Brandt 719	-	71.5
Celite 20 lb Brant 719	-	66.0
Celite 40 lb Brant 719	-	81.8
ThymeGuard 64 floz/ac	40.0	50.0
Dormant Oil 4%	100.0	100.0
Summer Oil 0.5%	100.0	100.0
Check average	9.0	17.6

Methods. Numerous field spray trials and laboratory bioassays for chemical control of pear psylla were completed in 2018. Laboratory assays focused on adults. Slide dip and Potter spray tower methods were used to measure acute mortality in the lab. Spray trials were conducted in small, replicated field plots (3-4 trees/plot; four replicates). All psylla life stages and natural enemies were counted weekly.

Results. *Bioassays*. Results of various bioassay were combined and are shown in Table 2. Overall, the Potter spray tower method consistently resulted in greater psylla mortality in both treatments and checks. This suggests that allowing psylla to move freely may lead to death, possibility due to greater energy use.

Field Trials. Early season field trials suggest that adult knockdown is best achieved with delayed dormant sprays, as opposed to sprays closer to popcorn. Adult densities are still increasing at delayed dormant; whereas by popcorn the are naturally declining. Bexar and Malathion at delayed dormant resulted in adult density reductions between 20-40%, while check densities increased 100% or more (Fig. 3).

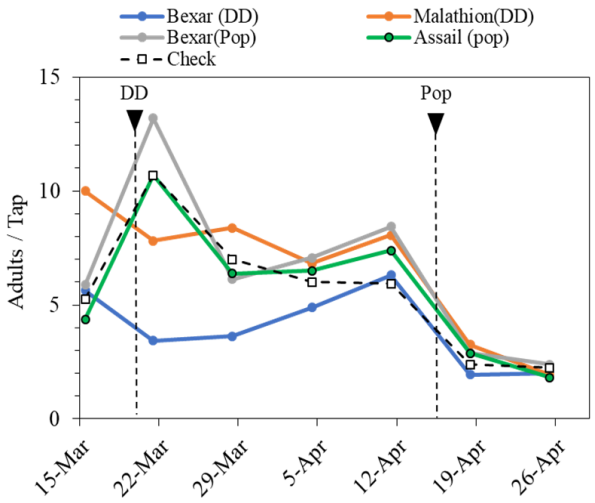


Fig. 3. Winterform psylla counts in plots sprayed at delayed dormant (Bexar or Malathion) or popcorn (Bexar or Assail)

Postbloom spray trials examined soft and conventional materials. Multiple sprays in close succession help improve psylla control. Celite (Fig. 4) Cinnerate, AzaDirect and Cinnerate-AzaDirect in rotation (Fig. 5) provided good control of psylla, while having low impacts on natural enemies. Celite at 40 lb/acre was more effective than 20 lb/acre. Ecotec was the least effective soft material, but still provided some population suppression, yielding ~50% fewer nymphs than the check. The conventional comparison of Assail, Delegate and Actara also suppressed psylla, but natural enemies were reduced in these plots.

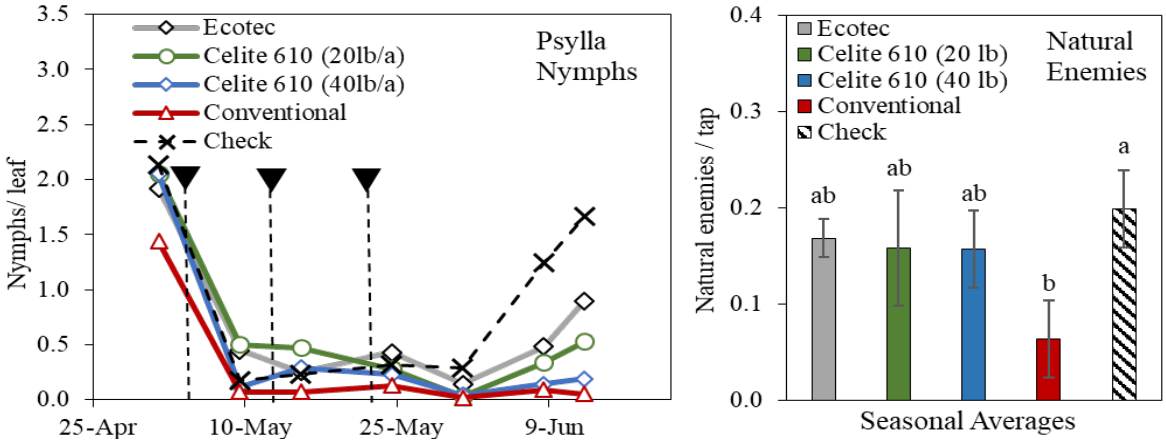


Fig. 4. Pear psylla 1st-5th instar nymphs by count (left), and season averages of combined natural enemies following application of various insecticides on field plots at the TFREC pear orchard in Wenatchee, WA.

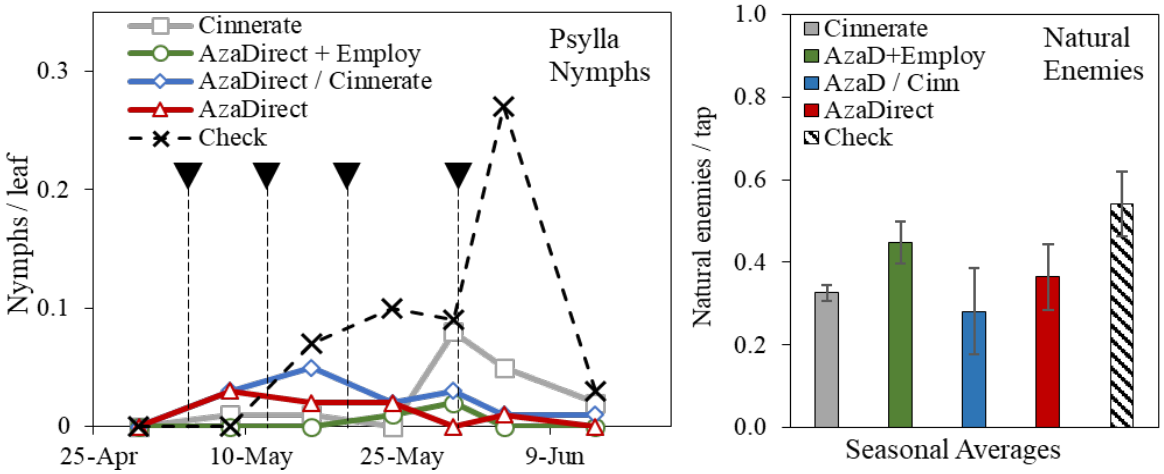


Fig. 5. Pear psylla 1st-5th instar nymphs by count (left), and season averages of combined natural enemies following application of various insecticides on field plots at the Sunrise pear orchard near Rock Island, WA.

Obj. 5. B. Non-insecticidal control tactics.

Plant defense elicitors (SARs) for control of pear psylla.

Methods. The plant defense elicitor products Actigard 50WG (Acibenzolar-S-methyl) and Employ (harpin protein) were applied to four-tree plots in various commercial orchards. Three application timings were tested independently: bloom, late May, and late July. For the first two timings, foliar applications were made by airblast sprayer for both products. The July application was made to foliage for Employ, and as a soil drench for Actigard (foliar application restricted at this timing). Sampling was performed bi-weekly on pear psylla and spider mites. To measure defense elicitation, leaves were collected and flash frozen in liquid nitrogen for RNA extraction and analysis. In cooperation with Dr. Rodney Cooper, USDA Wapato, a technique using qPCR was developed to measure expression of various defense genes (Chitonase and PR genes).

Results. No differences were observed among treated or untreated plots for psylla life stages or mites. Psylla and mite populations were low overall in these orchards, reducing potential resolution for treatment effects. The qPCR technique to detecting expression of defense genes was developed in the fall of 2018, but has not yet been used to measure expression trees from our experimental plots. Additional preliminary testing is still necessary before this method can be used reliably to quantify

expression of defense genes elicited by SAR product. Once this method is deemed reliable, we will have the ability to examine how different rates and timings of SARs effect the quality of systemic defense mechanisms in pears.

Fall applied Surround.

Interest was raised regarding the potential to use Surround as a post-harvest application. This tactic may benefit orchard blocks that are hard to access at delayed-dormant due to ground moisture/snow.

Methods. Surround WP 100 lb/acre was applied to 2-acre commercial orchard blocks in October 2017. Some sprays also included the sticker NuFilm 17 128 oz/acre. Psylla adults were counted in the fall and the spring in these plots and untreated check plots. Potted Anjou trees were sprayed with the same treatments in early November and left outside for the winter. In early March, another set of plants were sprayed with Surround CF 50 lb/acre. Cage choice tests were performed in the greenhouse. Psylla adults were released into cages with trees from of each treatment and checks; adult location and oviposition was measured. To measure the decline of Surround residues over the winter, tree branches from both field plots and greenhouse plants were photographed immediately following Fall Surround applications and immediately prior to February counts. Images were processed using Image J, measuring branch whiteness (Fig. 6).

Results. Surround residues declined significantly over the winter, but detectable levels remained (Fig. 7). Commercial plots sprayed with Surround in October had fewer adults in March than untreated checks. In greenhouse choice tests, trees with fall-applied Surround had significantly fewer psylla adults and eggs (Fig. 8) than checks, and numbers were similar to surround applied immediately prior to the experiment in March. These results suggest that 100 lb/acre of Surround WP applied in the fall will help protect blocks the following spring. Although this should not be used a substitute for spring Surround sprays, it is a viable option for specific blocks that are difficult to access by tractor-sprayer in the Spring.

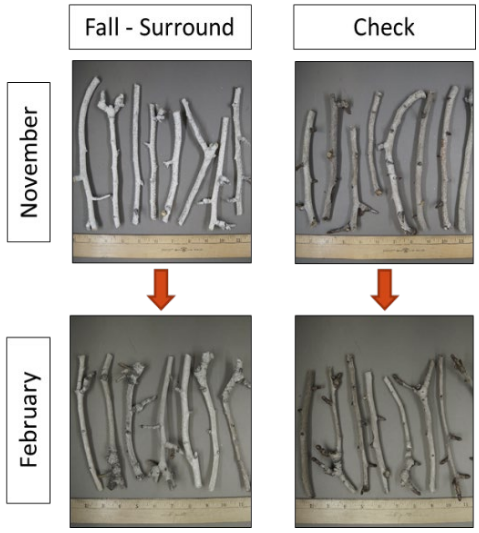


Fig. 6. Photos of cut branches from commercial plots in the November and February; analyzed for Surround residues using Image J.

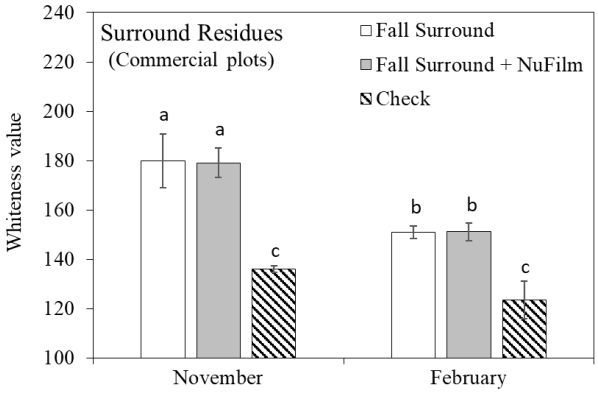


Fig. 7. Whiteness values (Surround) on branches in the fall and spring.

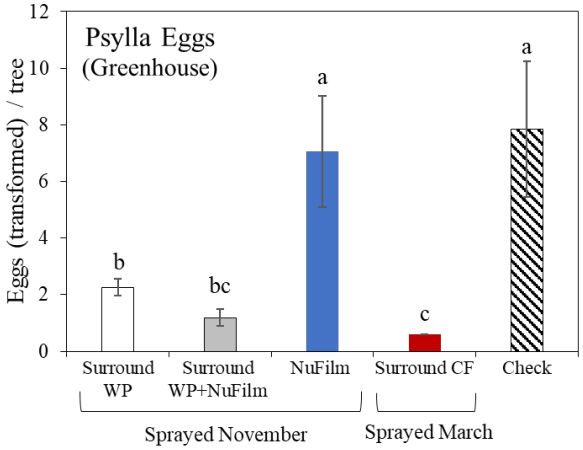


Fig. 8. Number of eggs laid on potted pear trees following Surround and NuFilm treatments.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2

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

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Budget: **Year 1**: $24,202 **Year 2**: **$24,686** (2% inflation)

Other funding sources: None

WTFRC Collaborative expenses: None

Budget

Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Russ Karow
Telephone: (541) 737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	**2018-19**	**2019-20**
Salaries Faculty Res. Assist. 2 mo	9,908	10,106
Benefits OPE 61%	6,044	6,165
Undergraduate labor (&OPE 12%)	1,000	1,020
Equipment	0	0
Materials and Supplies	750	765
Local Travel	750	765
Plot Fees	750	765
Medford russet trials	5,000	5,100
Total	$24,202	**$24,686**

OBJECTIVES:

1) Evaluate rate of pH-buffering on oxytetracycline-mediated suppression of fire blight pathogen populations on flowers and incidence of fire blight infection (Corvallis).

2) Evaluate effect of pH-buffering on finish quality of Comice and Bartlett pear fruit (Medford).

3) Evaluate if oxytetracycline formulation ('-hydrochloride' or '-calcium complex') influences the pH-buffering enhancement of oxytetracycline.

SIGNIFICANT FINDINGS:

- Fire blight pathogen populations on pear and apple flowers continued to increase during the post-petal fall period.

- In both apple and pear, pH-buffering improved the efficacy of oxytetracycline for fire blight suppression

- Based on measurement of fire blight pathogen populations in flowers, pH-buffering appears to prolong the inhibitory residual of oxytetracycline.

- pH-buffering of oxytetracycline caused negligible to slight effects to fruit finish (russeting).

- Before pH-buffering, FireLine reduced incidence of blight greater degree (75%) than Mycoshield (58%) but after buffering, both materials suppressed blight similarly (80 to 84%).

METHODS:

Rationale. We observed previously that the potency of oxytetracycline for fire blight suppression was sensitive to the pH of the spray suspension. In 2018, field experiments were designed to determine if a pH-buffering adjustment could optimize the potency of oxytetracycline. In addition, corresponding trials in Medford pear orchards evaluated treatments of pH-reducing buffers on russet-sensitive cv. 'Comice' and on russet-tolerant cv. 'Bartlett' to assess risk to fruit finish.

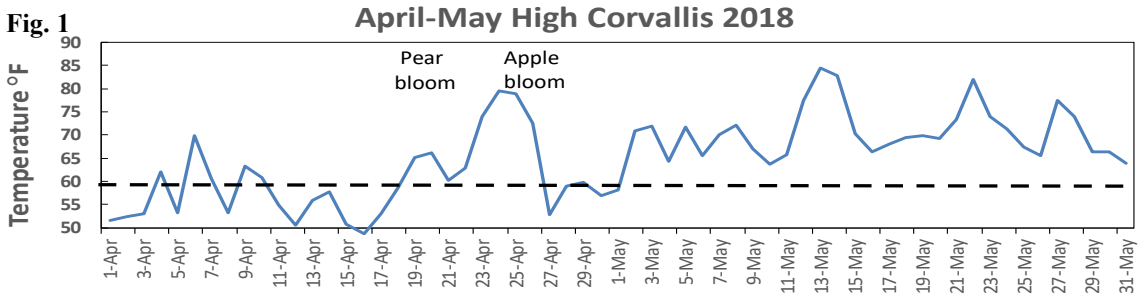
Experimental design. Experimental orchards were located at Oregon State University's Botany & Plant Pathology Field Laboratory near Corvallis (pathogen-inoculated), and at the OSU Southern Oregon Research and Extension Center near Medford, OR (fruit finish). Experiments were arranged in a randomized complete block designs with 4 replications. Treatment suspensions were sprayed to near runoff with backpack sprayers during early morning hours. To enumerate pathogen and yeast populations on flowers, five flower clusters were sampled from each replicate tree at full bloom, petal fall, and one-week post-petal fall, which was followed by washing the flowers, recording the pH of the wash, and dilution plating the wash on a selective culture media. In Corvallis trials, incidence of fire blight was determined by counting and removing the blighted flower clusters at 2- to 4-weeks after bloom. Number of blighted clusters per tree were divided by total clusters, which were counted before bloom. In Medford, in late August the proportion of the fruit surface with symptoms of russeting was scored with a modified Horsfall-Barratt rating scale.

Data summary and analysis. Measured population sizes for *E. amylovora* were $\log_{10}$-transformed and plotted in graphical arrays. The effect of the treatments on the pH of floral surfaces were plotted similarly. The effect of sprayed treatments on incidence of fire blight and on fruit finish were subjected to analysis of variance (ANOVA).

RESULTS:

Weather in spring 2018. During pear bloom, temperatures were generally unfavorable for fire blight development until near petal fall. Consequently, epiphytic pathogen populations were low during most of bloom. Nonetheless, fire blight incidence was remarkably high owing to a heavy bloom and warm temperatures after petal fall when pathogen populations increased to > 1 million cells per flower. In apple, temperatures were most favorable for fire blight development during the early and late portions of the bloom period. Maximum daily high between 9 April and 10 May averaged 63ºF; maximum daily high from 23 to 26 April averaged 76ºF. Fire blight risk as determined by the heat unit model, COUGARBLIGHT, was 'high' to 'exceptional' from 24 to 27 April. In Bartlett pear, the water-controls averaged 440 infections per tree (44% of total clusters), and 223 infections per tree in Gala apple (73% of total clusters).

Fig. 1



Objectives 1:

Infection suppression. In Corvallis, for both pathogen-inoculated trials, all treatments significantly suppressed fire blight ($P \leq 0.05$) compared to the water control including the treatment of 'citrate (16 oz.) plus disodium phosphate (8 oz.)' without the addition of oxytetracycline (Table 1). **In Bartlett pear**, outstanding suppression (84 to 85% relative to water control) was observed with the '½ rate of Buffer Protect' applied with either of the two oxytetracycline materials, FireLine or Mycoshield. Moreover, this treatment of 'oxytetracycline plus ½ rate of Buffer Protect' provided suppression superior to oxytetacycline only ($P \leq 0.05$). The addition of 'citrate (32 oz.) plus disodium phosphate (16 oz.)' to FireLine and of 'citrate (16 oz.) plus disodium phosphate (8 oz.)' to Mycoshield also significantly enhanced suppression of infection ($P \leq 0.05$) relative to the respective antibiotic material by itself. **In Gala apple**, all treatments that included a citrate-based buffer with FireLine significantly enhanced fire blight suppression (93 to 95% suppression) ($P \leq 0.05$) compared to FireLine by itself (85% control) (Table 1). Mycoshield was not evaluated in the apple trial.

Pathogen populations in flowers. **In pear**, on trees treated with FireLine (with or without buffer), pathogen populations on flowers were nearly undetectable from full bloom to petal fall (Fig. 2A). In contrast, for these same FireLine treatments, pathogen populations increasing rapidly during the post-petal fall period. For example, the pathogen populations on flowers treated with FireLine by itself increased from 2.0 to 6.5 $\log_{10}$ (CFU per flower) (i.e., from100 cells per flower to 5 million cells per flower) from petal fall to 3 days post-petal fall. FireLine plus '½ rate of Buffer Protect' or FireLine plus 'citrate (32 oz.) plus disodium phosphate (16 oz.)' showed the slowest rates of pathogen population increase during the post-petal fall period. **In apple**, the pattern and magnitude of measured pathogen population sizes over the bloom period were similar to those observed in pear with most of the pathogen growth on FireLine-treated flowers occurring after petal fall (Fig. 2B). An exception was FireLine with 'citrate (32 oz.) plus disodium phosphate (16 oz.)' treatment, which showed almost no increase in the pathogen population over the sampling period.

Floral pH. **In both pear and apple,** relative to other treatments, lower floral pH measurements were associated with treatments of 'FireLine with ½ rate of Blossom Protect', 'FireLine with 'citrate (32 oz.) plus disodium phosphate (16 oz.)', and 'citrate (16 oz.) plus disodium phosphate (8 oz.)' by itself (Fig. 3A&B). Apple flowers sampled from these treatments had a lower a minimum pH (4.9 to 5.3) than correspondingly treated flowers of pear (minimum pH of 5.5 to 5.6).

Table 1. Evaluluation of pH-bufferd oxytetrcycline materials for fire blight control in Bartlett pear and Gala apple, Corvallis, 2018.

Treatment	Rate per 100 gallons water	Stage treatment applied*		PEAR		APPLE	
		Full Bloom	Petal Fall	Percent blighted floral clusters**		Percent blighted floral clusters**	
Water (BioLink)		**X**§	**X**	**44.0**	**a**	**73.4**	a
FireWall 50	2.7 oz.	X	---§	5.5	d	3.3	c
Citrate Na_2PO4	16 oz. 8 oz.	X	X	23.9	b	15.1	b
FireLine	16 oz.	X	X	10.9	c	11.1	b
FireLine 1/2 Buffer Protect	16 oz. 75 oz.	X	X	6.4	d	2.2	c
FireLine Citrate Na_2PO_4	16 oz. 32 oz. 16 oz.	X	X	5.3	d	2.3	c
FireLine Citrate Na_2PO_4	16 oz. 16 oz. 8 oz.	X	X	8.9	cd	3.9	c
FireLine Citrate Na_2PO_4	16 oz. 12 oz. 12 oz.	X	X	9.6	cd	3.9	c
Mycoshield	16 oz.	X	X	18.4	b	--	
Mycoshield 1/2 Buffer Protect	16 oz. 75 oz.	X	X	7.0	cd	--	
Mycoshield citrate Na_2PO_4	16 oz. 16 oz. 8 oz.	X	X	7.2	cd	--	

*** Trees inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at 1 x 10^6 CFU/ml on 12 April (pear) and 24 April (apple). ** Trees used in the experiments averaged 1012 and 375 flower clusters per tree for pear and apple, respectively. For each treatment, percent blighted flower clusters was transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. § X indicates material was sprayed at that bloom stage date; --- indicates material was not applied at that bloom stage. Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$.**

Objective 2:

Fruit russeting. Application of citrate-based pH-buffering treatments at full bloom and petal fall resulted in slight effects on percent fruit russeting for both Bartlett and Comice pear fruit grown near Medford, OR. In Bartlett, a few significant differences ($P \leq 0.05$) were observed with treatment means for '½-rate of Buffer Protect', 'citrate (32 oz.) plus disodium phosphate (16 oz.)', and 'citrate (16 oz.) plus disodium phosphate (8 oz.)' being significantly higher than treatment means for 'citrate only (16 oz.)' and 'water control'. The difference between the most russeted treatments and the least russeted treatments was ~0.5%. The same trends of treatment effects occurred in the Comice trial with '½-rate of Buffer Protect', and citrate combined with higher rates of disodium phosphate' showing slightly more fruit russeting (~0.75%) compared to 'water' or 'citrate only'; these differences were not significant ($P > 0.05$).

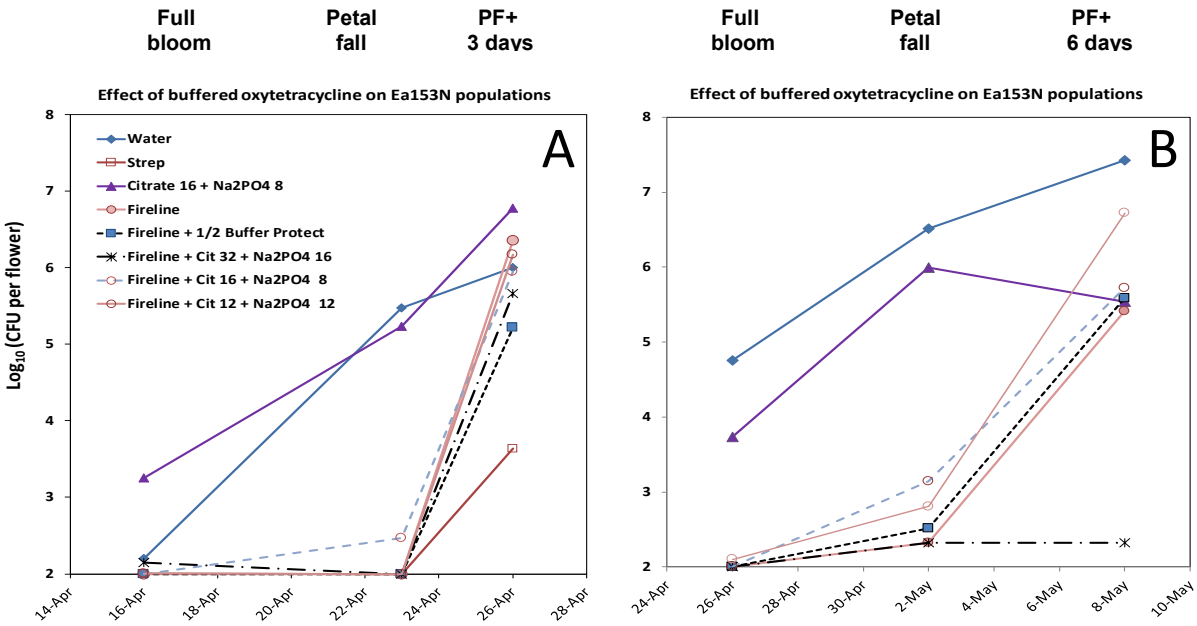


Fig. 2. Effect of treatments applied to A) Bartlett pear and B) Gala apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers during April and May 2018. Pathogen populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree, and plating the wash onto a selective culture medium. Data depict mean of each treatment program on each sampling date.
Note: Y-axis is log scale: a value of '2.0' is 100 pathogen cells/flower (the detection limit) and a value of '6.0' is one million cells per flower.

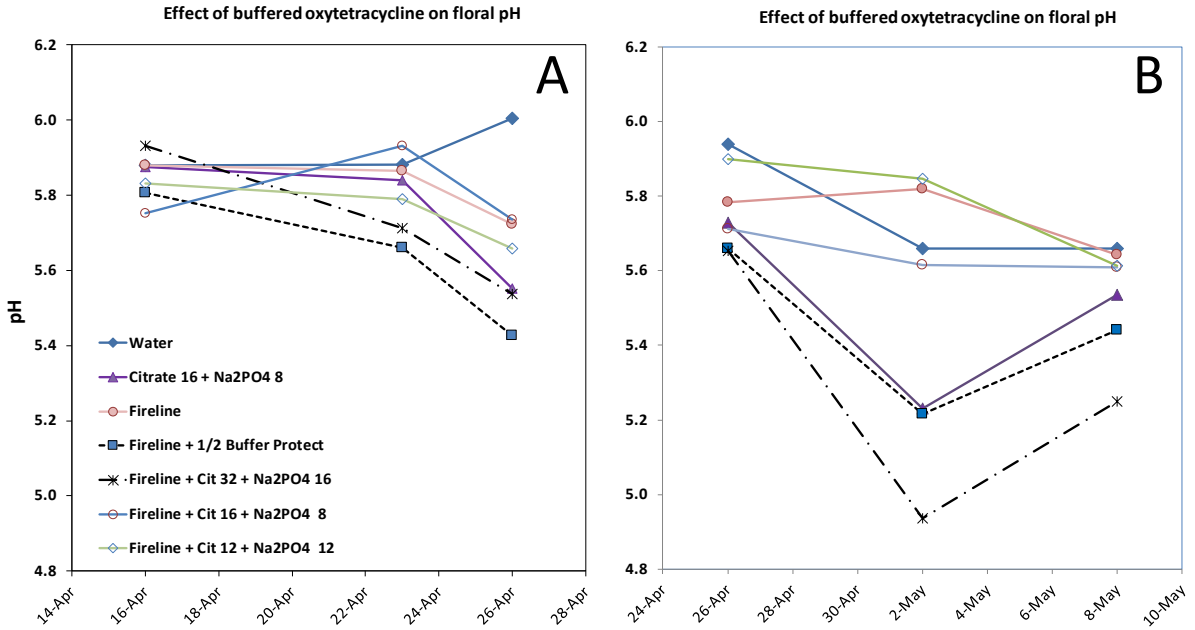


Fig. 3. Effect of treatments applied to A) Bartlett pear and B) Gala apple trees to suppress fire blight on the pH of floral surfaces during April and May 2018. A hand-held pH-probe was placed in a deionized-water wash of five flower clusters (~25 flowers, bulked in 25 ml of water) from each replicate tree. Data depict mean of each treatment program on each sampling date.

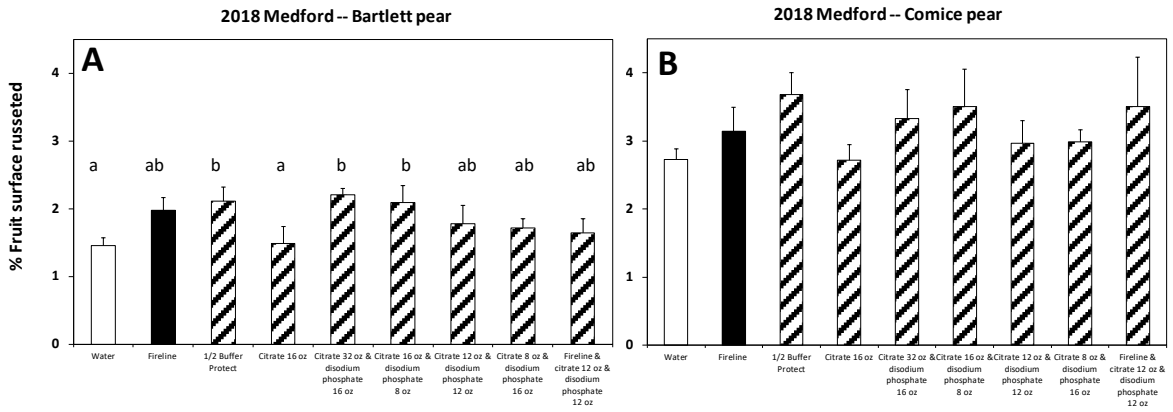


Fig. 4. Effect of citrate-based buffers applied to A) Bartlett and B) Comice pear trees on severity of fruit russeting (%) in orchards located near Medford, OR. Treatments were applied at full bloom and at petal fall (April). In late August, 30 fruit from each replicate tree were rated for russeting severity. Data depict mean and standard error from four replicate trees that received each treatment. In A), bars labeled with same letter are not significantly different according to Fischer's protected LSD at $P = 0.05$.

Objective 3:

Comparison of FireLine and Mycoshield. The effectiveness of both FireLine (17% oxytetracycline-hydrochloride, 17% a.i.) and Mycoshield (oxytetracycline-calcium complex, 17% a.i.) were enhanced by pH-buffering (Table 1 and Fig. 5). Without buffering, FireLine reduced incidence of blighted clusters to a significantly greater degree (75%) than Mycoshield (58%) ($P \leq 0.05$) (Table 1), but after buffering with Buffer Protect or citrate (16 oz.) plus Na_2PO_4 (8 oz.), both materials suppressed fire blight similarly (80 to 84%). Furthermore, both FireLine and Mycoshield suppressed pathogen populations on floral surfaces compared to the water-treated control (Fig. 6). Without buffering, the pathogen population suppression achieved by FireLine at full bloom and petal fall was about a log unit (~90%) better than Mycoshield (Fig. 6). With pH buffering, both materials showed similar effects on pathogen suppression. At 3 days after petal fall, the most suppressive treatments were 'FireLine plus ½ Buffer Protect' and 'Mycoshield plus citrate (16 oz.) and Na_2PO_4 (8 oz.)'.

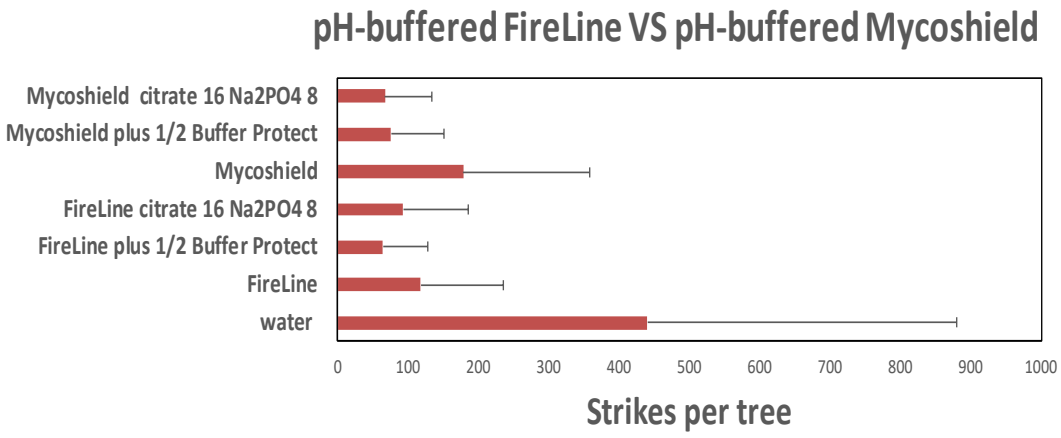
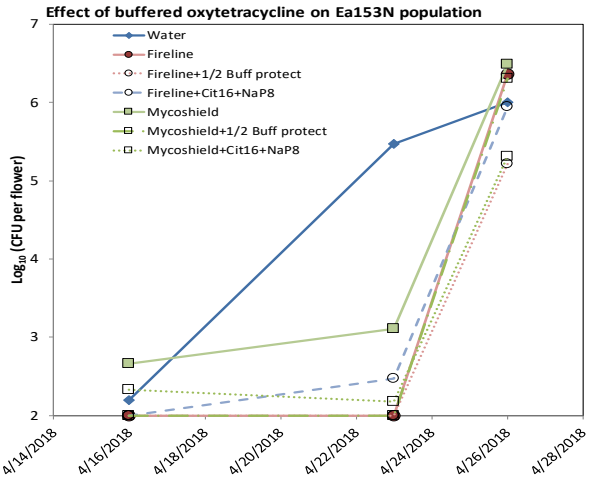


Table 5. Effect of pH-bufferd oxytetrcycline materials for fire blight control in Bartlett pear expressed as strikes per tree. Data depict mean and standard error from four replicate trees.

Fig. 6. Effect of pH-buffered oxytetracycline treatments applied to Bartlett pear on the population size of *E. amylovora* strain 153N on flowers during April 2018. Pathogen populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree, and plating the wash onto a selective culture medium. Data depict mean of each treatment program on each sampling date.
Note: Y-axis is log scale: a value of '2.0' is 100 pathogen cells/flower (the detection limit) and a value of '6.0' is one million cells per flower.



Discussion

Inhibiting growth of the fire blight pathogen on pome flowers has the immediate benefits of reducing floral infection and the longer-term benefit of reducing the amount of epiphytic inoculum carried into late- and rattail-phases of bloom. In enhancing these goals, pH-buffering of oxytetracycline appears to improve the stability of the antibiotic in the spray tank, or increase the duration of the effective residual on floral surfaces, or both. In aqueous solution (22ºC), oxytetracycline has a half-life of 41 hours at pH 7, which is increased to 171 hours at pH 5. In addition to direct effects on oxytetracycline, more acidic conditions also have direct, negative effects on the fire blight pathogen. *Erwinia amylovora* cannot grow at pH $\leq$ 5. Moreover, at pH 7.0 (22ºC), the doubling time for a growing population of *E. amylovora* is 1.4 hour, but at pH5.5, the doubling time is increased to 3.2 hours.

With regard to chemical-induced fruit russeting, moderately-sensitive Bartlett pear and highly-sensitive Comice pear provided an indication of relative safety of a pH-buffering adjustment to the spray suspension. Surprisingly, in Comice, no significant effects of the treatments were observed, but a few treatments slightly increased fruit russeting on Bartlett. In both trials, treatments with higher amounts of buffer in the spray suspension were correlated with increased fruit russeting, but citrate alone, which did not increase russeting in either cultivar, had the lowest pH (Table 2). Overall, compared to other materials we trialed for fruit russeting risk, the degree of injury induced by citrate-based buffers was very small (see 2019 WTFRC apple crop protection continuing report).

Table 2.

Antibiotic	Rate/100 gal	Buffer (rate 100 gal)	pH in well-water
FireLine	16 oz.	--	6.3
FireLine	16 oz.	½ rate Buffer Protect (75 oz)	3.8
-	-	½ rate Buffer Protect (75 oz)	3.7
-	-	citrate (32 oz.) plus Na_2PO_4 (16 oz.)	3.3
-	-	citrate (16 oz.) plus Na_2PO_4 (8 oz.)	3.6
-	-	citrate (12 oz.) plus Na_2PO_4 (12oz.)	4.6
-	-	citrate (16 oz.)	3.0
Mycoshield	16 oz.	citrate (16 oz.) plus Na_2PO_4 (8 oz.)	3.9
Mycoshield	16 oz.	½ rate Buffer Protect (75 oz)	4.2
Mycoshield	16 oz.	--	5.8

A problem encountered at the time of the treatment applications was that Na_2HPO_4 dissolved very slowly in the cold, well water used for spraying. We solved this by pre-dissolving Na_2HPO_4 in hot water before adding to the spray tank. Because this is impractical at large scale, 2019 trials will utilize **1)** dibasic potassium phosphate ($K2HPO_4$), which reduces pH similarly and readily dissolves in cold water, and **2)** the commercial acidifier/spray adjuvant, LI 700 (Loveland Products, Inc., Loveland, CO, lovelandproducts.com/product/li-700). FireLine and Mycoshield acidified with citrate only (16 oz.) also will be evaluated.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3 (No-cost extension)

Project Title: Functional genomics of 'D'Anjou' pear fruit quality and maturity

PI: Loren Honaas
Organization: USDA-ARS
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Address: 1104 North Western Ave
City/State/Zip: Wenatchee, WA 98801

Cooperators: Stefano Musacchi & Sara Serra (WSU-TFREC), David Rudell & Jim Mattheis (USDA-ARS), Claude dePamphilis (PennState)

Total Project Request: Year 1: $52,707 **Year 2:** $33,488 Year 3: $0

Other funding sources: USDA-ARS technician salary and benefits - $31,734

Budget 1
Organization Name: USDA, ARS **Contract Administrator: Chuck Myers**
Telephone: 510-559-5769 **Email address: chuck.myers@ars.usda.gov**

Item	2017	2018	2019
Wages[1]	$12,500	$12,500	
Equipment[2]	$1,980	NA	
Supplies	$8,407	$5,483	
Miscellaneous[3]	$29,820	$15,505	
Total	$52,707	$33,488	**$0**

Footnotes:
[1] Cooperative Agreement to Penn State for data processing and data analysis
[2] Service contract for CLC genomics workbench support
[3] Illumina sequencing & library prep at Penn State Genomics Core via Cooperative Agreement

Objectives:
1) **identify gene activity** correlated with fruit quality and maturity as it relates to on-tree fruit position
2) **discover genes** in 'D'Anjou' pear for comparative genomics with 'Bartlettt' pear
UPDATE: gene discovery via whole genome sequencing
3) **generate a list of potential biomarkers** for use in research and fruit production

Year 3 goals:
In year 3 we will re analyze the 'D'Anjou' gene activity (i.e. RNA-Seq) data with our new 'D'Anjou' genome that contains all of the genes in 'D'Anjou' (see objectives 1 & 2). We anticipate that this approach will resolve issues related to genetic mismatches between 'Bartlett' and 'D'Anjou' pear. With higher quality gene activity data, we can develop a higher resolution and more complete picture of gene activity that is associated with postharvest fruit quality, leading to a higher confidence list of potential fruit quality biomarkers (objective 3).

Significant Findings:
- 'D'Anjou' pear genome has been sequenced with cutting-edge 3rd gen technology
- Gene activity data has structure that is consistent with findings of Musacchi, Serra, and Rudell
- Genetic differences between D'Anjou and Bartlettt hinder gene activity measurements

Methods:
Update regarding gene discovery efforts for Objective 2 A combination of ***1)*** improvements to our methods for DNA preparation with ***2)*** very recent decreases in costs for 3rd generation sequencing technology have put full genome sequencing with-in reach for this project – which is synergistic with Honaas' WTFRC Technology Committee project "Enhancing reference genomes for cross-cultivar functional genomics." This is a preferred gene discovery approach to using raw gene activity data obtained from fruit because the collection of 'D'Anjou' gene models will be more accurate and complete.
Genome sequencing and gene activity analysis
We obtained 'D'Anjou' pear trees from Van Wells (Wenatchee, WA) and dormancy was broken in the USDA green house. Young leaves approximately 2 cm in length were harvested and flash frozen on liquid nitrogen. Frozen tissue was sent to cooperator dePamphilis for DNA extraction methods testing, genomic DNA quality evaluation, sequencing sample preparation tests, and then genome sequencing at Penn State's genomics core facility. Genome data were used to survey for genome differences between 'D'Anjou' and 'Bartlett' pear. Pilot genome assembly is underway and assemblies are being evaluated. Extensive gene activity analyses have been run for 'D'Anjou' pear, including validation, using the 'Bartlett' genome reference.

Results & Discussion:
Streamlined genomic DNA prep led to successful genome sequencing in 'D'Anjou' pear Very high molecular weight DNA (HMW DNA - e.g. ~100,000bp pieces of DNA) is one essential component to accessing 3rd generation sequencing technology. Cooperator dePamphilis's group has successfully developed an extraction and purification protocol that results in good yields of HMW DNA (Figure 1). Long genomic fragments are extremely fragile such that mixing or shaking the sample creates shear forces that will break the very long and delicate strands. Other groups have used more expensive and elaborate methods to get DNA of sufficient quality (as in the recent double haploid 'Golden Delicious' genome). Our group, using specialized techniques like Pulsed Field Gel Electrophoresis (PFGE), has demonstrated that more rapid and cost-effective methods from flash-frozen young leaves yield suitable DNA for 3rd generation sequencing.

A second hurdle to getting valuable 3rd generation genome data is the unpredictable success rate during sequencing sample preparation. The only effective way to determine if HWM DNA samples

will work is to attempt test DNA preparations. At the Penn State genomics core we successfully generated data from multiple approaches and selected the highest performing strategy to sequence 'Granny Smith' and 'D'Anjou' - each yielding millions of reads that were >10,000bp (50x longer than 2nd gen technology). Using the same DNA we also generated 2nd generation genome data (shorter, but more numerous reads). These 2nd gen data are useful to quantify differences between reference and cultivar-specific genomes, as well as error-correct 3rd generation data during the iterative process of building and evaluating genome assemblies.

Contrasts reported by Musacchi, Serra, and Rudell are supported by gene activity data
We examined our samples from Stefano Musacchi's project "Improving Quality and Maturity Consistency of 'D'Anjou'" to determine if reported peel chemistry differences between fruit were accompanied by gene activity differences. This serves as a sort of validation, and indeed a principle component analysis (PCA) shows changes in gene activity during postharvest storage and gene activity differences depending upon fruit position in the canopy (Figure 2). Additionally, a gene co-expression network analysis performed by Dr. Stephen Ficklin (WSU Department of Horticulture) also shows that correlated gene activity in peel differs between fruit from different tree canopy positions (Figure 3).

While these analyses indicate our gene activity (i.e. RNA-Seq) data captures gene activity differences that we can relate to differences in fruit quality, other aspects of our analysis indicate the picture is incomplete. Even though we see clear agreement with previous work by Drs. Musacchi, Serra, and Rudell, it is also clear from steps we take to validate our analyses that issues arising from use of the 'Bartlett' genome are present.

These data quality checks include examining data usage and validation via independent techniques (like PCR). Because data usage in experiments like these should be 70-80%, our current rate of ~50% data usage indicates gene activity signals are likely missing or suppressed. The marginal agreement between qPCR and RNA-Seq (Figure 4, using our published protocol: Hargarten et al. 2018 - https://doi.org/10.21273/JASHS04424-18) also points to issues with the use of the 'Bartlett' genome as an RNA-Seq reference. This disagreement is likely due to false negatives, that is, missing or suppressed gene activity signatures in the analysis, that occur due to genetic differences between 'D'Anjou' and 'Bartlett.'

Genetic differences between 'D'Anjou' and 'Bartlett' We examined genetic differences between 'D'Anjou' and 'Bartlett' as part of our WTFRC Tech Committee project "Enhancing Reference Genomes for Cross-cultivar Functional Genomics." As expected, we found ~5.6 million small genetic differences that likely interfere with interpretation of gene activity measurements (Figure 5). These differences highlight the need to develop a 'D'Anjou' specific set of gene models, which we can extract from a draft assembly of the 'D'Anjou' genome.

Stakeholder perspectives:
The quality of an RNA-Seq reference (genome or *de novo* transcriptome) has a substantial impact on the reliability of RNA-Seq data analysis. Because our work shows that use of the 'Bartlett' genome for RNA-Seq (i.e. gene activity) analysis presents serious hurdles, we are focused on the 'D'Anjou' gene discovery objective of this project. Recent advances have made genome sequencing with 3rd generation sequencing technology a viable option for this project. This cutting-edge type of genome data (vs. raw gene activity data) greatly improves our ability to discover accurate and complete 'D'Anjou' gene sequences. Plus, it provides a valuable resource for the incoming USDA ARS pear genomics scientist, who will have full and unlimited access to these data.

Our preliminary checks and validations indicate that our RNA-Seq analysis, though incomplete, shows gene activity that is associated with fruit flavor, quality, appearance, and, most of

all ripening rate. As we make progress a clearer and more complete picture of gene activity will emerge, which we can then confidently relate to postharvest fruit quality. Linking fruit quality to gene activity signatures is a key first step in biomarker development.

Figures and Tables

Figure 1. Very long fragments of genomic DNA from our stream-lined protocol. 18 hour Pulsed Field Gel Electrophoresis (PFGE) of genomic DNA shows that our modified genomic DNA protocol was successful for both apple and pear and is comparable to high quality samples of *Theobroma cacao* (chocolate tree) DNA.

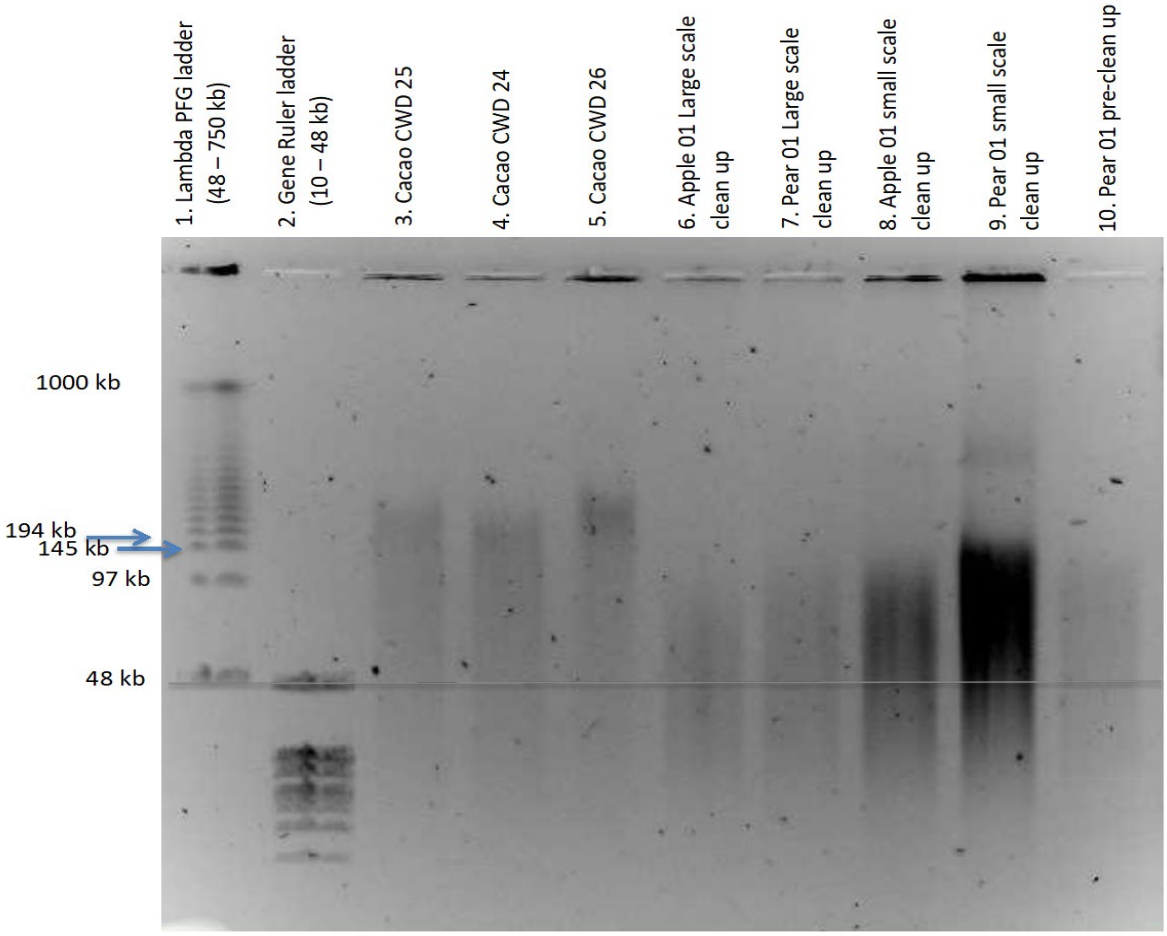


Figure 2. **Principle Component Analysis** shows there are clear differences between gene activity signals from fruit picked from external canopy positions vs. internal canopy positions. This suggests that the gene activity analysis, though incomplete, will reveal gene activity differences that can be related to differences in fruit quality.

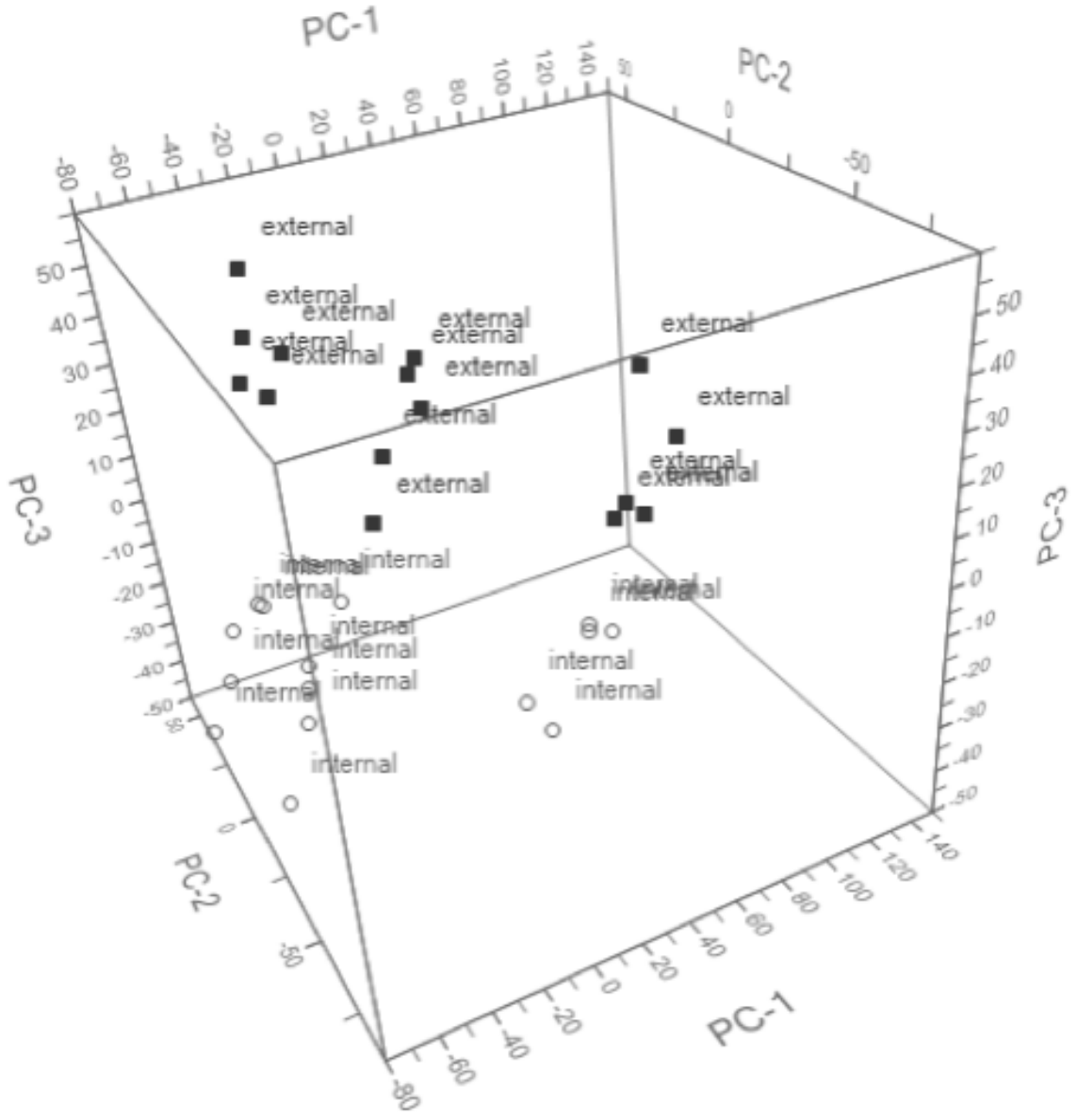


Figure 3. **Gene co-expression network analysis** shows clusters of genes that are unique to fruit picked from external (light grey) vs. internal (dark grey) canopy positions. This, like the PCA, also suggests that the gene activity analysis, though incomplete, shows gene activity differences that can be related to differences in fruit quality.

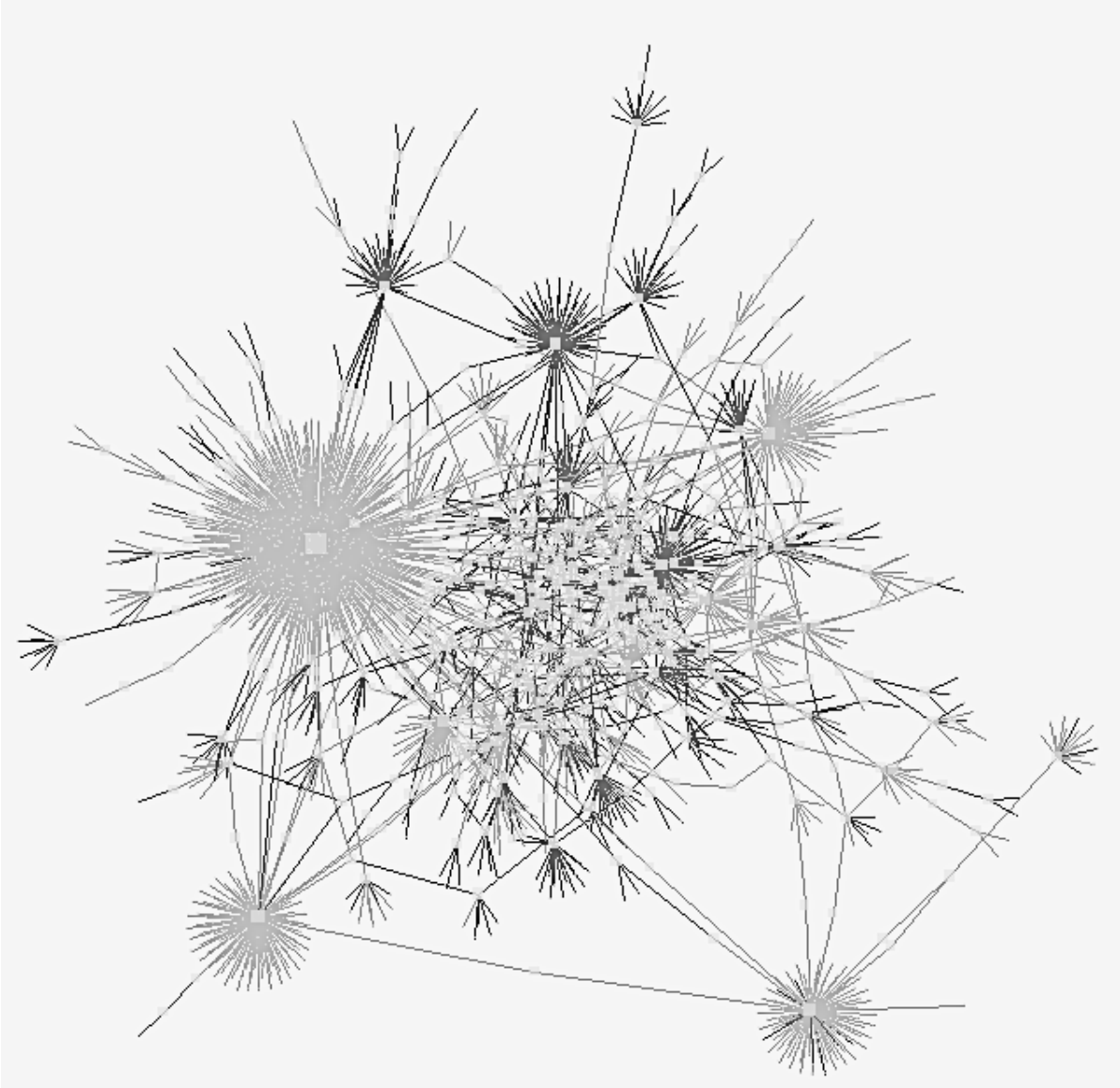


Figure 4. **Marginal agreement between RNA-Seq and qPCR estimates of gene activity.** Pearson's correlation of RNA-Seq vs. qPCR should show an average R^2>0.8 - for 'D'Anjou' pear the average is ~0.6, indicating inaccurate or incomplete estimates of gene activity via RNA-Seq.

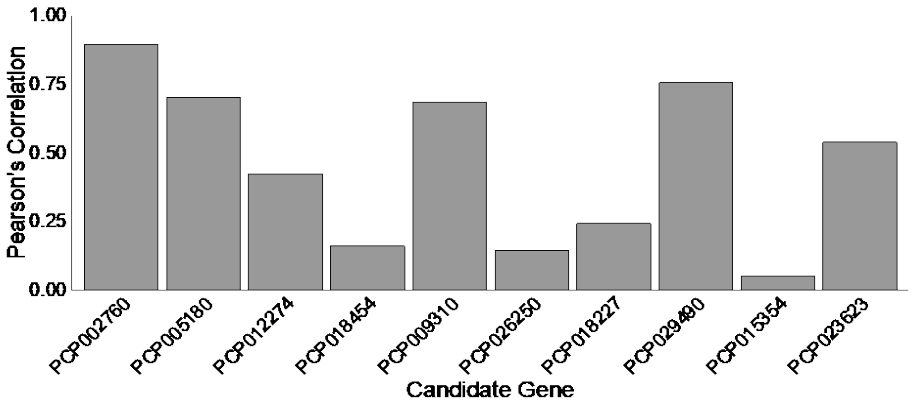
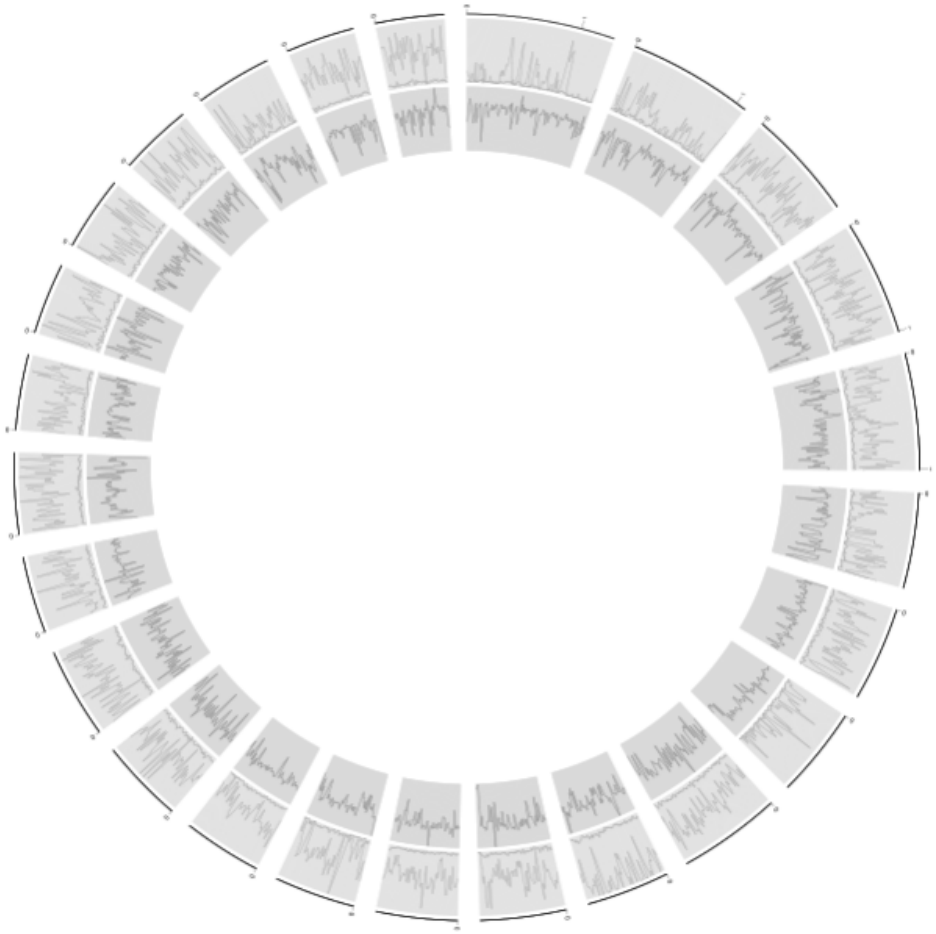


Figure 5. **Genome CIRCOS plot** summarizing 'D'Anjou' polymorphisms relative to the 'Bartlett' genome. Each block is a large genomic fragment. The outer trace shows the frequency of differences, while the inner trace shows the amount of genome data across each block. Genetic differences are widely distributed across the whole genome, totaling roughly 5.6 million.



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

Project Title: Acoustically based mating disruption of winterform psylla

PI: David Horton
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Co-PI (2): Elizabeth Beers
Organization: Washington State University
Telephone: (509) 663-8181
Email: ebeers@wsu.edu
Address: WSU-TFREC
Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

Co-PI (3): David Crowder
Organization: Washington State University
Telephone: (509) 335-7965
Email: dcrowder@wsu.edu
Address: 166 FSHN Building
Address 2: PO Box 646382
City/State/Zip: Pullman, WA 99164

Total Project Request: **Year 1:** $52,761 **Year 2:** $49,733 **Year 3: $53,166**

Other funding sources: None

Budget 1
Organization Name: WSU Pullman **Contract Administrator:** Ben Weller
Telephone: 509-335-0052 **Email address:** grants.fsclark@wsu.edu

Item	2018	2019	2020
Salaries[1]	$28,417	$29,554	$30,736
Benefits[2]	$2,580	$2,683	$2,791
Wages[3]	$11,040	$11,251	$11,471
Benefits[4]	$1,124	$1,145	$1,168
Equipment			
Supplies[5]	$6,000	$3,000	$3,000
Travel[6]	$3,600	$2,100	$4,000
Miscellaneous			
Plot Fees			
Total	$52,761	$49,733	**$53,166**

Footnotes:
[1] Salary for the PhD student for the academic year
[2] Benefits for the PhD student for the academic year include health insurance and fringe
[3] Wages for the PhD student for the summer; also includes a time-slip employee who will work 40 hours a week for 12 weeks each summer during the project
[4] Fringe benefits for the PhD student and time-slip employee during the non-academic year
[5] Yr 1 – acoustics equipment for conducting the vibrational studies (Objective 2). Yrs 2 and 3 - Experimental supplies for Objectives 3 and 4
[6] Yr 1 – Funds will support travel to the USDA-ARS facility in Gainesville, FL. Yrs 2/3 - Vehicle lease through the state motor pool; this vehicle will be used to complete field research objectives

OBJECTIVES

1. Recruit Ph.D. student (co-supervisors E. Beers and D. Crowder). COMPLETED
2. Describe vibrational signals used by psylla in mate location activities. ONGOING
3. Show (in large cage studies with potted trees) that it is possible to slow or disrupt mating by mechanically transmitting these signals to the tree substrate.
4. Show that it is possible to slow or disrupt mating in a field setting by mechanically transmitting signals through the support wires of a trellised pear orchard.

SIGNIFICANT FINDINGS

- Ph.D. student (Ms. Dowen Jocson) arrived in summer 2018 (Pullman campus).
- Pear psylla colonies were established at the Pullman location; pear whips were planted and are being maintained in a greenhouse at the Pullman location.
- Acoustics equipment was purchased and set-up at the Pullman location. Assays with summerforms and winterforms began in December 2018.
- Acoustic signals from male psylla detected, quantified, and described. This is the first evidence that this species (like many other psyllids) communicates acoustically. The signal is superficially similar to that in a closely related pear psyllid (the European pear psylla; *Cacopsylla pyri*), but of higher pitch and with generally larger numbers of individual pulses at the beginning of a signal.
- Playback tests to confirm that the male signal induces female acoustic response (duetting) will begin in early 2019.

METHODS

Source of insects and plants. We are using field-collected and lab-reared winterforms and summerforms in recording acoustic cues and for eventual testing of synthesized mimics of those cues.

Objective 1. Recruitment of Ph.D. student. COMPLETED (see Results and Discussion).

Objective 2. Describe vibrational signals.

Detecting and recording vibrational signals. ONGOING. We are recording vibrational signals of pear psylla using an accelerometer as described below in Results and Discussion. ***Playback tests of signal***. TO BEGIN IN EARLY 2019 (see Results and Discussion). Synthetic mimics of male-produced vibrational signals will be assayed in playback tests to confirm that the signal does indeed prompt vibrational response by female psyllids (duetting).

Objective 3. Large cage studies to prove disruption. We will use a cage study to examine the effects of synthetic mimics of vibrational signals on mating success of winterform psylla. The tests will be done out-of-doors in large "Bugdorm" cages (6 x 4 x 4 foot) each containing a potted pear tree 4-5 foot in height. A minishaker will be used to transmit the female-signal to trees. Fifty virgin female winterforms will be introduced into each cage and allowed to settle on trees. After 48 hrs, 50 male winterforms will be added to each cage, and the buzzer apparatus activated. Females will be collected from each cage after 2 days and dissected to determine mating status. Control cages will be treated identically to treatment cages, with the exception that no buzzer system will be present.

Objective 4. Field tests in trellised pear orchard. We will conduct a field test of the disruption concept under an orchard situation. Tests will be done in March at a high density pear orchard under a wire trellis system. Electromagnetic minishakers attached to trellis wires will be used to disseminate the acoustic signals to trees. A laptop computer will control the minishakers and signal production. We will collect winterforms from target trees (those receiving the signal mimics) and control trees located a few rows away. Females will be dissected to determine mating status.

RESULTS AND DISCUSSION (YEARS 1-2)

Objective 1: Recruitment of Ph.D. student. COMPLETED. Ms. Dowen Jocson, an M.S. graduate of St. Louis University, arrived at the Pullman campus in late summer 2018 to begin a Ph.D. program in Entomology at WSU (co-supervised by Crowder, Beers, Horton).

Objective 2: Describe vibrational signals. ONGOING. *Insects and plants*. Winterform psylla and pear whips were forwarded by Drs. Horton and Beers to Ms. Jocson in November 2018 (Fig. 1). The insects were separated by sex and placed in single-sex colonies (to prevent mating) on whips. A mixed-sex colony was also set-up to allow mating for production of the summerform morphotype. *Acoustics equipment*. Equipment required to record and describe acoustics signals was purchased and set-up in a semi-soundproof room at the Pullman campus (Fig. 2A). We are using an accelerometer to detect and record signals (Fig. 2B). The accelerometer detects vibrations in the plant surface produced by signaling insects much as a seismograph is used to detect earth tremors. The insect-signal is detected by the accelerometer and sent to a computer, where the signal is then translated into a readable form. Analysis of signals is then done using freely available software (Raven, Audacity).

Male acoustic signal. Assays were initiated in December 2018 with both summerform and winterform males. We have successfully detected and described the male signal (Fig. 3: upper panel), the first evidence showing that this species like other psyllid species does indeed communicate acoustically. The male signal consists of a series of 15-25 "pulses" lasting about 10 seconds, followed by a longer phrase of more tightly packed syllables (Fig. 3: upper panel). Dowen characterizes the combined "pulse + syllable" signal as being somewhat like the sound produced by a car engine trying to start (series of pulses or "cranking" sounds), followed by the "purring" of the engine once the engine is running ("syllable" phase of the signal). Duration of an individual call was about 30 seconds, with consecutive calls (3 calls shown in Fig. 3: upper panel) separated by 10 to 15 seconds. The signal shown here for our pear psylla (*Cacopsylla pyricola*) is superficially similar to that exhibited by its close relative, the European pear psylla (*Cacopsylla pyri*; Eben et al. 2014). One noticeable difference is that the signal of our pear psylla has substantially higher average frequency ("pitch"; 1320 Hz [Fig. 3: lower panel]) compared to the much lower frequency of the *C. pyri* signal at approximately 690 Hz (Eben et al. 2014). I.E., the male signal from our psylla is of considerably higher pitch than the signal of male European pear psyllid. The biological significance of this difference is as yet unknown. It is possible that the difference in the two signals contributes to mating isolation between species in geographic regions where the two psyllids co-exist.

Figure 1. Establishing plant and insect cultures at Pullman location.

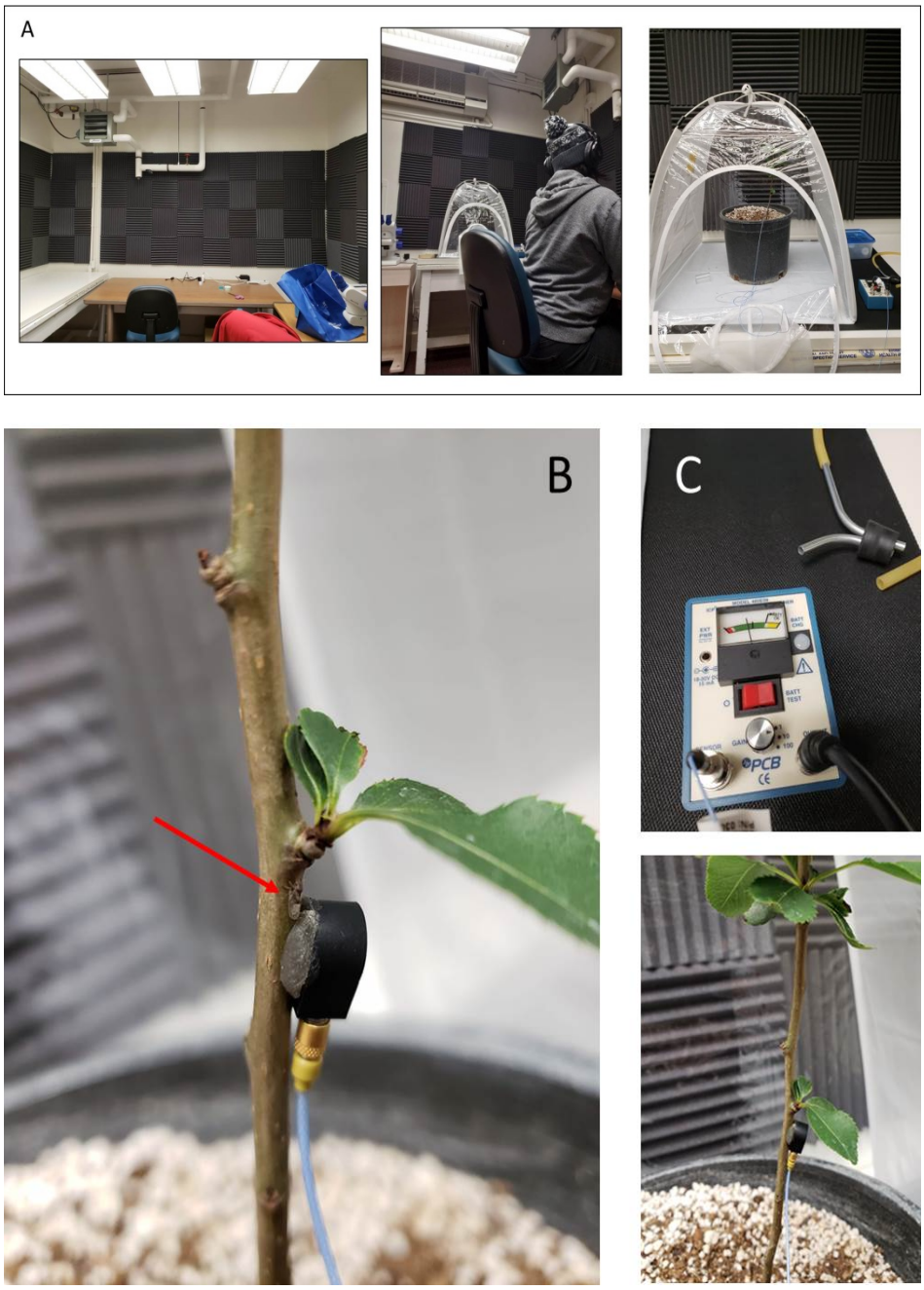

Figure 2. (A) Semi-soundproof room at Pullman location being used in our acoustics assays. (B) Head of accelerometer attached to pear whip (red arrow shows location of a psyllid). (C) Signal conditioner used to power accelerometer head and translate the vibrations.

Playback tests of signal and description of the female response. The next step is to confirm that the male signal prompts a return signal from the female psyllid, and to describe that female signal. Our long-term practical aim for this project is to show that a mimic of the female signal, transmitted through trellised pear trees under field conditions, disrupts the mate-seeking behavior of males. For that purpose, we need a description of the female signal. Eben et al. (2014) showed that female *C. pyri* rarely signaled spontaneously but required the male signal to induce her acoustic reply. We are

following methods of Eben et al. (2014) in using synthesized playbacks of the male signal to entice females to call. The signal (male) mimic is being transmitted to stems of pear seedlings using a Linear Resonance Actuator (the same technology that is used to make your cellphone vibrate), connected to a computer, with the other end attached to the stem of a plant hosting one or more female psylla. These playback trials are to begin in early 2019.

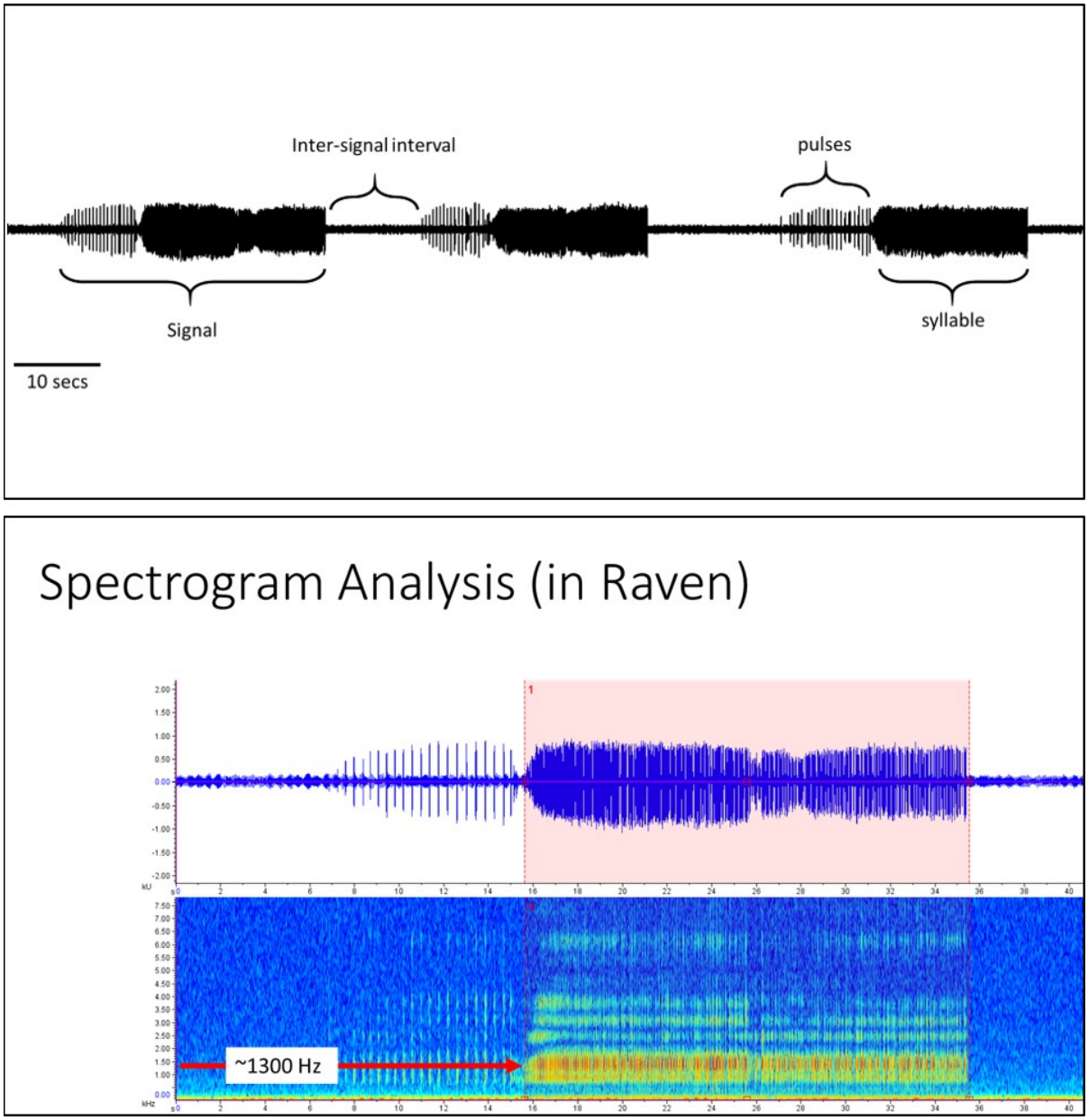

Figure 3. Upper panel shows oscillogram for signaling male pear psylla; lower figure shows sonogram and spectrogram analysis depicting signal frequency concentrated at 1300 Hz.

Reference

Eben, A. et al. 2014. First evidence of acoustic communication in the pear psyllid *Cacopsylla pyri* L. (Hemiptera: Psyllidae). J. Pest Sci. DOI 10.1007/s10340-014-0588-0.

CONTINUING PROJECT REPORT **YEAR**: 2

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

PI: W. Rodney Cooper
Organization: USDA-ARS, Wapato, WA
Telephone: 509/454-4463
Email: Rodney.Cooper@ars.usda.gov

Cooperators: David Horton and Stephen Garczynski, USDA-ARS in Wapato, WA; William Walker, Swedish University of Agricultural Sciences

Budget: **Year 1**: $12,000 **Year 2**: **$10,000** **Year 3**: $7,500

Other funding sources

Agency Name: USDA-ARS Research Associate Program
Amt. awarded: $163,635
Notes: Funding for a USDA-ARS Research Associate

Agency Name: Northwest Potato Research Consortium
Amt. requested: $36,000
Notes: Study on potato psyllid saliva

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

Item	2018	2019	2020
Supplies	$11,000	$9,000	$6,500
Plot Fees	$1000	$1000	$1000
Total	$12,000	**$10,000**	$7,500

Footnotes:

OBJECTIVES

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

SIGNIFICANT FINDINGS

- Genes involved in reproduction, photoreception, muscle, and immunity were more highly expressed in summerform psylla than in winterform psylla, consistent with previously documented biological differences between these populations.
- Genes associated with bacterial endosymbionts were more highly expressed in winterform psylla than in summerform psylla, which is consistent with the previous report that endosymbiont infection is higher in winterforms.
- Genes that are homologous to odorant-binding protein chordotonal receptor genes were identified from the pear psylla transcriptome. The putative identification of these genes will enable further study on how psylla locate host plants and mates.

METHODS

Collection of summerform, diapausing winterform, and post-diapause winterform pear psylla. We have collected adult pear psylla monthly from a pear orchard located at the USDA experimental farm near Moxee, WA (Figure 1) since August of 2017, and will continue to collect psylla until at least July of 2018. Summerform and winterform psylla will be separated during autumn when populations of the different morphotypes overlap. The specimens will be stored in -80°C in RNAlater to preserve the RNA. These collections will provide us with about 4-5 months of summerform collections, 3-4 months of diapausing winterform psylla, and 4-5 months of post-diapause winterform psylla (Table 1).

Collection of winterform psylla from overwintering shelter hosts. Post-diapause winterform psylla will be collected from pear trees (non-dispersing) and from various shelter hosts including Juniper, Pine, Spruce, *Salix*, and apple in early-February. Collections will be made from plants located at the USDA experimental farm near Moxee, WA (Figure 1). Winterform psylla have been collected from these shelter hosts in previous years, and results of gut content analysis indicate that psylla visit and feed upon these trees at this location (see 2018 Final Report by Cooper and Horton). Specimens will be stored in -80°C in RNAlater until they are processed for analyses.

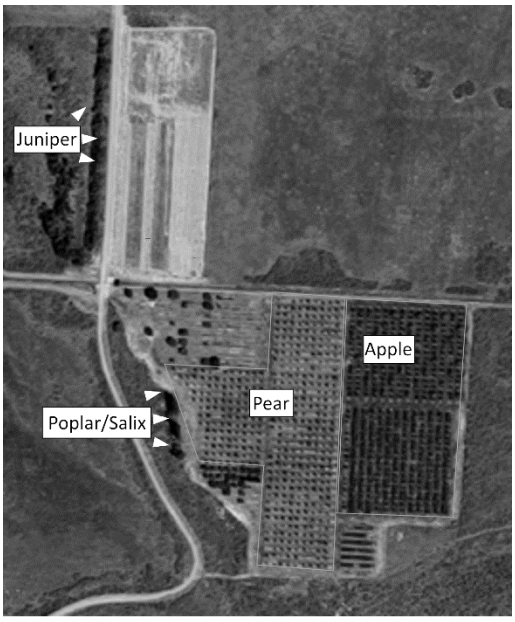

Figure 4. Winterform pear psylla will be collected from a pear orchard at the USDA experimental farm, and from surrounding shelter hosts including apple, Juniper, Poplar, and Salix.

Transcriptomics. RNA from whole bodies of at least 10 pear psylla in RNAlater will be extracted using a commercial kit. Two replications will be included for each treatment (12 months for Objective 1, at least five overwinter hosts for Objective 2). Samples will be shipped to Novogene for RNA sequencing (www.novogene.com; cost of $225 per transcriptome). Whole transcriptomes will be assembled using the online bioinformatics software, EGassembler. Annotation of all transcripts will be performed using Blast2GO software (Conesa et al. 2005) that categorizes putative biological functions to genes by

identifying similar sequences of known function within publicly available databases. Quantitative analyses of transcript expression levels will be determined with standard abundance expression software, such as RSEM, and differential expression analysis will be conducted with DESeq to assess expression levels across samples.

Differential expression of at least 10 genes will be confirmed using quantitative real time PCR (qPCR). Based on gene annotations and homologies to other insects, qPCR analysis will be performed on genes that are predicted to be involved with diapause, sensing (visual or olfactory), or basal immunity. Primers and probes specific for each target gene will be designed from sequences obtained from the transcriptome. cDNA libraries will be constructed from RNA from each sample. qPCR will be performed on cDNA using a Roche Lightcycler real-time PCR machine located at the ARS laboratory in Wapato. Ribosomal protein 3 and Actin gene will be used as control genes to standardize gene expression among samples.

Table 1. Summary of psylla collections and transcriptome comparisons.

Collection	Description	Phenological traits
Objective 1		
Sept. - Nov.	Diapausing winterform -Morphotypes overlap in September	-Reproductive diapause; lack of mating and ovarian development -Attracted to the color of foliage -More susceptible to insecticides
Dec - Feb.	Post-diapause winterform -January collection may include a mixture of diapausing and post-diapausing adults	-Reproductive development is slow due to cold temperatures -Not attracted to the color of foliage
March		-Mating and egglaying activities -Not attracted to the color of foliage
April		-Mating and egglaying continue -Attracted to the color of foliage
May - Aug.	Summerform	Reproductive, attracted to pear and the color of foliage
Objective 2		
Pear	Non-dispersing winterform	Overwinters on developmental host
Apple	Dispersing winterform	Overwinters on deciduous fruit tree
Juniper Pine		Overwinters on conifer
Salix		Overwinters on deciduous wind break

RESULTS & DISCUSSION

Dr. Karol Krey was hired as a Post-Doctoral Associate and was onboarded in September of 2018. Dr. Krey will be responsible for the completion of this project under the direction of Cooper. RNA has been extracted from three populations of pear psylla: summerform, diapausing winterform, and post-diapause winterform (Table 1). We initially sequenced RNA from only one replication per psyllid population to ensure that sequencing parameters were suitable for analysis. An additional three replications per population have been sequenced and are currently being analyzed.

Initial results demonstrate variable gene expression among summerform, diapausing winterform, and post-diapause winterform pear psylla, and results are consistent with observed differences among the populations in behavior and biology (Table 2; Ullman and McLean 1988, Krysan and Higbee 1990, Horton et al. 1998, Horton et al. 2007, Civolani et al. 2011, Cooper et al. 2017). The top 50 highest expressed genes from each population were identified and compared to sequences deposited in the NCBI database. Genes were then categorized based on their putative function (immunity, muscle, photoreception, vitellogenin, or other biological processes) and origin (ribosome, mitochondria, *Arsenophonus* or *Carsonella*), or were designated as unknown.

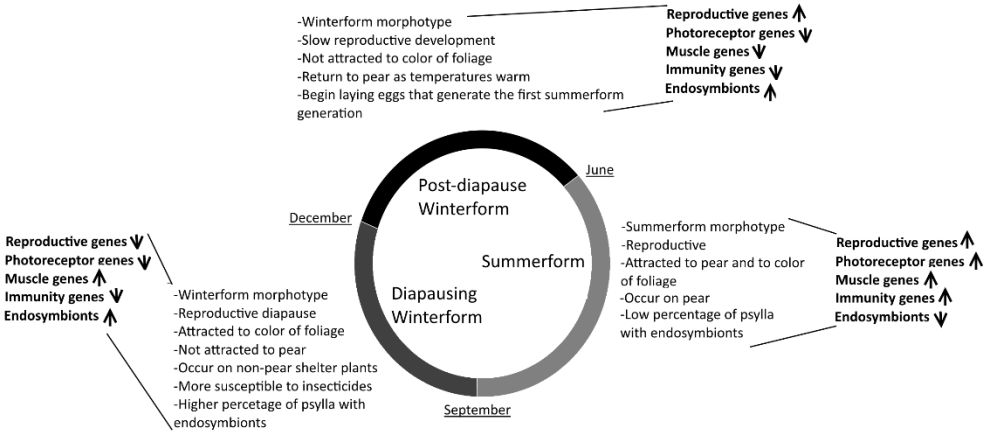

Figure 5. Annual occurrence and phenological traits associated with summerform (light grey), diapausing winterform (dark grey), and post-diapause winterform (black) pear psylla. Arrows denote relative up- or down-regulation of genes involved in reproduction, photoreception, muscle function, immunity, or endosymbionts.

Biological Processes. Genes assigned to the "biological processes" ontology category include those that are responsible for normal cell function. Our initial results found variation in expression of genes involved with reproduction, photoreception, muscle function, and immunity.

Vitellogenin genes are responsible for **egg development and reproduction** and were detected in summerforms and winterforms, which are the psylla reproductive forms (Table 2; Figure 2). Vitellogenin was not detected in diapausing winterform psylla underscoring the lack of reproductive development within this population (Table 2; Figure 2) (Horton et al. 1998).

Expression of genes involved in **photoreception** (sight) were only detected in summerform psylla (Table 2), which are highly attracted to the color of foliage (green and yellow). Winterforms are also attracted to the color of foliage in autumn and late spring but lose this attraction during winter and early spring (Figure 1; Fye 1982, Kaloostian 1970). This would suggest that expression of photoreceptor genes is turned off during the winter and expressed again in late spring. In follow-up analyses, we will determine the seasonal timing of expression of photoreceptor genes in winterform psylla.

Muscle protein genes were among the top 50 most highly expressed genes in all three populations but were more highly expressed in summerforms (Table 2). Nine of the top 50 most highly expressed genes in diapausing winterform psylla encoded muscle proteins, versus only three for summerforms and one for post-diapause winterforms. The larger number of muscle transcripts in

diapausing winterform psylla is likely due to a decrease in expression of other genes. The specimens used in this analysis were collected in November during a relatively inactive period between the autumn and spring dispersals (Horton et al. 1994). Transcription of genes does not always correspond with immediate changes in phenotype. RNA will often accumulate in an organism to be translated into functional proteins at a later time, and this accumulation of RNA allows for the more rapid production of proteins. The synthesis of muscle RNA in inactive diapausing winterform may be in preparation for post-diapause spring dispersal.

Our transcript analysis found that genes that are involved in **immunity** were among the top 50 most highly expressed genes in all three populations, but a greater diversity and a higher transcript count of immunity genes was detected in summerforms than in winterforms (Table 2). Compared with winterforms, summerforms are more resistant to certain classes of insecticides (Unruh et al. 1994) and are more likely to harbor certain bacterial endosymbionts (Cooper et al 2017). These seasonal differences in biological traits may be due to the observed changes in the relative expression of immune response genes (Figure 2).

Ribosomal genes are involved in translation of proteins and were more highly expressed in summerform psylla than in winterform psylla (Table 2). In addition, genes that were categorized as "**other biological processes**" were also more highly expressed in summerforms than in winterforms. The overall higher expression of genes involved in protein translation and cell function in the summerforms likely corresponds with higher metabolic activity within this population.

Mitochondrial genes. Mitochondria are organelles responsible for energy metabolism. These cell structures have their own genome that are separate from the insect's genome. Most of the transcripts expressed in all three populations of psylla were associated with mitochondria (Table 2). The lower expression of mitochondrial genes in diapausing winterform psylla is consistent with the relative inactivity of these psyllids compared with summerform and post-diapause winterform psyllids.

Table 2. Summary of the top 50 most expressed genes in summerform, diapausing winterform, and post-diapause winterform psylla.

	Summerform			Diapausing Winterform			Post-diapause Winterform		
Category	**No. genes**	**TPM[a]**	**%[b]**	**No. genes[c]**	**TPM[a]**	**%[b]**	**No. Genes**	**TPM[a]**	**%[b]**
Vitellogenin	5	28,995	6.3	0	0	0.0	1	7396	1.3
Photoreception	1	997	0.2	0	0	0.0	0	0	0.0
Muscle	3	3393	0.7	9	1300	1.1	1	820	0.1
Immunity	4	11,118	2.4	1	86	0.1	2	2332	0.4
Other Biol. Processes	6	13,666	3.0	3	302	0.3	2	4232	0.7
Ribosomal	10	11,128	2.4	12	1297	1.1	31	3158	0.5
Mitochondria	7	361,627	78.2	7	93,447	77.5	6	541,804	94.1
Carsonella	1	948	0.2	5	17,330	14.4	3	8642	1.5
Arsenophonus	1	1734	0.4	1	4962	4.1	1	3459	0.6
Unknown	12	28,994	6.3	11	1859	1.5	3	4114	0.7

[a] *Transcripts per million-measure of relative gene expression within each category.*
[b] *Percentage of gene expression within each category.*
[c] *Plant RNA was detected among the top 50 expressed genes, so only 49 psylla genes were detected.*

Bacterial Endosymbionts. Pear psylla are associated with several bacterial endosymbionts including *Carsonella* and *Arsenophonus*. *Carsonella* is an obligate endosymbiont that synthesizes essential amino acids that are lacking in psylla's diet. The biological role of *Arsenophonus* is not yet known. Genes from both endosymbionts were more highly expressed in winterform psylla than in

summerform psylla (Table 2; Figure 2). We previously reported that endosymbiont infection is higher in winterform psylla than in summerform psylla (Cooper et al. 2017). The observed decrease in expression of immunity genes in winterform psylla could provide more suitable environment for bacterial growth and function (Table 2). We will explore this possibility in future analyses.

Other identified genes. We identified twelve pear psylla genes that are homologous to odorant binding protein genes of aphids and citrus psyllid which may be involved in host finding. We also identified five pear psylla genes that are homologous to chordotonal genes of fruit fly which may be involved in detection of acoustic mating signals. The putative identification of these genes will allow us to study the molecular basis for host and mate finding by pear psylla.

Anticipated benefit to the industry. Although changes in behaviors and phenotypes associated with summerform, diapausing winterform, and post-diapause winterform psylla are well-documented, the timing for these behavioral changes and mechanisms controlling behaviors are not currently understood. The results of our studies will allow us to pinpoint the exact timing for these changes and will contribute to our long-term goal of providing a better understanding of winterform biology and improve management of this bottlenecked population.

Results have a very high potential to lead to practical tools for the pear industry. Cooper is collaborating with citrus researchers to develop non-GMO gene silencing therapy to kill potato psyllid. This technology, call FANA, was developed against citrus psyllid and the citrus greening disease (http://www.aumlifetech.com/gene-silencing-technology-2/), and does not involve genetically-modified organisms. The transcriptomes obtained from our proposed study will enable us to adapt this technology to target genes that are critical to the survival of pear psylla.

REFERENCES CITED

Civolani, S., M. Leis, G. Grandi, E. Garzo, E. Pasqualini, S. Musacchi, M. Chicca, G. Castaldelli, R. Rossi and W. F. Tjallingii. 2011. Stylet penetration of *Cacopsylla pyri*: an electrical penetration graph (EPG) study. J. Insect Physiol. 57: 1407-1419.

Conesa, A., S. Gotz, J. Garcia-Gomez, J. Terol, M. Talon, and M. Robels. 2005. Blast2GO: A universal tool for annotation and visualization in functional genomics research. Bioinformatics. Bti610. Cooper, W. R., J. W. Dillwith, and G. J. Puterka. 2010. Salivary proteins of Russian wheat aphid (Hemiptera: Aphididae). Environ. Entomol. 39: 223-231.

Cooper, W. R., S. F. Garczynski, D. R. Horton, T. R. Unruh, E. H. Beers, P. W. Shearer and R. J. Hilton. 2017. Bacterial endosymbionts of the psyllid *Cacopsylla pyricola* (Hemiptera: Psyllidae) in the Pacific Northwestern United States. Environ. Entomol. 46: 393-402.

Fye, R. E. 1982. Dispersal and winter survival of the pear psylla. J. Econ. Entomol. 76: 311-315.

Horton, D. R., E. C. Burts, T. R. Unruh, J. L. Krysan, L. B. Coop, and B. A. Croft. 1994. Phenology of fall dispersal by winterform pear psylla (Homoptera: Psyllidae) in relation to leaf all and weather. Can. Entomol. 126: 111-120.

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.

Horton, D. R., C. Guedot, and P. J. Landolt. 2007. Diapause status of females affects attraction of male pear psylla, *Cacopsylla pyricola*, to volatiles from female-infested pear shoots. Entomol. Exp. Appl. 123: 185–192.

Kaloostian, G. H. 1970. Transitory hosts of the pear psylla. J. Econ. Entomol. 63: 1039-1041.

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

Ullman, D. R. and D. L. McLean. 1988. Feeding behavior of the winter-form pear psylla, *Psylla pyricola* (Homoptera: Psyllidae), on reproductive and transitory host plants. Environ. Entomol. 17: 675-678.

Unruh, T. R., and J. L. Krysan. 1994. Reproductive diapause and host plants affect insecticide tolerance of adult pear psylla (Homoptera: Psyllidae). J. Econ. Entomol. 87: 858-865.

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

Project Title: Field evaluation of pear cultivars on cold hardy quince rootstocks

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Cooperators: Sara Serra, Kristal Dowell, Mateus Pasa, Joseph Postman, Mike McCarthy, Dale Goldy

Total Project Request: **Year 1:** $58,110 **Year 2: $70,585** **Year 3:** $84,421

Other funding sources: None

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: Russell Karow**
Telephone: 541 737-4866 **Email address: Russell.Karow@oregonstate.edu**

Item	2018	2019	2020
Salaries	5,536	5,702	8,810
Benefits[1]	4,464	4,598	7,104
Wages[2]	1,300	1,300	1,300
Benefits	130	130	130
Supplies	0	5,500	7,500
Travel[3]	3,316	3,316	3,316
Plot Fees[4]	5,000	5,000	5,000
Total	19,746	**25,546**	33,160

Footnotes:
[1] Benefits were calculated from actual OPE rates (OSU technician). An annual increase of 3% was applied to years 2 and 3.
[2] Wages are for part-time employee to help with general maintenance during the season; 100 hours at $13/hr. Part-time employee benefits are calculated at 10%.
[3] Travel to cover mileage to plot for measurements and one trip per year (4 days) for Einhorn to travel to plots to perform pruning and training tasks and meet with S. Musacchi and grower collaborators (airfare was estimated at $1,000 roundtrip, four nights hotel ($150/night), car rental ($500) and per diem ($54/day).
[4] Plot fees are to compensate growers for land, resources and fruit.

Budget 2 (Musacchi)
Organization Name: WSU **Contract Administrator: Joni Cartwright**
Telephone: 509-633-8181 **Email address: joni.cartwright@wsu.edu**

Item	2018	2019	2020
Salaries	21,000	21,840	22,714
Benefits[1]	8,364	8,699	9,047
Supplies	0	5,500	10,500
Travel[2]	4,000	4,000	4,000
Plot Fees[3]	5,000	5,000	5,000
Total	38,364	**45,039**	51,261

Footnotes:
[1] Benefits were calculated from actual WSU rates. An annual increase of 3% was applied to years 2 and 3.
[2] Travel is to cover mileage to plot for measurements to travel to plots to perform data collection
[3] Plot fees are to compensate growers for land, resources and fruit.

Objectives:
Evaluate vegetative and fruiting performance of Bartlett and d'Anjou pear trees on promising, cold-hardy quince rootstocks.

Significant Findings:
- Total tree survival was high across both plots: on average, 5% to 6% mortality was observed in WA and OR, respectively.
- Sources of mortality will require additional years to determine. Tree mortality was not always associated with a given scion (i.e., incompatibility) though the accession 99.002 suffered the highest overall mortality at both sites.
- In addition to possible graft incompatibility, 2017 gopher damage at the OR site contributed to tree losses.
- Training began in 2017 at both sites. Trees were 'restarted' by removing most lateral branches using bevel cuts to renew new shoots. Trees were also headed to increase uniformity and encourage leader development and branching. Due to observed gopher damage at the OR site, a decision was made to maintain a few limbs per tree in an effort to stimulate new root development. Intensive tree training will continue this dormant season. The gopher situation appears to be under control.
- Variation in trunk size (a good overall indication of canopy size and vigor) was evident across genotypes. However, because tree size was variable at planting and trees were renewed in spring 2018, we attribute the observed disparity to carryover effects from the nursery and reserve our inferences on dwarfing effects for future years.

Methods:
Planting design. For each of the rootstock/scion combinations, trees were divided evenly between the two planting sites, irrespective of differences in the quantity of trees among rootstock/scion combinations. We selected three multi-tree replications per treatment. While more replicates would have increased our statistical power to detect treatment differences, we were compelled to have a minimum of four trees per replicate in order to eliminate confounding effects on vigor from neighboring trees possessing different rootstocks and growth habits. When tree numbers limited replicates to four trees, the center two trees will be used for data collection and the outer trees will be treated as guard trees. For the remaining 2/3rd of rootstock/scion combinations, however, there are between 6 and 15 trees per replicate. For these replicates, only the first and last tree will be excluded from data collection. Both plots are equipped with trellis and supplemental irrigation. The irrigation and fertilization regime will be discussed and agreed upon given quince's relatively high demand for nutrients (especially N). All other inputs (pesticides, herbicides, frost protection, etc.) will be according to commercial standards.
Training system. Trees are being trained to a central leader, spindle architecture. Branches will be initiated using a combination of horticultural techniques (heading of leaders, girdling, and scoring above individual buds). While 2017 tree survival was high, variability in tree size and branch number within a given rootstock/scion combination was also high. Therefore, 2017 was regarded as an establishment year to assess survivability, develop root systems and determine the appropriate training strategy for 2018. Trees were headed to encourage uniform canopy development. In cases where one or more branches had developed in 2017, limb removal was accomplished using a bevel cut to encourage generation of a horizontal replacement limb. Restarting trees will not eliminate tree-to-tree variability, but we expect greater canopy uniformity with respect to the number fruiting units. Further, trunk cross sectional area can be assigned as a covariate in the statistical analysis to correct for the effect of tree size on the first production years. Newly emerged limbs considered too upright or vigorous will be removed using a bevel cut in 2018. Limb positioning (i.e., tying) will be performed if necessary.

Results and Discussion:
Mortality was generally low across all trees and both sites. Inconsistencies among the type of graft (Comice or direct) and the scion influence limit inferences on graft incompatibility. For example, in WA, quince CYD 99.002 had 67% mortality when directly grafted to Anjou, 0% mortality on Bartlett, and ~5% mortality using Comice (when summing Comice interstem trees) (Table 2). These data appear to indicate potential incompatibility with Anjou. However, the highest mortality levels for 99.002 in OR occurred with Comice (~17% when summing Comice interstem trees) and only one tree was lost when direct grafted to Anjou (Table 1). Graft incompatibility often takes several years to display and we expect these relationships to become clear in the future. In addition, there was a confounding influence of gopher predation to roots in OR.

Table 1: Number of trees planted, trees still alive and percent mortality at the end of 2018 in Parkdale, OR for the 2 cultivars Anjou and Bartlett grafted on 9 different quince accessions (table sorted by cv and CYD acc. #). Type of graft was either a direct graft (no interstem between scion and roots) or with an interstem of Comice.

Cv	quince rootstock (CYD accession)	type of graft	Count of trees planted June 2017	Count of alive trees Jan 2019	%Mortality
ANJOU	22.001	Comice	22	22	0
		Direct graft	22	22	0
	23.001	Comice	12	11	8
		Direct graft	10	10	0
	57.001	Comice	17	15	12
		Direct graft	10	10	0
	65.001	Comice	20	20	0
		Direct graft	13	12	8
	67.001	Comice	12	12	0
	68.002	Comice	15	14	7
	70.001	Comice	42	42	0
		Direct graft	10	9	10
	99.002	Comice	56	55	2
		Direct graft	12	11	8
	118.001	Comice	11	10	9
		Direct graft	10	7	30
BARTLETT	22.001	Comice	24	24	0
		Direct graft	15	15	0
	23.001	Comice	12	12	0
	57.001	Comice	16	16	0
		Direct graft	14	13	7
	65.001	Comice	19	19	0
		Direct graft	11	9	18
	67.001	Comice	14	13	7
	68.002	Comice	16	15	6
	70.001	Comice	43	41	5
		Direct graft	16	15	6
	99.002	Comice	57	37	35
		Direct graft	12	12	0
	118.001	Comice	48	41	15
		Direct graft	12	11	8

Table 2: Number of trees planted, trees still alive and percent mortality between the end of 2018 and planting (06/06/2017) in Entiat (WA) for the 2 cultivars Anjou and Bartlett grafted on 9 different quince accessions (table sorted by cv and CYD acc. #). Type of graft was either a direct graft (no interstem between scion and roots) or with an interstem of Comice.

Cv	quince rootstock (CYD accession)	type of graft	Count of Tree planted (06/06/2017)	Count of alive trees (11/20/2018)	Count of dead trees (11/20/2018)	% failure in 17 M
ANJOU	22.001	comice interstem	22	22	0	0
		direct graft	20	20	0	0
	23.001	comice interstem	12	12	0	0
	57.001	comice interstem	17	17	0	0
		direct graft	11	10	1	9.1
	65.001	comice interstem	17	17	0	0.0
	67.001	comice interstem	12	12	0	0.0
	68.002	comice interstem	14	14	0	0.0
	70.001	comice interstem	39	34	5	12.8
		direct graft	13	12	1	7.7
	99.002	comice interstem	42	42	0	0.0
		direct graft	12	4	8	66.7
	118.001	comice interstem	11	10	1	9.1
		direct graft	10	10	0	0.0
BARTLETT	22.001	comice interstem	24	24	0	0.0
	23.001	comice interstem	12	12	0	0.0
	57.001	comice interstem	15	15	0	0.0
	65.001	comice interstem	17	17	0	0.0
	67.001	comice interstem	13	13	0	0.0
	68.002	comice interstem	16	12	4	25.0
	70.001	comice interstem	29	29	0	0.0
	99.002	comice interstem	54	49	5	9.3
		direct graft	10	10	0	0.0
	118.001	comice interstem	35	35	0	0.0
Total			***477***	***452***	***25***	***5.2***

For OR, fairly good shoot production was observed across all rootstocks and scion combinations in 2018 (Table 3). On average, ~10 shoots per tree generated new growth segments averaging ~35 cm (~1.1 ft.) in length. There were few notable differences among rootstocks and scion combinations. Further, during 2018/2019 dormant season, we will renew or remove shoots deemed suboptimal for future productivity. This will also serve to encourage leader development. In cases where more than 10 shoots were generated per tree, several will require removal so leader growth is not stunted. The goal is to fill space in these formative years, which too many branches will counteract. Pruning will take place in February and we will additionally weigh all prunings removed from trees, as was done in WA in 2018 (Table 4).

For WA, due to the limited amount of material removed in winter 2017-2018, pruning data in Table 4 were collected by plot as opposed to single trees. Average pruned wood in kg/tree was obtained by dividing the total amount of material removed in the plot by the number of trees belonging to the plot (Table 4). From the amount of wood removed per graft combination and type of graft, we can extrapolate the vigor that the rootstock is going to induce on the scion. For both cultivars, CYD accession 57.001 with the interstem showed the highest pruning weights/tree (129 g

for Anjou and 165 g for Bartlett). Slightly lower amounts for the combinations with and without interstem grafted on CYD accessions 22.001 and 23.001 for both cultivars. Combinations of both cultivars with CYD acc# 118.001 with interstems registered the lowest amount of cut wood from pruning, suggesting a low vigor of the scion.

Table 3: Average number of new shoots per tree, total annual 2018 shoot growth, average shoot length (cm), trunk cross-sectional area (TCA) and TCA increase over 2018 for the 2 cultivars Anjou and Bartlett grafted on 9 different quince accessions (table sorted by cv and CYD acc. #) in Parkdale, OR. Type of graft was either a direct graft (no interstem between scion and roots) or with an interstem of Comice.

Cv	quince rootstock (CYD accession)	type of graft	Avg # new shoots	TTl new growth (cm)	Avg shoot length (cm)	Avg TCA Jan 2019 (cm^2)	Avg Absolute Increase from spring 2018 (cm^2)	Avg Relative Increase (%)
Anjou	22.001	Comice	8.86	463.01	52.3	6.84	3.58	63.42
Anjou	22.001	Direct graft	7.84	343.04	43.8	5.02	1.75	30.52
Anjou	23.001	Comice	13.2	416.86	31.6	6.63	3.05	51.48
Anjou	23.001	Direct graft	8.06	282.99	35.1	5.81	1.62	23.66
Anjou	57.001	Comice	7.11	318	44.7	5.87	2.65	46.87
Anjou	57.001	Direct graft	11.22	583.85	52.1	7.09	2.56	37.45
Anjou	65.001	Comice	10.97	489.59	44.6	8	3.67	58.58
Anjou	65.001	Direct graft	5.4	236.75	48.8	6.33	2.11	32.19
Anjou	67.001	Comice	11.25	424.22	37.7	6.05	2.71	47.39
Anjou	68.002	Comice	5.61	181.12	32.3	2.77	1.18	24.89
Anjou	70.001	Comice	10.05	393.14	39.1	5.19	2.36	42.08
Anjou	70.001	Direct graft	15.17	493.43	32.5	5.71	1.58	22.85
Anjou	99.002	Comice	9.69	462.44	47.7	5.42	2.58	47.85
Anjou	99.002	Direct graft	19.97	1154	57.8	9.81	3.14	40.53
Anjou	118.001	Comice	7	246.42	35.2	2.95	1.51	33.97
Anjou	118.001	Direct graft	7.17	408.25	56.9	5.83	2.06	33.52
Bartlett	22.001	Comice	10.63	279.42	26.3	5.36	2.82	54.41
Bartlett	22.001	Direct graft	10.47	275.48	26.3	6.17	2.36	36.9
Bartlett	23.001	Comice	12.75	333.52	26.2	7.5	3.28	51.89
Bartlett	57.001	Comice	13.2	348.89	26.4	7.01	2.73	41.77
Bartlett	57.001	Direct graft	10.88	279.14	25.7	6.96	2.51	37.73
Bartlett	65.001	Comice	15.9	364.4	22.9	6.53	2.83	45.89
Bartlett	65.001	Direct graft	10.72	250.95	23.4	6.51	2.5	38.75
Bartlett	67.001	Comice	9.44	212	22.5	4.64	1.73	29.51
Bartlett	68.002	Comice	6.4	91.74	14.3	3.98	1.25	22.52
Bartlett	70.001	Comice	15.03	356.5	23.7	5.02	2.01	34.54
Bartlett	70.001	Direct graft	11.42	271.46	23.8	4.72	1.35	21.86
Bartlett	99.002	Comice	15.98	606.35	37.9	6.4	2.95	50.22
Bartlett	99.002	Direct graft	12.75	500.58	39.2	9.13	2.62	32.95
Bartlett	118.001	Comice	3.61	56.03	15.5	2.45	0.88	19.37
Bartlett	118.001	Direct graft	5.92	141.81	23.9	4.78	1.84	31.18

*Table 4: Weight of winter pruning material as kg/tree (pruning on 03/16/2018) in Entiat (WA) for the 2 cultivars Anjou and Bartlett grafted on 9 different quince accessions (table sorted by cv and CYD acc. #). Type of graft was either a direct graft (no interstem between scion and roots) or with an interstem of Comice. Where reps were fewer than 3, the combination was removed from the statistical analysis (see "." in SNK column and N= column for number of reps generating the average). Significance of the model for combination (Cv x quince rootstock x graft type) was *** if $p<0.001$, ** $p<0.01$, * $p<0.05$, NS=not significant, post doc means discrimination done with SNK.*

Cv	quince rootstock (CYD accession)	type of graft	pruning wood (March 2013) in kg/tree	SNK	N=
ANJOU	**22.001**	comice interstem	0.074	abc	3
ANJOU	**22.001**	direct graft	0.072	abc	3
ANJOU	**23.001**	comice interstem	0.103	ab	3
ANJOU	**57.001**	comice interstem	0.129	a	4
ANJOU	**57.001**	direct graft	0.135	.	1
ANJOU	**65.001**	comice interstem	0.108	ab	3
ANJOU	**67.001**	comice interstem	0.031	c	3
ANJOU	**68.002**	comice interstem	0.016	c	3
ANJOU	**70.001**	comice interstem	0.022	c	7
ANJOU	**70.001**	direct graft	0.013	.	2
ANJOU	**99.002**	comice interstem	0.053	bc	4
ANJOU	**99.002**	direct graft	0.025	.	1
ANJOU	**118.001**	comice interstem	0.016	c	3
ANJOU	**118.001**	direct graft	0.065	.	1
Significance combination			***		
BARTLETT	**22.001**	comice interstem	0.112	ab	7
BARTLETT	**23.001**	comice interstem	0.108	ab	3
BARTLETT	**57.001**	comice interstem	0.165	a	3
BARTLETT	**65.001**	comice interstem	0.152	a	3
BARTLETT	**67.001**	comice interstem	0.033	c	4
BARTLETT	**68.002**	comice interstem	0.021	c	3
BARTLETT	**70.001**	comice interstem	0.021	c	4
BARTLETT	**99.002**	comice interstem	0.064	bc	9
BARTLETT	**99.002**	direct graft	0.077	.	1
BARTLETT	**118.001**	comice interstem	0.009	c	6
Significance combination			***		

Table 3 also provides trunk cross sectional areas (TCSA) at the OR site as an overall integrative measure of tree vigor. Again, we limit inferences until future years of data are recorded, since differences among rootstocks and rootstock-graft combinations on tree growth are mainly associated with a combination of nursery effects and restarting of canopies. A two-fold difference between the smallest and largest trees is, however, encouraging, since a range of vigor in any collection of material is desirable. WA data is presented in Table 5 where scions were measured at 10 cm above the graft union (or direct graft of above the interstem but always on the scion) in July 2017 (approx.1 month after planting) and in November 2018 (approx.17 months after planting are reported. For Anjou, the largest TCSA after planting in 2017 was the direct graft with CYD#70.001, while the lowest the graft with interstem with CYD#68.002 (Anjou/Comice/68.002). Seventeen months after planting the situation slightly changed, Anjou/57.001 reported the highest TCSA in November 2018 significantly different from all the other combinations, while again the combination Anjou /Comice/68.002 had the lowest TCSA (similar to other combinations, see Table 3). For Bartlett, the largest TCSA after planting in 2017 was the direct graft with CYD#99.002, while the lowest the graft with interstem with CYD#118.001 (Bartlett/Comice/118.001). In 2018, the previous trends (TCSA2017) were confirmed with Bartlett/99.002 the most vigorous combination and Bartlett/Comice/118.001 and Bartlett/Comice/68.002 the least vigorous.

*Table 5: Average trunk cross sectional area (TCSA in cm^2) in July 2017 and November 2018 in Entiat (WA) for the 2 cultivars, Anjou and Bartlett, grafted on 9 different quince accessions (table sorted by cv and CYD acc. #). Type of graft was or direct graft (no interstem between scion and roots) or with interstem of Comice. N= number of values generating the mean. Significance of the model for combination (Cv x quince rootstock x graft type) *** if p<0.001, ** p<0.01, * p<0.05, NS=not significant. Different letters within individual columns are significantly different.*

Cv	quince rootstock (CYD accession)	type of graft	N=	TCSA cm^2 (July 2017)	SNK	N=	TCSA cm^2 (Nov 2018)	SNK
ANJOU	**22.001**	comice interstem	22	0.38	e	22	3.63	de
	22.001	direct graft	20	2.28	b	20	6.06	b
	23.001	comice interstem	12	0.63	de	12	4.26	cd
	57.001	comice interstem	17	0.52	de	17	4.35	cd
	57.001	direct graft	10	2.60	b	10	7.50	a
	65.001	comice interstem	17	0.68	de	17	4.33	cd
	67.001	comice interstem	12	0.60	de	12	3.09	def
	68.002	comice interstem	14	0.27	e	14	1.84	f
	70.001	comice interstem	39	0.41	e	34	2.67	def
	70.001	direct graft	13	3.40	a	12	6.30	b
	99.002	comice interstem	42	0.51	de	42	3.25	def
	99.002	direct graft	11	1.11	cd	4	4.51	cd
	118.001	comice interstem	11	0.33	e	10	2.31	ef
	118.001	direct graft	10	1.43	c	10	5.53	bc
Significance combination				***			***	
BARTLETT	**22.001**	comice interstem	24	0.64	c	24	4.49	b
	23.001	comice interstem	12	0.76	bc	12	5.13	b
	57.001	comice interstem	15	0.92	b	15	5.31	b
	65.001	comice interstem	18	0.84	bc	17	5.21	b
	67.001	comice interstem	13	0.71	bc	13	3.24	cd
	68.002	comice interstem	15	0.62	c	12	2.67	d
	70.001	comice interstem	29	0.58	c	29	3.07	cd
	99.002	comice interstem	54	0.65	c	49	4.07	bc
	99.002	direct graft	10	1.56	a	10	8.63	a
	118.001	comice interstem	33	0.36	d	35	2.07	d
Significance combination				***			***	

Timeline and expected activities:

Fall/Winter 2018/2019:

- End of season trunk measurement
- Quantify the number and length of individual fruiting units
- Measure tree height
- Conduct dormant pruning to encourage uniform branching, spur development and eliminate undesirable branches

Spring/Summer 2019:

- Meet with grower collaborators to discuss season and plan activities
- Quantify the number of flower clusters per tree and determine if bloom should be removed or maintained based on canopy volume
- Continue training trees to initiate branching on 2019 new leader growth
- Achieve target tree height

Fall/Winter 2019/2020:

- End of season trunk measurement
- Count number of fruiting units per tree and quantify annual growth
- Dormant pruning to encourage uniform branching, fruit size and fruit set on spurs and eliminate undesirable branches

CONTINUING PROJECT REPORT **YEAR**: 2 of 2 (No-cost extension)

Project Title: Epidemiology and management of postharvest decay on pears

PI: Achala N KC
Organization: Oregon State University
Telephone: 541-772-5165 Ext 222
Email: achala.kc@oregonstate.edu
Address: 569 Hanley Rd.
City/State/Zip: Central Point, OR-97502

Cooperators: Mike Naumes (Naumes Inc, Medford, OR), Matt Borman (Harry&David, Medford, OR),

Total Project Request: Year 1: 44,698 **Year 2: 46,039**

Other funding sources: None

WTFRC collaborative expenses: None

Budget

Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2017	2018	2019
Salaries Faculty Research Assistant	22,500	23,175	
Benefits OPE 63%	14,198	14,624	
Wages	0	0	
Benefits	0	0	
Equipment	0	0	
Supplies	6,000	6,180	
Travel	2,000	2,060	
Miscellaneous	0	0	
Plot Fees	0	0	
Total	$44,698	$46,039	**$0**

Footnotes: Annually: FRA 6 mo + fringe, 6K supplies and consumables, 2K local and in-state travel, 3% inflation

OBJECTIVES

1. Monitoring prevalence of major fungal pathogens throughout the pear growing season towards understanding postharvest disease epidemiology
2. In vitro sensitivity of postharvest decay pathogens to currently available fungicides and efficacy of new fungicides toward resistance management
3. Manipulating postharvest storage conditions to reduce the susceptibility of fruit infection

SIGNIFICANT FINDINGS

- Postharvest rot pathogens, *Botrytis cinerea, Cladosporium herbarum, Penicillium expansum,* and *Alternaria* spp. are prevalent in orchards at the initial stages of fruit development
- Three new pathogens, *Dothiorella iberica*, *Diaporthe rudis,* and *Fusarium avenaceum* were isolated from bloom time samples and occasionally isolated from culled samples in packing houses (not included in this study)
- *Botrytis cinerea* inoculum causing gray mold on postharvest fruits reside on fruit tissues as early as flower bud development
- Some isolates of *B. cinerea* tested in this study are resistant to the fungicides for scab management; triflumizole, cyprodinil, and dodine that may result in indirect selection of resistant *B. cinerea* population to these groups of fungicides
- One hundred percent of isolates tested in this study were sensitive to a group of fungicide, iprodione (FRAC 2) that is not registered for pear in PNW

METHODS

Objective 1.

Experimental Design: Two commercial Bosc orchards in Southern Oregon were included for periodic monitoring of postharvest rot pathogens. Stratified random sampling method was used for sampling the tissues. There were five strata with four trees in each strata. Twenty trees in each location with three branches per tree were marked before sample collection. Samples were collected from the same branch throughout the season. Altogether 60 samples were collected from each stage of fruit development, white bud, full bloom, petal fall, fruitlets, and field bins at harvest.

Pathogen isolation and identification: Samples were processed with an initial rinse in sterile water, surface sterilization for one minute in 1% sodium hypochlorite solution, and a final rinse in sterile water. Samples were then blotted on a lab wipe to dry and cultured on half-strength potato dextrose agar (PDA) amended with streptomycin and ampicillin. For the white bud, full bloom, and petal fall stages, the entire sample was cultured. For the fruitlet stage, where disease symptoms were evident, the edge of the affected area was cultured, otherwise the blossom and stem end of the fruitlets were cultured. The culture plates were incubated at room temperature (70^{o} F) under 12 hrs light and dark cycles. Based on the culture morphology, each unique culture was sub-cultured on full-strength potato dextrose agar. Pure culture of each fungus was obtained by single spore culture or hyphal tip method in water agar. The obtained pure culture was transferred to full strength PDA and the fungi was identified based on culture and spore morphology. After identification, the culture was air dried under laminar flow hood and stored in -112^{o} F for long-term storage. The fungi that could not be identified based on culture and spore morphology were marked as unknowns and proceeded for DNA extraction and sequencing. Genomic DNA extraction followed a CTAB protocol. The ITS and elongation factor 1-alpha (EF1-α) genes were amplified, and sequenced. The

sequence data were compared with NCBI fungal database using BLAST tool and the fungal species were identified based on percent identity.

The field bin samples in cold storage were monitored every week for prevalence of any disease symptoms. The symptomatic tissues were cultured on half-strength potato dextrose agar (PDA) amended with streptomycin and ampicillin. The pure culture, identification, and storage followed the similar methods as described before.

Objective 2

Fungicide sensitivity tests: Frequently prevalent pathogens *B. cinerea* were tested for their sensitivity against fungicides from four different FRAC groups (2, 3, 9, and U12; Table 1). Commercial formulations of products were used to reach specified concentrations of each active ingredient (Table 1). The fungicides were diluted in sterile distilled water to obtain stock solutions and were added to potato dextrose agar (PDA) to obtain final concentrations of 0, 0.01, 0.1, 1, 10 µg ml^{-1} active ingredient. Cyprodinil (group 9) and dodine (group U12) were tested at the additional concentrations of 100 µg ml^{-1} and the maximum labeled rate for each of these products. Nineteen *B. cinerea* isolates were tested for their sensitivity against these fungicides. Mycelial plugs 5 mm in diameter from actively growing hyphal tip were excised from the inoculum plate, inverted, and placed on three replicate plates of each concentration of fungicide. Plates were incubated at ambient laboratory temperature (approx. 70°F) for three days under fluorescent lights on a 12h on – 12h off cycle. Colony diameter was measured across two axes, averaged and the mycelial plug diameter was subtracted from the average value. The percent inhibition was calculated in relation to colony diameter in no-fungicide control plates and the effective concentration to reduce growth by 50% (EC_{50}) was calculated by fitting the dose response to a sigmoidal function. Each experiment was repeated and the mean, range, and standard error of EC_{50} values were calculated for each fungicide and isolate combination.

Fungicide efficacy tests for resistance monitoring: Whole plant resistant screening assay for *B. cinerea* developed by FRAC was used for resistance screening of twenty isolates to the same four groups of fungicides. Bosc fruits from SOREC research orchard were harvested at their commercial maturity. The fruits were surface sterilized with 1% sodium hypochlorite (household bleach) and wounded with a sterile nail head. The wounds were treated with 50 µl of fungicides adjusted to their respective highest labeled rate and were left to dry for two hrs after the treatment. Once the fruits absorbed the fungicides, spore suspension of isolates (50 µl of 1 x 10^5 spores/ml) were dropped to the wounds. Thus inoculated fruits were incubated at room temperature for 5 days. The control fruits did not receive any treatments but were inoculated with the spore suspension. After five days, lesion diameter were measured and the fungicide efficacy were calculated as:

$$\% \text{ efficacy} = [(\Phi \text{ control} - 5) - (\Phi \text{ treated} - 5)] / (\Phi \text{ control} - 5) \times 100 \ldots\ldots [\text{eq.1}]$$

Where Φ = lesion diameter, and 5 is the diameter of the wound.

If the % efficacy was less than 50% for a particular fungicide, the isolate was considered resistant, otherwise sensitive. Once, the efficacy data was calculated, co-relation between efficacy and EC_{50} were determined to identify the discriminatory dose for each fungicide (Table 1).

Objective 3

Preharvest 1-MCP application: The trial was conducted in Southern Oregon Research and Extension Center, pear research orchard. The 2017 trial included Harvista application a week and two weeks prior to commercial harvest at minimum (48 fl oz/acre) and maximum rates (96 fl oz/acre). The treatments were applied in Bosc and Comice pears. The treatments were arranged in randomized complete block design with four replications. Rears Harvista kit and the product were supplied by AgroFresh. The kit was attached to Rears air blast sprayer and applied per AgroFresh recommendations. Ninety fruits were harvested from each treatment, of which 80 fruits were divided into four boxes of

20 fruits each. The fruits were stored in cold storage at 30° F and each box was labeled as 2, 4, 6, and 8 months. The fruits were examined for disease incidence and fruit texture at 2, 4, 6, and 8 months after storage. The rest ten fruits were surface sterilized and artificially inoculated with conidial suspension of *B. cinerea.* The inoculated fruits were then stored in cold storage at 30° F and the lesion diameter was recorded following the same method as described under preharvest fungicide application trial.

Based on 2017 trial, two rates of Harvista (minimum and maximum) were applied two weeks prior to commercial harvest on Bosc and Comice. The Harvista application and their ability to keep fruits firmer after a week and two weeks of commercial harvest were tested. Ten fruits from each treatment and a control treatment were pressure tested at the day of application. Two hundred comice fruits were harvested from every treatment at commercial harvest, a week and two weeks after commercial harvest. Out of which 40 were used to pressure test at harvest, two sets of 40 each were packed in two boxes to be tested after 2 months and 4 months after storage in normal atmosphere cold storage and CA rooms at Naumes Inc. Similarly, 280 bosc fruits were harvested from every treatment at commercial harvest, a week and two weeks commercial harvest. Out of which 40 were used to pressure test at harvest, three sets of 40 each were packed in two boxes to be tested after 2 months, 4 months, and six months after storage in normal atmosphere cold storage and CA rooms at Naumes Inc. The stored fruits from two months boxes were analyzed for fruit firmness, quality, and sensory evaluation at Harry and David facility in Medford, OR. The stored fruits from four months boxes are being analyzed for fruit firmness and quality. The stored fruits from six months boxes are still in storage to be evaluated at the mid of March.

RESULTS & DISCUSSION

Objective 1

Based on culture and spores morphology four pathogens previously reported as postharvest rot pathogens, *Botrytis cinerea, Penicliilum expansum, Caldosporium herbarum,* and *Alternaria* spp. were identified. These pathogens were consistently isolated from full white, full bloom, petal fall, fruitlets, and field bins at harvest. Several fungal species were isolated along with the pathogenic species, the identity of which could not be established based on culture and spore morphology. They were grouped into thirteen unique species based on cultural characteristic on PDA media. The molecular diagnostic methods using the ITS and elongation factor 1-alpha (EF1-α) sequencing, thirteen unique fungi were identified. The thirteen species were tested in surface disinfected wound inoculated fruits for their ability to cause disease. Out of thirteen, three species were identified as pathogenic on wound inoculated fruits (Fig. 1). The three new species were identified as *Dothiorella iberica, Diaporthe rudis, and Fusarium avenaceum.* Interestingly, the former two species also cause trunk disease in grapes (a complex of many fungal pathogens). With the increasing wine grape acreage in the area, we might not only be sharing the acreage but also the pathogens to some extent. It will be important to keep monitoring for newer pathogens as the area's agricultural practices change.

At full white stage, all the pathogens were isolated in similar frequencies. At full bloom until fruitlets stage *Alternaria* spp. were most frequently isolated, followed by *B. cinerea* and *C. herbarum.* From the field bin samples, *C. herbarum* were frequently isolated. The isolates of *B. cinerea* were isolated mostly from full bloom and petal fall samples. The three new pathogenic species were present at full white, full bloom, and petal fall and later tapered off (Fig. 2).

Figure 1. Fruits after a week and two weeks of inoculation. A: Control; B: *Dothiorella iberica* C. *Fusarium avenaceum;* D. *Diaporthe rudis*

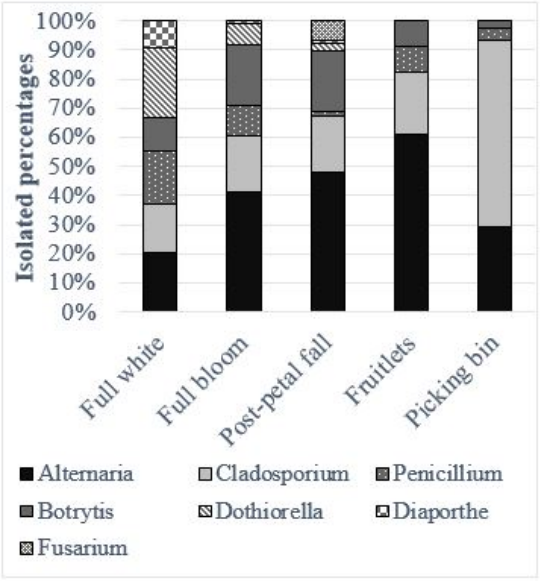


Figure 2. Seasonal distribution of post -harvest rot pathogens isolated from earlier stages of fruit development

Objective 2.

The effective concentration to reduce radial growth by 50% (EC_{50}) was calculated for each pathogen-fungicide combination. The EC_{50} for isolates against cyprodinil ranged from 1.35 to 26 µg ml^{-1}. No discriminatory dose for use on pears was found in the literature, but a 1 µg ml^{-1} was considered a discriminatory dose for *B. cinerea* on New Zealand wine grapes (Beresford et al 2017), and all isolates except one exceeded this limit by at least threefold. The range of EC_{50} values against dodine was 73.4 to 195.7 µg ml^{-1}. No baseline or discriminatory dose information for *B. cinerea* was found in the literature. While the EC_{50} values seem high, the labeled rate for the product used to supply this active ingredient was also high, with a maximum of 1,485 µg active ingredient ml^{-1} applied. The range of EC_{50} values against iprodione was 0.53 to 1.32 µg ml^{-1} No discriminatory dose for use on pears was found in the literature, but all isolates were below the 10 µg ml^{-1} concentration determined in a study of *B. cinerea* on Southern US strawberries (Fernández-Ortuño et al 2014). The range of EC_{50} values against triflumizole was 0.38 to 1.53 µg ml^{-1}. Except for cyprodinil, the tested isolates were sensitive within labeled field rates. Two isolates (10%) were not sensitive against cyprodinil at the maximum labeled field rates and EC_{50} for 85% isolates were greater than 5 µg/ ml.

Among, the isolates tested for fungicide efficacy, 100% of the tested isolates were sensitive to iprodione, a fungicide group that is not registered for pear in PNW (Fig. 3 and 4). Only 30% of the isolates were sensitive to triflumizole and all the sensitive isolates were collected from SOREC research orchards. The remaining of other isolates (70% of the tested isolates) with reduced sensitivity to triflumizole were collected from conventionally managed commercial orchards. Triflumizole (FRAC group 3) includes a common group of fungicides, including Inspire Super for managing pear scab diseases and powdery mildew. Among the tested isolates, 65% and 75% of the isolates were sensitive to dodine and cyprodinil respectively. Even though dodine is currently not used for commercial application, it was a fungicide of choice for scab management until the resistance became an issue. Similarly, cyprodinil (FRAC group 9) is also an important component of pear scab management. While the resistance frequencies to these groups among the tested isolates are relatively low precautionary measures should be taken for fungicide rotations. Identification of a

group of fungicide with high efficacy, iprodione (FRAC group 2) is one of the major accomplishments of this project. However, the results need to be verified with larger number of isolates and further steps needs to be taken toward registration and labeling.

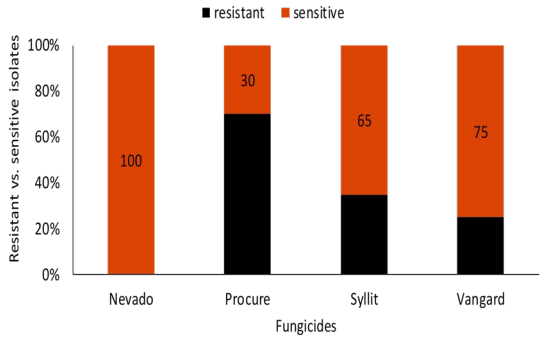


Figure 3. Frequency distribution of resistant and sensitive isolates to four fungicides included in this study.

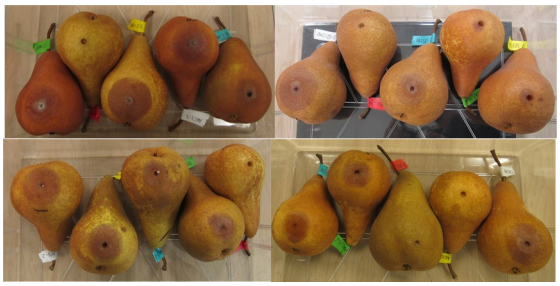


Figure 4. Fruits treated with fungicides and inoculated with *B. cinerea* spore suspension. Fruits with White tape: Control; Yellow: Vangard; Red: Nevado; Green: Syllit; and Blue: Procure. Each block was inoculated with different *B. cinera* isolates.

Objective 3

Preharvest 1-MCP application: Preharvest application of foliar 1-MCP alone did not significantly reduce the wound initiated *B. cinerea* infection in cold storage for both bosc and comice pears. The disease progress over time was lower on Bosc fruits treated with 1-MCP a week prior to harvest at minimum rate; however, it was not statistically significant. Disease progress on other treatments were significantly higher than water control treatments. Similar result was observed on comice fruits treated with 1-MCP. Application of 1-MCP at preharvest is not fungicidal enough to control the disease caused by wound initiated *B. cinerea*.

The fruits stored in normal atmosphere cold storage from 2017 pre harvest 1-MCP application resulted comparable firmness relative to control treatments. At two months after storage, Harvista applied comice fruits two weeks prior to harvest were firmer than fruits with Harvista treatments one week prior to harvest and control treatments with no Harvista application. However, all fruits were below 2.5 lbs after five days of ripening. At four months after storage, no significant differences were observed on any of the Harvista treated fruits. All fruits were below 1.5 lbs after five days of ripening (Fig. 5). Similar results were observed on bosc fruits (Fig. 6). At two months after storage, Harvista treated fruits were firmer compared to no harvista treated control fruits. At four months after storage, no significant differences were observed among the treatments. At six months after storage, fruit texture data were difficult to interpret due to hardening of outer layer. That could be due to loss of moisture resulting rubbery texture of fruit periderm and subsequent difficulties for the probe to puncture fruits for texture data. Differences in percent senescence were observed among the treatments. In bosc after six months of storage, the percent senescence on the fruits treated with Harvista two weeks prior to harvest resulted in zero senesced fruits with minimum rate and 35% senesced fruits with maximum. However, on fruits treated a week before harvest resulted in 50 and 55% senesced fruits with minimum and maximum rate. The fruits with no Harvista treatment resulted 80% senesced fruits (Fig. 7)

The results were promising from this study as the firmness/ripeness of the fruits were not affected by pre harvest application of 1-MCP and that the fruits can be stored longer with less loss to senescence. However these data was generated from only one years of study. We repeated the two weeks prior to harvest application with two rates in 2018 and other objectives were added for the

treatments effect. Fruits were harvest at three different time points (commercial harvest, a week and two weeks post commercial harvest) and looked at the efficacy of 1-MCP to maintain fruit texture in tree for better harvest planning. The fruits from this study are still in cold storage under normal and CA rooms which will be analyzed for their ripening and other fruit quality parameters and will be presented in final report.

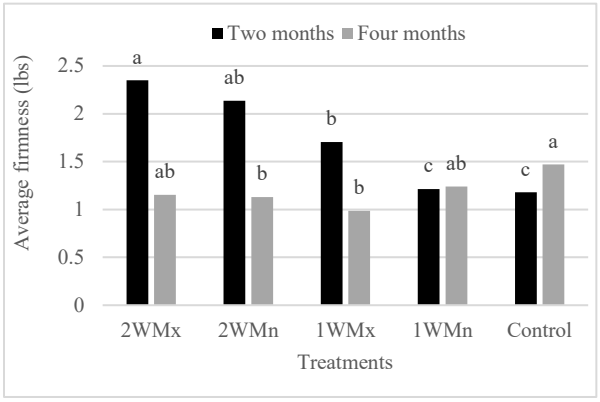


Figure 5: Fruit texture on comice fruits with Harvista application. The bars with same letters are not significantly different (*P*<0.05*)*

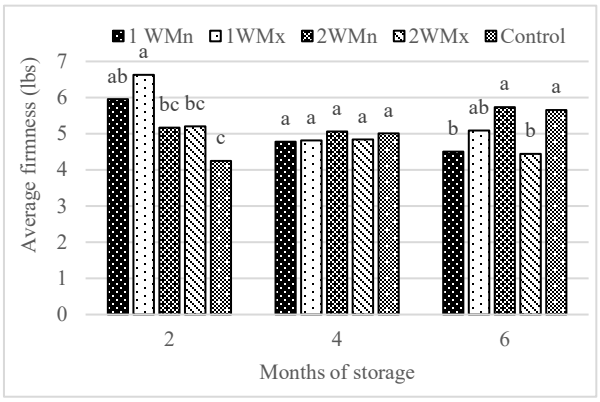


Figure 6: Fruit texture on bosc fruits with Harvista application. The bars with same letters are not significantly different (*P*<0.05*)*

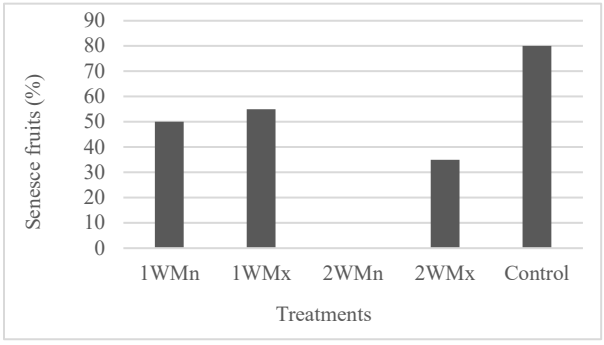


Figure 7: Percent senescence on bosc fruits stored in normal atmosphere cold storage for six months.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-17-102

Project Title: Greenhouse screening of 49 dwarf rootstock candidates

PI:	Amit Dhingra	**Co-PI**:	Kate Evans
Organization:	Washington State University	**Organization**:	Washington State University
Telephone:	509 335 3625	**Telephone**:	509-663-8181
Email:	adhingra@wsu.edu	**Email**:	kate_evans@wsu.edu
Address:	155 Johnson Hall	**Address**:	1100 N. Western Ave
City/State/Zip:	Pullman, WA 99164	**City/State/Zip**:	Wenatchee, WA 98801

Cooperators: UC Davis project funded by Pear Bureau NW and Cal Pears.

Total Project Request: Year 1: 34,133 Year 2: 19,289 **Year 3**: **20,037**

Other funding sources

Agency Name: USDA SCRI Preapplication
Amount Pending: $2,520,000 (2019-2023)
Notes: "Phenotypic and Genomic Characterization of Pyrus Germplasm for Development of Dwarfing Rootstocks for Sustainable Pear Production in the USA" (PI Dhingra, Co- PI Evans). Synergistic project to characterize diverse set of Pyrus germplasm via large scale phenotyping and genotyping.

Agency Name: USDA NIFA
Amount Pending: $497,997 (2019-2021)
Notes: "Characterizing the Genomic Basis of Compactness and Dwarfing-Induction in Pyrus communis" (PI Dhingra). Synergistic project to understand the genomic basis of dwarf stature and dwarfing in a grafted pear plant.

Agency Name: PNW Pear Bureau
Amount Pending: $322,003 (2019 – 2022)
Notes: "Pear Rootstock Breeding" (PI: Evans; Co-PI: Dhingra, Co-PI: Soon Li Teh)
Synergistic project to develop and establish pear rootstock seedlings to develop dwarfing rootstocks that are suited for high-density pear production.

Agency Name: PNW Pear Bureau
Amt. awarded: $273,253 (2015-2018)
Notes: "Pear Rootstock Breeding" PI Evans, Co- PI Dhingra. Synergistic project to advance the selected pear rootstock seedlings via phenotyping and propagation.

Agency Name: Washington State University Graduate school
Amt. awarded: $34,000 (2017)
Notes: Support for Danielle Guzman, Graduate student in the Dhingra lab.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: Washington State Univ **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2017	2018	**2019**
Salaries[1]	21,000	10,920	11,357
Benefits	10,133	5,269	5,480
Supplies[2]	1,000	1,000	1000
Travel	500	500	500
Plot Fees[3]	1,500	1,600	1,700
Total	34,133	19,289	**20,037**

Footnotes:
1 – Support for technical help to multiply rootstock selections, graft with scions and manage plants
2 – Greenhouse soil and supplies
3 – Greenhouse space usage fee per year

OBJECTIVES

1. Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform rooted material for subsequent grafting experiments.
2. Graft 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

This project represents one of the three distinct but complementary approaches to identify a source of dwarfing within *Pyrus communis*. The aim of the project is to evaluate if the dwarf habit of the seedlings will transmit to the scion. Promising selections out of the 49 dwarf seedlings are expected to be used as a rootstock selection, or a parent for the pear rootstock breeding program.

The project plan is to introduce all selections into tissue culture and establish enough clones for each selection in the greenhouse. These will then be grafted over with budwood from Bartlett and Anjou. OHxF 87 rootstock will be used as a control. The grafted plants will be grown and maintained in the WSU greenhouse to assess if the dwarfing trait is transmitted to the scion. Data on internode length, height and ratio between the two; crotch angle will be recorded. Seedlings that impart dwarfing to the scions will be evaluated as rootstock candidates in field trials to be performed after the completion of this project.

SIGNIFICANT FINDINGS

- The seedlings being cycled through rapid growth process in the greenhouse have achieved a height of 18-36 inches depending on the seedling they were derived from.

- Budwood from each of the seedling has been initiated and established in the micropropagation system.

METHODS

Objective 1: Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform material for subsequent grafting experiments.

For the greenhouse screening, each of the 49 dwarf seedlings will be established in vitro to establish a source of developmentally and physiologically uniform, clean and genetically true to type plant material. These dwarf rootstocks have already been genetically screened and DNA fingerprint for each of the selections is already available which will be utilized to ensure genetic uniformity.

Briefly, axially buds from dormant or actively growing plant material will be surface sterilized with bleach and washed with autoclaved water prior to being initiated on to the basic pear initiation media standardized in the Dhingra lab. Once the buds have swollen and elongate into an initial shoot, the nodes would be excised and placed onto the pear bud multiplication media. Usually, a 3-4x rate of multiplication, obtained via suckering and elongation, per 4-5 weeks is achieved routinely in the lab. Give the genetic variability of the material being used, it is expected that the media may need to be standardized for some of the genotypes to achieve optimal growth and multiplication.

The goal would be to have a minimum of 50 plantlets per seedling established in tissue culture. This will provide a good and constant source of plant material for subsequent steps. For this experiment, 25 plantlets will be moved from bud multiplication media to rooting media. The rooted plantlets will be moved to the greenhouse, acclimatized and grown to a height of 18-24 inches in the greenhouse to achieve a minimum caliper of $1/4^{th}$ inches. Thereafter the rootstock plants will be forced into dormancy

and maintained at 42 degree Fahrenheit till they are ready to be budded. Along with the 49 dwarf aneuploid selections, the current industry standard rootstock OH × F 87 will also be processed in a similar way and will be used as a reference material in the experiment. Therefore, there will be a total of 50 selections each with 50 plants each in tissue culture which totals to 2500 plants. In the greenhouse, 1250 rootstocks (50 selections x 25 plants each) will be prepared for objective 2.

Objective 2. Graft or bud 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

Virus and disease free, genetically true to type Bartlett and Anjou budwood will be used to perform chip budding of 10 clones for each of the 50 rootstocks. Once the buds have callused and swollen, only 5 plants of each selection per scion will be maintained in the greenhouse for phenotyping of the habit imparted to the scions. The budded plants will be screened for number of nodes produced and height of the plant achieved over a set period of time till the plants go into paradormancy. Thereafter the plants will be provided 1000 hours of chilling and placed back in the greenhouse to initiate another spurt of growth. This aspect will be repeated for 2-3 cycles to identify the potential rootstock selections that are not dwarf on their own but also transmit the trait to the scion. The desirable aneuploid rootstocks will then be selected for field based evaluations as this project nears its conclusion.

RESULTS AND DISCUSSION

Objective 1: Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform material for subsequent grafting experiments.

The 49 dwarf seedlings were obtained from crosses made in 2013. The growth of these seedlings has been fast tracked using horticultural rapid cycling process which includes providing ecodormancy (cold requirement) treatments in a cold room. The plant material was incubated in the cold for 4 months and has been recently moved to the green house, where the plant material is undergoing active growth (Figure 1). We initiated nearly 50 buds per selection. All of the selections have been successfully introduced and established into the micropropagation system.

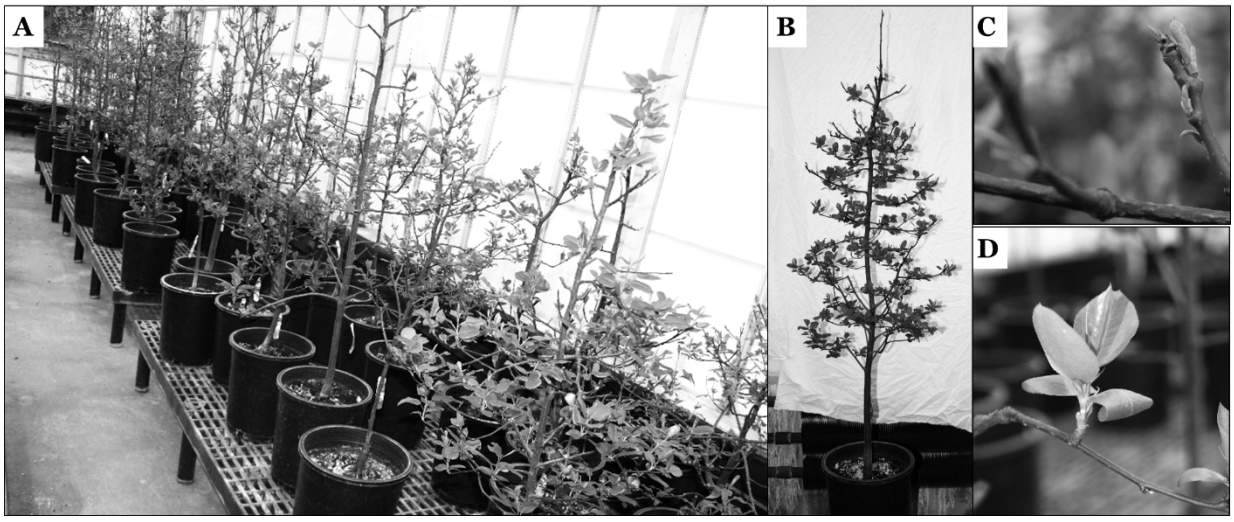


Figure 1: 49 dwarf seedlings in the greenhouse. A. An overview of all the seedlings. B. One of the selections exhibiting a compact growth habit. C and D – actively growing shoots that are being processed to be initiated in the micropropagation system.

Objective 2. Graft or bud 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

All of the plant material has been cloned and the plants are currently growing in the greenhouse. The plants have reached a height of 18-36 inches depending on the seedling they have been cloned from. The plants shall undergo dormancy cycle in fall 2019. They will be chip budded with 'Bartlett' and 'D'Anjou' budwood and grown in the greenhouse.

Figure 2: Twenty clones representing each of the 49 seedlings growing actively in the greenhouse. The plants have reached a height of 18-36 inches depending on the parent seedling.

Outreach

Presentation by Amit Dhingra - 'The foundation for the future of pear production in the PNW' Research News Flash talk at the Washington State Tree Fruit Association Meeting, December 2017.

CONTINUING PROJECT REPORT **YEAR**: 2/3 years

Project Title: Mechanisms and practical solutions to control scald of pears

PI: Yu Dong
Organization: OSU MCAREC
Telephone: 541-386-2030 (EXT. 38229)
Email: dongyu@oregonstate.edu

Cooperators: Steve Castagnoli, Paul Chen, Craig Mallon, Grady Leiblein, Allison Walston, Arden Reed

Total Project Request: **Year 1:** 36,916 **Year 2:** 39,011 **Year 3: 40,061**

Other funding sources
none

Budget:
Organization Name: Agricultural Research Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2017	2018	2019
Salaries	20,222[1]	20,829	21,454
Benefits	1,950[2]	2,009	2,069
Wages	10,744[3]	11,066	11,398
Benefits	1,074[4]	1,107	1,140
Equipment			
Supplies	3,500[5]	3,500	3,500
Travel	500[6]	500	500
Miscellaneous			
Total	37,990	39,011	**40,061**

Footnotes:
[1]Postdoctoral Research Associate: 1/2 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 800hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 10% of the wage, with a 3% annual increase.
[5]Supplies: maintaining cold storage and CA storage rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

OBJECTIVES

1. Understand completely the physiological mechanisms of scald development; understand growing season conditions and harvest maturity effects on the natural antioxidant capacity associated with the oxidation of α-farnesene into conjugated trienols (CTols) and therefore scald susceptibility of Anjou pear.
2. Study commercially-feasible methods for controlling scald of susceptible Anjou pear; the potential of the combination treatments of Harvista/ReTain + ethoxyquin + low-O_2.
3. Study the potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coatings as alternatives to ethoxyquin for controlling scald of Anjou pear.
4. Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.

SIGNIFICANT FINDINGS

- Pre-harvest calcium (Ca) spray increased fruit calcium content, antioxidant metabolites, antioxidant enzymes, and total antioxidant capacity of 'd'Anjou' pears and resulted in a slight reduction in superficial scald after 4 and 5 months in storage.
- Regardless of NAA application rate and timing, the incidence of superficial scald was not inhibited by NAA. After 4 months of storage, all NAA treatments had relatively high incidence of superficial scald, with no significant differences between treatments and the untreated control.
- O_2 concentrations of 0.5 and 1% inhibited the development of superficial scald, with 0.5% O_2 stored fruit remaining free of scald over the entire storage period, whereas 2% O_2 stored fruit developed 68% scald at 6 months and increased to 90% after 10 months.
- AVG applied 1 WBH at 60 or 120 ppm resulted in reductions in scald compared to the untreated control after 3 and 5 months of RA storage. Incidence of superficial scald of all treatments increased through the storage period and reached 100% by month 7.

RESULTS

1. Understand completely the physiological mechanisms of scald development

α-Farnesene metabolism (Data were shown in 2017-2018.)

Fruit with varied accumulated cold units (ACU) on fruit scald susceptibility

Fruit was collected at commercial harvest maturity from 5 orchards located at elevations ranging from 500 to 2,000 ft. and placed in RA storage. Fruit will be evaluated after 3, 5, and 7 months of storage and results will be reported in the final report.

Fruit with varied Ca concentration on fruit scald susceptibility

$CaCl_2$ was applied at 3.47 pound/acre (100 gal/acre, Ca rate at 0.15%) 6 times from 1 month after full bloom to 1 week before harvest. Fruit were collected at commercial harvest maturity, then placed in RA storage for up to 5 months. Superficial scald and fruit physiological and biochemical responses were evaluated after 2, 3, 4, and 5 months of storage. Pre-harvest Ca sprays resulted in higher calcium content of fruit flesh at harvest, but no significant difference in fruit skin Ca content (Table 1). Ca sprays resulted in a slight reduction in superficial scald after 4 and 5 months in storage (Fig. 1). Although Ca treatment resulted in reduced firmness at harvest, after 3 months in storage, Ca treated fruit had higher firmness than the untreated control. Additionally, Ca treated fruit showed trends towards reduced EPR, α-farnesene, and CTols as the storage duration increased. RR showed no response to Ca treatment. Ca sprays resulted in an increase in the antioxidant metabolite TP but no

difference in TFO, or the antioxidant enzymes SOD and CAT or APX (Table 2). GR was higher in Ca treated fruit as was FRAP. DPPH showed no response to Ca treatment.

Different sunlight exposure on fruit scald susceptibility (Data were shown in 2017-2018.)

Influence of harvest maturity on fruit scald susceptibility (Data were shown in 2017-2018.)

Influence of NAA on fruit scald susceptibility

NAA was applied at 20 ppm 1 and 2 WBH and 40 ppm 1, 2, and 3 WBH. At harvest, fruit were placed in RA storage. Non-treated and NAA-treated fruit were evaluated after 2, 3, and 4 months of RA storage plus 7 d at 68 °F. After 4 months of storage, all NAA treatments had relatively high incidence of superficial scald, with no significant differences between treatments and the untreated control (Fig. 2). Regardless of NAA application rate and timing, NAA-treated fruit had lower EPR than non-treated fruit. Following 4 months of storage, fruit treated with NAA at 20 and 40 ppm applied 1 WBH showed significantly lower EPR than fruit treated with NAA at 20 and 40 ppm applied 2 WBH and 40 ppm applied 3WBH. RR, α-farnesene content, and CTols content of NAA-treated fruit and the untreated control increased during storage, with no significant differences between treatments and untreated control.

2. Study commercially-feasible methods for controlling scald of susceptible Anjou pear.

Effects of low-O_2 CA on fruit scald susceptibility

'D'Anjou' pears were harvested at commercial harvest maturity from MCAREC, and after 2 d of cold storage, fruit were loaded into gas-tight cabinets. The cabinets were flushed with purified nitrogen and then CO_2 concentration was adjusted to < 0.5% by adding hydrated lime. O_2 concentration was adjusted to 2.0, 1.0, or 0.5% within 6 d of sealing the cabinets. O_2 and CO_2 concentrations were monitored every two days using an O_2/CO_2 analyzer. Air-stored fruit was placed in a cold storage room at 30 °F. After 6, 8, and 10 months of storage, fruit were removed and held at 68 °F for 7 days. Superficial scald incidence of air stored fruit was 100% following 6 months of cold storage (Fig. 3). The 2% O_2 stored fruit developed 68% scald at 6 months and increased to 90% after 10 months. The lower O_2 concentrations of 0.5 and 1% inhibited the development of scald, with 0.5% O_2 stored fruit remaining free of superficial scald over the entire storage period. Ethylene production in air-stored fruit decreased from 5 to 1 ng kg^{-1} s^{-1}, while respiration rate increased from 11 to 13 μg CO_2 kg^{-1} s^{-1} from month 6 to 10. Low O_2 treatments had lower ethylene production and respiration rates at month six, then increased thereafter. The ethylene production rate of 2% O_2 stored fruit increased, reaching a maximum level of 7 ng kg^{-1} s^{-1} after 8 months of storage, before decreasing to 4 ng kg^{-1} s^{-1}. The ethylene production rate of 0.5 and 1% O_2 stored fruit increased between 6 and 10 months of storage, reaching 3 and 5 ng kg^{-1} s^{-1}, respectively. Total antioxidant capacity of air and CA stored fruit decreased during ripening. Reducing O_2 concentration from 21% to 0.5% inhibited the reduction of total antioxidant capacity, but no significant differences were observed among 2, 1 and 0.5% O_2 treatments. The effect of low O_2 treatments on α-farnesene content were similar to those on ethylene production. CTols of air and CA stored fruit gradually increased, and the 0.5 and 1% O_2 stored fruit had lower CTols contents than other treatments. These results indicate that reducing O_2 concentration can reduce superficial scald of 'd'Anjou' pears with 1 and 0.5% O_2 providing nearly complete and complete control.

Effects of pre-harvest Harvista on fruit scald susceptibility (Data were shown in 2017-2018.)

Effect of pre-harvest Retain on fruit scald susceptibility

AVG (Retain) was applied at 60 ppm 1 WBH and 120 ppm applied 1 and 2 WBH. After harvest, fruit were placed in RA storage. Fruit were evaluated after 1, 3, 5, and 7 months of RA storage plus 7 d at 68 °F. Incidence of superficial scald of all treatments increased through the storage period and reached 100% by month 7 (Fig. 4). After 3 and 5 months of RA storage AVG applied 1 WBH at 60 or 120 ppm provided reductions in scald compared to the untreated control. All treatments were producing a significant amount of ethylene by month 3 of storage. By month 5 non-treated fruit and fruit treated with AVG at 120 ppm 2 WBH showed higher ethylene production rate than fruit treated with AVG at 60 or 120 ppm 1 WBH. Fruit treated with 60 and 120 ppm AVG 1 WBH had lower CTols and incidence of superficial scald than the non-treated fruit during 5 months of storage, but no difference was observed between non-treated fruit and fruit treated with 120 ppm AVG 2 WBH. Beyond 5 months of RA storage, EPR of non-treated fruit decreased, while AVG-treated fruit increased. After 7 months of RA storage fruit treated with 120 ppm AVG 1 WBH had lower EPR than fruit treated with 60 ppm AVG 1 WBH or 120 ppm AVG 2 WBH. In contrast, total antioxidant capacity in all treatments decreased through the 7 month storage period, with no significant differences among the non- and AVG-treated fruit. α-Farnesene content of non-treated fruit and fruit treated with 120 ppm AVG 2 WBH increased, reaching a maximum level after 5 months of storage, before decreasing. Fruit treated with 60 and 120 ppm AVG 1 WBH showed an increase of α-farnesene content through the storage period. CTols content of all treatments increased through the 7 month storage period. AVG applied 1 WBH at 60 or 120 ppm maintained lower CTols content than the control and AVG applied at 120 ppm 2 WBH.

Effect of the combination of pre-harvest AVG + ethoxyquin + 1 % O_2 CA on fruit scald susceptibility

AVG was applied at 60 and 120 ppm 1 WBH. Fruit was harvested at commercial harvest maturity from MCAREC, and after 2 d of cold storage fruit from each treatment was treated with 1000 ppm ethoxyquin, and then loaded into gas-tight cabinets. The cabinets were flushed with purified nitrogen and then O_2 concentration was adjusted to 1% within 6 d. Concentrations of O_2 and CO_2 were monitored every two days using an O_2/CO_2 analyzer. Fruit were evaluated after 6, 8, and 10 months of storage and results will be reported in final report.

The potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coatings as alternatives to ethoxyquin for controlling scald of Anjou pear.

After ‘d’Anjou’ pears were harvested at 14.23 lbs in 2018, fruit were treated with Lovastatin at rates of 0.6 and 1.2 mmol L^{-1} and ascorbic acid (AsA) at 5 and 10 mmol L^{-1} for 10 min, then stored in RA storage for 5 months. Evaluation of superficial scald was carried out every month and results will be reported in final report. In 2019, other naturally-occurring food-grade antioxidants, such as Semperfresh, carnauba, chitosan, or sodium alginate will be employed in this trial.

Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.

This summary will be developed for the final report.

Table 1. Effects of Ca treatment on calcium content of skin and flesh tissue in pears at harvest.

	Calcium content (ppm)	
	Skin tissue	Flesh tissue
Control	377.63 a	1258.53 b
Ca treatment	396.60 a	1401.00 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

Table 2 Total polyphenols (TP), total flavonoids (TFO), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, and ferric reducing antioxidant power (FRAP) of flesh tissue in pears with and without Ca treatment after 5 months of storage plus 7 d at 68 °F.

	TP (mg g^{-1})	TFO (mg g^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	APX (U g^{-1})	GR (U g^{-1})	DPPH (mM g^{-1})	FRAP (mM g^{-1})
Control	0.6 b	1.2 a	1.4 a	0.9 a	78.0 a	9 b	2.5 a	1.4 b
Ca treatment	0.8 a	1.1 a	1.5 a	1.1 a	68.0 a	14 a	2.8 a	2.2 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$**.**

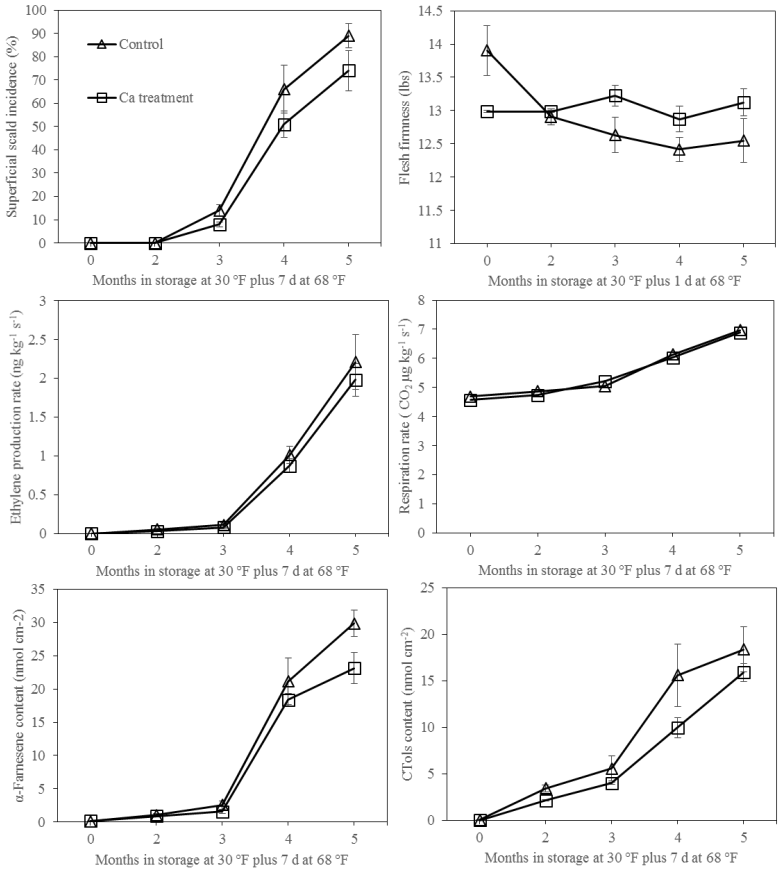


Figure. 1. Effects of Ca treatment on superficial scald incidence, flesh firmness, ethylene production rate (EPR), respiration rate (RR), α-farnesene content, and CTols content of 'd'Anjou' pears during 5 months of storage at 30 °F. Values are means ± standard deviation (SD).

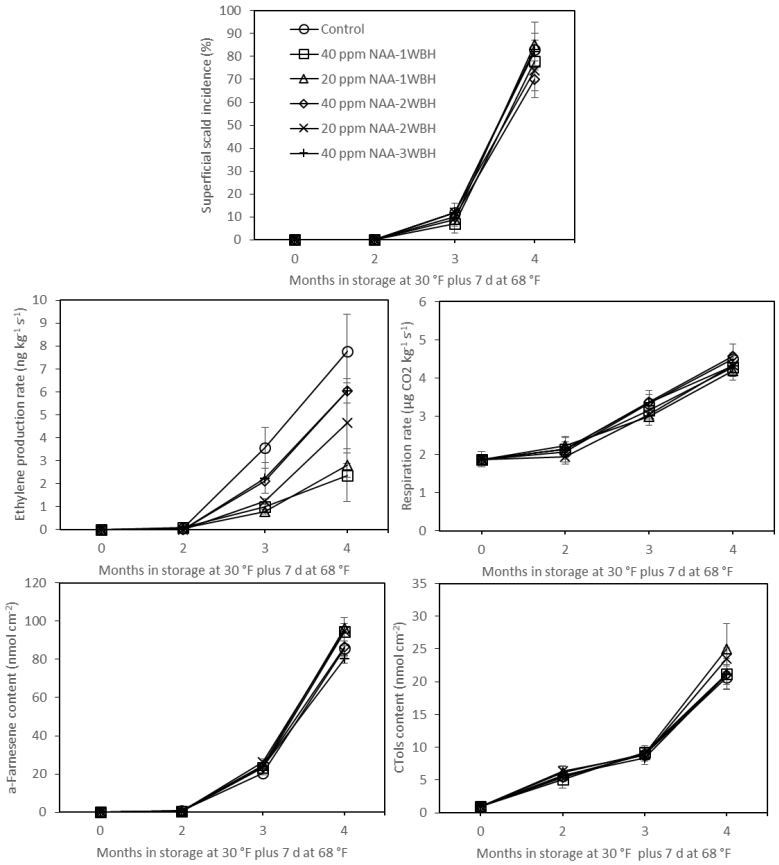
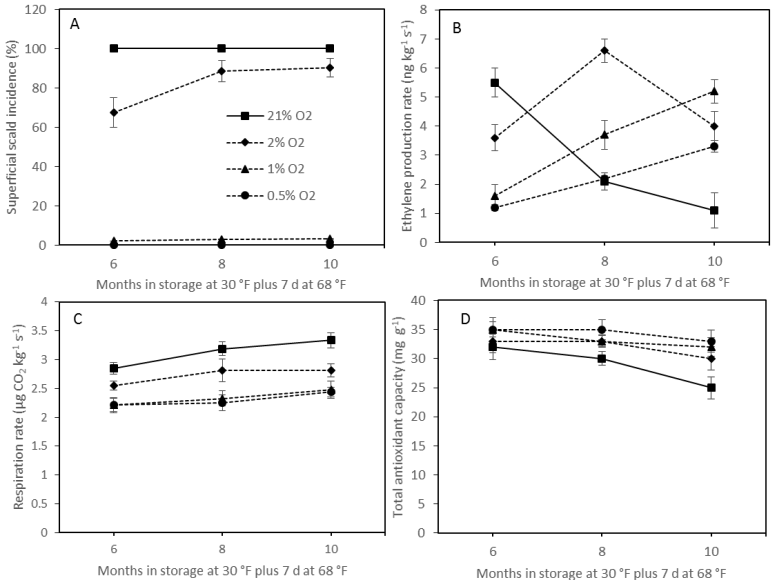


Figure. 2. Effects of pre-harvest NAA spray at rate of 20 and 40 ppm applied at 1, 2, or 3 weeks before commercial harvest on EPR, RR, α-farnesene content, CTols content, and superficial scald incidence of 'd'Anjou' pears on day 7 at 68 °F for 4 months of storage at 30 °F. Values are means ± SD.



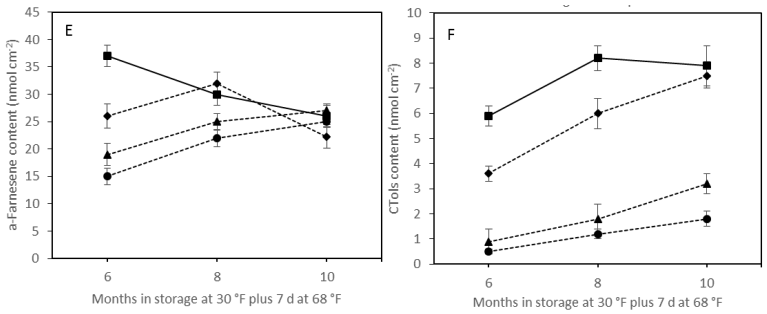


Figure. 3. Effects of different O_2 regimes on superficial scald incidence, EPR, RR, total antioxidant capacity, α-farnesene content, and CTols content of 'd'Anjou' pears after 6, 8, and 10 months of storage at 30 °F plus 7 d at 68 °F. O_2 concentrations were 21 % (air), 2 %, 1 %, and 0.5 % with CO_2 < 0.5 %. Values are means ± SD.

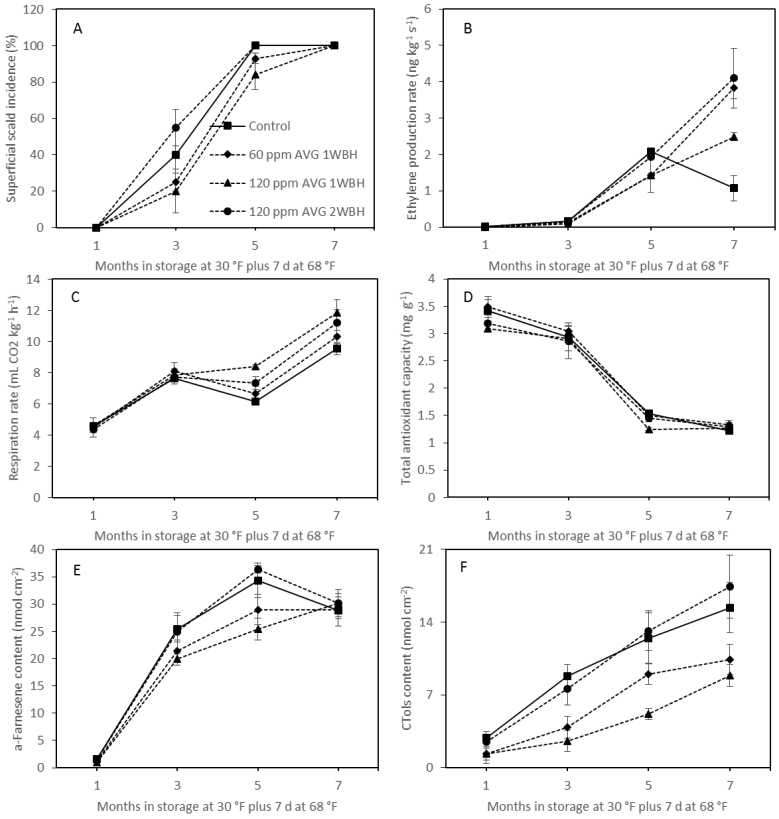


Figure. 4. Effects of preharvest treatments of AVG at rate of 60 and 120 ppm applied at 1 and 2 weeks before commercial harvest on EPR, RR, α-farnesene content, CTols content, and superficial scald incidence of 'd'Anjou' pears on day 7 at 68 °F for 10 months of storage at 30 °F. Values are means ± SD.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2 Years
WTFRC Project Number: PR-18-103

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

PI: Lee Kalcsits
Organization: Washington State University
Telephone: 509-293-8764
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Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI: S. Tianna DuPont
Organization: Washington State University
Telephone: 509-293-8758
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Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI: Troy Peters
Organization: Washington State University
Telephone: 509-786-9247
Email: troy_peters@wsu.edu
Address: 24106 N Bunn Rd.
City/State/Zip: Prosser/WA/99350

Cooperators: Bob Gix (Blue Star Growers); Chet Walker (S & W Irrigation)

Total Project Request: Year 1: $118,792 **Year 2: $64,537** Year 3: $89,794

Other funding sources

Agency Name: Bonneville Environmental Foundation water stewardship
Amt. requested: $30,000
Notes: Since this was awarded, we reduced our requested budget request by $20,000 in 2019 to $64,137. The remaining $10,000 in supplies will allow us to install better instrumentation at grower sites

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509-293-8803 **Email:** arcgrants@wsu.edu/kim.rains@wsu.edu

Item	2018	**2019**	2020
Salaries[1]	45,503	**47,723**	49,216
Benefits[2]	18,119	**18,844**	19,598
Supplies[3]	~~47,170~~[4] 27,140	**9,970**	12,970
Travel[5]	8,000	**8,000**	8,000
Total	118,792	**~~84,537~~ 64,537**	89,784

Footnotes:
[1] Salaries to support a technician at $3500/month at 75% FTE in the Kalcsits lab and a technician at $3500/month at 33.34% FTE in Tianna DuPont's program. The budget includes a 4% salary increase per year.
[2] Benefits for both technicians calculated at 39.8 %
[3] Supplies include irrigation supplies for objective 1, lab and field consumables, extension materials, analysis costs for nutrient analysis and fruit storage costs.
[4] $30,000 of supplies in year 1 is requested for irrigation supplies to retrofit commercial blocks for testing. Funding for this is also included in the grant application to Bonneville Environmental Foundation.
[5] Travel includes mileage for Kalcsits, DuPont, and Peters for regular trips to commercial orchards and the Sunrise Research Orchard and for hotel and meal per diems for overnight trips to the Wenatchee region for Dr. Peters and his M.S. student to make measurements.

OBJECTIVES

1. Test whether increasing the frequency of irrigation or changing irrigation volume applied during specific times during the season affects fruit productivity and quality.
2. The extension portion of the project will establish demonstration which showcase irrigation optimization strategies to show versus tell growers how changes to irrigation are critical to impact yield and pack out.
3. Conduct a cost-benefit analysis comparing potential increased revenue from changes to irrigation strategies with the costs of making the change.

From the completion of these objectives, we will: a) document the ability of strategies to improve yield, fruit size and fruit quality; b) document the return on investment of these strategies; c) document the changes in the water efficiency of each of these strategies. Demonstration on-farm will create advocates among stakeholders who can tell the story of adoption.

SIGNIFICANT FINDINGS

In sandy soils, pear trees respond quickly to water restrictions and can show signs of stress in short periods of time after an irrigation event.

These stresses can impact yields through decreases in fruit size but in 2018, did not appear to have an appreciable impact on fruit quality or cork spot incidence after storage.

Through evaluations done by Dr. Troy Peters and Tianna DuPont, growers experiencing irrigation-related issues could be experiencing several different problems that require specific changes in each scenario.

Through a grant from the Bonneville Environmental Foundation and in cooperation with the Cascadia Conservation District, the team is working with growers experiencing water-based problems to make changes to help improve water distribution and management of their systems.

In 2019, we will continue with research experiments focusing on identifying soil and plant-based thresholds that are critical for water management in pears to improve fruit size and reduce stress. We will monitor fruit quality in grower orchards on a spatial scale to evaluate the impact of changes to these systems.

METHODS

Objective 1

For this objective, the research was conducted at the Sunrise Research Orchard in Rock Island, WA in a semi-mature block of Anjou and Bartlett pears that was planted in 2007 at a spacing of 6’ between trees and 14’ between rows. The orchard was irrigated using microsprinklers hooked up to a variable speed drive system that allows for flexibility in water schedules. The soil in this site is a sandy loam soil with a high percentage of sand. The poor water holding capacity of the soil makes this an excellent location to manipulate soil water content and ensure that we are getting enough variation to achieve the desired effects on the trees. There were four treatments applied. The first was where soil moisture levels were maintained near field capacity for the entire irrigation season. The second was limiting irrigation to 60% field capacity from 15-60 DAFB. The third treatment was limiting soil moisture to 60% of field capacity from 60-105 DAFB. The last treatment was modified

from the original proposal. We opted to implement a stem water potential based irrigation scheme where irrigation was triggered when the mean stem water potential for sampled trees was more negative than -1.0 mPa. This strategy reduced overall water use by more than 40%.

Fruit was harvested on September 2, 2019 from sample trees. Fruit was stored in regular atmosphere at 33 °F for 12 weeks. After storage, fruit quality was assessed including fruit size, weight, firmness, and soluble solids content. Cork spot incidence was also assessed in these same fruit samples. Subsamples were then taken for nutrient analysis for N, P, K, Ca, and Mg to look for changes in the ratios among these competing nutrients that may correspond to differences in cork spot, fruit size, or vegetative vigor. We are in the process of analyzing these samples. Additionally, the project team will track return bloom in 2019 to look at the influence of irrigation frequency on return bloom in Anjou pears.

During the season, we measured plant indicators of water stress during the growing season to relate to horticultural responses such as vegetative and fruit growth. Physiological measurements were made including mid-day stem water potential and stomatal conductance. Plant water status, measured as Ψ_{md} will be assessed using a 3005 Series Plant Water Status Console (Soilmoisture Equipment Corp, Goleta, CA, USA). Leaves used for measurement of Ψ_{md} will be bagged for at least one hour in silver reflective bags to equalize the leaf and xylem water potential before readings are taken. Ψ_{md} will be measured around solar noon. Stomatal conductance (mmol m^{-2} s^{-1}) was measured on mature, sun-exposed leaves on the upper half of the canopy using a LiCor-6400XT Gas Exchange System.

Soil moisture was monitored using Decagon 5TM soil moisture and temperature sensors in each plot over the entire season to capture seasonal changes in soil moisture profiles in addition to the treatment level variations in soil moisture. In the early and late withholding treatments, volumetric soil water content was used to guide irrigation events where volumetric water content below 13% vol/vol triggered a small irrigation set to bring soil moisture levels above that threshold.

RESULTS & DISCUSSION

Objective 1. Test whether increasing the frequency of irrigation or changing irrigation volume applied during specific times during the season improves fruit productivity and quality. Fruit weight averaged between 210 and 220 g per fruit for all treatments (Figure 1). Fruit weight was significantly larger when trees were watered using a stem water potential (SWP) based irrigation strategy, essentially providing water at the right time when it is physiologically needed rather than based on timing. This meant less water early in the growing season, when it was cool in June, and when it was smoky in early August. Interestingly, cork spot incidence was quite high in this block compared to a relatively low incidence across orchards in the PNW in 2018. This could be driven by several factors including the absence of calcium sprays or the high proportion of sand in the soil.

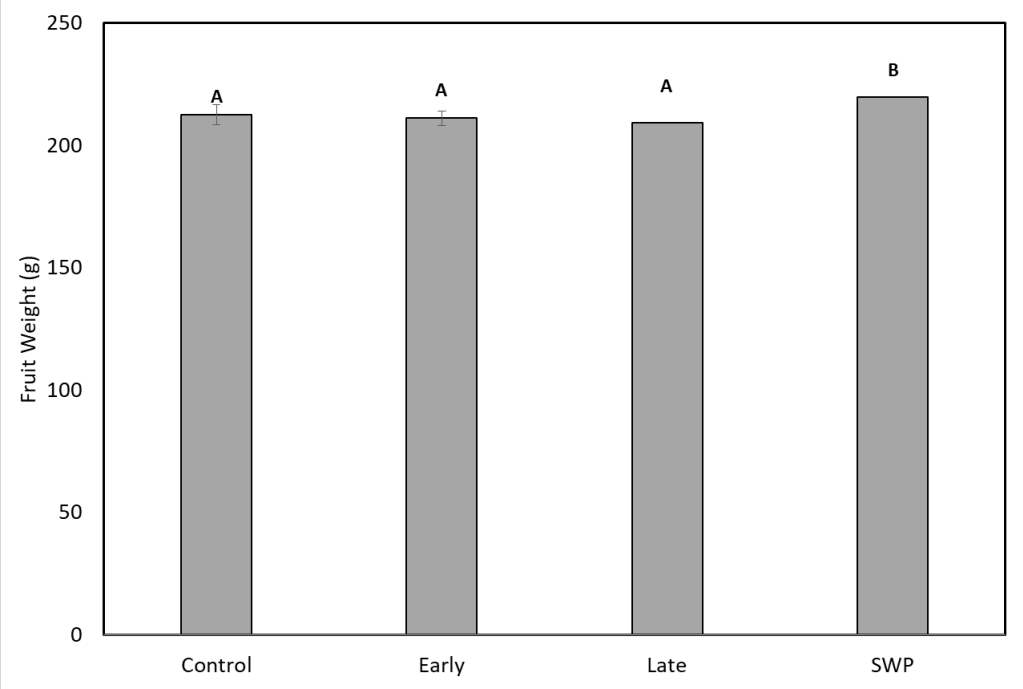

Figure 6. Mean fruit weight (g) of D'Anjou pears either irrigated fully near field capacity (Control), deficit irrigated early (Early), deficit irrigated late (Late), or irrigated based on midday stem water potential (SWP). Letters denote significant differences between means determined using a Tukey's HSD test.

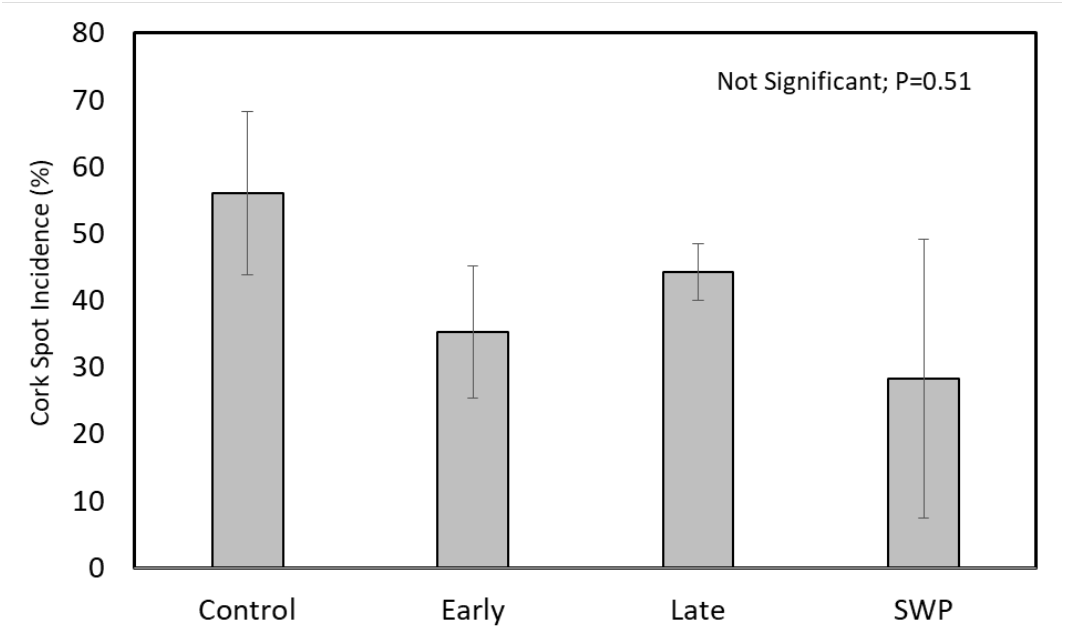

Figure 7. Mean cork spot incidence (%) of D'Anjou pears either irrigated fully near field capacity (Control), deficit irrigated early (Early), deficit irrigated late (Late), or irrigated based on midday stem water potential (SWP)

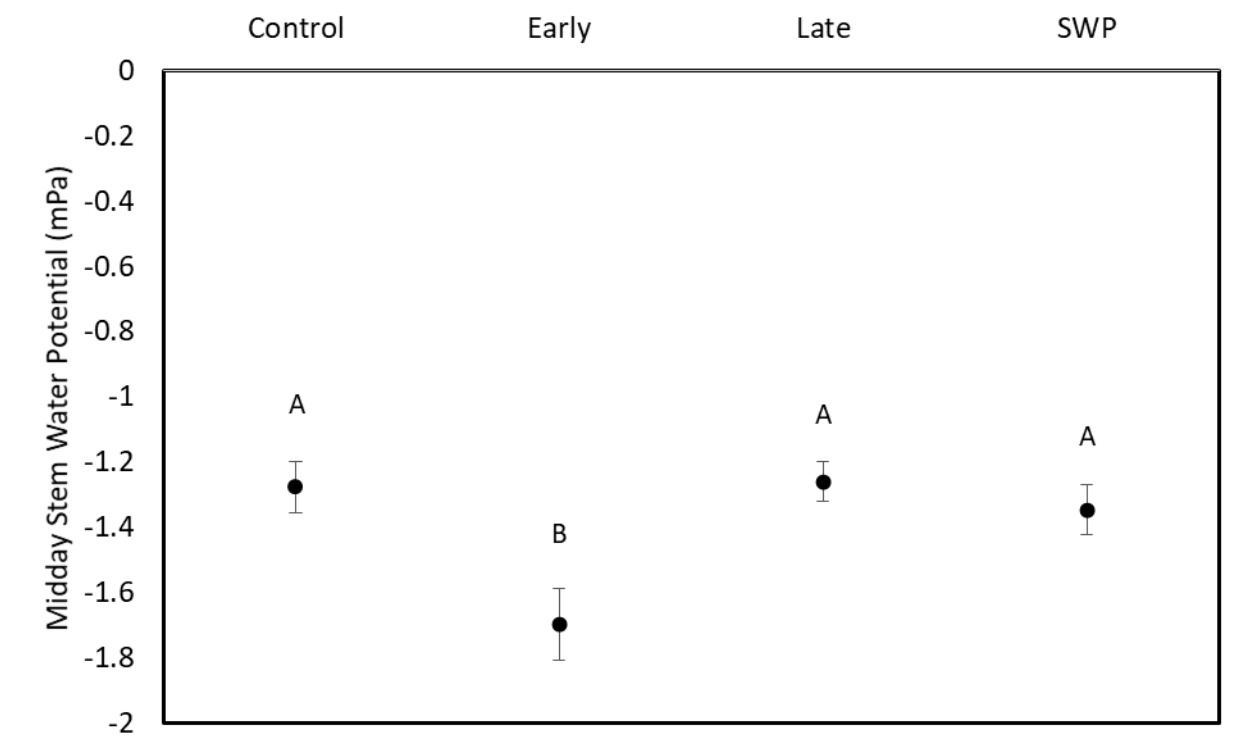

Figure 8. Midday stem water potential (-mPa) for D'Anjou pears measured on July 6, 2018 (91 °F)from trees either irrigated fully near field capacity (Control), deficit irrigated early (Early), deficit irrigated late (Late), or irrigated based on midday stem water potential (SWP). Letters denote significant differences between means determined using Tukey's HSD test.

Objective 2a. Identify common challenges for irrigation efficiency. DuPont, Peters

Interviews with growers and consultants identified common challenges to irrigation efficiency in pears. These challenges include: lack of sufficient irrigation; uneven pressure and distribution due to hills; irregular water distribution across the block due to old, inappropriate or malfunctioning equipment; sandy soils with low water holding capacity; heavy soils with limited drainage; insufficient or excess watering due to inability to time water applications; and/or system inefficiencies.

Subobjective 2b. Identify potential demonstration plots. DuPont, Peters

A call for applications was sent out in spring 2018. All eight applicant sites were evaluated by the irrigation specialist in June/July 2018 through half day on-site evaluations. Site evaluations included measurement of pressure, flow, and distribution at various points in the block. Site evaluation reports were shared with each grower involved. Based on site evaluations two blocks were eliminated due to the fact that irrigation systems appear to be working efficiently. One site was eliminated due to challenges beyond the scope of this project. The selected sites include Selfs Rd (challenge over-irrigation from scheduling and irrigation design); Flowery Divide Har (challenge uneven distribution due to lack of pressure and multiple nozzle sizes); Flowery Divide Lo (challenge irrigation

scheduling); and Williams Canyon (challenge clogging filters). An eighth site (Goat Hill) is still under evaluation.

Subobjective 2c & 2d. Draft efficiency strategy plans. Irrigation designs. Peters, Walker, DuPont

Recommendation reports were created for each site and discussed with grower collaborators. For sites with irrigation design needs Chet Walker from S&W engineered plans (Flowery Divide Har, Goat Hill).

Subobjective 2e. Designs installation. DuPont, Growers

Sites Selfs Rd, Flowery Divide Har, Flowery Divide Lo, and Williams Canyon have plans in place to install changes in early spring 2019 before irrigation water flows in the canals. An additional site at Caudle Dryden was installed in spring 2018 with non-research commission funds.

Caudle-Dryden Case Study

The challenge at the Caudle Dryden site was run off and small fruit size. The site consists of two side by side 10-acre blocks: 'hill' and 'clover.' The existing irrigation system consisted of Rainbird impact sprinklers on a 36' x 36' spacing (34 heads/ A). The application rate was approximately 0.3 inch/ hr or 0.14 inch/ hr at 50% efficiency. The new system consists of R10 micro-sprinklers with a lower output per sprinkler (0.43 gph) compared to impact sprinklers installed at a 20' x 20' spacing (109 heads/ A). While application rate per hour was similar (0.12 inch/hr at 70% efficiency), smaller droplet size and less output per sprinkler should result in a larger percentage of water infiltrating vs running off the soil. Block 'hill' was designated as the 'Standard' treatment and not changed, block 'clover' was designated the 'new' treatment with R10s installed in June 2018.

After the first year the grower collaborator's impression of the new system was that there was *"Zero run off in the new system. Leaf color was more uniform."* He was happy that "*Before the quickest we could water was 9 days. Now if we want to, we can water the whole block in 2 days (20 lines at a time)"* This gives them more flexibility.
Measurements were taken in 'Standard' versus 'New' blocks to compare tree water stress, soil moisture and fruit quality. Please note as un-replicated blocks information comparative not statistical.

Tree water stress measurements were taken on July 16, 2018 measuring leaf water potential using a pressure bomb. Measurements were taken from one tree in every other row at the top of the hill. In the 'New' system trees displayed less stress with all values falling under the -1.2 mpa threshold considered to be water limited. In comparison in the 'Standard' block leaf water potential had more variation and more trees above the -1.2 mpa threshold (Figure 1).

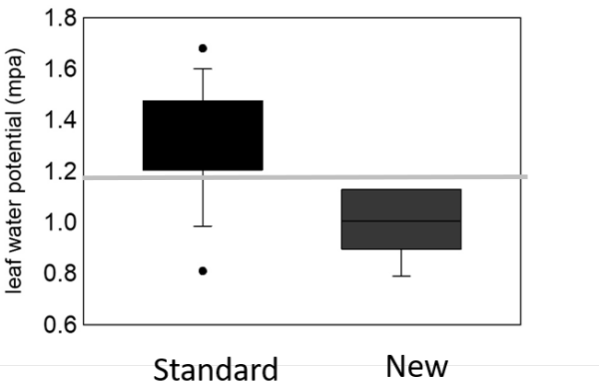

Figure 9 Leaf water potential of trees sampled from the upper slope from the standard system compared to the new irrigation system

20 Fruit were harvested from 8 trees on a grid pattern across the top and bottom of 'Standard' and 'New' plots. Fruit were stored for 12 weeks and then evaluated for size and quality. Fruit size was more

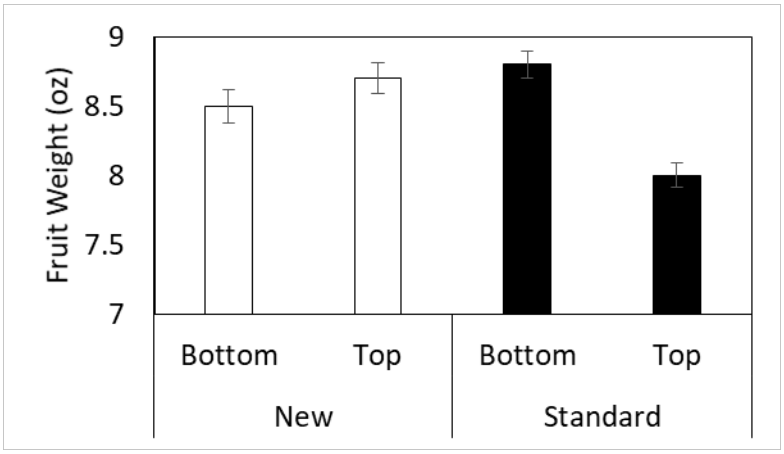

Figure 10 Fruit weight for D'Anjou pears with either the new system (white bars) or old system (black bars).

uniform in the 'New' plot compared to the 'Standard' plot (Figure 2).

Fifty-six bins were tagged separately, and pack-out data compared for each plot. The percent packout was higher in the 'New' plot at 95.6% compared to 92.7% in the 'Standard' block with 22.95 packs per bin in 'New' and 22.27 in 'Standard.' **This resulted in 820 packs of US #1 per acre in the new system compared to 788 in the standard system.** The size distribution of US #1s included slightly more large fruit in the 'New' with 736 vs 734 packs of 90+ size fruit. These were primarily in the 60 and 70 class fruit with 73 vs 53 60 class and 210 vs 201 70 class. Using average FOB prices from the January 9, 2018 (Washington Tree Fruit Association Weekly Grower's Bulletin) dollar values were assigned to each size class for US #1 fruit. Based on these estimates the new system would receive approximately $700 per acre more than the standard. This would result in a reasonably quick return on the investment of $1,000 per acre. More specific estimates and return on investment will be calculated based on actual returns.

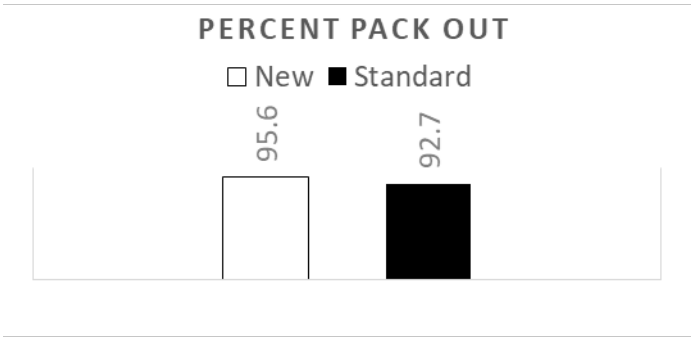

Figure 6 Pack out (%) based on packinghouse data.

2019 Plans

- Return bloom, vigor, and nutrient analysis from 2018 experiment
- Repeat experiment at Sunrise in 2019 focusing on tree response to deficit water to better develop thresholds that can help growers make better irrigation decisions
- Continue with the installations at grower sites to solve several common irrigation-related issues
- Sample fruit and physiological measurements to assess how effective changes were to tree health and productivity

CONTINUING PROJECT REPORT **YEAR**: 2 of 3 (No-cost extension)

Project Title: Interstem grafts to evaluate pear germplasm for dwarfing potential

PI: Joseph Postman
Organization: USDA Agricultural Research Service
Telephone: 541-738-4220
Email: Joseph.Postman@ars.usda.gov

Cooperators: Kate Evans, Washington State University

Total Project Request: **Year 1**: $18,000 **Year 2**: $9,000

Budget:

Organization Name: USDA-ARS **Contract Administrator: Richard Kimball**
Telephone: 510-559-6019 **Email address: Richard.Kimball@ars.usda.gov**

Item	2017	2018	2019
Salaries			
Benefits			
Wages	$12,000	$9,000	
Benefits			
Equipment			
Supplies	$6,000		
Travel			
Miscellaneous			
Plot Fees			
Total	**$18,000**	**$9,000**	**$0**

OBJECTIVES

Clonal propagation of pear clones as self-rooted trees for rootstock trials is very challenging, and a procedure to pre-screen selections for dwarfing ability will help focus resources. The USDA living pear germplasm collection has numerous pear selections identified as potential rootstocks, and includes a very large and diverse assortment of pear selections and species that have never been evaluated as rootstocks or for dwarfing potential. The objective of this project is to evaluate whether interstem grafts can be used to identify pear selections that have dwarfing potential, and provide a relatively rapid assay for screening pear germplasm for prospective rootstock candidates. The goal was to top-work 10 grafts of 30 interstem selections with Bartlett and 10 with Bosc in order to obtain 8 successful combinations for field planting in year 2.

SIGNIFICANT FINDINGS

- The concept of generating interstem trees in the greenhouse in one season was accomplished, but a single season was not adequate for evaluating dwarfing potential.
- In 2017, 194 Bosc trees and 204 Bartlett trees on 29 different interstems grafted onto seedling pear rootstocks in the greenhouse were evaluated (see January 2018 Report).
- In 2018, 174 Bosc trees and 184 Bartlett trees on 28 different interstems were field planted in early July, and growth data was taken in November.
- Rootstock, interstem, and cultivar stem diameters; cultivar stem heights; and number of side branches was recorded.
- Mean cultivar stem heights on different interstems ranged from 18 to 59 cm for Bosc, and from 50 to 92 cm for Bartlett. Mean cultivar stem diameters ranged from 4.6 to 8.1 mm for Bosc and from 7.0 to 9.4 mm for Bartlett.
- Despite the range of tree heights and stem diameters, statistical significance could not be demonstrated (HSD .05) due to within treatment variation.
- Although this trial failed to detect significant differences in dwarfing effect of pear interstem selections after 1 year in containers or after a second season in the field, we would like to follow the growth of this replicated field planting for two more seasons at no cost to the tree fruit industry.

METHODS

Interstem Grafts. Scions were collected in January 2017 from interstem candidates and from virus free mother trees of Bartlett and Bosc (for top-working) and stored at 4 °C (40 °F). After Tree Fruit Research Commission funding was awarded in April 2017 pear seedling rootstocks were planted in 2” x 10” deepots. An undergraduate student intern was hired for the summer to graft and manage the trees. Beginning in late May, 15 cm (6 in) long interstems were grafted onto seedling rootstocks in a cool greenhouse. Twenty grafts were made with each of 31 interstem candidates. Approximately 2-3 weeks after interstem grafts were made, 10 were chip-budded with Bartlett and 10 with Bosc at the top of the interstem. Grafted trees were maintained in pots and flood irrigated in a shade-house during the growing season. Rootstock and interstem shoots were regularly removed during the growing season to force the cultivar buds. Interstem graft survival and cultivar top-graft survival was assessed in mid-July and again in mid-October. Length of cultivar bud growth was measured in mid-October. Results from 2017 were reported at the pear research review in February, 2018.

Tree Architecture. Funding from USDA-ARS in 2017 allowed collection of tree architecture traits for the suite of interstem candidates, continuing the work of Richard Bell to evaluate for traits potentially correlated with dwarfing potential. Amount and angle of side branching, tree form, stem spininess, fruit crop, and mean interstem length was measured on 10 current season shoots per rootstock candidate.

Field plot established in 2018. In early July 2018, Bosc and Bartlett trees on 28 different interstems were field planted in a randomized block design with two blocks per treatment. A narrow irrigation pipe trencher was used to prepare planting furrows for easily lining out the trees on 24 inch centers (Figure 1). Trees were fertilized, regularly irrigated, and weeds were controlled for the remainder of the growing season. After leaf-fall in late October, the total height of Bartlett and Bosc shoots was measured from the bud union and the number of side branches was counted. Stem diameters were measured in mm using a digital caliper below the interstem graft union (rootstock), at mid-interstem (interstem), and above the interstem (cultivar). Analysis of variance was conducted and Tukey's HSD was used to compare means.

RESULTS & DISCUSSION

At the end of year 2, 174 Bosc trees and 184 Bartlett trees on 28 different interstems had survived with the number of reps for each cultivar/interstem combination ranging from 4 to 8 for Bartlett and from 3 to 8 for Bosc (Tables 1-4). Using Bosc on Bosc interstems (Tables 1-2) and Bartlett on Bartlett interstems (Tables 3-4) as reference controls. While some cultivar/interstem combinations resulted in trees that were generally smaller or larger than controls none of the differences were statistically significant. Results were not what would be expected for several rootstocks. BU 2/33 (Pyro II) should be somewhat dwarfing, however with Bartlett scions the trees were more vigorous than controls, and with Bosc they were essentially no different than controls. For several of the OHxF selections, OHxF 97 should be more vigorous, with OHxF 333 and OHxF 69 increasingly more dwarfing. This dwarfing relationship was not observed. The lack of expected or of significance in the observed results was likely due to a combination of relatively large within treatment differences, and a lack of normal new growth given the late season planting time.

No-cost extension. Since the interstem field plot is now well established on the USDA germplasm farm in a location adjacent to other long term plantings, the maintenance costs will be negligible for irrigation and weed control. We anticipate much more uniform growth during full growing seasons in the field rather than the partial growing seasons experienced in the first two years. We hope to collect more useful data for two additional growing seasons before trees become too crowded on the close spacing. While this trial did not demonstrate that interstems can be used to predict the dwarfing capability of rootstock candidates after a single growing season in containers, or after a second year in the field, the use of interstems may still be a practical method to assess the dwarfing capacity of pear rootstock candidates. We hope that this will be demonstrated after one or two additional growing seasons.

Figure 1 – Interstem trees field planted in early July 2018.

Table 1. Mean stem diameter (mm) of Bosc grafts.

interstem	n	diam
OHxF 97	5	4.6
Gasparian 38	5	5.1
P. regelii	4	5.1
P. fauriei 12-14	6	6.0
OHxF 69	8	6.0
P. spinosa ALB-038	6	6.0
P. betulifolia (Shaanxi)	5	6.1
P. salicifolia hyb.sdlg.5	8	6.2
P. sachokiana	6	6.2
P. calleryana D6	6	6.2
Bartlett	6	6.4
QR 708-12	7	6.4
P. syriaca (Armenia)	5	6.6
OHxF 333	7	6.7
BU 2/33 - Pyro II	4	6.7
BP-2	8	6.8
P. nivalis compact	4	6.9
P. korshinskyi	8	7.1
P. salicifolia hyb.sdlg.1	5	7.2
BP-1 B92	7	7.2
OHxF 87	8	7.3
Bosc	8	7.5
Granatnaya	6	7.6
Passe Crassane	8	7.7
P. syriaca (Israel)	1	7.8
Sbkta (P. elaeag.)	7	7.9
Mustafabey	8	8.1
Pyronia	8	8.1

Table 2. Mean height (cm) of Bosc grafts.

interstem	n	height
OHxF 97	5	18.4
Gasparian 38	5	22.7
P. calleryana D6	6	26.3
P. fauriei 12-14	6	29.3
OHxF 69	8	32.0
P. regelii	4	33.3
P. spinosa ALB-038	6	38.7
P. salicifolia hyb.sdlg.5	8	38.8
P. betulifolia (Shaanxi)	5	42.4
P. syriaca (Armenia)	5	42.8
OHxF 87	8	45.1
QR 708-12	7	46.0
P. korshinskyi	8	47.4
P. salicifolia hyb.sdlg.1	5	47.4
Sbkta (P. elaeag.)	7	48.1
OHxF 333	7	48.2
Mustafabey	8	48.3
BU 2/33 - Pyro II	4	48.5
P. sachokiana	6	48.8
Bartlett	6	49.7
Granatnaya	6	50.2
Bosc	8	50.5
BP-2	8	51.0
P. nivalis compact	4	51.8
BP-1 B92	7	52.4
Passe Crassane	8	52.8
Pyronia	8	57.0
P. syriaca (Israel)	1	59.0

Table 3. Mean stem diameter (mm) of Bartlett grafts.

interstem	n	diam
P. sachokiana	7	7.0
Granatnaya	3	7.1
P. betulifolia (Shaanxi)	7	7.6
QR 708-12	8	7.6
P. spinosa ALB-038	3	7.7
BP-1 B92	6	7.8
P. nivalis compact	3	8.0
P. korshinskyi	8	8.0
Pyronia	7	8.2
OHxF 69	8	8.3
Mustafabey	8	8.3
Le Nain Vert	4	8.3
OHxF 97	8	8.4
P. salicifolia hyb.sdlg.5	8	8.5
OHxF 333	8	8.6
Bosc	7	8.6
BP-2	8	8.6
Passe Crassane	8	8.9
Gasparian 38	4	8.9
Bartlett	8	8.9
OHxF 87	8	9.0
P. calleryana D6	8	9.1
P. fauriei 12-14	8	9.2
P. regelii	3	9.2
P. syriaca (Armenia)	6	9.2
P. salicifolia hyb.sdlg.1	8	9.3
Sbkta (P. elaeag.)	8	9.3
BU 2/33 - Pyro II	4	9.4

Table 4. Mean height (cm) of Bartlett grafts.

interstem	n	height
Granatnaya	3	50.3
P. nivalis compact	3	50.3
P. sachokiana	7	53.6
QR 708-12	8	60.9
P. calleryana D6	8	62.0
BP-1 B92	6	64.0
Le Nain Vert	4	64.0
OHxF 97	8	68.3
P. betulifolia (Shaanxi)	7	68.7
BP-2	8	68.8
Passe Crassane	8	68.8
Sbkta (P. elaeag.)	8	69.4
Mustafabey	8	72.9
Bartlett	8	73.3
OHxF 87	8	73.3
OHxF 69	8	74.8
P. korshinskyi	8	77.4
Pyronia	7	77.6
Bosc	7	79.9
Gasparian 38	4	80.0
P. salicifolia hyb.sdlg.5	8	81.3
P. salicifolia hyb.sdlg.1	8	82.0
P. fauriei 12-14	8	84.5
P. syriaca (Armenia)	6	85.3
OHxF 333	8	86.7
P. regelii	3	89.0
BU 2/33 - Pyro II	4	91.8
P. spinosa ALB-038	3	92.0

CONTINUING REPORT **Year: 2018**

Project Title: WTFRC Internal Program – Food Safety Efforts

PI: Ines Hanrahan
Organization: WTFRC
Telephone: 509 669 0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S.18th St., Suite 100
City/State/Zip: Union Gap, WA, 98903

Cooperators: Jacqui Gordon (WSTFA), Faith Critzer & Girish Ganjyal (WSU), Manoella Mendoza and Mackenzie Perrault (WTFRC), Rob Atwill, Missy Partyka, and Ronny Bond (UC Davis), Bonnie Fernandez-Fenaroli (CPS)

Other funding sources

Agency Name: WA SCBGP
Amt. requested/awarded: $ 216,682 Title: Enhanced food safety education and training for tree fruit producers (awarded to WSTFA with Jacqui Gordon as PI)
Agency Name: FDA
Amt. requested/awarded: $243,651 for FY18 awarded to WCFSS (Atwill et al.) Title: Facilitating implementation of FSMA regulations for agricultural water quality
Agency Name: CPS
Amt. requested/awarded: $290,000 to Zhu and Suslow; Title: Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention

WTFRC internal program budget:

Item	2018	2019
Salaries[1]	2,975	3,100
Benefits	1,220	1,325
Wages[2]	6,000	7,500
Benefits	3,180	3,230
RCA Room Rental		
Shipping		
Supplies[3]	200	350
Travel[4]	3,500	3,100
Plot Fees		
Miscellaneous		
Total	**17,075**	**18,605**

Footnotes:
[1]Salaries: 5% of Mendoza (with 41% benefits), not included in salaries: 15% of Hanrahan time, 1% Schmidt
[2]Wages: 53% benefit rate
[3]Supplies include 1 poster for ASHS and misc.
[4]Travel includes: CPS annual meeting, 3 trips to WSU in Pullman, in state day travel to attend trainings, Annual NW Food Safety and Sanitation Conference in Portland

OBJECTIVES

1. Enhance collaboration in all areas of food safety (research, policy, FSMA implementation)
    a. Participate in development of training for industry
    b. Develop an effective food safety outreach program

SIGNIFICANT ACCOMPLISHMENTS IN 2018

Food safety remains one of the highest priority items within the industry. As some compliance dates of FSMA have been effective, it is of utmost importance to continue to provide the Washington growers with timely assistance. Further, in order to attract microbiologists to work on problems related to food safety for tree fruit, a strong collaboration from scientists with a horticulture background is of great advantage to ensure that project goals and outcomes reflect immediately actionable items. Lastly, translating research into layman's terms and providing a bridge between science, politics and farming is another important goal of this project.

Research:

We participated in a number of on-going and new collaborative projects, funded by WTFRC, CPS, SCBG and FDA (see Table 1). Notably, WTFRC is increasingly sought as collaborator in national grant applications to NIFA or SCRI.

The WTFRC, under leadership of Ines Hanrahan, continued to serve as a partner in research for the Center for Produce Safety (CPS) and she attended the annual meeting in Charlotte, NC. Tree fruit specific research priorities were developed and integrated into the annual CPS call for proposals, with the help of the NHC Food Safety Committee. During the proposal process Dr. Hanrahan frequently serves as specialist to answer questions asked by scientists preparing to propose new research projects. Currently one project involving local scientists has been funded by CPS: 'Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention' (Zhu/Suslow; $290,000). WTFRC has developed and executed a packing line survey for this project in 2017 to determine the current industry practices related to spray manifold interventions and is currently assisting Dr. Zhu's team to set-up packingline validation studies with industry collaborators. The team is also sourcing fruit for the experiments. In January of 2018 Hanrahan has also participated in a SCRI industry relevance review of 11 proposals submitted to USDA NIFA in the area of food safety.

Table 1: Summary of WTFRC collaborations* in food safety research in 2018 and pending research for 2019

Keyword	PI's	Affiliation(s)	Funding Source	Amount
Continuing/finishing/new in 2018				
Listeria storage*	Zhu/Amiri/Hanrahan	WSU, WTFRC	WTFRC	195,414
Imp. Dryer	Ganjyal/Zhu	WSU	WTFRC	57,000
Water sampling	Partyka/Bond	UC Davis, WTFRC	FDA	243,651
Food Safety Training	Gordon	WSTFA	SCBG	216,682
List. Cleaning*	Zhu/Hanrahan	WSU, WTFRC	WTFRC	306,285
FMSA PCHF	Ganjyal	WSU, WTFRC	WTFRC	98,971
Brush bed sanitation*	Critzer et al.	WSU, WTFRC	WTFRC	51,967
Listeria monitoring	Kovacevic et al.	OSU	ODA SCBG	174,540
Packing sanitation*	Critzer et al.	WSU, WTFRC	WTFRC	203,000
Rapid detection tools*	Critzer	WSU, WTFRC	WTFRC	112,000

Ozone in storage*	Zhu	WSU, WTFRC	WTFRC	300,000
E.Faecium as surrogate	Zhu et al.	WSU	WA-SCBG	250,000
Water treatment	Critzer	WSU	WA-SCBG	194,000
Pending for 2019				
Apple slices*	Zhu	WSU	WTFRC	TBD
Microbiome*	Zhu	WSU	WTFRC	TBD
PSR cost effective mgt.*	Danyluk et al.	U. Florida	SCRI-CAP	TBD
Antimicrobial coatings	Wang et al.	UC-Davis	USDA-NIFA	TBD
Lm survival/biocontrol	Amalaradjou et al.	U.Conn.	USDA-NIFA	TBD

*collaborations involve a separate WTFRC internal budget

FSMA implementation: In order to best serve the needs for timely information distribution related to new laws pertaining to food safety, WTFRC staff (Hanrahan) leads efforts to coordinate all outreach activities by the various industry organizations. Specifically, NHC (policy), WSTFA (education) and WTFRC (research) efforts have continued to be combined and talking points were coordinated to prevent further confusion, when learning how to implement the already complicated laws. Further, the WSTFA continued to host numerous PSA training sessions in 2018, and Hanrahan served as a trainer for two modules in April. WTFRC staff assisted in meeting logistics and Hanrahan frequently serves as expert to help field questions. In February, Hanrahan attended a two-day listening session related to the ag water rule and in March she was invited to present at the annual Western Regional Center to Enhance Food Safety Meeting at UC Davis. In May, Hanrahan attended an OFRR (on farm readiness review) training in Oregon and in November she participated in an FDA listening session on the draft guidance for the PSR by participating in a panel discussion.

Development of industry training modules: In collaboration with WSTFA, NHC, and WSU, we repeated the existing workshop module "Putting Cleaning and Sanitation Programs into Practice (in Spanish)" and "Verification of cleaning and sanitation programs for tree fruit packinghouses: a hands-on environmental monitoring workshop". These workshops provide a combination of classroom and hands-on activities and take place in collaborating packing facilities (Table 2). Dr. Hanrahan's contributions to these workshops include: leading of general curriculum development, being a trainer, delivering talks, and helping with logistical support (including staff). Secondly, the same group, in collaboration with UC Davis will hold a workshop named: FSMA water quality testing (moved to 2019). This module will also be a repeat of a curriculum originally developed in 2016. It is the first of its kind in the nation to address practical considerations for water testing under FSMA. Workshops were designed to give participants theoretical background in combination with outdoor activities geared towards learning based on examples coupled with hands on training (Table 2).
In addition, WTFRC is collaborating with the WSFTA to develop a series of food safety videos. In 2017 we finished and the WSTFA distributed two videos: Hand Washing Training, and Cross Contamination vs. Cross Contact. These videos are available in both English and Spanish upon request from Jacqui Gordon (jacqui@wstfa.org). For a 2018 release, we have finished a video on Good Agricultural Practices in the orchard and another video in the packinghouse (the four zones). WTFRC personnel contributes to content development, video shooting, voice over, and development of a training module to teach growers how to best use these materials when training their crews.

Table 2: WTFRC staff involvement in WSTFA sponsored food safety trainings in 2018

Name of Workshop/Training	**Date**
FSMA Water Quality Testing Workshop Wenatchee	In 2019
FSMA Water Quality Testing Workshop Yakima	TBD
Putting cleaning and sanitation programs into practice – Yakima (Spanish)	April
Environmental monitoring	July

Food Safety outreach:

Based on industry feedback, Dr. Hanrahan developed a Q&A series in collaboration with the Good Fruit Grower to answer frequently asked questions related to food safety. In 2017, a total of 5 pieces were published, and for 2018 another article has been published in the February 15th issue. More pieces will be written as needed.

In addition, Ines Hanrahan is serving as an adjunct faculty member for the WSU School of Food Science. She is currently participating as a committee member on two Ph.D. and two MSc. committees in the Food Science Department. All students will work in the general area of food safety on very relevant tree fruit industry topics and are interested in a career in tree fruit upon graduation.

Further, Dr. Hanrahan is serving on the stirring committee of the PNW Food Safety and Sanitation Conference, a regional conference with 450 attendees annually. Other outreach activities may include but are not limited to: posters at national/international meetings, invited talks, lectures for WSU classes.