Apple Crop Protection Research Review

Yakima Convention Center

			Thursday, January 30, 2020	
Time	Page	Presenter	Project Title	Yrs
8:30		Hanrahan	Introduction/Video presentation: 50th Anniversary of WTFRC	
			Continuing Projects	
8:45	1	Beers	Optimizing sterile insect release of codling moth in Washington	18-20
8:55	8	Gut	Optimization of release strategies for sterile codling moths	19-20
9:05	14	Knight	New attractants for monitoring MD and mass trapping of codling moth	19-20
9:15	22	Beers	Integrated control of BMSB **(funded off-cycle)**	19-21
9:25	3	Amiri	Rapid lab and field detection of two major apple quarantine pathogens: ***NCE***	17-19
9:35	36	Amiri	Understand the epidemiology of Botrytis to curb gray mold postharvest	18-20
9:45			**Coffee break with scientists**	
10:15	41	Hopkins	Using cold storage to increase the stability of honey bee supply: ***NCE***	18-20
10:25	46	DuPont	Integrated fire blight management	19-20
10:35	56	DuPont	Implementation of alternative methods to control replant disease: ***NCE***	17-19
10:45	64	Amiri	Outreach program for apple decays management in WA	19-20
10:55	68	Amiri	Pre- and postharvest disease management in organic apple systems	19-21
11:05	76	Schmidt	Apple pesticide residue studies	18-20
11:15			**Coffee break with scientists**	
11:45			**Committee lunch/Continuing Report Discussions**	
			Final Reports	
1:30	79	Khot	Data to model apple airblast spraying drift exposure levels	18-19
1:45	91	Johnson	Refinement of practical fire blight control: Non-antibiotic and SAR	18-19
2:00	104	Jones	Optimizing control for leafrollers and western tentiform leafminer	17-19
2:15	113	Jones	Evaluating and improving biological control of WAA	17-19
2:30	124	Beers	Brown marmorated stink bug control in Washington	16-18
	136	Nabity	Assessment of apple immune responses to WAA saliva**:** ***Written report only***	16-18
	149	Knight	Improved monitoring and lure and kill for cm management**:** ***Written report only***	18

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project: CP-18-104

Project Title: Optimizing sterile insect release of codling moth in Washington

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Cooperators: Bill Brauchla (Wilbur Ellis), Jay Brunner (WSU Professor Emeritus), Melissa Tesche (OK-SIR)

Total Project Request: **Year 1: $**98,947 **Year 2:**$98,359 **Year 3: $102,711**

Other funding sources: **Awarded**
Amount: $29,724
Agency Name: Western IPM

WTFRC Budget: ***None***

Budget 1
Organization Name: Washington State Univ. Contract Administrator: Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 Email address: arcgrants@wsu.edu/ shelli.tompkins@wsu.edu

Item	2018	2019	**2020**
Salaries[1]	58,940	61,298	63,750
Benefits[2]	20,046	20,847	21,681
Wages[3]	6,240	6,500	7,020
Benefits[4]	591	616	665
Equipment	0	0	0
Supplies[5]	5,767	2,200	2,200
Travel[6]	7,363	6,898	7,395
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	98,947	98,359	**102,711**

Footnotes: [1]Salaries for project manager (1 FTE) and technician (0.075 FTE); [2]benefits at 33.5% (project manager) and 41.8% (technician); [3]Wages for time slip: $12.50/hr (yr 2), and $13.50/hr (yr 3) for 13 weeks/summer; [4]benefits at 9.5%; [5]Supplies: computer, printer/software; lab/office supplies, electronics; video camera/accessories, sterile moths and release stations, bands. [6]Travel to plots, motor pool rental, fuel, per diem, travel to industry meetings to present results

Objectives:

1. *Determine the effect of fixed vs variable release rate on efficacy of SIR*.
   We have completed the second year of releases in the replicated plot study in the Tonasket/Malott area. We will continue releases in 2020 using the same treatment scheme and sampling methods as 2019.
2. *Compare the non-target effects of broad-spectrum pesticide use versus SIR as a supplement to mating disruption in organic orchards*.
   This objective was discontinued in 2019 based on 2018 results.
3. *Examine the synergy between SIR and other tactics using modeling techniques*.
   Models that look at the interaction of multiple mortality/fecundity factors must first be underpinned with field or laboratory estimates of the effects. Initial attempts to investigate the complementarity of mating disruption and SIR using mating tables failed, and an alternative method using molecular markers is currently ongoing. If this method proves reliable, field testing will commence in 2020.
4. *Investigating sterile moth recapture and behavior.*
   We investigated recapture of sterile insect near net installations and the effect of high densities of sterile females. Additionally, we investigated the apparent longevity/viability of sterile moths in the field.

Significant Findings

- Recapture of sterile moths peaked during July and late August but was much lower in spring and fall.
- The recapture rate of sterile moths was higher in the 1x compared to the 3x rate.
- Sterile:wild (overflooding) ratios ranged from 1-15 depending on treatment and time of season.
- Codling moth fruit damage did not differ among SIR treatments at the end of the first generation, but all treatment means were <0.5% damage.
- Damage by codling preharvest was numerically similar in all treatments
- Fruit damage maps revealed the spatial pattern of damage, with the possibility of treating 'hotspots' with higher rates of SIR in 2020.
- Fewer released moths were recaptured inside a full net enclosure compared with an open orchard; recapture under overhead nets was intermediate between the two. The highest number of both sterile and wild moths were recaptured in the check plots compared to the full cage and overhead net plots. The fewest sterile moths were found in the full net enclosures.
- When higher densities of female moths were released, the % recapture of females was significantly lower than the lower release density treatments.
- Moth recapture peaked at three days post-release and declined over time. Sterile moths were detectable for nine days post release.

Obj. 1. Determine the effect of fixed vs variable release rate on efficacy of SIR

The plots identified in 2018 were used in 2019, with the exception of one orchard which was removed; a different orchard on the same ranch was substituted. Otherwise, the same treatments were applied to the same plots as the previous year (Table 1). Each of the three treatments had four replicate plots, for a total of 12 plots. All orchards are organic apple orchards in the Tonasket/Malott area. Plots range from 4-8 acres in size, with range of 0.1 to 1.8 miles between plots to minimize moth spillover. All plots use codling moth mating disruption and received a complete organic control program (petroleum oil, CM virus, and optionally, Entrust) at the grower's discretion.
Moths were transported from the Osoyoos facility on Sundays and released on Mondays by an unmanned hexacopter (Hermes V2 UAS) by M3 Consulting of Phoenix, AZ to the 8 SIR plots

(treatments 1 and 2). Moths were released weekly for 22 weeks (22 April through 16 September) using a release device on the aircraft calibrated to deliver the specified rate evenly over the plot. On a per-acre basis, the 1x (constant) treatment received 22 dishes (17,600 moths) during the season, and the 3x (varying) treatment received 53 dishes (42,400 moths); because of the varying rate, the 3x treatment actually received only 2.4-fold the number of moths as the 1x treatment.

Table 1. Treatments tested for codling moth SIR

Trt.	Description	Sterile moth rate
1	1x SIR	Std. CM program + std. rate of SIR (800 sterile moths/acre/week)
2	3x SIR	Std. CM program + gradated rate of SIR Base rate increased to 2x (1,600 moths) and 3x (2,400 moths) following wild moth phenology
3	Check	Std. CM program + insecticides; no SIR moths

Moth densities and distribution were sampled using plastic Delta traps baited with the CM-DA+AA lure (codlemone, pear ester, and acetic acid) (Fig. 1A). Traps were deployed at a density of 1/acre in

Fig. 1. Plastic delta trap with CM+DA+AA lures (A); codling moth genitalia, male (B) and female (C); note internal red dye showing through integument of the female.

a grid pattern. Liners are changed weekly, and moths categorized by sex (Fig. 1B,C), mating status, and origin (sterile, wild). Sterile moths were identified by crushing the abdomen to see the internal red dye used in the larval diet. Lures were changed every 6 weeks as per manufacturer's recommendations. Traps were counted the entire release season plus one week before and after the initial and final releases. Trap results were summarized and mapped using GIS software (Fig. 2) and sent to grower-cooperators weekly along with information on the sterile:wild ratio.

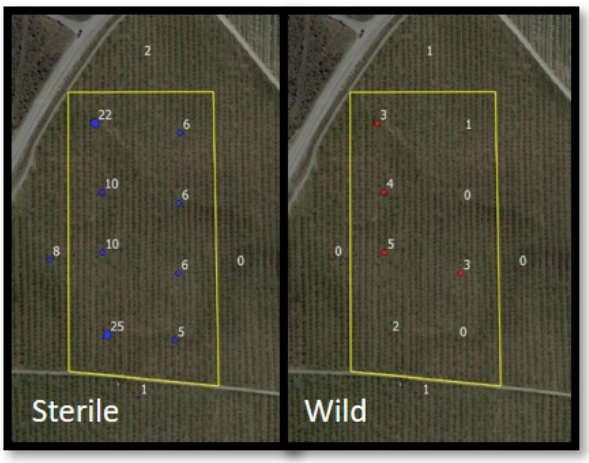

Fig. 2. Bubble map of weekly trap catches.

The success of the treatments was measured with fruit damage samples (codling moth stings and entries) after the completion of the first generation (ca. late June), then again pre-harvest for each variety (August-October). The location (latitude/longitude) of each damaged fruit was recorded and mapped to determine spatial patterns and relationship with trap captures.

Results. Sterile moth recapture peaked in mid-summer (July-August) in the 1x release rate and in late June and late August in the 3x release rate (Fig. 3). Studies by Canadian researchers identified

the issue of relatively poor performance of the sterile moths at cooler temperatures, possibly related to rearing conditions. Overall, the interior traps in the 3x treatment recaptured 1.05-fold as many moths as the 1x treatment. The percentage recapture of sterile moths was more than twice as high for the 1x (1.15%) compared to the 3x (0.48%). The sterile:wild ratios fluctuated during the season, influenced by the response of sterile moths to traps and the generational peaks of the wild population. Ratios were generally highest during mid-summer, and somewhat higher in the 3x rate (0.8-15, mean 6.4) than in the 1x rate (0.5-15.4, mean 6.1). The proportion of moths captured by the interior vs exterior traps was consistently higher over time, irrespective of treatment (data not shown).

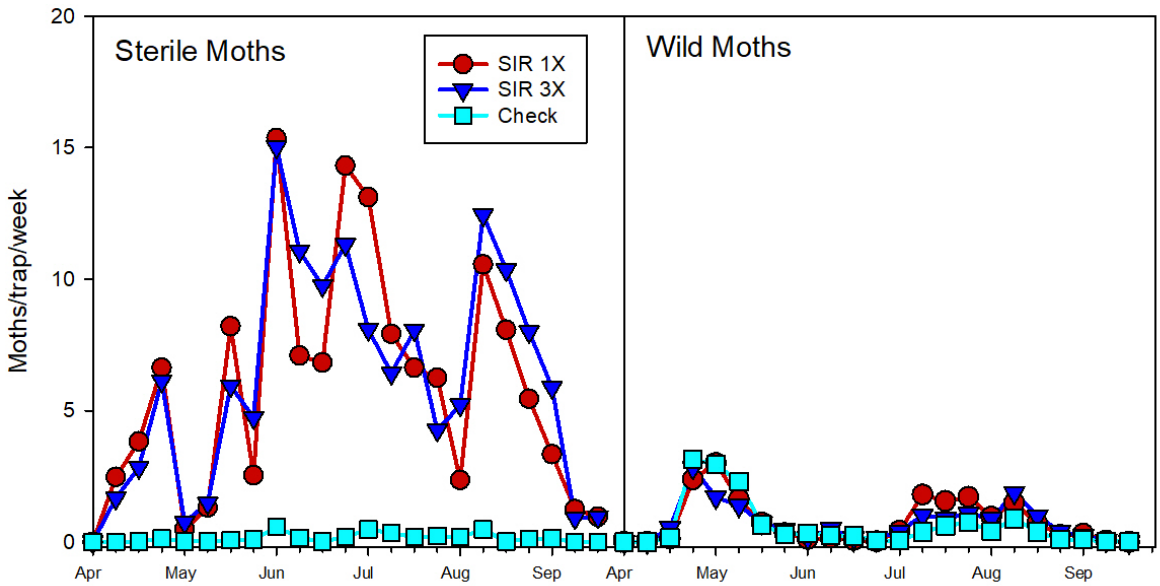

Fig. 3. Capture of sterile (left) and wild (right) moths, 2019

Wild moth densities showed generational peaks, with the strongest peak (late May) in the first generation. There were <4 moths/trap/week throughout the season, in contrast to the 2018 season, when the first generation peaked at about 10 wild moths/trap/week (data not shown). There were no significant differences between wild moth capture between the treatments.

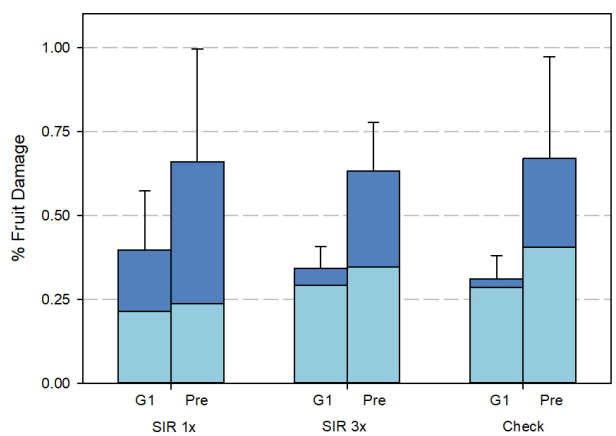

Fig. 4. Codling moth fruit damage, 1st generation (G1) and preharvest (Pre), 2019.

Codling moth damage was ≤0.4% at the end of the first generation (late June), and averaged 0.65% in the preharvest evaluation. (Fig. 4). Damage levels among treatments was similar. Additionally, total codling moth damage was positively related to wild moth capture (higher fruit damage levels where higher wild moth numbers) (Fig. 5). Fruit damage levels below 0.5% were associated with weekly moth captures of <0.5 wild moths/trap. Note that the trap captures are specific to our rather high trap density of 1/acre.

The intensive, spatially referenced fruit damage sample at preharvest provides confirmation of trap data regarding areas of higher damage in some of the plots. Many of the plots had damage fairly evenly distributed (Fig 6A), while in others, damage was concentrated in one area of the orchard (Fig. 6B). Information of this type will allow future moth releases to be increased in high-damage areas in an effort to reduce overall damage.

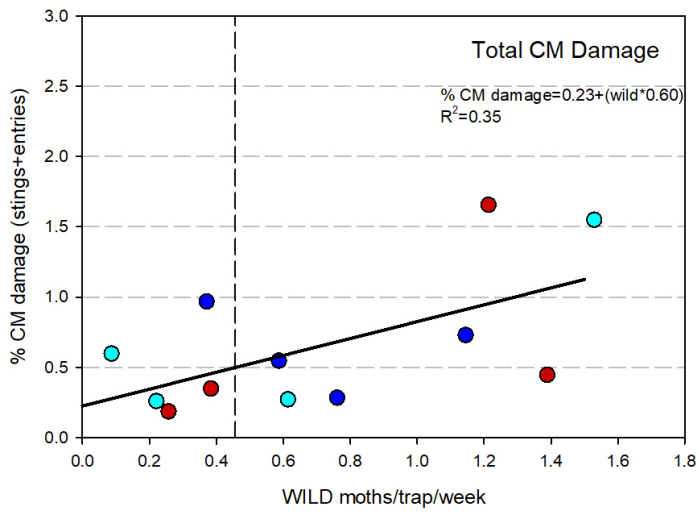

Fig. 5. Relationship between wild moth densities and fruit damage, 2019.

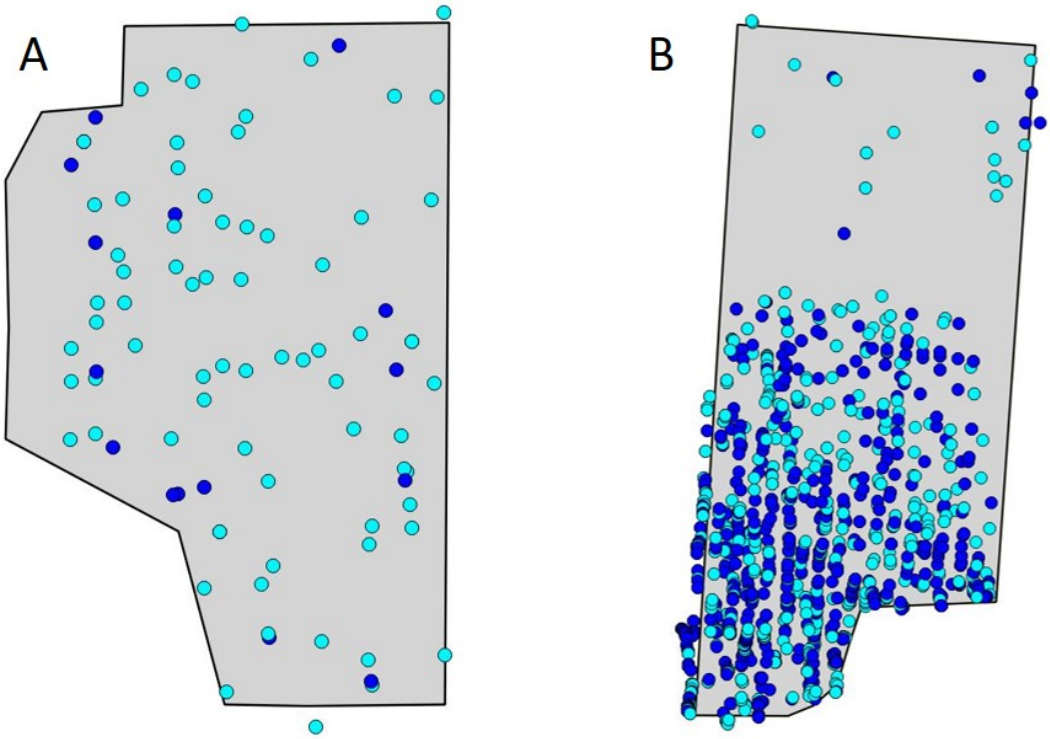

Fig. 6. Spatial pattern of fruit damage. A, evenly distributed, B, clustered.

Obj. 3. Examine the synergy between SIR and other tactics using modeling techniques.

In order to construct a predictive model of the interacting effects of multiple control tactics, the magnitude of those effects must first be estimated from laboratory or field data. Development of predictive models dating back to the 1950's have suggested that a critical component to SIR success is the ability of sterile males to compete with wild males for mates. One complicating factor for application to tree fruit pests is the interaction between SIR and mating disruption, which both reduce successful matings, but by very different methods. While pheromone trap captures (the ability to locate a phermone source) are frequently used as a proxy for mating success, there may be great disparities between the total trap capture and the male who mates first with a wild female. Because moths typically only mate once, this order of arrival is critical, and ultimately determines whether mating of wild females leads to fruit damage by larvae. Mating tables are considered the most accurate measure of this rate of larval production, but are laborious to deploy, and the low proportion of mating of tethered females on a given night makes sufficient replication challenging. We propose a more direct measure, that of examining the spermaophore in mated wild females, and determining whether it came from a wild or sterile male. This is based on the assumption that wild and sterile moths have genetic differences that can be detected using molecular methods.

Resuls. In 2019, we sampled the Osyoos colony periodically and preserved moths for future molecular diagnosis to look at variation over time. We also have genetic material from various regions in the state from wild codling moth populations. To date, we have not found a method that will successfully differentiate between a moth from the Osoyoos colony (before or after irradiation) and a wild moth. This work will continue in 2020.

Obj. 4. Investigating sterile moth recapture and behavior

Moth releases near overhead nets or net enclosures
Net installations, both overhead and full cage, are becoming more common in Washington orchards. The numerous benefits of such installations, coupled with the prevalence of sunburn-sensitive cultivars, make them an attractive option. In addition to horticultural benefits, net installations will have implications (both positive and negative) for pest control. While previous research has been aimed at excluding codling moth, the availability of SIR means we need to explore the question of dispersal, retention and behavior of codling moth inside or under nets

This experiment was conducted in an orchard in Douglas County, WA which had an existing installation of both a full net cage and an overhead net. These two treatments were compared with a check which had no nets (10-26 acres/plot). We deployed traps (CM-DA+AA lures in an orange plastic Delta trap) 50 ft inside the perimeter of the blocks, and marked sterile moths from the OK-SIR facility in Osoyoos, BC, were released 50 ft outside of the perimeter of the blocks. There were about two-thirds as

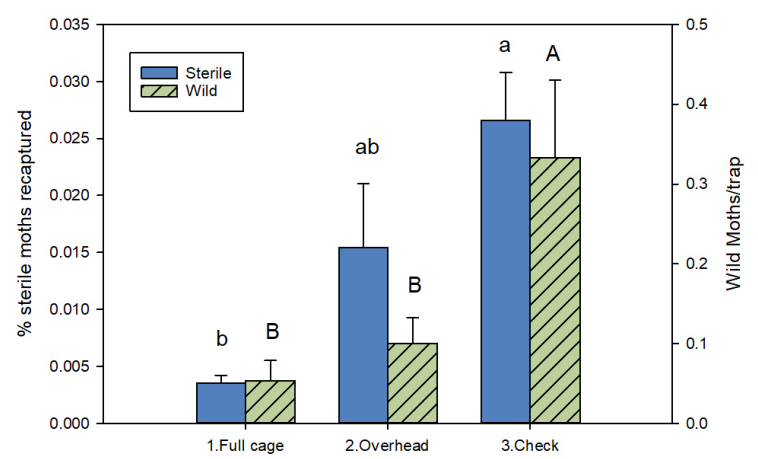

Fig. 7. Recapture of sterile and wild codling moth inside full cages or under overhead nets compared to a check.

many traps as release points, plus a trap in the center of the block. Both sterile and wild moths were counted. Treatments were replicated over weeks (n=7); the number of moths available in a given week were divided evenly among release cups. The total number of moths per week released was the product of moths/cup x release points/block. The recapture was expressed as the percentage of released moth recaptured on a per-trap basis.

Results. Overall, the percentage of sterile moths recaptured was low (<0.4%); likewise, wild moth densities were also low (<0.35 moths/trap/week) (Fig. 7). For the recapture of sterile moths, the highest numbers were caught in the check/no net plot, the lowest in the full cage plot, with the overhead net intermediate. Interestingly, the same trend occurred for wild moths, although the underlying population was unknown at the beginning of the experiment.

Varied sterile female density releases
This experiment was conducted in an orchard in Douglas County, WA. Three treatments were compared to each other and replicated over 4 weeks. The treatments were deployed in plots that were at least 0.1 miles from each other. We deployed traps (CM-DA+AA lures in an orange plastic Delta trap) in radii of increasing distance from a release point (~ 25 m, 50 m, and 75 m). The treatments were varying densities of female moths (1x females (800), 2x females (1600), 3x females (2400) with number of males held constant (800). The moths were released and traps checked every Tuesday. Both sterile and wild moths were counted.

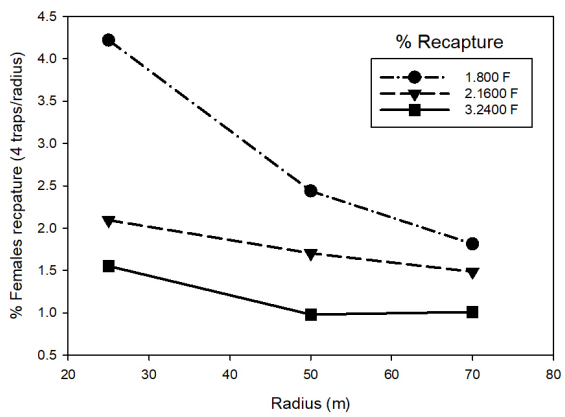

Fig. 8. Percent recapture of sterile female moths under different release densities, 2019.

Results. Overall, the percentage of sterile moths recaptured was low (<1.2%). The percent recapture was significantly higher in the low female density treatment in the trapping radius closest to the release point. Recapture decreased with increasing trap distance from the release point (Fig. 8).

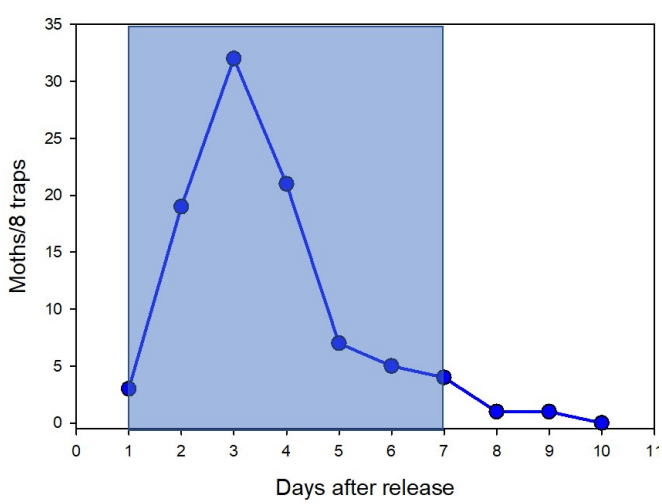

Fig. 9. Recapture of sterile moths over time, 2019.

Sterile moth accumulation in traps
This experiment was conducted in a 2-acre pear block in Chelan County, WA. Eight traps (CM-DA+AA lures in an orange plastic Delta trap) were deployed in the block and there were four moth release points (800 moths per release point, 3,200 total). Trap liners were counted daily to assess the duration of recapture. The traps were checked until recapture ceased (10 days post-release).

Results. The total recapture of the moths was 2.9%. Moth recapture was very low on day 1 and peaked on day 3. Recapture steadily decreased after day 3 (Fig. 9). Overall, the majority of recaptured moths (98%) were caught within a week (shaded area).

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

Project Title: Optimization of release strategies for sterile codling moth

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Cooperators: Dustin Krompetz (M3), Julianna Wilson (MSU)
Total Project Request: **Year 1:** $125,000 **Year 2: $125,000**

Other funding sources: **None**
WTFRC Budget: None

Organization Name: Michigan State University **Contract Administrator:** Diane Cox
Telephone: 517-884-4243 **Email address:** contractteam2@osp.msu.edu

Item	2019	2020
Salary: Project Manager[1]	$25,168	$25,671
Benefits: Project Manager[1]	$3,084	$3,270
Supplies: Project Manager[1]	$4,248	$3,559
Wages: Time Slip Staff[2]	$19,040	$19,040
Benefits: Time Slip Staff[2]	$4,809	$4,809
Project Vehicle[3]	$4,900	$4,900
Fuel[3]	$7,000	$7,000
Misc. Field Supplies[4]	$4,751	$4,751
Travel[5]	$7,000	$7,000
SIR CM, delivery and release[6]	$45,000	$45,000
Total	$125,000	**$125,000**

Footnotes: [1]Project Manager: Rob Curtiss: 50% Salary ($25,168 +2% increase in year 2) + Fringe ($3,084 yr 1; $3,270 yr 2) + supplies ($4,248 yr 1; $3,559 yr 2). [2]Time Slip Staff: Two technicians at $14/hr for 40 hr/wk 17 weeks ($19,040) + 0.0765 fringe rate ($1,456.56)+Health @419/mo. [3]Project vehicle: One rental vehicle @$1,225/month for 4 months (Enterprise Car Rental) + Fuel: 240-300 miles/day*3 days/week* $0.54/ mile (MSU 2019 Mileage rate). [4]Misc. Field Supplies: Traps, Liners, lures, etc. [5]Travel: PI and Key person 2 travel to WA field sites 2x/year @$1,750.00/ trip/ person. [6]Sterile CM Delivery and Release: M3 Consulting Group Sterile Codling Moths + Delivery, and UAS release missions throughout field season and LIDAR missions for 3 orchards.

OBJECTIVES: Recap project objectives. Delineate the goals and activities for the next year. Include schedule of activities and anticipated accomplishments. Explain any deviations from original objectives or schedule.

Original Project Goal and Objectives

The overall project goal is to provide information that forms the basis of a best management practices recommendation for the use of Sterile Codling moths on a farm-scale in Washington. If farmers are to use this technology at their own cost, they will need to know the most efficient and cost-effective way to implement this method; we plan to provide that information by the conclusion of this project.

1 – Determine if orchard factors impact dispersion of SIR CM – **Completed 2019**

a) Determine impact of orchard architecture on distribution of released CM,
b) Determine impact of topography on distribution, and
c) Correlate topography and architecture impacts on distribution of CM.

2 – Determine if release factors impact dispersion of SIR CM – **To be completed 2020**

a) Determine the optimal target release altitude,
b) Determine if distributed or point releases are optimal for dispersion, and
c) Determine the optimal time of release.

2020 Project Goals and Activities

Washington apple orchards from George to Tonasket will be used as sites for releases of sterilized codling moths from the Osoyoos-Kootenay Sterile Insect Release (OKSIR) facility. There are varying topographies, architectures, and other orchard factors throughout the area. Release plots will be 10 acres in size, and sufficiently separated to minimize interference from each other. All plots will receive standardized release rates of 800 sterile moths/acre (400 males and 400 females) for every release. The following treatments testing release factors will be replicated in test plots in 2020:

1) Release altitude
2) Distributed or point releases
3) Time of release
4) Unmanned Aircraft System (UAS) release or hand release

Release altitude:

Preliminary data from 2018 indicates that sterile CM released from a single point 35m above an orchard follow a skewed dispersion. In order to determine if the altitude of release can be optimized to reduce loss of moths out of the target area, sterile CM will be released in replicated plots at varying altitudes: 0-5m, 10-15m, 20-25m, and 30-35m. The 0-5m release will be performed by hand and used as the control for the other releases. All other releases will be performed using a GPS targeted UAS at the test altitude.

Moths will be recaptured in CM-DA+AA baited orange Delta traps placed in a grid around the central release site of each test orchard. Trap liners will be checked and replaced as needed or at least weekly and lures will be replaced every six weeks. Marked sterile CM captures will be sexed and counted for each trap and summarized in 3d maps to provide visual representations of dispersion.

Distributed or point release:

Preliminary data from 2018 experiments indicate that evenness of sterile CM dispersion may be impacted by the location of releases in the orchard. In this experiment we will test dispersion from a central point versus nine evenly distributed release locations in each 10-acre orchard. Moths will be released by hand and will be recaptured in CM-DA+AA baited orange Delta traps placed in a grid throughout the test plots. Trap liners will be checked and replaced as needed or at least weekly and lures will be replaced every six weeks. Marked sterile CM captures will be sexed and counted for each trap and summarized in 3d maps to provide visual representations of dispersion.

Time of release:

Data from preliminary experiments conducted in 2018 suggest that sterile CM dispersion may be impacted by release time of day. Additional data are needed to determine the optimal time to release sterile CM. This experiment will hand release marked moths in 10-acre orchards at varying times of the day. Depending on logistics of obtaining moths from Canada, moths will be released at time intervals from 9:00 am – 9:00 pm. Released moths will be recaptured in CM-DA+AA baited orange Delta traps placed in a grid throughout the test plots. Trap liners will be checked and replaced as needed or at least weekly and lures will be replaced every six weeks. Sterile CM moth captures will be sexed and counted for each trap and summarized in 3d maps to provide visual representations of dispersion.

UAS or hand release:

As efficiencies are identified for sterile insect release, one potential area is the method of release. More ground can be covered in less time using a UAS to release moths, but it is not known if there are other beneficial or detrimental effects of releasing moths using these technologies. This experiment seeks to compare the capture and dispersion of moths released at standard UAS mission altitude (35m) and ground releases. Moths will be released by hand at the center of three 10-acre orchards and by UAS at the center of three 10-acre orchards and recaptured in CM-DA+AA baited orange Delta traps placed in a grid throughout the test orchards. Liners will be replaced as needed or at least weekly and lures will be replaced every six weeks. Sterile moths will be marked as in other experiments and captures will be sexed and counted and summarized in 3d maps to provide visual representations of dispersion from the central release point.

Data for all experiments will be analyzed as described in project proposal.

<u>SIGNIFICANT FINDINGS: Provide a bulleted list of significant findings during the prior year(s) of the project.</u>

Objective 1 – Orchard Factors 2019:

1. Release in standard planting (20 trap control), 15 replicates
    - Moderately high aggregated dispersion (index=1.94), low recapture (0.83%)
    - Higher sterile moth release rates may be required in standard plantings

2. Release in netted orchards (20 traps), 15 replicates
    a. 8' net height
    - Highly aggregated dispersion (dispersion index=2.8), high recapture (5.36%)

- Short nets impede dispersion of released sterile moths

b. 20' net height (20 traps), 15 replicates
   - Moderately aggregated dispersion (index=1.73), high recapture (3.14%)
   - Good dispersion of sterile moths under tall nets

3. Release in varying topographic conditions of orchards (20 traps), 18 replicates
   - Slightly aggregated dispersion (index=1.53), high recapture (4.75%)
   - Hilly topography did not impede dispersion of sterile moths

4. Release in trellised orchards (32 traps)
   a. No trellis standard planting (control), 19 replicates
      - Highly aggregated dispersion (dispersion index=2.56), low recapture (1.67%)
      - Sterile moth dispersion somewhat impeded in standard plantings

   b. V – trellis, 18 replicates
      - Slightly aggregated dispersion (index=1.59), very high recapture 9.15%)
      - V – trellis system highly conducive to the sterile moth technique

   c. Vertical trellis, 18 replicates
      - Near even dispersion (dispersion index=1.39), very high recapture (9.08%)
      - Vertical trellis system highly conducive to the sterile moth technique

METHODS: Outline the methods to be employed.

Orchard characteristics

Apple orchards used for all trials are in Washington State and experiments will be subject to variable management conditions and practices, including various forms of irrigation, mating disruption and insecticide treatments. Orchard blocks for these experiments will be 8-10 acres and receive releases of 800 sterile moths/acre/release.

Source of codling moths

Sterile CM will be obtained from the OKSIR facility in Osoyoos, British Columbia, Canada. Recently eclosed mixed-sex , internally-marked sterile CM will be placed in petri dishes at an approximate 1:1 ratio with 400 males and females, treated with 33 krad of gamma radiation from a Cobalt-60 source, immediately packed into battery-powered coolers (2.8 Cu. Ft. Portable Fridge/Freezer: Edgestar Co. Austin, Texas) held at approximately 5°C, and shipped to Washington State. Sterile moths will arrive at field sites by noon the day after they are packed, and immediately released into field plots. Sexes will not be separated prior to release.

Sterile codling moth dispersion experiments

Handling of Codling Moths: Upon arrival at field sites, moths will be dispensed into 540-ml polystyrene cups (Fabri-Kal Corp. Kalamazoo, MI) in batches of up to 4000/cup, colored using a unique Dayglo florescent dye (DayGlo Color, Cleveland, OH), allowed to warm to ambient temperature, and then released at pre-marked central locations in the blocks. Released moths alight on the leaves and stems of the surrounding trees in all directions. Environmental characteristics will be recorded at time of release based on readings from the nearest Washington State University weather station.

Assessing treatments: Marked moths will be recaptured in Orange Pherocon VI delta traps (Trece Inc., Adair, OK) baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) bisexual lure. Traps will either be placed in a 20-trap grid pattern in order to measure dispersion of moths released from the center of the block, or 32 traps in eight transects radiating from the center to measure direction and distance of dispersion (after Turchin and Thoeny, 1993). Traps placed in grids will be approximately 30 meters apart, while traps in transects will be placed approximately 10 meters apart. Lures will be replaced every 6-7 weeks. Traps will be placed within the top 1/3 of pre-marked trees. Trap liners will be collected once weekly throughout the study period for examination in the laboratory. Wild-type and sexed sterile moths captured in traps will be recorded.

Data Analysis: Average sterile moth recapture will be reported as a percent of total released for each treatment and significant differences will be determined. Sterile moth recapture data will be presented in 3d bar charts and bubble/heat plots as visual representations of average moth recapture. Morisita's index of dispersion will be used to measure degree of evenness or aggregation of sterile moth dispersion for each replicate. Percent recapture will be used to measure the overall effectiveness of the release strategy. An analysis of variance will be conducted on Morisita's indices and % recapture calculated for replicates to compare treatments and determine if there are significant treatment differences. Morisita's index will only be calculated when a replicate captures >0.25% of released moths in 20-trap grids and >0.4% of released moths in 32 trap patterns because below this level, 1 moth/trap+1, the index is not meaningful (Amaral et al., 2014).

RESULTS & DISCUSSION: Focus on the findings during the prior year(s) of the project. Discuss significance to the industry and potential economic benefits. Use summary graphics.

Sterile moth dispersion in trellised blocks was more even and recapture was higher than in systems where trees were large and stand-alone. Moths clustered more in these standard plantings. Trellised systems are highly conducive to sterile moth dispersion (Figure 1).

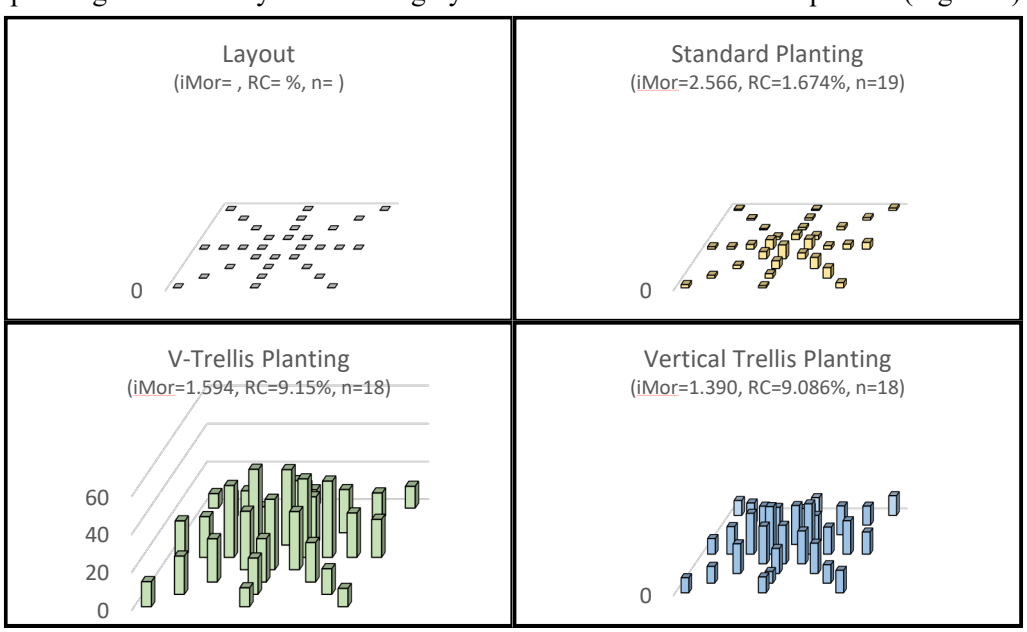

Figure 1. Sterile moth dispersion in several training systems.

Release on steep hills do not appear to prevent downhill spread, and moths released at the center of the block spread across the slope in a manner similar to flat orchards (Figure 2).

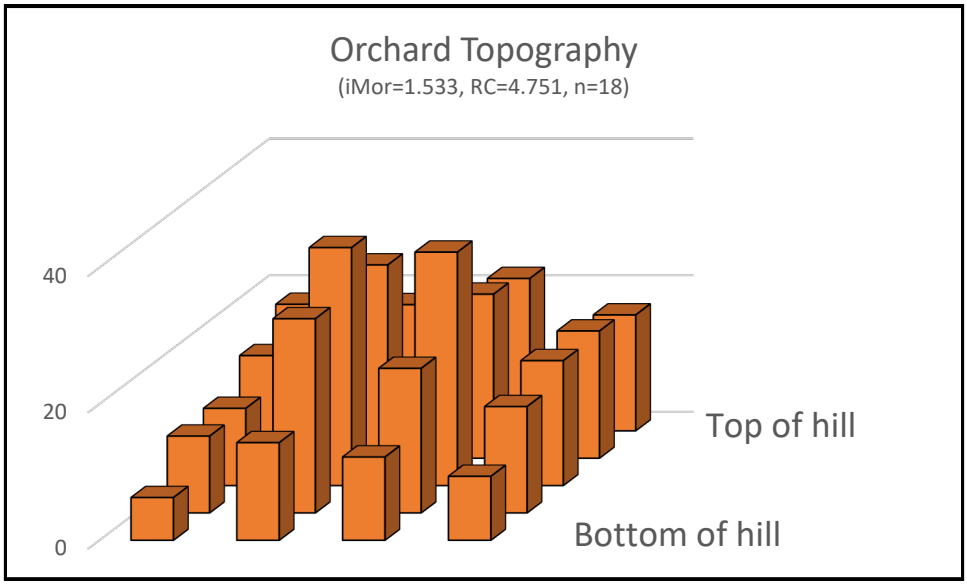

Figure 2. Sterile Moth recapture on a steep hill.

Short nets impede dispersion, and moths dispersed under tall nets in a manner similar to control blocks (Figure 3).

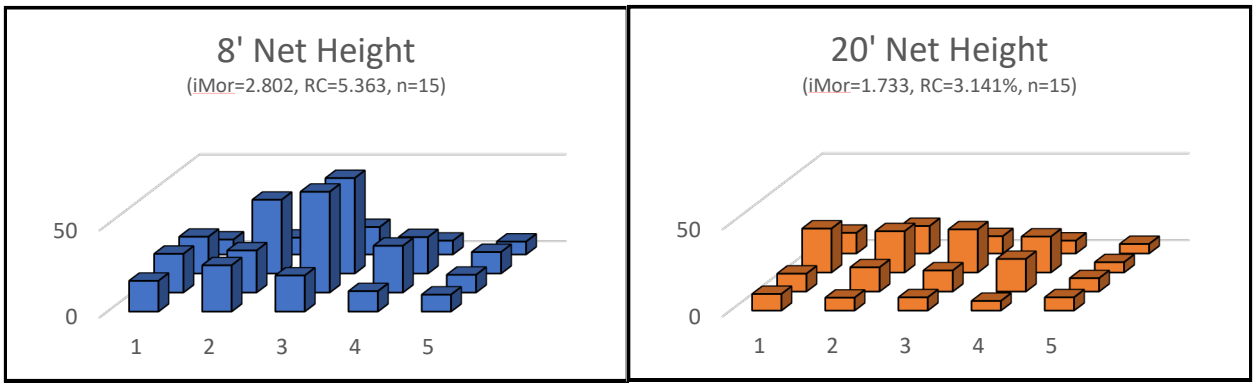

Figure 3. Sterile moth capture under two net heights.

These findings suggest that if Washington farmers use the sterile insect technique to control CM on individual farms, it would be most effective if moths are released in trellised training systems, outside of or under tall nets, and at the center of blocks with steep slopes.

REFERENCES CITED

Amaral , M.K.; Péllico Netto, S.; Lingnau, C.; Figueiredo Filho, A. 2014. Evaluation of the Morisita index for determination of the spatial distribution of species in a fragment of araucaria forest. Applied Ecology and Environmental Research 13(2): 361-372.

Turchin, P. and Thoeny, W.T. 1993. Quantifying dispersal of southern pine beetles with mark-recapture experiments and a diffusion model. Ecological Applications 3(1): 187-198.

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

Project Title: New attractants for monitoring, MD, and mass trapping of codling moth

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Cooperators: Gary Judd, Ag and Agri-Food, B. C., Canada, Bill Lingren, Trécé Inc., OK

Total Project Request: **Year 1:** $53,000 **Year 2: $56,000**

Other funding sources: California Pear Research Commission **Awarded Amount:** **$6,000**

Notes: Trécé Inc. provided *gratis* all of the lures (value estimated at $30,000) used in the project.

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

Item	2019	2020
Salaries	-	
Benefits	-	
Wages	~~18,000~~	
Benefits	~~3,000~~	
Equipment	-	
Supplies	~~7,000~~	
Travel	~~3,000~~	
Miscellaneous	-	
Plot Fees (Research Farm)	~~4,000~~	
Total	**~~$35,000~~ 0**	

Footnotes: Dr. Knight retired from the USDA in June 2019 and the awarded funds were not transferred for his use. Nevertheless, the project was conducted with the approved funds for the three visiting scientists.

Budget 2
Organization Name: Instar Biologicals
Contract Administrator: Alan L. Knight
Telephone: (509) 910-1570 **Email address:** uncfencer76@hotmail.com

Item	2019	2020
Salaries		19,500
Benefits		-
Wages		6,500
Supplies		8,500
Travel		2,000
Miscellaneous		-
Total		$36,500

Footnotes: The funds awarded to Dr. Knight with USDA were not transferred in 2019 due to his retirement. Full funding for Year 2 of this project is requested in 2020.

Budget 3
Organization Name: ASTRA
Contract Administrator: Nicola Minerva
Telephone: +39 335 5974964 **Email address:** Nicola.minerva@astrainnovazione.it

Item	2019	2020
Travel (visit USDA lab July - August)	6,000	6,500
Total	$6,000	$6,500

Footnotes: Mr. Preti is a PhD student in Bolzano, Italy. The requested support will be used both for his research in the USA and in Italy related to the project.

Budget 4
Organization Name: Universidad Austral de Chile
Contract Administrator: Ricardo Leal
Telephone: +56 63 222 1778 **Email address:** rleal@uach.cl

Item	2019	2020
Travel (visit USDA lab June -July)	6,000	6,500
Total	$6,000	$6,500

Footnotes: Dr. Basoalto is an assistant professor at Austral University in Valdivia, Chile. The requested support will be used both for his research in the USA and to continue the project in our off-season in South America.

Budget 5
Organization Name: Instituto Nacional de Investigación Agropecuaria
Contract Administrator: Esteban Cisneros
Telephone: +59 82 3677641 **Email address:** ecisneros@inia.org.uy

Item	2019	2020
Travel (visit USDA lab July - August)	6,000	6,500
Total	$6,000	$6,500

Footnotes: Dr. Mujica is a government researcher in Uruguay and funding supports her work in both the USA and to continue the project in our off-season in South America.

OBJECTIVES:

1. Evaluate the ***4-way K*** compared with standard lures (Combo and Combo plus acetic acid) across a range of orchards including key cultivars of both apple and pear.
2. Continue to test the effectiveness of additional plant volatiles in various combinations compared with the ***4-way K*** and standard lures.
3. Validate the female codling moth-based Biofix approach to time egg hatch using the ***4-way K*** and/or other new lures compared with DAS predictions for several weather stations.
4. Evaluate mating disruption with dispensers loaded with pheromone plus the addition of attractive plant volatiles.
5. Evaluate the use of ***4-way K*** and standard lures in mass trapping of codling moth in organic orchards.

SIGNIFICANT FINDINGS:

- Two binary lures, *MegaLure 4K* ™ and *MegaLure 5K* ™ were formulated by Trécé Inc. based on our characterization of a new more potent lure for both male and female codling moth. The first lure is black PVC and is loaded with pear ester, nonatriene, and linalool oxide or these three plus the addition of sex pheromone in the 4K and 5K lures, respectively. The second white membrane lure is loaded with acetic acid.
- Twenty volatiles were evaluated, and one new volatile was identified that was marginally more effective when substituted in the 4K blend for linalool oxide. Studies in South America are continuing to evaluate new blends.
- Seasonal studies demonstrated that these new lures are effective for at least 8 weeks.
- The 4K lure outperformed the Combo + AA binary lure and the 4K lure caught more female moths than the 5K lure.
- A new Combo-P lure caught the greatest number of total and male moths.
- A clear bucket traps loaded with the 4K lure caught 97-times more females than an orange delta trap baited with the Combo lure.
- Moth catch in 1-gallon milk jugs with two 3” holes was greater than in any commercial bucket trap type tested.
- Three sizes of milk jugs (gallon, half gallon, and quart) each with a pair of holes from 1.5 – 3.0” performed similarly.
- Increasing density up to at least 40/acre did not decrease the efficiency of individual traps.
- Traps can be loaded with mineral oil to retain moths though adding 5% vegetable glycerin improves the preservation of the catch and could diminish off-odors impacting catch.
- Adding acetic acid co-dispensers next to MD dispensers loaded with sex pheromone, pear ester, and two plant volatiles increased moth catches in replicated plots.
- Biofix determination with either 4K or 5K lures in monitoring traps placed at head height (6’) outperformed standard combo lure-baited traps attached to poles and placed in the upper third of the canopy.
- 3,800 traps in 26 paired studies were established in apple/pear blocks across WA, OR, CA, and CO to evaluate the effectiveness of mass trapping to manage codling moth.
- All traps were baited with the Combo-P plus AA lures in the first half and these were replaced with either 4K or 5K lures at mid-season.
- All moths were counted (64,202) and a subsample of moths were sexed (39,510) and females were dissected (4,214) to determine their mating status.
- Fruit injury prior to harvest was up to 70% lower in the trapped blocks compared with the paired untrapped blocks. Similar results were obtained in both apple and pear.

METHODS:
Five types of studies were conducted in the first year of this project. 1. Seasonal comparison of new lures. 2. Evaluation of new dispensers for mating disruption. 3. Evaluating the effectiveness of the new lures to establish a Biofix. 4. Studies to optimize the use of female removal for management. 5. Grower-based field trials to evaluate the effectiveness of female removal in pest management.

Male, female, and total moth catch in orange delta traps baited with four different types of lures were evaluated. The four lures were provided by Trécé Inc. and included the standard grey septa *Combo DA* lure in combination with an acetic acid co-lure, the new *Combo-P* pvc lure in combination with acetic acid, the *Megalure 4K* comprised of two lures the acetic acid lure and a pvc matrix loaded with pear ester, nonatriene, and linalool oxide; and the *MegaLure 5K* which was similar to the 4K lure but had the sex pheromone added to the lure. Eight replicates of each lure were randomized in an untreated apple block beginning in late April. Traps were checked weekly for 11 weeks. The study was repeated in the second flight for 8 weeks in orchards either treated with pheromone or pear ester/pheromone dispensers or untreated.

Experimental 'Meso' dispensers were formulated with sex pheromone and pear ester alone and in combination with either farnesol or linalool. These latter two dispensers were also hung in combination with a high-load acetic acid co-dispenser (TRE1531). Studies were run with 32 dispensers per acre in replicated (N=4) 1-acre plots. Each plot was monitored with two sex pheromone-baited traps loaded with either 0.1 or 3.0 mg active. Traps were checked weekly and the 0.1 mg lures were replaced every two weeks. The study was ended after 8 weeks.

Studies were conducted to assess whether the *MegaLure 4K* could allow pest managers to monitor codling moth at a more convenient height than having to use poles. These studies also looked at the establishment of a Biofix to time the start of egg hatch of codling moth based on either male or female catch.

Several studies were conducted to improve trap performance. We compared seven types of traps baited with either the *Combo-P + AA* lures or the *Megalure 5K*. This study had five replicates with each lure and was run for one month. The second study evaluated the effect of trap density. Three replicates of three trap densities (15, 25, and 40 traps per acre) were established in 1-acre plots. All moths were collected from traps in each replicate after eight weeks, counted and sexed. A third study including 10 replicates examined an orange trap baited with a *Combo-P* lure versus the use of clear bucket traps baited with the *4 K* lure. The liners in the delta trap were replaced after one week and the study concluded after two weeks. A separate study examined moth catch in clear bucket traps that were partially filled with either propylene glycol (PG), 20% PG in mineral oil, and 1.5% neem oil in mineral oil. Six studies were conducted to evaluate new volatile blends with 20 compounds being substituted for linalool oxide in the 4-Way K blend. Eight to 10 replicates were included in each study and moth catch was compared with the 3K and 4K blends.

Extensive studies were conducted to evaluate the use of female removal as a tactic to manage codling moth primarily in organic orchards. Blocks were placed within orchards treated with sex pheromone dispensers, including Isomate Flex, Isomate Mister, or Cidetrak CM-DA Combo PP dispensers or left untreated. All paired blocks were treated with the same growers' program. Plots (1--4 acres) were established with or without the supplemental use of female removal. All female-removal treated plots received 24 traps per acre. All traps were initially baited with the *Combo-P* + AA lures. At mid-season, traps were rebaited with either the 4K or 5K lures depending on our lure supply. All moths were collected from traps at mid-season and prior to harvest. Moths were counted and a subsample of moths were sexed, and females were dissected to determine their mating status. Levels of fruit injury (800-2,400 fruits) were sampled in each of the paired blocks at mid-season and prior to harvest.

RESULTS & DISCUSSION:

All three new lures (Combo-P, 4K, and 5K) outperformed the standard, grey septa Combo lure during the first moth flight (Table 1). The 4K lure caught the greatest number of female moths. A similar pattern was seen during the 8-week period during the second moth flight in MD orchards.

Table 1 Mean moth catches in orange delta traps over an 11-week period during the first flight of codling moth in an apple block not treated with sex pheromone dispensers.

	Mean (SE) moth catch per trap per week		
Lure	Males	Females	Total
Combo + AA	4.0 (0.4)d	2.8 (0.3)c	6.8 (0.6)b
Combo-P + AA	16.4 (1.1)a	3.7 (0.4)bc	20.1 (1.3)a
Megalure 5K	10.9 (0.8)b	5.7 (0.6)b	16.7 (1.3)a
MegaLure 4K	7.7 (1.0)c	9.8 (0.8)a	17.4 (1.7)a
ANOVA df = 1, 30	$F = 36.47$, $P < 0.0001$	$F = 30.74$, $P < 0.0001$	$F = 21.35$, $P < 0.0001$

Two new formulations of dispensers for mating disruption of codling moth were evaluated to see the effect of adding plant volatiles and acetic acid to dispensers already loaded with sex pheromone and pear ester. Linalool and farnesol were chosen based on 2018 data, though neither of these compounds are as attractive as linalool oxide. Unexpectedly, a significant increase in male catch occurred in low-load sex pheromone-baited traps compared with the standard Combo dispensers. These data suggest that the use of the high-load AA dispensers may have attracted males into these plots. Future studies will look at the use of volatiles shown to be more attractive when used in combination with pear ester.

Table 2 Comparison of moth catch in orange delta traps loaded with sex pheromone.

	Mean (SE) moth catch per trap over 8-week period	
MD Treatment	0.1 mg PH lure	1.0 mg PH lure
Untreated	28.4 (9.1)a	96.4 (22.1)
CMDA-Meso	2.4 (0.7)c	59.6 (7.5)
CMDA/Linalool + AA Meso	4.4 (0.7)bc	96.0 (23.0)
CMDA/Farnesol + AA Meso	13.2 (3.9)ab	104.2 (11.9)
ANOVA df = 3, 16	$F = 9.89$, $P < 0.001$	$F = 1.28$, $P = 0.31$

Studies demonstrated that the 4K lure can be used with traps placed low in the canopy without the use of poles (Table 3). This also avoids placing traps near MD dispensers. Male and female moths were caught 1-week earlier in the 4K traps and provided a better prediction of first egg hatch.

Table 3 Comparison of lures and trap height to monitor codling moth.

Lure	Trap height	Mean (SE) moth catch		
		Male	Female	Total
Combo-P	Low	9.0 (6.0)b	0.5 (0.5)b	9.5 (6.5)c
Combo-P	High	47.5 (4.5)a	3.0 (1.0)b	50.5 (3.5)b
MegaLure 4K	Low	25.0 (2.0)b	27.5 (1.5)a	52.5 (0.5)b
MegaLure 4K	High	77.0 (20.0)a	31.0 (5.0)a	108.0 (15.0)a
ANOVA		Height: $P < 0.05$	Lure: $P < 0.01$	Height * lure: $P < 0.01$

Initial studies demonstrated that clear delta-shaped traps outperform the standard orange delta trap when baited with either the Combo-P or 5K lures. Next, we showed how much progress has been made in capturing female codling moth by comparing a Combo-P lure in an orange delta versus 4K in a clear bucket trap (Table 4). The latter trap-lure combination caught 97-times more females than the standard trap-lure combination. A third study compared drowning solutions used in bucket traps. No differences were found with adding Neem oil to mineral oil but it did not preserve samples and allowed microbial growth. Future work will evaluate the addition of vegetable glycerin (organic approval is likely) as a preservative added to the oil.

Table 4 How much better can we catch female codling moths?

Trap	Lure	Mean (SE) catch per trap		
		Male	Female	Total
Clear bucket	4K	194.6 (15.2)a	289.7 (30.7)a	484.3 (42.8)a
Orange delta	Combo	98.2 (10.1)b	3.0 (0.8)b	101.2 (10.8)b
ANOVA df = 1, 18		$F = 30.41$ $P < 0.0001$	$F = 262.5$ $P < 0.0001$	$F = 116.9$ $P < 0.0001$

Studies were conducted early in the season to compare homemade traps with several commercial non-saturating traps. This included a clear peanut butter jar with two 2” holes and milk jugs with two 3” holes. The milk jug caught the greatest number of females and total moths.

Table 5 Trap comparisons with the MegaLure 5K, N = 5, May 2019.

Trap, N = 5	Mean (SE) moth catch per trap		
	Male	Female	Total
Mini bucket – green/clear	54.0 (8.2)ab	74.6 (6.2)bc	128.6 (11.3)ab
Homemade clear jar	84.4 (12.7)ab	83.6 (5.2)ab	168.0 (15.7)ab
Clear bucket	55.2 (15.8)b	79.2 (13.5)bc	134.4 (29.2)ab

Homemade milk jug	133.0 (32.1)a	116.2 (20.3)a	249.2 (50.0)a
Green/Yellow/clear bucket	48.0 (5.2)b	41.4 (6.7)c	89.4 (9.5)b
Green/white bucket	60.2 (9.6)ab	70.0 (15.2)bc	130.2 (23.6)ab
All green bucket	46.0 (9.2)b	45.6 (7.9)bc	91.6 (16.1)b
ANOVA df = 6, 28	$F = 3.37$ $P < 0.01$	$F = 8.69$ $P < 0.01$	$F = 4.05$ $P < 0.01$

Limited studies were conducted with three sizes of milk jugs with a range of hole sizes. No clear pattern was found in these tests and the three jug sizes with a range of hole sizes were significantly more effective than the clear bucket trap.

The key to the effectiveness of FR technology is the ability to remove as many virgin females as possible. However, it is important to match the number of traps (cost of program) with the pest threat. Our initial trap density is based on a consideration of potential cost and earlier work on the range of activity of pear ester on moth catch. Our studies examined three trap densities, 15, 25, and 40 per acre. We found that female catch per trap only declined slightly across these three densities. This shows that adding additional traps is cost effective and should be used to treat higher moth densities.

We evaluated 20 new volatiles in combination with pear ester, nonatriene, and acetic acid. One candidate was found that outperformed the current 4K blend. Further studies are in place in South America this winter to examine various blend substitutions and this new volatile. We also found several volatiles associated with rosy apple aphid damage that reduced moth catch and field evidence that codling moth behavior is severely disrupted in heavily aphid-infested orchards. Further studies will address the use of repellent blends to disrupt moth sexual behaviors.

The project was able to work with growers in four states and helped to establish 26 paired studies. Three groups of these studies are presented in Table 6 as six apple orchards in the Yakima Valley, five pear orchards in CA, OR, and WA, and six apple orchards situated between Quincy and Tonasket. Pest pressure was deliberately high in nearly all of these blocks and growers all used their typical organic spray and MD programs. Data showed that switching from the Combo-P + AA lure to the 4K or 5K lure at mid-season significantly increased the proportion of females caught. Fruit injury across the paired blocks ranged from zero up to 70% lower in the block trapped. The two trials in northcentral Washington that had no difference in fruit injury between the paired blocks were likely the result of the traps being deliberately placed at the higher end of a pest gradient in the orchard. Thus, the equal results across the paired blocks showed that the traps were able to reduce the 'hot spot' down to the background level in the orchard.

Table 6. Summary of field trials using female removal technology to manage codling moth.

		Range among trials				
Region, # trials	Crop	Moths per trap	Prop. female	Prop. virgin female	% fruit injury	Prop. reduction in injury
Yakima, 6	Apple	15 - 56	0.11 – 0.76	0.25 – 0.65	0.5 – 3.1	0.41 – 0.76
CA/OR/WA, 5	Pear	3 - 24	0.11 – 0.73	0.00 – 0.51	0.3 – 8.2	0.34 – 0.75
Northcentral WA, 6	Apple	11 - 36	0.34 – 0.79	0.07 – 0.45	0.2 – 3.2	0.00 – 0.69

Significance / Economics

Tremendous progress was made during 2019 in the development of female removal as a viable tactic to manage codling moth in problem organic blocks. This progress is foremost based on our discovery in 2018 of a new more potent female attractant blend, now coined *MegaLure 4K*. A range of studies were conducted early in the summer to examine various factors critical to improving the effectiveness of female removal, including lure type, trap type, trap density, drowning solutions, and identification of new attractants. Growers will directly benefit from these extensive studies and the effectiveness removing female codling moths achieved next season should be even greater.

Beginning in 2020 the *Megalure 4K* lure will be available for growers to use over the entire season. This lure catches 3x the number of females as the Combo-P + AA. Trapping grids can be improved from this year and treated areas in each block should be expanded to cover the entire area of moderate to high pressure codling moth. Also, trap density will be better matched to pest pressure.

Growers in 2020 can use any trap design, including delta traps, but non-saturating designs such as bucket traps require less maintenance. Milk jugs appears to be a lower cost and more effective approach and are available in a range of sizes. An organic grower in Colorado was able to purchase jugs for as low as $0.20 each and used 1,000 to manage his organic Gala/Fuji block. We also expect that plastic jugs can be reused a few times and hopefully recycled at the end of their useful life. Eliminating the use of Neem oil as an antimicrobial agent and switching to vegetable glycerin will likely improve the performance of traps, perhaps by 25%. While, fruit injury from codling moth achieved in trapped blocks were up to 70% lower than in the comparison untrapped blocks, our data suggests that adopting the suite of improvements validated during the 2019 season will further improve the program as much as 4-fold next season.

Female moth removal would appear to be a more rational approach to manage codling moth than trying to prevent moth mating or to expect wild moths to mate with mass-reared Canadian sterile variants. Every female codling moth, whether virgin or mated, caught in a trap is no longer able to lay eggs in the grower's orchard and is no longer producing larval offspring intent on fouling fruit to munch on apple seeds. Thus, with some accuracy, it is possible based on the known biology of this pest, favorability of the weather, and the value of individual fruits to assess the return to the grower of removing female moths from their orchard. We have prepared a simple spreadsheet that includes the number of eggs laid per female in cool/wet or warm/dry springs and for either two or three moth generations per season. Values for life stage mortalities are added to the model. The price paid to growers for individual apples is the final variable and we have considered a return of $0.05 or $0.25 per fruit. This simple and straightforward analysis suggests that the removal of a single female codling moth early in the summer can be worth $2 to 43 or $34 to 697 if the pest has two or three generations, respectively. One real-world example from an organic apple block in Tieton with two generations found that the value of individual traps ranged from $7.50 to $750 depending on the moth reproduction prevented and apple price. Clearly, this approach makes economic sense to WA growers!

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

Project Title: Integrated control of brown marmorated stink bug

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Cooperators: Dave Crowder (WSU Department of Entomology, Pullman, WA; co-PI and Objective 1 leader of the NIFA SCRI grant on BMSB); Dr. Tracy Leskey (Research Leader, Appalachian Fruit Research Station, Kearneysville, WV; Dr. Rodney Cooper, USDA-ARS Wapato lab; tree fruit grower(s)

Total Project Request: **Year 1:** $96,326 **Year 2: $99,851** **Year 3: $**103,517

Other funding sources: **Awarded**
Amount: $156,047 (Beers program)
Agency Name: NIFA-SCRI
Notes: Five years, 2016-2020**.** Grant #2016-51181-25409

Other funding sources: **Awarded**
Amount: $26,675
Agency Name: Washington State Commission on Pesticide Registration
Notes: One year, July 1, 2019-June 30, 2020. Project #19AN023

Other funding sources: **Awarded**
Amount: $119,667
Agency Name: AFRI Pre-doctoral Fellowship
Notes: Two years, May 1, 2019 – April 30, 2021

Other funding sources: **Requested**
Amount: $16,505
Agency Name: Washington State Commission on Pesticide Registration
Notes: One year, May 1, 2020 – 30 April 30, 2021. Project #20AN014

WTFRC Budget: None

Budget 1
Organization Name: Washington State Univ. Contract Administrator: Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 Email address: arcgrants@wsu.edu/ shelli.tompkins@wsu.edu

Item	2019	**2020**	2021
Salaries[1]	53,395	55,531	57,752
Benefits[2]	21,166	22,012	22,893
Wages[3]	7,800	8,112	8,436
Benefits[4]	725	754	785
Equipment			
Supplies[5]	3,000	3,000	3,000
Travel[6]	5,200	5,200	5,200
Miscellaneous			
Plot Fees[7]	5,040	5,242	5,451
Total	96,326	**99,851**	103,517

Footnotes: [1]Research Technician (Smytheman), 1.0 FTE, [2]Benefits 39.6%. [3]Time-slip wages 13 weeks@$15/hr, [4]Benefits, 9.3% [5]Laboratory, field and office supplies, electronics. [6]Motor Pool rental, April-October. [7]Plot fees for Sunrise Orchard apples.

Objectives

1. Investigate the efficacy and non-target effects of insecticide infused netting as a means of monitoring and control of BMSB
2. Redistribute *Trissolcus japonicus* (the samurai wasp) where established BMSB populations are identified, and monitor its establishment and non-target effects
3. Determine development of BMSB on shrub-steppe plants
4. Track the invasion of BMSB in Washington State

Significant findings

- The poncho trap caught ~3x more BMSB than the ghost trap; however, non-target captures of Hymenoptera were also higher in the poncho trap.
- BMSB were caught in similar numbers inside the orchard in plots bordered by ghost or poncho traps and the control (no trap).
- *T. japonicus* may have been recaptured at two of the 2018 release sites on yellow sticky cards.
- *T. japonicus* attacked *Podisus maculiventris* egg masses at similar rates as BMSB (ca. 22%); attack rates of *Chinavia hilaris* (2%) and *Euschistus conspersus* (3%) were much lower than BMSB.
- A *T. japonicus*-specific primer was designed and optimized for use in molecular diagnosis where the adult fails to emerge. DNA can be detected as soon as eggs are laid in the BMSB egg mass through all points up to 10 days after oviposition.
- BMSB was able to complete development (egg to adult) on an assemblage of shrub-steppe plants at mid- and late season; however, survivorship was lower, and development was slightly slower, than on the colony diet.
- The range of BMSB in Washington State has expanded to 29 counties as of December 2019.

Obj. 1: Investigate the efficacy and non-target effects of insecticide-enhanced netting as a means of monitoring and control of BMSB

1a. Attract-and-kill trap design. A promising control strategy for BMSB being developed in the eastern US is the use of traps constructed of insecticide-infused netting (IIN) placed adjacent to orchards. With the recent confirmed reports of BMSB populations and damage in orchards in Eastern Washington, we began field trials to examine the efficacy of attract-and-kill IIN traps to prevent fruit damage. Preliminary testing in fall 2018 suggested an alternative trap design is up to 3× more effective than the one used in the eastern US. In this experiment, two traps were tested: 1) the current standard 'ghost trap', consisting of IIN draped over a 6 ft post with a tarp underneath to capture dead insects; and 2) a novel design, or 'poncho trap' (Fig. 1), which supports the IIN in a panel shape (7 ft x 3 ft), has flaps sewn in the netting at the middle and top on each side, and is fitted with collection tubs at the bottom. Each trap was baited with three BMSB

Fig. 1. Poncho trap. Inset: BMSB adult caught in flap.

aggregation dual pheromone lures to promote capture and retention.

These traps were deployed along 100 m (328 ft) orchard borders with 3 traps/section at 30 m (98 ft) intervals. Each replicate orchard had three treatments: 1) ghost trap; 2) poncho trap; 3) check (no trap). The ghost and poncho traps were baited with 3 dual BMSB lures (Trécé, Adair, OK), and were place in the bordering vegetation across a farm road from the orchard's edge. Sticky traps (3/plot) baited with a single dual lure each were place 15 m (50 ft) into the orchard interior to detect incursion of BMSB into the orchard. Traps were counted 4 times during the deployment period (July-October), recording BMSB adults and nymphs, and in the case of the attract-and-kill traps, non-target species. Fruit damage (Fig. 2) was evaluated *in situ* using visual scan by two people walking on both sides of a row ca. 40 m (131 ft) from the border and recording damaged fruit that appeared to be caused by stink bugs. In addition, a picked fruit sample was taken from the upper canopy of 8 trees (10 fruit/tree), or a total of 80 fruit/plot. The picked fruit were placed in common cold storage for ca. 4.5 months to allow damage symptoms to develop, and then peeled and examined for stink bug feeding wounds.

Fig. 2. BMSB on neck of pear (left); fruit damage (right)

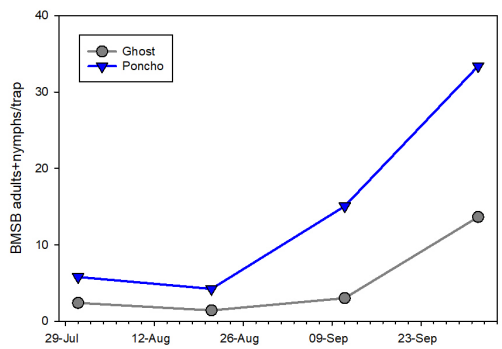

Fig. 3. BMSB captured in attract-and-kill traps, 2019.

Results: The poncho traps caught nearly 3-fold more BMSB than ghost traps, however non-target Hymenoptera captures were also higher. Trap catch was low in late July and August, but rose dramatically in Sept-October (Fig. 3). Incursion trap catch did not differ significantly among treatments. Preliminary assessment of fruit damage did not show any treatment differences; stored fruit have not yet been evaluated.

1b. Physical exclusion, net barriers. Previous trials with net barriers (white, 20% shade, 2 x 5 mm openings;12 ft high × 150 ft long) near the borders of apple orchards in north central Washington have been shown to intercept stink bugs migrating into orchards from the surrounding habitat. To test if the addition of IIN into the net barrier can further reduce stink bug migration, half of each barrier was retrofitted with IIN attached to the existing flaps facing the native vegetation. Three treatments were tested: 1) net barrier with IIN, 2) net barrier alone, and 3) check (no net). To determine the efficacy of the net barrier, stink bug densities were sampled with a beating tray in the orchard border and surrounding vegetation for each treatment area once per week while adults are active (May-Sep). To evaluate the effects of the net barrier and IIN, tarps were deployed against the net on the vegetation side (treatment 1 and 2) and on the ground (treatment 3) to catch dead arthropods. All specimens were collected from the tarps into vials of 70% ethanol weekly from April – October. Species-level identification of stink bugs, and family-level identification of other arthropods in these samples will provide insight into the impacts of physical barriers and IIN on both target and beneficial arthropods.

Results. Stink bug populations in the orchard were lower in the control plots than in the two barrier treatments, although the differences were not statistically different. Fruit damage was lowest in the deltamethrin net treatment, again without statistical differences. About 6-fold more stink bugs were killed by the deltamethin vs plain net barriers (non-significant), however, higher numbers of some non-target species (Neuroptera, Coccinellids, and Hymenopter) were also killed by the deltamethrin-augmented nets.

Objective 2: Redistribute *Trissolcus japonicus* (the samurai wasp) where established BMSB populations are identified, and monitor its establishment and non-target effects

2a. Redistribute the samurai wasp in Washington State. Candidate release sites with a persistent and high density of BMSB (sufficient to sustain the parasitoid) will be identified using the information generated by Obj. 4. We will rear the parasitoid using egg masses from our BMSB colony, increasing the numbers during the growing season. If necessary, additional wasps will be obtained using sentinel egg masses (SEM) placed in Vancouver, WA. Classical biological control theory suggests that 'propagule size', or the number of natural enemies released, may affect the success of establishment. Therefore, we will make repeated releases of the samurai wasp over 3-5 weeks in mid-late summer, when their preferred host, BMSB egg masses, are most abundant in the field. Preliminary studies at the Vancouver sites will compare yellow sticky cards to SEM for their ability to detect the samurai wasp; if these methods are comparable, then follow-up monitoring of releases will be conducted using this method. In addition, sites where the samurai wasp were released in the past will also be monitored for overwintering and establishment in eastern Washington.

Results. The 2018 season was the first widespread release of *T. japonicus* in the state, and the 2019 season was devoted to determining whether it had successfully overwintered in the release sites. The 2019 monitoring used yellow sticky cards, which are less labor intensive than sentinel egg masses survey, but with unknown sensitivity. Sample processing is still in progress, but preliminary scans of the yellow sticky cards indicate at least two sites recovered *T. japonicus* at the release sites. In addition, 960 female *T. japonicus* were released in Douglas County as part of the foraging experiments.

2b. Determine permeability of net enclosures to *T. japonicus*. Shade netting orchard enclosures have the potential to disrupt biological control by altering the behavior of and/or excluding natural enemies. This question is fairly specific to Washington, where net enclosures are becoming more common for exclusion of direct pests including stink bugs. We deployed BMSB egg masses inside and outside small cages enclosing 3 apple trees, and compared them to a check (no cage). *Trissolcus japonicus* was released in the next row (height ca. 2 m, in a cup clipped to the trellis wire) to the east (3 m/10 ft) away from the BMSB egg mass. After three days, the egg masses were retrieved, and incubated to determine parasitism.

Results. No *T. japonicus* were recovered either inside or outside net cages.

2c. Determine the effects of host plant and canopy height by *T. japonicus*. The samurai wasp is thought to be an arboreal species, and the impact of host egg mass position on parasitism success at different heights within the canopy in an orchard context has not been investigated. In addition, the effect of host may influence foraging success.

To address this knowledge gap, we deployed BMSB egg masses and released *T. japonicus* adults on the same day in the next tree row to the east (3 m/10 ft away) of the egg masses. In the host plant test, we placed the egg mass either low on the apple tree, or on a potted pepper plant wired to a tree at the same height to test the effect of host. In the canopy height test, we placed the egg masses at either

1 m or 3 m from the ground pinned to the underside of apple leaves. Each treatment for each test was replicated 6 times, with one fresh BMSB sentinel egg mass per replicate, and 20 female +1-2 male parasitoids per release cup. Egg masses were retrieved 3 days after deployment (their putative period of attractiveness) and incubated to determine parasitism.

Results. Only a single BMSB egg mass (of 6) produced adult *T. japonicus* on the pepper plants, with an abnormally low attack rate (6 adults/13 eggs). No parasitoid attack occurred in egg masses deployed at 1 or 3 m in the apple canopy.

2d. Determine the non-target effects of the samurai wasp on native stink bugs. Research investigating the possible non-target effects is considered an essential part of a classical biological control program, and is crucial to efforts to obtain permits to release biological control agents. While current regulations allow redistribution of the samurai wasp within the state, a national permit may allow the introduction of multiple strains of this parasitoid to optimize biological control against different haplotypes of BMSB.

Thus far, the majority of research on non-target effects of the samurai wasp in the US has been limited to laboratory studies. Preliminary studies of non-target effects in the field were conducted in Washington in 2017-2018 (using sentinel egg masses, or SEM), and established a low to moderate impact on native stink bugs. However, in these studies the SEMs were placed in unnaturally close proximity to one another. Furthermore, studies in the PNW have only examined native pest stink bugs, and the potential nontarget impacts on beneficial stink bugs in field settings remain unknown. In order to test nontarget effects in a more realistic manner, we evaluated the ability of the samurai wasp to parasitize native stink bug egg masses that were temporally separated from BMSB egg masses.

Our previous studies used the native pest stink bugs *Thyanta pallidovirens* (red-shouldered stink bug), *Chlorochroa ligata* (Conchuela bug), and *Euschistus conspersus* (consperse stink bug). In 2019, we tested egg masses of a predatory stink bug *Podisus maculiventris* (spined soldier bug) and two pest stink bugs, *Chinavia hilaris* (green soldier bug) and consperse stink bug.
Sentinel egg masses were deployed in two treatments: 1) native stink bug complex and 2) BMSB. The egg masses were deployed at the same location, but temporally separated (never present at the same time). Egg masses were taken from laboratory colonies and exposed in the field for 3-4 days, after which they were collected and observed to determine egg fate (proportion of egg masses attacked, % eggs attacked/parasitized). Eggs were dissected after all parasitoids or stink bug nymphs had hatched, and molecular diagnosis will be used to determine the status of unhatched eggs. If eggs were killed due to parasitoid activity, this constitutes non-reproductive impacts on the host species, which may contribute significantly to mortality.

Results. Trissolcus japonicus attacked BMSB egg masses and those of the spined soldier bug at similar rates (22-23%), and with about the same level of success (64-69%) (defined as the % eggs producing adult parasitoids) (Fig. 4). In contrast, only 2-3% of the pest stink bug egg masses were attacked, and of those attacked, only 21-23% produced adult *T. japonicus*. We designed a primer specific to *T. japonicus*, and used it to verify the fate of eggs which did not produce an adult wasp. PCR analysis is still ongoing and will enable us to determine total parasitoid impact (non-reproductive effects) in addition to reproductive success.

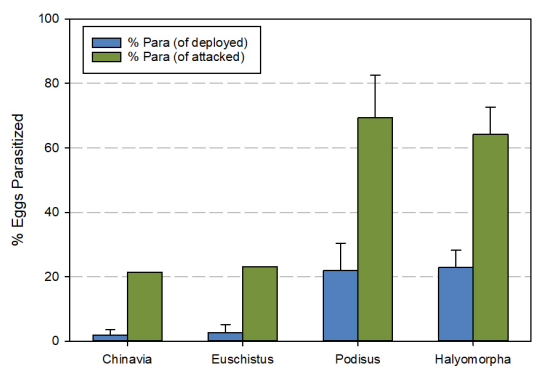

Fig. 4. Samurai wasp attack rate on native stink bugs compared to BMSB.

Objective 3: Determine development of BMSB on shrub-steppe plants

In a preliminary study conducted with our laboratory colonies, we found that BMSB was able to complete a full generation (egg to adult to egg) when fed only on mixed cuttings of Oregon grape, serviceberry (Fig. 5), and bitterbrush. We followed up on this finding by evaluating BMSB developmental rates and survivorship on cuttings from similar combinations of native plant species to those often found in orchard borders.

BMSB egg masses were placed in cages containing cuttings from multiple shrub-steppe plant species during two periods: mid-season (June-July) and late season (Aug-Sept), coinciding approximately with the timing of the two generations of BMSB in the field. The plants used during each period were taken from the same location, which was known to harbor native stink bugs, and at the field-relevant phenological stage. The plant assemblage for the mid-season test consisted of bitterbrush, elderberry, wax currant, chokecherry, snowberry, serviceberry, and poplar. The late season assemblage was the same except it lacked wax currant and poplar, but included wild rose. The check plant assemblage was the colony diet, composed of hosts known to be favorable to BMSB development: peach cuttings, carrots, sunflower seeds, pumpkin seeds, Spanish peanuts (all but peach are used as the colony diet). Four to five egg masses/cage (<4 days old) were counted and left to hatch. After hatch was complete, the egg masses were retrieved and the number of successfully hatched eggs recorded. The cages were checked regularly for newly eclosed adults, which were removed and weighed, and the date of eclosion recorded to determine developmental time. Survivorship was calculated as the number of adults/number of initial hatched eggs.

Fig. 5. BMSB nymph on chokecherry.

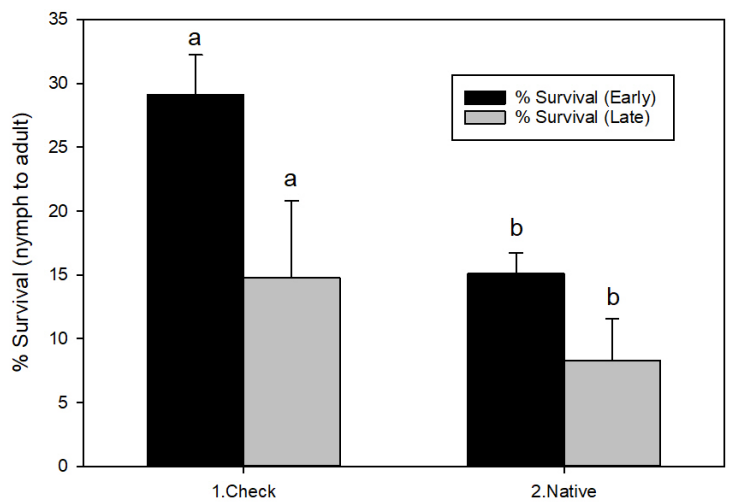

Fig. 6. Survivorship of BMSB on native plants vs colony diet at two time periods.

Results. BMSB were able to complete egg to adult development on both the colony diet and on native shrub-steppe plant assemblages. Developmental time was little affected by the host plant diet, with only a few days difference the number of days from egg to adult. Similarly, adult weights (a proxy for future reproductive success) was similar on both diets. However, there was significantly lower percentage survivorship on the native host plants than on the colony diet (Fig. 6). Survivorship was relatively low overall, with only 27-48% in the mid-season assessment, and 8-15% in the late season assessment. It should be noted that the 'colony diet' was comprised of plants that BMSB might not normally encounter in wild habitats, especially carrots and peanuts (underground plant parts). Comparison of shrub-steppe plant assemblages with those from the mid-Atlantic area might be a more meaningful comparison.

Objective 4: Track the invasion of BMSB in Washington State

Since BMSB's original discovery in Clark County in 2010, it has continued to spread throughout eastern and western Washington. BMSB has perennial and growing populations in Washington's primary agricultural regions (Chelan, Yakima, and Walla Walla Counties). Although the majority of reports are from urban areas, the BMSB invasion in the eastern US shows us the next step is movement into crops, followed by economic damage to crops. Tracking the spread of BMSB across Washington will continue to identify new at-risk areas, and give growers insight into current BMSB pressure. Conversely, it identifies areas with a relatively low detection levels of BMSB, where growers of at-risk crops should monitor, but not need to control this pest at present.

To determine the presence and relative abundance of BMSB in both new counties and new areas within counties we used pheromone trap surveys and homeowner reports. The surveys were conducted using sticky traps baited with the dual BMSB pheromone (Fig. 7). The survey was conducted in the fall to maximize the probability of detection, targeting areas of interest to the tree fruit industry. The homeowner reports are solicited on websites and Extension meetings to cover urban areas in the state. An email address (tfrec.reportbmsb@wsu.edu) has been created to collect such reports, with requests that they be accompanied by a location (street address or city) and photo for identification. The latter is necessary in that a number of related Hemiptera have been submitted, including Coreidae and other Pentatomids. The distinctive features of the adult BMSB make photo ID relatively straightforward. Verified finds will be recorded in a database available to BMSB researchers, and the results available in map form (http://tfrec.cahnrs.wsu.edu/beers-tfentomology/bmsb/bmsb-wa/) which is updated periodically, including the end of the calendar year.

Fig. 7. Sticky trap for detection of BMSB.

Results. BMSB has been found in 29 (out of 39) counties in Washington state as of November, 2019 (Fig. 8). Two additional counties (Pacific and Jefferson) had the first detection in 2019, both on the Olympic Peninsula (homeowner reports). Seventeen counties with previous detections had positive finds again in 2019. Traps placed in fruit growing regions near Oroville, Brewster, and Mattawa were negative, but there was a new detection near Rock Island (Douglas County).

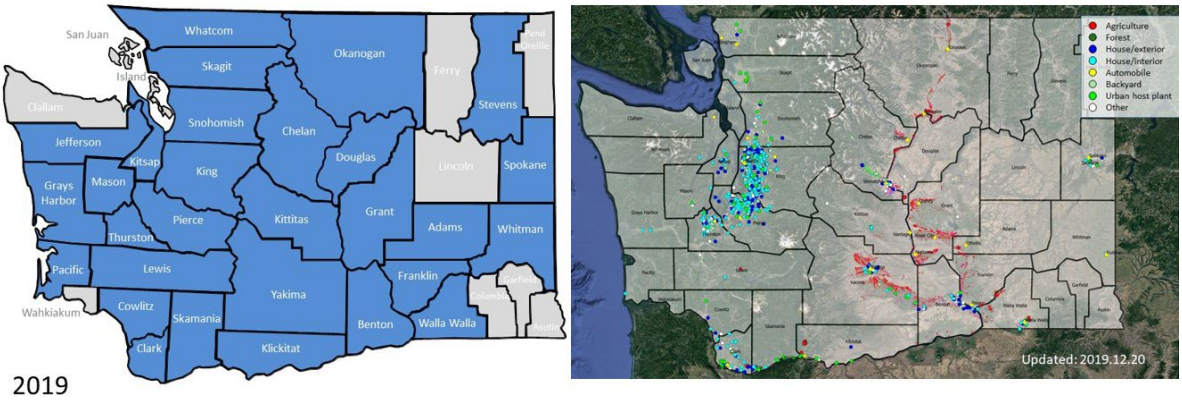

Fig. 8. BMSB detection by county (left) and by GPS location (right). Red, green and magenta pixels (right) represent apple, pear, and sweet cherry acreage, respectively.

CONTINUING PROJECT REPORT **YEAR**: No-cost extension
WTFRC Project Number: CP-17-100

Project Title: Rapid lab and field detection of two major apple quarantine pathogens

PI: Achour Amiri
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P.O Box 646430
City/State/Zip: Pullman/WA/99164

Cooperators: Stemilt Growers, Borton Fruit

Total Project Request: **Year 1:** $35,211 **Year 2:** $15,429 **Year 3:** $3,800

Other funding sources None

WTFRC Collaborative Expenses: None

Budget 1: Amiri
Organization Name: WSU **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu/ shelli.tompkins@wsu.edu

Item	2017	2018	2019
Salaries[1]	12,635	9,371	
Benefits	4,449	3,358	
Wages			
Benefits			
Equipment[2]	13,735		
Supplies[3]	4,000	2,000	3,000
Travel[4]	392	700	800
Miscellaneous			
Plot Fees			
Total	35,211	15,429	3,800

Footnotes:
[1] Salaries are Rachel Bomberger (30% FTE in 2017 and 10% FTE in 2017) and for 6 months 20% FTE for Research Intern (Amiri lab) for 2018.
[2] Equipment will include costs for portable Genie II instrument to be used in and outside lab environments.
[3] Supplies include reagents for LAMP assay optimization and field use.
[4] Travel to Wenatchee for Rachel Bomberger and for Amiri lab to field and packinghouse samplings and testing.

OBJECTIVES:

1. Laboratory development and optimization of the LAMP assay to:

   -Identify and distinguish the different species causing in the bull's eye rot complex, including *Neofabraea perennans*, *N. malicorticis*, *N. alba* and *N. kienholzii.*
   Deviation from the original objective: The initial objective was to develop primers for all the four *Neofabraea* species, however, for the sake of time and funds, we focused on the *N. perennans*, which is the most important and frequent species causing bull's eye rot in Central Washington. Future work, beyond this project, will focus on developing the LAMP assay for the three other species and the findings will be provided to the industry once available.

   - Identify the causal agent of speck rot (*Phacidiopycnis washingtonensis*).

2. Evaluate the sensitivity and reliability of the LAMP assay for early detection of Neofabraea and *Phacidiopycnis washingtonensis* on artificially inoculated (Sunrise) and naturally infected (commercial orchards) fruit before and after harvest.

SIGNIFICANT FINDINGS:

Objective 1.

- A primer set was developed for the detection of *Neofabraea perennans*.
- This primer was specific to *N. perennans* only and did not react to other species.
- The LAMP assay was very sensitive as it detected as low as 1 pg of fungal DNA.
- A Gennie II portable instrument (Figure 2) which is battery-powered has been acquired and was optimized to use without DNA extraction.
- One set of primers was developed for *Phacidiopycnis washingtonensis.*

Objective 2.

- The LAMP assay was successfully used to detect *N. perennans* on Golden Delicious apples inoculated with *N. perennans* at the Sunrise orchard and in storage for up to 90 days.
- The LAMP assay was able to detect as low as 1,000 spores/ml of *N. perennans* on Golden Delicious fruit.
- Commercial fruit were sampled 90, 60, 30 and 0 days preharvest in 2018 from one conventional and one organic commercial orchard (cv. Pinata) in Quincy. Fruit, from the same orchards, were sampled after 30 and 90 days of storage in RA at 34°F.
- The LAMP assay used the portable device Genie II detected *Neofabraea* inoculum on fruit from both commercial orchards at low (10%) frequency 90 days preharvest.
- The frequency of samples positive to *N. perennans* increased though the growing and storage season to reach about 65% after 90 days of storage.

METHODS:

Objective 1. Laboratory development and optimization of the LAMP assay to detect the causal agent of speck rot: *Phacidiopycnis washingtonensis.* This assay has already been developed for *Neofabraea*. Therefore, work in the third year will focus on *Phacidiopycnis*.

Lamp primers design and LAMP assay optimization. One set of six primers has been designed for *P. washingtonensis* using the β-tubulin gene.

Pure culture of isolates previously characterized as *P. washingtonensis* will be used to extract pure DNA. This DNA will be used to test the specificity of the primers and the sensitivity of LAMP assay at different DNA concentrations (1, 2, 4, 8, 16 and 32 ng/µl).

Objective 2. Evaluate the sensitivity and reliability of the LAMP assay for early detection of latent infections of *P. washingtonensis* on fruit in the orchard and in storage.

2a. Detection of Phacidiopycnis on artificially inoculated

Trials will be conducted at the Sunrise Orchard WSU-experimental orchard. The cultivar Red Delicious, one of the most susceptible cultivars to this disease. Fruit will be inoculated on tree by sprays of spore suspensions at 0 (water), 100, 1,000, and 10,000, spores/ml 60 days before commercial maturity. Four replicate trees will be used for each spore concentration. Six fruit/tree will be harvested at 60, and 30 days before commercial maturity and at commercial maturity. Forty fruit from the same trees will be harvested and stored at 34°F and samples of 10-fruit each will be sampled at 30, 60, 90, and 120 days postharvest. The fruit samples at each time will be blended in 1 g of the blend will be suspended in in water or lysate buffer and used for LAMP reactions. Different dilution factors; 10, 100, 500, 1000 will be tested to optimize detection. This dilution step is necessary because apple samples contain several chemical compounds that may interfere or inhibit the LAMP reaction.

2b. Detection of Phacidiopycnis on commercial fruit.

1-c LAMP assay reactions

LAMP identification using purified DNA of on fruit will be assessed using the portable device Genie II instrument (Figure 1). The LAMP portable device battery-powered heat consisting of a thermocycler necessary for the LAMP assay and a screen that show real-time amplification of positive DNA if present in the sample. At each detection a positive sample consisting of DNA of N. perennans will be used as a check-up.

Figure 1. Portable Genie II instrument, battery-enabled aquired by Pathology lab at WSU-TFREC to be used LAMP detection of pathogens without prior DNA extraction.

RESULTS AND DISCUSSION

Objective 1: Primer design and LAMP assay optimization for *N. perennans*

<u>*Specificity of LAMP primers to detect N. perennans only*</u>: Initial reactions using template DNA at 100 ng/µl provided negative reactions for all fungal species tested except for *N. perennans* and one *N. kienholzii* isolate (Nk-4480) (Figure 2-left). Although the primer designed to detected *N. perennans* only continued to amplify *N. kienholzii*-4480 in further reactions, the amplification was 6 times lower and significantly delayed (15 min) after that of *N. perennans* (Figure 2, right).

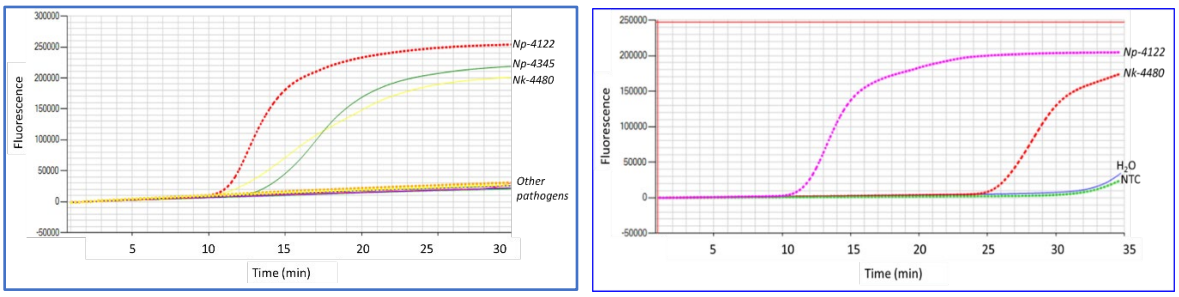


Figure 2. Initial specificity LAMP assessment using the portable LAMP Genie® II instrument to amplify *N. perennans* and other pathogens (left) and in comparison, with *N. kienholzii* (Nk.4480) (right). All reactions were run at 65°C and a DNA concentration of 1 ng/µl for each pathogen. Np and Nk indicate *N. perennans* and *N. kienholzii*, respectively. H_2O and NTC are negative controls without DNA.

<u>*Sensitivity of LAMP to detect different DNA concentrations of N. perennans*</u>: A 30 min reaction in a traditional thermocycler followed by gel electrophoresis analysis showed that DNA concentration as low as 0.1 ng/µl of DNA could be detected (Fig. 2A). LAMP reactions conducted in the portable Genie® II instrument using a fluorescent dye was able to detect *N. perennans* DNA concentrations as low as 0.01 ng/µl within 17 min and 0.001 ng/µl after 45 min (Fig. 2B). When the DNA concentrations were higher than 0.1 ng/µl, amplifications occurred between 9 and 15 min after the reaction start.

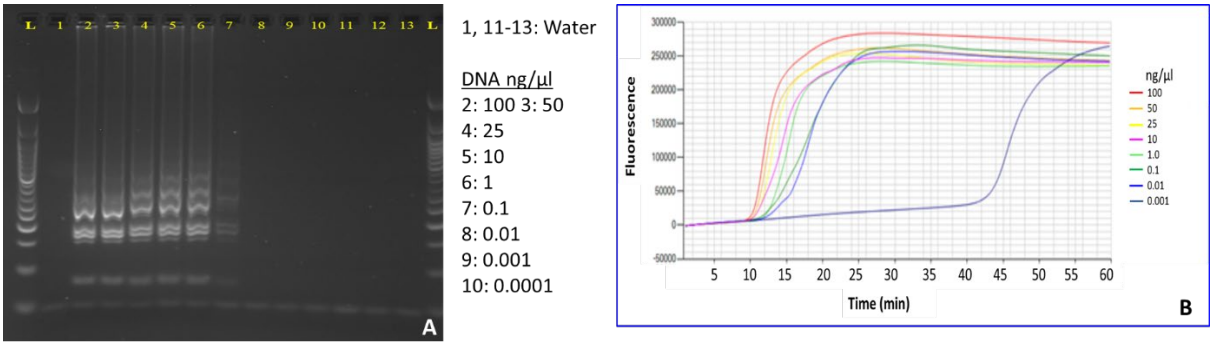


Figure 3. (**A**) Image of 1% agarose gel demonstrating the sensitivity of LAMP assay using the primer set 22 at 65°C to detect different DNA concentrations of *N. perennans*. (**B**) LAMP sensitivity in Genie® II portable instrument at 65°C for detection of *N. perennans* at different DNA concentrations between 100 and 0.001 ng/µl. Negative control reactions are not shown as they were included with reactions with DNA concentrations from 1.0 to 0.0001 ng/µl.

Objective 2. Detection of *Neofabraea perennans* on fruit using LAMP

Detection of Neofabraea perennans in artificially-infected fruit. Apple trees of Golden Delicious, a highly susceptible cultivar to *Neofabraea* spp., were inoculated with spore suspensions of *N. perennans* at concentrations of 0, 100, 1000, and 10,000 spore/ml. Only 5 to 20% of samples were positive to *N. perennans* in non-inoculated fruit or those inoculated with a spore suspension at 100 spores/ml regardless of the inoculation or sampling time (Figure 4). On apples inoculated at 1,000 spores/ml, LAMP detected *N. perennans* in samples collected 0 DPI as well as those sampled during storage, whereas *N. perennans* was detected at all sampling times on almost all fruit inoculated with a spore suspension at 10,000 spores/ml (Figure 4).

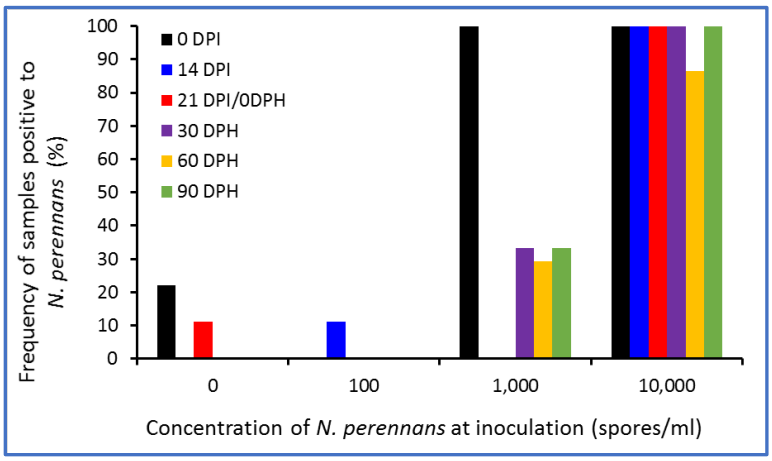
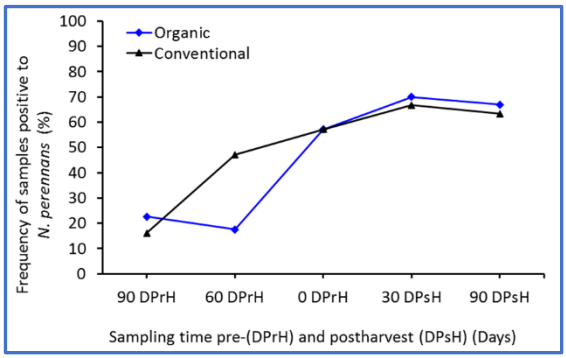


Figure 4. Mean frequency (%) of Golden Delicious samples positive to *N. perennans* detected by LAMP in Genie® II from fruit inoculated with different spore concentrations of the pathogen. DPI and DPH indicate day post-inoculation and day postharvest, respectively.

Detection of Neofabraea perennans on fruit from commercial orchards: naturally-infected fruit. The portable LAMP assay was used to detect *N. perennans* in asymptomatic Piñata apples from commercial conventional and organic orchards in Quincy. Natural infections of *N. perennans* were detected in samples collected 90 days preharvest in both organic and conventional orchards and the frequency of fruit carrying *N. perennans* increased during the growing season and reached the maximum values of 66 and 70% in conventional and organic orchards, respectively, 30 days postharvest and remained steady for up to 90 days postharvest (Figure 5, on right).



Ongoing and future work:

Objective 1.

- Complete the LAMP optimization for *Phacidiopycnis* in the lab and test for specificity.
- Complete the LAMP optimization for *Phacidiopycnis* in the lab and test for sensitivity Complete visual assessment methods for the LAMP assay using turbidity or specific dyes

Objective 2.

- Conduct LAMP detection of *Phacidiopycnis washingtonensis* on artificially- and naturally-infected apples using the portable LAMP device Genie II

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CP-18-102

Project Title: Understand the epidemiology of Botrytis to curb gray mold postharvest

PI: Achour Amiri
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PI: Tobin Peever
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Cooperators: Chelan Fruit, Stemilt

Total Project Request: **Year 1:** $32,360 **Year 2:** $34,943 **Year 3: $33,371**

Other funding sources

Agency Name: WSDA-Specialty Crop Block Grant program
Amt. awarded: $170,195
Notes: "Strategies to enhance pre- and postharvest management of gray mold in pome fruit" PI: Amiri, co-PI: Tobin Peever

WTFRC Collaborative Expenses: None

Budget 1: (Achour Amiri)
Organization Name: WSU **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu/ shelli.tompkins@wsu.edu

Item	2018	2019	**2020**
Salaries[1]	14,400	14,976	15,575
Benefits[1]	6,385	6,640	6,906
Wages[2]	5,760	5,990	6,230
Benefits[2]	545	567	590
Equipment			
Supplies[3]	1,500	3,000	3,000
Travel[4]	1,070	1,070	1,070
Miscellaneous			
Plot Fees[5]	2,700	2,700	
Total	32,360	34,943	**33,371**

Footnotes:
[1] Salaries are for a Research Intern (0.3 FTE) at 44.3% benefit rate.
[2] Wages are for non-student temporary employee for summer help at 9.5% benefit rate.
3 Supplies include reagents for PCR and qPCR, agar media, plates and sampling materials.
[4] Travel to commercial and experimental orchards and packinghouses in WA for trials set -up, sampling and data collection.
[5] Plot fees for a block to be used for preharvest trial on gray mold in years 1 and 2.

OBJECTIVES:

Objective 1. Investigate infections timing of fruit by *Botrytis* in Washington apple orchard to determine critical timing of infections and subsequent disease expression in storage.

Objective 2. Evaluate the effect of preharvest weather conditions (rain and temperature) on infections and gray mold development in organic and conventional orchards.

Objective 3. Define the genetic structure of the *Botrytis* populations in pre- and postharvest environments and its impact on fitness, pathogenicity and resistance to commonly used fungicides.

SIGNIFICANT FINDINGS:

- *Botrytis* was detected on flowers and fruit collected throughout the season from bloom to harvest.
- *Botrytis* was detected in the of orchard atmospheres throughout the season from bloom to harvest at low frequencies and variable among locations.
- The size of *Botrytis* inoculum was greater in organic orchards compared to conventional orchards.
- The inoculum size decreased from bloom to fruit set in conventional but then increased toward maturity and harvest. In organic orchards, the inoculum size increased throughout the season.
- Variabilities in inoculum size and dynamic throughout the season has been observed between orchards located in different districts.

METHODS

Objective 1. Investigate infections timing of fruit by *Botrytis* in Washington apple orchard to determine critical timing of infections and subsequent disease expression in storage. **April 2020-April 2021**

In spring of 2019, 96 apple blossoms were collected from two conventional orchards located in Chelan County (Orondo) and in Grant County (Quincy). During the summer, 96 fruit were collected from the same trees and orchards used for flowers sampling on a monthly basis from fruit setting to harvest. Blossom and fruit samples were transported in separate clean bags to the Pathology Lab at Washington State-Tree Fruit and Extension Center (TFREC) in Wenatchee. Additional fruit were harvested from the same orchards and stored in RA at 34°F to assess the incidence of gray mold in storage. Flowers were freeze-dried and stored at -80°C. Fruit were peeled and the peel and the flesh of the fruit will be freeze-dried separately and stored at -80°C. The separation of the peel from the flesh will help separate between infestation (spores present on the surface) from endophyte infections (present inside the fruit). DNA is being extracted from freeze-dried samples and the presence of *Botrytis* will be detected using a quantitative polymerase chain reaction (qPCR) assay (Diguta et al. 2010). Alternatively, spores of *Botrytis* will be enumerated from fresh (non-dried samples) on a *Botrytis* semi-selective artificial agar medium (Edwards and Seddon 2001). This trial will be reconducted in 2020 to obtain third year of data in order to make stronger conclusion with regard to the occurrence and evolution of Botrytis in the orchard and how it will impact the development of gray mold postharvest.

Objective 2. *Evaluate the role of preharvest weather conditions (rain and temperature) on infections and gray mold development in organic and conventional orchards.* **April 2020-April 2021**

Temperature and wetness are the two main factors that drive *Botrytis* infections. In central Washington, wetness (>10 hours) can be primordial in early season and at bloom when flowers can be very susceptible to *Botrytis* infections and between September and November for fruit, whereas temperatures of late spring and early summer (65 to 78°F) and at harvest in September through October combined with rain can be very conducive for fruit infections. The objective is to make a correlation between temperatures and wetness and *Botrytis* infections throughout the season. Protocols and trials described in Objective 1 will be used for this objective. Weather data (rain and temperature) will be collected from the Washington State University-AgWeaterNet (http://www.weather.wsu.edu/) in way to obtain data for all and each sampled orchard from the closet weather station. A correlation between rain and temperature occurring at bloom and at each period of fruit sampling and *Botrytis* incidence on flowers and fruit from the orchards will be analyzed. We will conduct trials for 3 years and we aim to collect enough consistent data that would help construct a predictive model for *Botrytis* infections in the future (not part of this project).

Objective 3. *Define the genetic structure of the Botrytis populations in pre- and postharvest environments and its impact on fitness, pathogenicity and resistance to commonly used fungicides.* **October 2019-April 2020**.

Botrytis isolates collected from flowers (early season: overwintered populations and those collected at bloom and throughout the growing season) and fruit in the orchard (summer and late-season) as well as from decayed fruit (after several months of storage) will be DNA fingerprinted using molecular markers. A set of 12 microsatellite markers developed by Fournier et al. (2002) and in the laboratory of Co-PI Peever will be used to fingerprint isolates. These markers are currently being used to fingerprint Pacific Northwest isolates of *B. cinerea* from small fruit in Peever lab. We will test the hypothesis that gray mold infections initiated in the orchard contribute substantially to packinghouse infections. The alternative hypothesis is that packinghouse infections although initiated in the orchard undergo a long period of cold temperature and controlled atmospheres (Low O_2 and high CO_2 concentrations) and may be genetically impacted. The use of these molecular markers will allow us to verify these hypotheses. We will also use these markers to investigate the impact of fungicide sprays (Organic vs. conventional orchards) and resistance levels on the evolution of *Botrytis* populations in apple orchards and packinghouses, as well as on their fitness and pathogenicity. Gray mold of fruit crops is known to be mainly caused by the species *Botrytis cinerea*. However, recent studies from strawberry, blueberry, blackberry, and grape have reported that gray mold can also be caused by species other than *B. cinerea*. The importance of these other species, including *Botrytis pseudocinerea, B. mali, B. group S, and B. californicae*, as decay agents in Washington apple storage facilities will be determined using genetic markers specific to these species (Dowling and Schnabel 2017; Fournier et al. 2003; Leroch et al. 2013; Li et al. 2012; Saito et al. 2016).

RESULTS AND DISCUSSION

Objective 1. Infection timing of *Botrytis* preharvest and postharvest

As shown on Figure 1 below, *Botrytis* was detected in orchards at almost all sampling times. There seem to be a carry-over from bloom to fruit and increases as the fruit mature. Fungicide spray programs for each orchard were obtained and are being analyzed to correlate with potential fungicide effect on reduction of Botrytis load on fruit as this can be explained by the slight reduction observed before harvest (Figure 1) following the preharvest spray. However, the incidence of fruit infected (not

decayed) with *Botrytis* increased significantly to 66% in Orondo and 58% in Mesa after 6 months of storage in CA. It is important to note that the fruits used in this study were not treated postharvest.

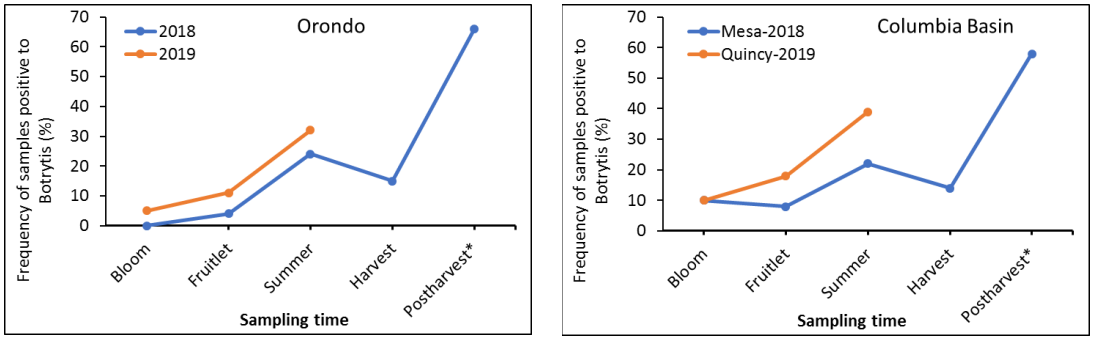

Figure 1. Evolution of *Botrytis* incidence throughout the preharvest growing season as detected by qPCR. 2019 Sampling after harvest are being analyzed.

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

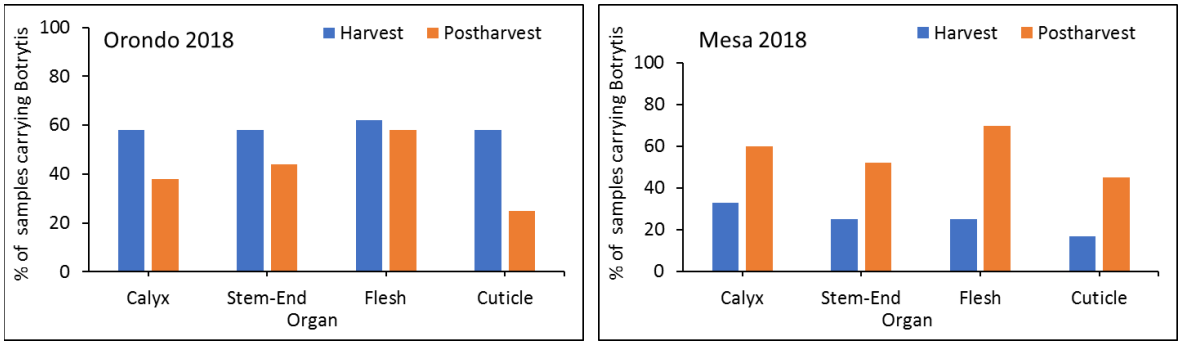

Figure 2. Incidence of *Botrytis cinerea* on different organs of the fruit at commercial maturity (harvest time) in organic and conventional orchards in 2018. Samples from 2019 Are being analyzed currently.

Impact of weather conditions on Botrytis incidence preharvest (ongoing): Weather parameters including temperature, wetness, and rainfall from all the four orchards used in 2018 and the two orchards used in 2019 were collected and are being analyzed to establish potential correlations with Botrytis incidences between locations and throughout the season. A third year of trials will be conducted in 2020 to compare to the two previous seasons.

Genetic population structure and sensitivity to fungicides: In total, 220 isolates of *Botrytis* were collected from the orchards used in this study in 2018 and 100 isolates were collected in 2019 so far. The isolates from 2018 were single-spored to start from the same genetic background. The isolates from 2019 will be single-spored once collection is completed in storage. DNA is being extracted to conduct a genetic analysis in order to detect the presence of species other than *B. cinerea* in populations from Washington.

In order to determine at which stage, orchard or storage, resistance to fungicides is selected, all the isolates collected in 2018 and 2019 will be tested for fungicide sensitivity to several pre and postharvest fungicides used in orchards and packinghouses.

Future work:

2019-2020: Conduct a 3nd year of field trials in the same orchards used in Year 1 & 2 for comparison.

Obtain data from cold storage facilities on samples collected from previous season

Analyze weather data and impact on botrytis incidence.

Conduct the genetic analyses of *Botrytis* collected so far.

CONTINUING PROJECT REPORT **YEAR:** No-Cost Extension
WTFRC Project: CP-18-105

Project Title: Using cold storage to increase the stability of honey bee supply

PI:	Brandon Hopkins	**Co-PI:**	Walter Sheppard
Organization:	Washington State University	**Organization:**	Washington State University
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Email:	bhopkins@wsu.edu	**Email:**	shepp@wsu.edu
Address:	Department of Entomology	**Address**:	Department of Entomology
Address 2:	PO Box 646382	**Address 2:**	PO Box 646382
City/State/Zip:	Pullman WA 99164-6382	**City/State/Zip:**	Pullman WA 99164

Cooperators: 2B Apiaries, Olson's Honey, Idaho Bee Storage

Total Project request: $128,314 Year 1 (2018): $100,000 Year 2 (2019): $28,314

Other funding sources

Agency Name: Google X
Amt awarded $35,000 + (~$150,000 in-kind for sensors)
Notes: Collaboration and sponsored research with Google's R&D company (X) began about the same time colonies were being prepared for this winter project. They wanted to help by proving sensors and in turn are getting some data about hive activity.

Agency Name: California Almond Board
Amt. awarded: $97,000
Notes: The award from the Almond Board was made possible by leveraging the money provided by the WTFRC to secure the remainder of the funding required to complete the construction of the research equipment needed for the research described in this proposal

Agency Name: USDA-NIFA
Amt. requested: $499,800
Notes: The proposal included research that is only possible because of the funding awarded by the WTFRC and the Almond Board of California. Provides funding for graduate students and bee research supplies to complement WTFRC funindg of the CA containers.

Organization Name: WSU **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email:** arcgrants@wsu.edu

Item	2018	**2019**
Salaries		15,290
Benefits		2464
Wages		6000
Benefits		1560
Goods and Services	100,000	
Total	100,000	28,314

OBJECTIVES

Goals
The initial goal of this proposal is to construct three controlled atmosphere rooms capable of holding a significant number of honey bee colonies for winter and summer experiments. The second goal is to utilize the controlled atmosphere facilities to address the following objectives:

Objectives:
1. Optimize controlled atmosphere storage conditions (CO_2, humidity, temperature) to maximize Varroa mite mortality while producing healthier bees following the winter storage period.
2. Determine ideal timing and storage conditions for mid-season (summer) honey bee colony cold-storage to create a break in the brood cycle that allows beekeepers to more efficiently and effectively control Varroa.
3. Utilize the combined findings from the objectives above to develop a whole season IPM strategy for commercial tree fruit pollinators. Implement that strategy in collaboration with a commercial beekeeping operation to demonstrate the feasibility and economic benefit to the commercial beekeeping industry.
4. Leverage the new facilities to attain additional funding to expand the research/utility of indoor controlled as it applies to the stabilization of the beekeeping industry for the benefit of the tree fruit industry

SIGNIFICANT FINDINGS
The most significant progress is related to Objective 4. We were able to leverage funding provided by the WTFRC to secure funding from the Almond Board of California to get enough funds to move forward with the facilities needed. The time to get these funds and the time for design and planning with WSU facilities has pushed the timeline of the original grant back approximately 1 year (updated in methods below).

- With the combined funds from WTFRC and the Almond Board for the facilities, we were able to apply for USDA-NIFA funding to expand the research associated with this project

- Continued to gather preliminary data regarding objective 2. In that we completed an observational study with a commercial beekeeper in Idaho where we were able to force colonies into a broodless state and significantly improve varroa control in those colonies compared to colonies with capped brood (Fig 1).

- Produced a 1st edition of an indoor wintering "best management practices" booklet that will soon become an online resource with continual updates on this management practice. A place where findings from this research will be redaly accessbale to beekeepers.

METHODS
The research laid out in this proposal is dependent on an initial acquisition of two 1280 ft^3 controlled atmosphere chambers. Each chamber will be capable of holding 80 colonies at adjustable temperature, CO_2 and humidity levels. Temperature adjustments will range from 35-55° F with a range of CO_2 levels from normal atmospheric (300 ppm) to 80,000 ppm (8%). These containers are now operational and have research colonies inside.

Year 1 (2018) – Procurement of additional funding and design of research chambers - **Completed**

Controlled atmosphere honey bee wintering-

Year 2- indoor wintering - 2019 – completed/underway

October -160 honey bee colonies were assessed. The number of frames of bees and brood were recorded. Samples of bees were collected in alcohol to determine the initial Varroa mite load in each colony. Those sample were used to determine tracheal mite and nosema infection (two economically important pests besides Varroa mite). 120 colonies were selected for the winter research from the initial 160 colonies screened. Colonies with too many varroa mites or too few were excluded.

December 2019- Present : Procurment and placement of CA experimental chambers (rerefigerated cargo containers.
Triton 20ft refrigerated cargo containers were placed at the WSU Irrigated Research Farm near Othello, WA

Using information about the initial Varroa mite loads for each colony; colonies were assigned to one of two controlled atmosphere rooms and a set of 40 colonies remains outdoors for the winter as an additional control. Thirteen colonies in each treatment was fitted with a hive scale and set of sensors (temperature, humidity, and CO_2) to continually log data through the trial period.
The colonies are distributed so that each group contains, on average, the same Varroa mite load. Both containers are set at 40°F with the manipulated manipulated variable being CO_2. One container is set to maintain normal atmospheric level of CO_2, and the second container is set at a maximum of 8% CO_2 (80,000 ppm). Each hive was fitted with a screened bottom (allows falling debris/mites to fall through) and a "sticky card". At the end of the 45 day storage period the colonies will be removed and placed outdoors where a miticide treatment will applied along with a fresh sticky card. The number of mites remaining after storage will be compared to the total number of mites gathered on the sticky cards during storage to determine Varroa mite mortality caused by the treatment period. All treatments will be compared for effects on population size, survival and mite loads.

Year 3 – Indoor wintering - 2020

October – The experimental set up from winter of 2019 will be duplicated in year 3, except the CO_2 level that provided the best control of Varroa will be used with the addition of a third chmaber. In year 3, we will evaluate the effects of humidity at three levels (45%, 65%, 80%) to enhance mite mortality. Previous research has suggested that Varroa mite mortality in the winter is increased due to dehydration because mites have a much larger surface area to volume ratio than honey bees. Carbon dioxide interferes with control of respiratory openings, which have a major effect on controlling water loss. It is our hypothesis that by increasing CO_2 and decreasing humidity we can increase mite mortality while bees are stored indoors for the winter months.

Controlled atmosphere Mid-season brood break for enhanced Varroa control
Year 3- Summer brood break – 2020

August – 160 honey bee colonies will have the honey crop removed and colonies assessed prior to the onset of the trial. The number of frames of bees and brood will be recorded. Samples of bees will be collected in alcohol to determine the initial Varroa mite load and tracheal and nosema infection in each colony. Each colony will be weighed before and after the trial period. Four colonies in each treatment will be fitted with a hive scale to continually log the weight change through the trial period. Those same colonies will be fitted with traps at the entrance to monitor the number of bees that die and are removed from the colony during the trial period.

With information about the Varroa mite loads for each colony; colonies will be assigned to one of three controlled atmosphere rooms and a set of 40 colonies will remain outdoors for the 3-week trial as an additional control. The colonies will be distributed so that each group contains, on average, the same Varroa mite load. They will be placed in the controlled atmosphere chambers for 18 days. One room will be set at 40°F and in complete darkness. The second room will be held at 50°F in complete darkness. The third room will be held at 40°F with a season normal diurnal light cycle. Each hive will be fitted with a screened bottom (allows falling debris to fall through) and under the screened bottom a "sticky card" will be placed. At the end of the 18-day storage period the colonies will be removed and placed outdoors where a miticide treatment will applied along with a fresh "sticky card". The number of mites gathered following the miticide treatment will be compared to the total number of mites gathered on all sticky cards to determine the Varroa mite mortality caused by the treatment period. All colonies will be assessed again as they were at the start of the experiment and health, colony size, mite loads will be compared. These colonies will then be followed through the rest of the season until it becomes too cold to work bees (below 50°F). They will be sampled and assessed at one and two months post-treatment date. They will again be assessed when they are moved to California for almond pollination the following year

Year 4 - 2021

Using findings from the experiments described above we will engage with commercial beekeeper collaborator to follow and study 400 honey bee colonies using the CA overwintering facilities of our collaborator (Olson's Honey) in Yakima WA. We will assess all 400 as described in methods above during the period of almond pollination in California. All colonies will be given a numbered tag. At the end of the almond pollination season, all colonies will be treated with the most widely used registered commercial product for Varroa control (Apivar). Hives will again be assessed while colonies are in apple orchards during pollination. Any colony issues (queenless, bacterial or fungal disease) other than those caused by Varroa mite will be remedied and recorded. Nutritional issues and swarm control will be decided on by the beekeeper, consistent with his normal operating procedures. Colonies will be assessed again in the summer after canola seed pollination during which time the honey crop will be removed. After this assessment colonies will be divided into 4 treatment groups with the average Varroa load and colony strength equally distributed across all 4 treatment groups. Treatment group 1 (commercial standard control) will be treated with industry standard miticide treatment in conjunction with rest of the commercial operation and will be moved to California "holding yard" in November when the rest of the groups are moved to controlled atmosphere storage for the winter. Treatment group 2 will be treated the same as group 1 except that it will be placed in controlled atmosphere storage for the winter months before almond pollination begins. Treatment group 3 will be moved into a controlled atmosphere facility for 18 days to create a break in the brood cycle. After which it will be treated with a single application of miticide. Treatment 4 will remain outside isolated from the rest of the commercial operation and left untreated. All colonies will be assessed again in October as they are prepared for winter. Colony strength, Varroa loads and survival will be compared between treatment groups after the October sampling and again in January as they are prepared for placement in almond orchards.

RESULTS & DISCUSSION

Focus on the findings during the prior year(s) of the project. Discuss significance to the industry and potential economic benefits. Use summary graphics.

The initial funding of this project allowed the for the ability to aquire additional funding and planning with to prepare for arrival of the new equipment. We utilized the funding provided by the WTRC to leverage the additional funds need for the controlled atmosphere chambers from the Almond Board of

California ($100,000). The chambers will provide a wealth of research potetial moving forward and the combined funding that provided these chambers allowed us to secure a USDA-NIFA funding that utilizes these chambers for honey bee research. All preliminary evidence suggests that this line of research will provide valuable information for the commercial beekeeping industry to help keep more colonies alive and stabilize the supply of commercial pollinators.

We now have colonies inside the containers and will be moving the colonies from inside the containers directly to California were they will be assessed and the first winter's experiment will be completed. There are a series of exciting experiments that will go through the containers through out the year and resylts from this winter will be used to design the experiments planned for winter 2019/2020.

One of the comments/feedback we received about the concept of forcing period of broodlessness was that our preliminary work was done in August and many beekeepers are still producing honey at that time. We performed an additional observational study with a commercial beekeeper in southern Idaho who allowed us to follow a set of his colonies after almond pollination (April). We demonstrated the ability to stop brood rearing and significantly increase Varroa mite control. It is likely that the use of controlled atmosphere or refrigerated spaces to hold bees at times other than winter months could become the most significant management tool for increasing colony survival.

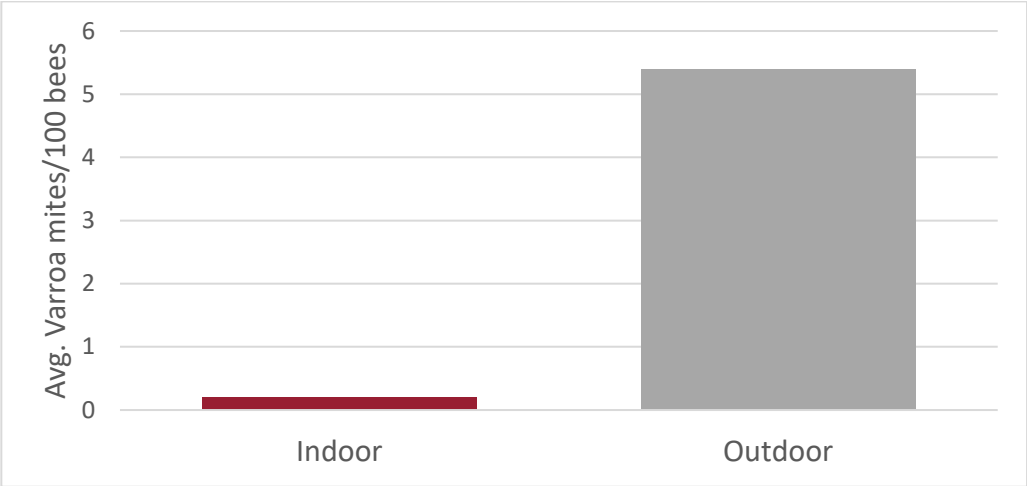

Figure 1: Average Varroa mite populations in colonies that experienced a break in brood production compared to colonies outdoors that continued brood rearing. All colonies experienced the same varroa treatment. The only difference being whether they were moved indoors for 18 days or remained outdoors.

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

Project Title: Integrated Fire Blight Management

PI: Tianna DuPont
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Co-PI (2): Ken Johnson
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Email: kenneth.johnson@oregonstate.edu
Address: Dept. Botany & Plant Pathology
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City/State/Zip: Corvallis, OR 97321-2902

Co-PI(3): Kerik Cox
Organization: Cornell University
Telephone: (315) 787-2401
Email: kdc33@cornell.edu
Address: Cornell AgriTech
Address 2: 630 West North Street
City/State/Zip: Geneva, NY, 14456

Co-PI (4): Kari Peter
Organization: Penn State University
Telephone: 717-677-6116 Ext. 223
Email: kdc33@cornell.edu
Address: Fruit Research & Extension Ctr
Address 2: PO Box 330, 290 University
City/State/Zip: Biglerville, PA 17307

Cooperators: WA: Sean Gilbert, Gilbert Orchards; Travis Schoenwald, Gebber Farms; Paul Stikama, Douglas Fruit; Doug Stockwell, Arrowhead.

Total Project Request: Year 1: $78,979 Year 2: **$77,323**

Other funding sources

Agency Name: Applications made to SCRI
Amt. requested/awarded: $346,000

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu/ shelli.tompkins@wsu.edu

Item	2019	**2020**
Salaries	$3,734[1]	$11,650[1]
Benefits	$1,421[2]	$4,433[2]
Wages		
Benefits		
Equipment		
Supplies	$14,324[3]	$1,000[4]
Travel	$500	$1000
Miscellaneous		
Plot Fees	$2,100	$2,100
Total	$22,079	**$20,183**

Footnotes: [1]Salaries for a scientific assistant one-month year 1 and 3 months year 2 (DuPont).
[2]Benefits at 38% for scientific assistant (DuPont).
[3]Trees, posts, wire etc and contract labor for planting 3 new blocks for a young tree trial ($6,155), and blossom blight trials ($8,169). [4]Trial supplies $1,000.

Budget 2

Organization Name: Cornell **Contract Administrator:** Donna Loeb

Telephone: (315) 787-2325 **Email address:** drr2@cornell.edu

Item	2019	**2020**
Salaries	$8,000	$8,320
Benefits	$5,200	$5,408
Wages		
Benefits		
Equipment		
Supplies	$2,000	$2,000
Travel		
Plot Fees	$1,700	$1,700
Miscellaneous		
Total	$16,900	**$17,428**

Footnotes: [1]Salaries for a temporary employee 2 months at $4,000 per month. Funds for temporary summer worker with experience in designing and conducting fire blight field trials in apples.
[2]Benefits at 65%.
[3] Materials: materials for conducting planting apples, including trees, flagging tape for treatment labeling. This would include materials for making pruning treatments and cleaning up after application of bactericides, and personal protection to be used during bactericide applications.
[3]Plot fees $1700.

Budget 3

Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Russ Karow

Telephone: (541) 737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2019	**2020**
Salaries FRA 3.5 mo	$5,827	$8,765
Benefits OPE 61%	$3,554	$5,347
Wages		
Benefits		
Equipment		
Supplies	$7,154	$2,500
Travel	$1,365	$1,000
Plot Fees	$2,100	$2,100
Miscellaneous		
Total	$20,000	**$19,712**

Footnotes: [1]Salaries for a senior faculty research assistant 1.2 mo in 2019, 1.6 mo in 2020 at $5000 per month.
[2]Benefits at 61% for faculty research assistant.
[3]Trees, posts, wire etc. and contract labor for planting a young tree trial ($6,155), trial supplies $1,000. [4]Trial supplies.

Budget 4
Organization Name: Penn State University **Contract Administrator:** Mary Masterson/Laura Reddington
Telephone: 814-865-9446; 814-867-0058 **Email address:** mmm183@psu.edu / lcr129@psu.edu

Item	2019	2020
Salaries	$7,358[1]	$11,370[1]
Benefits	$2,867[2]	$4,430[2]
Wages		
Benefits		
Equipment		
Supplies	$7,275[3]	$1,700[4]
Travel	$1,000	$1,000
Plot Fees	$1,500	$1,500
Miscellaneous		
Total	$20,000	**$20,000**

Footnotes: [1]Salaries for a research technician, 2 months in year 1; 3 months in year 2.
[2]Benefits at 38.97% for scientific assistant.
[3]Trees, posts, wire etc and contract labor for planting a young tree trial ($7,275), trial supplies $1,000.
[4]Trial supplies.

OBJECTIVES

1. Test materials to prevent bloom infections including biologicals, tank mixes, and mixes with bioregulators.
2. Demonstrate management strategies for young trees including coppers, plant defense elicitors, and Prohexodine Calcium (PhCa).
3. Test strategies to manage blocks once they are infected. Treatments will address how far back to cut, the utility of stub cuts, timeliness of cutting and the use of plant defense elicitors.
4. Provide outreach on fire blight prevention and management.

SIGNIFICANT FINDINGS

- Alum performed well in blossom blight prevention trials in WA, NY, PA and OR.
- Prohexadione Calcium (Apogee/Kudos) performed best when applied 2 weeks before inoculation. 6 oz or higher rates may be important in WA/OR compared to success at the 3 oz rate in NY.
- The 40 oz rate of Serenade Opti performed no better than the 20 oz standard for blossom blight control.
- Systemic acquired resistance products Regalia and Lifegard performed well in New York.
- For protection of young non-bearing trees flower removal was best followed by weekly applications of soluble copper (Previsto) at 2-3 qt/A.
- In a replacement tree trial in Oregon only 42% of trees treated 3 days before infection with actigard (vs 88% untreated, 79% preplant) developed trunk cankers.
- In cutting trials non cut trees died (100% case study 2 & 3 trial) or developed new strikes (7 new vs 1 in case study 1). Cutting fire blight quickly is essential.
- In cutting trials breaking back to the joint at the first year wood more often resulted in cankers forming on structural wood (case study 1 & 4) and leaving more canker to (case study 1,2,4).
- There were few significant differences between treatments with and without tool disinfection.

METHODS

Objective 1: Test materials to prevent bloom infections. This objective took place at research farms in Washington, Oregon, New York, and Pennsylvania. *Wenatchee, WA site*: 40-yrs old 'Red Delicious' apple at the Columbia View Research Orchard. Individual trees were marked as plots in a randomized complete block where suitable trees are selected based on sufficient bloom (100+ flowers), 4 replications, single tree plots. *Corvallis, OR site:* 60-yrs old 'Bartlett' pear orchard and 5-yr-old 'Gala' apple orchard at the OSU Botany and Plant Pathology Field Laboratory near Corvallis OR. The pear experiment was a randomized complete block design with 4 replications of 15 treatments applied to single tree plots. The apple experiment was arranged in a randomized complete block with 6 replications applied to single trees. *Biglerville, PA:* Twelve-year-old 'Cameo' trees on B.9 rootstocks were used and two-tree treatments were arranged in a randomized complete block with four replications. *Geneva, NY:* The orchard site is a planting of 18-yr-old 'Gala' trees on B.9 rootstocks trained to a vertical axis system. The experimental block was a arranged as a randomized complete block with replicate tree blocks.

Products were applied by tree to the area of the tree to be inoculated (whole tree OR, PA and NY; 100 clusters WA) according to manufacturer recommendations using a Stihl SR420 or Solo 451 mist blower backpack sprayer with a wetting agent. 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. An untreated-inoculated check treatment (water applications) was included. At 100% bloom (of the king blooms) *Erwinia amylovora* was applied at $1x10^6$ CFU ml^{-1} dilution ($1x10^7$ PA) to lightly wet each cluster on April 24, 2019 Oregon gala apples, April 18, Oregon bartlett pear , April 26, Pennsylvania Cameo apple. Whole trees (OR, NY), 100 clusters (WA), bottom 8 feet (PA) were inoculated.

Trees were visually evaluated for flower cluster infection every week following treatment. Cluster infection counts will be summed across all dates. Fruit will be evaluated for russet fruit skin marking during the third week in July. Statistical analysis will be performed using an mixed models, analysis of variance ANOVA, and multiple means comparison T test (LSD) in SAS v 9.4.

Objective 2: Young Tree Trials
Wenatchee: 610 Aztec Fugi on M9, 500 Aztec Fugi on G-935, 500 Anjou Pear on OHXF87, 135 WA38 on G935 were planted at the Columbia View Research Station for fire blight trials.

Oregon: Application timing of concentrated Actigard 50WG (acibenzolar-S-methyl (ASM), Syngenta Crop Protection, Greensboro, NC) treatments was evaluated on 1st-leaf Fuji apple trees as either a pre-plant or post-plant trunk spray for protection from fire blight infection. The experiment was arranged in a randomized block design with three treatments and 33 replications of single-tree plots. Treatments consisted of two trunk-paint treatments -- concentrated Actigard (30 g/liter) applied prior to or after planting (just prior to inoculation) -- and an untreated control treatment. Trees were planted on 3 May 2019. For the pre-plant treatment, Actigard plus 1% Break-Thru S 240 (polyether-modified polysiloxane, Evonik Corp., Richmond, VA)50W (30 g/liter) was applied to trunks trees by spraying the central leaders with the mixture in a 1-liter, hand held pump sprayer (model 418, Solo Inc., Newport News, VA). The sprayer was equipped with a cone-shielded nozzle, and during application, the nozzle tip was positioned a distance of 1-cm from the trunk surface spraying a 100-cm length of the central leader (126 cm avg. trunk height) on two opposing sides of trunk; approximately 60 ml of suspension was sprayed onto each tree. The pre-plant spray was allowed to dry before trees were planted. The post-plant application of Actigard 50WG was applied similarly to a different set of 33 trees on 4 June. On 7 June, all trees were inoculated with a mixture of four *Erwinia amylovora* isolates suspended in water at concentration of 10^9 CFU per ml. To inoculate a tree, the youngest three leaves on five actively growing shoot tips were cut along the mid-rib with a scissors that had been dipped in the pathogen suspension. One of inoculated shoot tips was covered in a plastic re-sealable bag containing 1 ml of SDW. Bags were removed from trees 3 days after inoculation. On 12 June, necrosis and ooze were visible on some inoculated shoots. Detailed disease assessments occurred on 24 July and 18 September. Measured variables included number of shoots infected, incidence of trunk cankers and canker length (cm) on trunks.

Objective 3: Test strategies to manage blocks once they are infected. This objective took place at one farm in East Wenatchee WA, two sites in western NY and one site in PA.

Wenatchee, WA site: A half-acre plot of 105 naturally infected trees located in a commercial orchard was used for this study. The experiment was arranged in a randomized, complete block design with 15 replications of 7 treatments applied to single tree plots where each tree had 1 to 14 naturally infected strikes per tree. Treatments included: **Best Management Practice** - Cutting back 12-18" from the end of the infected area into 2-year old wood and sanitizing loppers with a 10% Clorox

solution; **No Sanitation -** Cutting back 12-18" from the end of the infected area into 2-year old wood *without* sanitation; **Aggressive -** Cutting back 30" from the end of the infected area; **Long Stub -** Cutting back leaving a 5" stub and sanitizing between cuts; **Short Stub -** Cutting back leaving a 1-2" stub and sanitizing between cuts; **Breaking -**Breaking back to the joint at the end of the first-year growth; **No-treatment control.**

New York site: The trial was conducted in two sites in western NY. One was a 0.75-acre planting of 120 4-year 'EverCrisp' trees that were inoculated at 80% bloom to ensure a high level of shoot blight. The experiment was arranged in a randomized, complete block design with 10 replications of 6 treatments applied to single tree plots where each tree had 10 to 20 strikes per tree. The second site was a 1.2-acre planting of 150 7-year 'Idared' apples that were also inoculated at 80% bloom. The experiment was arranged in a randomized, complete block design with 10 replications of 5 treatments applied to single tree plots where each tree had 5 to 20 strikes per tree. Treatments (same as WA).

Pennsylvania site: 36 Gala trees planted in 2015 on M7 rootstock were used for the trial. Due to limited source of trees with fire blight for the cutting trials, treatments were adapted. Treatments included with and without sterilization leaving a 2-inch and 5-inch stub, in addition to breaking the branch and to control of leaving the fire blight in the tree. Cuts performed on July 19, 2019. Ratings in October included % of the cuts forming cankers at the site of the cut; % of the cankers formed progressing into the current season wood; and then % of the cankers formed progressing through to last season's wood. Each cut was rated using a 0 – 1 rating (0 = no; 1 = yes). Stats were performed on the level of % incidence of yes/no.

RESULTS

Objective 1. Blossom Blight Trials

Table 1: Fire Blight Materials 2019 Red Delicious Apples, Wenatchee WA. DuPont, T. Washington State University [a]

Treatment	Strikes per 100 Clusters				rate per 100 gal	Timings***
Kudos 6oz	24.0	±	6.9	a	6 oz	pink
Kudos 3oz	21.8	±	12.5	a	3 oz	pink
water	21.0	±	11.0	a	---	full bloom
Serenade 40oz	20.3	±	8.2	ab	40 oz	day before and day after 100% bloom, petal fall
Serenade 20oz	16.0	±	3.2	abc	20 oz	day before and day after 100% bloom, petal fall
Cueva	11.5	±	4.1	abc	4 qt	day before and day after 100% bloom, petal fall
Previsto	8.0	±	3.7	bc	3 qt	day before and day after 100% bloom, petal fall
Organic Control**	6.0	±	1.1	c	**	LS: 70%, BP 20%, 80%; PR 100%, petal fall
Fireline	5.7	±	3.1	c	24 oz	50% bloom, 100% bloom, petal fall
Firewall	4.8	±	2.8	c	28 oz	50% bloom, 100% bloom, petal fall
Alum	4.3	±	2.7	c	1%	100% bloom, petal fall

[a] Inoculation was conducted on the evening of April 27, 2019 at full bloom (of king blooms), and May 1 petal fall using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain). **Organic control: Lime sulfur (6%) at 70% bloom; Blossom Protect + Buffer Protect (1.24 lb + 8.75 lb) 20% and 80% bloom; soluble copper (Previsto 3qt) 100% bloom & petal fall. ***2019 application dates were: April 21 (pink), April 23 (20% bloom), April 24 and 25 (50% bloom), April 26 (full bloom minus 1 day), April 27 (full bloom), April 28 (full bloom plus 1 day), May 1, 2019 (petal fall), May 2, May 4 and May 6, and May 10, 2019.

Table 2. Resistance Inducers for Apple Fire Blight Suppression 2019

Gala apples, Corvallis, Oregon, K. B. Johnson & T. N. Temple, Oregon State University

Treatment	Strikes per 100 Clusters				rate per 100 gal	Timings***
Water check	26.67	±	4.25	a#	---	10% bloom, full bloom, petal fall
Kudos 3oz x, y	17.00	±	1.21	ab	3oz	10% bloom
Untreated check	13.83	±	1.58	bc	---	-----
Actigard 6oz x, y	12.17	±	4.38	bc	6 oz	10% bloom
Kudos x, y, Actigard y	11.17	±	3.53	bc	2 oz, 3.2oz	10% bloom
Kudos 6oz x, y	10.17	±	3.42	bc	6 oz	10% bloom
Actigard 3xz	5.33	±	2.04	c	2 oz	10% bloom, full bloom, petal fall

* Trees inoculated on 24 April with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain). ***10% bloom (April 23), full bloom (April 26), petal Fall (May 1)
'---' indicates material was not applied on that specific date. # Means within a column and within a section followed by same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference.
x Amended 1:1 with ammonium sulfate. y Amended with Regulaid: 16 fl. oz. per 100 gallons. z Amended with BioLink Spreader-Sticker: 4 fl. oz. per 100 gallons.

Table 3. Non-Antibiotic Materials for Control of Pear Fire Blight 2019
Bartlett pear, Corvallis, OR, K. B. Johnson & T. N. Temple, Oregon State University

Treatment	Strikes per 100 Clusters				rate per 100 gal	Timings***
Water	9.0	±	1.3	a#		full bloom, petal fall
Serenade Opti	5.1	±	1.3	b	20 oz	full bloom, petal fall
Blossom Protect + Buffer Protect	2.7	±	0.7	bc	21.4 oz, 150 oz	70% bloom, full bloom
Blossom Protect + Buffer Protect, Alum	2.3	±	1.4	bc	21.4, 140 oz, 133.5 oz	70% bloom BP, full bloom, petal fall Alum
FireWall	1.7	±	0.5	c	8 oz	full bloom

* Trees inoculated on 18 April with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain). ***70% bloom (April 18), full bloom (April 20), petal fall (April 24).
--- indicates material was not applied on that specific date. # Means within a column followed by same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference.

Table 4. 2019 Evaluation of Programs to Manage Blossom Blight on 'Cameo' at Penn State FREC in the Aville Dwarf Cameo Block[3]

Treatment & Amount/A (in 100 gal)	Timing[1]	% Blossom blight incidence[4]	% Blossom blight control
Untreated	--	94.1 a[2]	--
FireWall 24 oz	50% Bl, 80% Bl, 100% Bl, PF	1.4 e	99
FireLine 24 oz	50% Bl, 80% Bl, 100% Bl, PF	10.2 e	89.2
Alum 8 lb	50% Bl, 80% Bl, 100% Bl, PF	40.1 d	57.4
Serenade Opti 20 oz	50% Bl, 80% Bl, 100% Bl, PF	66.5 c	29.4
Serenade Opti 40 oz	50% Bl, 80% Bl, 100% Bl, PF	70.6 bc	25
Apogee 6 oz	P	65.3 c	30.7
Regalia 64 fl oz	P, PF	88.3 ab	6.2
Regalia 32 fl oz	50% B		

[1]Treatments were applied using a backpack mist blower until mist run-off. Application timings: Pink (P; 17 Apr); 50% Bloom (24 Apr); 80% Bloom (26 Apr); 100% Bloom (29 Apr); Petal Fall (PF; 2 May).
[2]Values within columns follow by the same letter(s) are not significantly different ($\alpha \leq 0.05$) according to Fisher's Protected LSD test. [3]Twelve year-old 'Cameo' trees on B.9 rootstocks were used and two- tree treatments were arranged in a randomized complete block with four replications. [4]All blossoms were inoculated on the tree, with the exception of the top 1-2 feet of the tree (could not be reached, unless with a ladder). Blossoms were inoculated late afternoon at 26 Apr with a bacterial suspension of 10^7 *Erwinia amylovora* cells/ml using a spray bottle. Blossom clusters were rated during the third week of May. Blossom clusters were rated infected if at least one blossom was dead. Due to the trees being overwhelming infection of blossoms for the majority of the treatments, shoot blight incidence was not counted.

Table 5. 2019 Evaluation of Programs to Manage Blossom Blight on 'Gala' at Cornell Agritech

Treatment programs (amt./A)*	Timing*	Incidence of blossom blight (%)**	Incidence of shoot blight (%)**
Non-treated	NA	88.1 ± 3.3 a	54.7 ± 5.4 a
FireWall 17WP 24 oz + Regulaid 3 pt	3	5.5 ± 2.1 de	1.3 ± 1.3 cd
Alum 8 lbs/100 gal	3,5	20.3 ± 5.5 bcd	10.1 ± 3.1 bcd
Blossom Protect 1.5 lbs + Buffer protect I 10.5 lbs	1,2,3	7.0 ± 2.5 de	1.6 ± 1.1 cd
Blossom Protect 1.5 lbs + Buffer protect II 7.5 lbs	1,2,3	8.0 ± 4.9 cde	3.5 ± 3.5 bcd
Serenade Opti 20 oz + Regulaid 3 pt	3,4,5,6	24.0 ± 5.6 bc	11.5 ± 1.6 bcd
Serenade Opti 20 oz + Regulaid 3 pt Actigard 2 oz/100 gal	3,4,5,6 1,6	15.0 ± 6.3 bcde	5.5 ± 2.0 bcd
Double Nickel LC 1 qt + Cueva 2 QT	1,3,5	19.0 ± 6.8 bcd	8.6 ± 5.1 bcd
Stargus 64 fl oz + Regulaid 48 fl oz	3	18.8 ± 6.4 bcde	11.6 ± 4.3 bcd
LifeGard 13.5 oz	1,3,5	16.3 ± 3.1 bcde	6.5 ± 2.0 bcd
LifeGard- 13.5 oz + Cueva 2 QT	1,3,5	12.0 ± 2.8 bcde	6.8 ± 3.0 bcd
Regalia 16 fl oz + Regulaid 48 fl oz	1,5	13.3 ± 6.3 bcde	7.6 ± 1.9 bcd
Regalia 16 fl oz + Regulaid 48 fl oz + Apogee 2 oz/100 gal	1,5	26.3 ± 8.5 b	7.3 ± 1.8 bcd
Apogee 2 oz/100 gal + Actigard 1 oz/100 gal	1,5	19.5 ± 8.6 bcd	6.3 ± 3.9 bcd
Apogee 3 oz/100 gal pink	1	17.8 ± 8.1 bcd	7.5 ± 2.0 bcd
Apogee 6 oz/100 gal pink	1	15.0 ± 4.9 bcde	6.1 ± 1.2 bcd
Apogee 3 oz/100 gal tight cluster	0	15.3 ± 6.3 bcde	9.7 ± 3.4 bcd
Apogee 3 oz/100 gal tight cluster	0	13.8 ± 0.5 bcde	8.9 ± 4.1 bcd

*Treatment timings were: 8 May "pink" (application 1) 13 May-40% bloom (application 2); 16 May- 80% bloom (application 3); 23 May-100% bloom (application 4); 30 May- petal fall/early terminal shoot growth (application 5); 5 Jun- terminal shoot growth (application 6). Rates are in amount per acre except where otherwise noted (see text above).
**All values represent the means and standard errors of 4 replicate trees. Values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to the LSMEANS procedure in SAS 9.4 with an adjustment for Tukey's HSD to control for family-wise error.

Objective 2. Young Tree Trials

Oregon. Overall, 99 of 100 (99%) of inoculated trees developed fire blight symptoms on at least one shoot. Number of infected shoots per tree was highest for untreated and pre-plant Actigard (4.1 of 5) and lowest for post-plant Actigard (3.1 of 5). By 18 September, trunk cankers developed and advanced on 88% of untreated trees and on 79% of trees treated with Actigard pre-plant. In contrast, trunk cankers developed on only 42% of trees treated with Acigard near post-plant (near inoculation). For those trees with trunk infection, by September, the average canker on a post-plant Actigard-treated trees was 78% smaller than the average canker on an untreated tree.

Table 6. Response of Fuji apples trees to inoculation with *E. amylovora* after trunk treatment of Actigard 50WG prior to or after planting.

	Untreated		Pre-plant Actigard		Post-plant Actigard	
Disease response	July 24	Sept 18	July 24	Sept 18	July 24	Sept 18
No. infected shoots post inoculation*	4.1± 1.1	-	3.9 ± 1.1	-	3.1 ± 1.2	-
Incidence of trunk canker**	85%	88%	65%	79%	39%	42%
Canker length infected trunks***	29 ± 17	49 ± 33	25 ± 20	46 ± 36	10 ± 5	11 ± 5

* Five shoots per tree were inoculated on 7 June with 1 x 10^9 CFU/ml *Erwinia amylovora* isolate mixture and were assessed for fire blight on 24 July and 18 September (± standard deviation).
** Percent of inoculated trees that developed a trunk canker (of a total of 33 trees per treatment).
*** Mean canker length (cm ± standard deviation) on trunks with symptoms; zero values not included.

Objective 3. Test strategies to manage blocks once they are infected. *Does the cutting treatment keep fire blight from spreading to form new strikes in the tree?* Fire blight bacteria is known to move through the tree's vascular system from initial infection sites to create new infections in other young susceptible shoot tips. Timely summer cutting of fire blight strikes is important to reduce the number of bacterial cells in the tree and the probability the cells remaining after cutting will be numerous enough to create new infections.

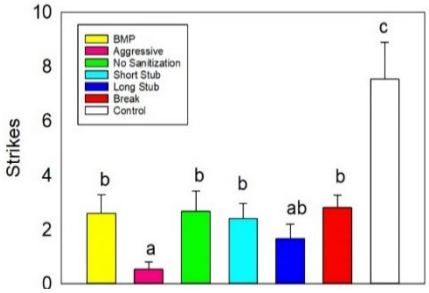


Figure 1 Number of New Strikes After Initial Cutting. Case Study 1 Washington.

Case study 1 (Wenatchee) All cutting treatments had significantly fewer additional strikes occur compared to no treatment controls (Figure 1). Aggressive cutting (more than 30 inches) had the lowest number of new strikes. However, the aggressive treatment had little tree remaining to initiate new strikes. Summer cutting greatly reduced the number of new strikes.

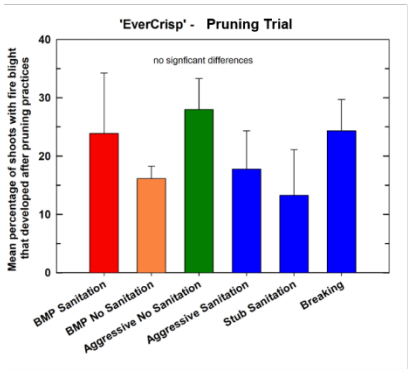
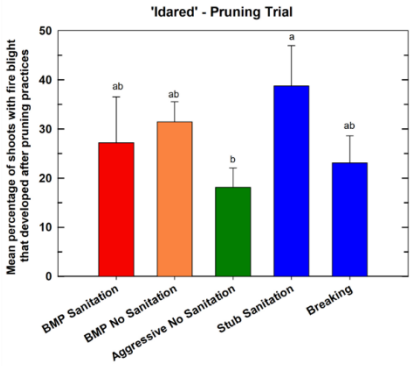


Figure 2 Case study 2 New York.



Case study 2 (New York). In both locations all trees that did not receive pruning all died. New strikes developed on all trees by the end of the season. In the EverCrisp trees, there were no differences among the programs in the percentage of strikes that developed after the pruning. In the Idared, block fewer strikes developed after pruning in the aggressive sanitation program (Figure 2.).

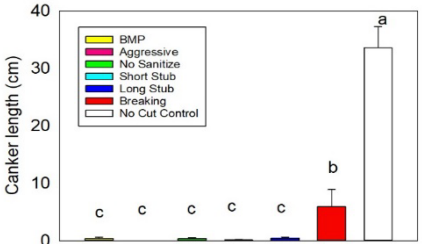


Figure 3 Average canker length left in trees end of the season (cm) (Wenatchee).

Which cutting treatments prevent rootstock infection and tree death?
Case study 1 (Wenatchee) No rootstock infections as indicated by oozing cankers, purpled leaves or early leaf drop were detected as of fall 2019. Additional evaluation will be made in spring 2020.

Case study 2 (New York). In both locations all trees that did not receive pruning all died, and all pruning treatments prevented rootstock infection and death.

How much canker is left to ooze next spring?
New fire blight infections in spring originate from ooze made by overwintering cankers. Fire blight cutting treatments which reduce the amount of canker tissue may reduce fire blight risk if winter cutting does not effectively manage remaining cankers. In the fall after cutting, treatments were evaluated for the length of remaining cankers to determine which treatments may most effectively reduce risk of new infections the following spring.

Case study 1 (Wenatchee) No cut control treatments averaged 34 centimeters of infected canker tissue (Figure 3). BMP, Aggressive, No Sanitation, Long Stub and Short Stub treatments ranged from 0 to

0.14 centimeters. Breaking averaged 6 centimeters, significantly higher than other cutting treatments. Breaking treatments frequently had a canker develop where the broken area meets larger diameter wood.

Case study 2 (New York). In Idared trees little infected tissue developed on pruned shoots after cutting. In EverCrisp breaking was significantly worse than aggressive cutting averaging 70 cm of infected canker tissue per shoot (Figure 4).

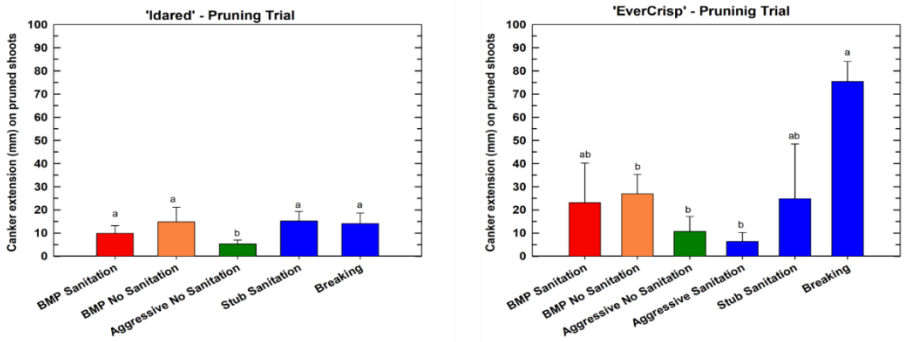


Figure 4 Case study 2 (New York) for Idared (left) and Evercrisp (right).

Can leaving a stub prevent cankers from reaching larger leaders?

High density apple plantings are often pruned to rejuvenate and maintain young productive wood growing directly from central leaders. This young one and two-year-old wood is susceptible to fire blight which can quickly travel to central or main leaders which when infected have to be stumped eliminating productive capacity until the tree regrows. A stub cut is hypothesized to prevent cankers from reaching structural wood. While bacterial concentrations are still sometimes high enough to initiate new infections, these new cankers are on a stub which can then be cut back in winter (Figure 3a) versus reaching the main leader (Figure 3b).

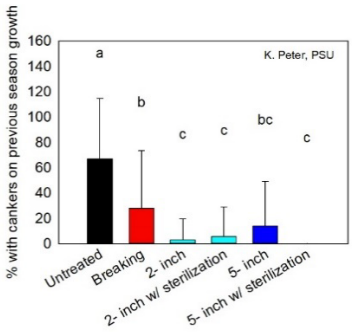


Figure 6 Pennsylvania Cutting trial.

Case study 1 (Wenatchee) Cutting treatments were evaluated as to whether cankers reached structural wood in fall 2019. Short Stub and Long Stub treatments both had significantly fewer cankers on structural wood (less than 1%) than Best Management Practice, Breaking and No Treatment Control (2, 6 and 7% respectively).

Case study 2 (New York). In all programs no cankers reached the central leader or expanded into the central leader when left flush cut.

Case study 3 (Pennsylvania). Leaving a 2 or 5 inch stub prevented more cankers from reaching previous year's wood compared to breaking flush to older wood (Figure 6)

Table 7. Results from Pennsylvania Case Study 3

Cutting treatment	% Cuts forming cankers	% Cuts progressing through current season growth	% Cuts progressing through previous season's growth
Untreated	97.2 ± 16.7 a	91.7 ± 28.0 a	66.7 ± 47.9 a
Breaking	26.1 ± 28.7 b	33.3 ± 47.8 b	27.8 ± 45.4 b
2- inch	16.7 ± 37.8 c	16.7 ± 37.8 b	2.8 ± 16.7 c
2- inch w/ sterilization	22.2 ± 37.8 bc	25.0 ± 43.9 b	5.6 ± 23.2 c
5- inch	22.2 ± 42.2 bc	25.0 ± 43.9 b	13.9 ± 35.1 bc
5- inch w/ sterilization	19.4 ± 40.1 bc	22.2 ± 42.2 b	0.0 ± 0.0 c

CONTINUING PROJECT REPORT **YEAR**: No-cost extension

Project Title: Implementation of alternative methods to control replant disease

PI:	S. Tianna DuPont	**Co-PI**:	Mark Mazzola
Organization:	Washington State University	**Organization**:	USDA-ARS
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Email:	tianna.dupont@wsu.edu	**Email**:	Mark.Mazzola@ARS.USDA.GOV
Address:	1100 N. Western Ave	Address:	1104 N. Western Ave.
City/State/Zip:	Wenatchee/WA/98801	City/State/Zip:	Wenatchee/WA/98801

Cooperators: Mike Robinson, BMR Orchards; Jim Baird, Baird Orchards; Sam Godwin, Box Canyon Orchard

Total Project Request: **Year 1:** $60,577 **Year 2:** $34,163 **Year 3:** $35,248

Other funding sources

Agency Name: USDA Crop Protection
Amt. awarded: $195,713
Notes: USDA Crop Protection Grant funds two additional sites. Thank you to in kind support from Progene Seeds, Trident Fumigation, Farm Fuel, Baird/BMR Orchards, Box Canyon Orchard, Gold Crown Nursery, Cameron Nursery.

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu/ shelli.tompkins@wsu.edu

Item	2017	2018	2019
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies[1]	23,609	2,000	2,000
Travel[2]	1,037	1,037	1,037
Plot Fees			
Miscellaneous			
Total	24,646	3,037	3,037

Footnotes:
[1]Plot set up and maintenance supplies over and above normal horticulture, i.e. seed meal, virtually impermeable plastic, etc.
[2]Travel to Othello for plots set up, maintenance and sampling.

Budget 2

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

Telephone: 510-559-6019 **Email address:** chuck.myers@ars.usda.gov

Item	2017	2018	2019
Salaries	19,800	20,592	21,416
Benefits	6,283	6,534	6,795
Wages[1]			
Benefits[2]			
Equipment			
Supplies[3]	9,848	4,000	4,000
Travel[4]			
Miscellaneous			
Plot Fees			
Total	35,931	31,126	32,211

Footnotes:

[1]Wages for a 33% scientific assistant to conduct microbial analysis of rhizosphere.

[2]Benefits at 31.7% for scientific assistant.

[3]Microbial analysis supplies.

OBJECTIVES

1. Conduct field scale experiments to test the efficacy of bio-renovation and anaerobic disinfestation as alternatives to soil fumigation for the control of apple replant disease. At each on-farm site four treatments (mustard seed meal bio-renovation, anaerobic soil disinfestation, fumigated control and non-fumigated control) will be applied in randomized strips in each of four blocks (four replicates each). Plant response to treatments will be assessed by measuring trunk cross sectional area and yield. In addition, microbial analysis of roots and soil will be conducted to determine treatment effects on target replant pathogens and overall composition of the microbiome including potential beneficial microbes.
2. Use field scale experiments to demonstrate to growers the steps to bio-renovation and anaerobic soil disinfestation. Each step will be documented with photos and video to create Extension factsheets explaining the process and lessons learned. Conducting trials at a large plot scale will allow us to use the same equipment growers would use, develop practical expertise, and work out the inevitable kinks with a new technique.

SIGNIFICANT FINDINGS

- **Tree growth of brassica seedmeal treatments was not significantly different than or greater than the fumigated control across all sites as measured by the difference in tree cross sectional area (TCSA) between 2018 and 2019, 2019 TCSA and tree height. Brassica seedmeal treatments had larger tree growth than no treatment controls in North and Central sites but not South site as measured by the difference in tree cross sectional area (TCSA) between 2018 and 2019, 2019 TCSA and tree height.**
- **Anaerobic soil disinfestation (ASD) tree growth was lower than fumigation and not different than no treatment control in Tonasket. In Rock Island ASD tree growth was not significantly different than fumigation and higher than no treatment control.**
- **In the Southern site (Othello) the anaerobic treatment was initiated again in 2018 in order to attempt to successfully reach anaerobicity. 2017 treatments did not keep the soil as wet as the protocol required. 2018 treatments reached anaerobicity. No significant differences were detected between ASD and control though the trend was towards larger growth in ASD.**
- **In the Othello site planted in 2018 fruit was harvested from second leaf trees. Brassica seedmeal yield in bins per acre was significantly lower in brassica seedmeal treatment compared to the fumigated control.**
- **At the end of the first growing season, Brassica seed meal amendment provided control of lesion nematode root populations at all three sites and was superior to pre-plant soil fumigation for nematode control at the Othello and Tonasket sites. ASD resulted in lesion nematode control that was similar to that attained with fumigation at the Othello and Tonasket sites.**

METHODS

Experimental site and design:
The experiment was implemented at three field sites including a 12-acre grower field in Othello WA (46.933876, -119.392096), a 1.5-acre field at Washington State University Sunrise research orchard at Rock Island, WA (47.31988,-120.0663747) and a grower 1 acre field in Tonasket WA (48.810692, -119.505724). All sites had a history of replant disease. Soil type at the Rock Island site is a Pogue fine sandy loam, at the Tonasket site are a Nighthawk loam, and Adkins is a very fine sandy loam, 0-5 percent slope. Sites were split into experimental blocks of 40 ft by 200 ft with 5 experimental

blocks in Rock Island and 4 in Tonasket and Othello. In Tonasket and Rock Island each of four soil treatments including anaerobic soil disinfestation (ASD), brassicaceae seed meal (BSM), fumigation, and no-treatment control were randomly assigned to 10 ft by 200 ft plots within each 8,000 square foot experimental block. In Othello BSM, ASD and fumigated treatments were assigned to 0.8 acre plots (3,300 to 3,600 tree row ft) in each block. The non-fumigated control treatment was assigned to an 80 by 5-foot plot nested within the fumigated control. Plots in Rock Island were further split based on the rootstock that will be planted into the soil (M9 or G41).

Treatments:

ASD treatment Rock Island and Tonasket: Soil moisture of the ASD plots was brought up to 17% volumetric moisture content (VMC) and 20.3% gravimetric moisture content using a sprinkler irrigation system and big gun irrigation prior to biomass application. Timothy hay was applied to the ASD plots at 8 tons/A, flailed to chop into small particles and sampled for particle size and nutrient levels. Biomass was incorporated into soil with a rototiller in two to four passes (July 5, 2018 Rock Island; August 8, 2018 Tonasket). Totally impermeable plastic film (Rock Island: TIF, 1.2 ml; clear, Vaporsafe, Trical, Gilroy, CA; Tonasket: TIF, Tri Est, Black, 1.2 ml) was applied using a plastic layer (Mechanical Transplanter) covering ASD plots. Soil moisture was then brought to and maintained above 30% VMC using a double drip line running constantly under the impermeable film. Treatments were maintained for 4 weeks. At the end of 4 weeks plastic was removed and irrigation turned off. Plots were allowed to aerate to reduce phytotoxicity for 4 weeks before samples were taken.

ASD treatment Othello: On June 28, 2017 triticale was cut and swathed into four-foot windrows using a John Deere R450 swather where swaths were lined up on future tree rows. Swathing concentrated plant biomass produced on a ten-foot width into a four-foot width area. As such 3.5 T/A field grown biomass averaged 10 ton/A applied biomass to the tree row. Six days (July 3) after cutting at 20% moisture, triticale was flail chopped using a Pak flail which left a six-foot wide swath of chopped biomass. A hay rake was used to re-concentrate chopped material into the four-foot wide treatment areas. Biomass was incorporated with a Celli rototiller to an eight-inch depth. Three-acre inches of irrigation (0.28 in/hr) were applied using hand lines with 6 gal/ min sprinkler heads to thoroughly wet the soil. Four to eight hours after irrigation, plastic was laid to seal the treatment area. Soil moisture averaged 24% in the top 5 cm and 25% at 15 to 20 cm at the time plastic laying began and 24 % (0-5cm) and 19% (15-20 cm) by the time plastic laying was finished in reps A&B. Soil moisture averaged 30% (0-5cm) and 32% (15-20 cm) as plastic laying commenced in reps C&D. Plots were irrigated with an additional 3 acre inches of water (11 hrs) resulting in soil moistures averaging 26% (0-5cm) and 34% (15-20 cm). One week after initial irrigation plots were re-wetted with an additional 1.7 acre inches of water.

Mustard meal treatment (BSM) Rock Island and Tonasket: Initial soil moisture in mustard treated plots was 25% and temperature was 81℉ (27℃) in Rock Island. Soil moisture was brought up by using both big gun and sprinkler irrigation systems (July 2-4, 2018). At soil moisture appropriate for tractor operation, Pescadero Gold Mustard meal (1:1 formulation of B. juncea and S. alba) (Farm Fuels Inc., Watsonville, CA) was applied using a Whatcom spreader at 1.7 lb per tree-row-foot (1.6 lb per tree-row-foot target) and raked to form 4-foot-wide strip (July 6, 2018 Rock Island; August 9, 2018 Tonasket). Mustardmeal was incorporated and mixed thoroughly into the soil using a rototiller to an 8 to 10-inch depth. The plots were sealed with totally impermeable film using a plastic layer within 20 minutes of incorporation.

Mustard meal treatment (BSM) Othello On July 15, 2017, 3.4 inches of irrigation water was applied (0.28 in/hr). On July 19 and 20, 2017 when soils had drained and dried to moisture appropriate for tractor implements Pescadero Gold Mustard seed meal (Farmfuels) was applied using

a Whatcom compost spreader at 1.6 lbs per tree-row-foot in a four-foot swath (6.8 T/treated acre, or 3.4 T/orchard acre). Mustard seed meal was incorporated into soil using a Celli rototiller within a maximum of 3 hours of spreading (average 30 minutes) and sealed with Totally Impermeable Film (TIF, Vaporsafe, Trident Inc) within ten to thirty minutes of incorporation. Soil temperature averaged 24˚ C (75 °F) at the time of treatment application.

Tree Establishment

Tonasket Lucy Rose (TC2) on Bud 10 Rootstock were planted on May 1, 2019 at a spacing of 10 by 2 ft by planting into a furrow created by a moldboard plow. All treatments received 1200 lb/A compost banded on a row and incorporated with a rototiller. Organic management commenced including mowing weed management. A small amount of damage from deer affected plots before deer fence was installed. **Central** WA38 on M9 and G41 rootstock trees were planted March 17, 2019 using hand planting on a 3 by 12 foot spacing. Trees were immediately clipped to trellis wires for support. Grass drive rows were established on May 7, 2019. Pre-plant fertilizer application was 360 lb per acre of a 15-15-7-3 K-S-Ca-Mg product (Wilbur Ellis Sieler Siding) supplying 55 lbs/A potassium, 56 lb/A Sulfur, 28 lb/A calcium and 14 lb/A Magnesium. Initial pruning, stubbing all branches to 20 to 15 cm was done May 20 to 30, 2019. 64 units of N (10 gal Calcium Ammonium Nitrate, 17% N per application) were applied in three applications 7 to 10 days apart (July and August) to all rows with the exception of mustard meal treatments (mustardmeal contained 306 T N/A). Thirty-eight trees (M9) were had low tree quality at planting resulting in death or the need for severe pruning and were excluded from data collection. **Othello** WA38 on G41 rootstock trees were planted in April, 2018 by hand on a 4 by 10 spacing. 2 Tons per acre of a 50/50 blend of mint compost (Soil Suplimint) and chicken compost (Nature's Nutrients) were applied. Trees were clipped to wires for support within 2 weeks. **Othello 2** WA38 on G41 were planted in April 2019 on a 4 by 10 spacing.

Site Maintenance

Othello Maintenance fertilizer included 25# N in the form of feather meal plus bone meal (Pro Natural Dry) in both May and June. A fall application of mint plus chicken compost was applied at 1 Ton/A and incorporated via rototill.

Field Measurements

Northern Site: Tonasket *Tree growth measurements:* In each plot containing approximately ten trees were marked at 20 cm above the graft union (every fifth tree with 5 buffer trees on each end of the row). Diameter measurements were conducted on June 28, 2019 using a tree caliper (2 perpendicular measurements) to establish baseline size and again on October 2, 2019 to measure first year growth. *Root and Rhizosphere soil sampling:* Five of 10 trees were randomly designated for root and rhizosphere soil sampling. Two to 4 sections of fine roots at a depth of 5-20 cm at a distance of 20 to 40 cm from the tree base were harvested with sanitized tools on October 2, 2019. Rhizosphere soil (approximately 4 to 6 grams) from immediately around roots sampled was gently removed from sampled root segments. Root samples were kept at 4° C until processing. Rhizosphere soil was kept at -20 C short term (extracted DNA, or long-term storage at -80 C).

Central Site: Rock Island *Tree growth measurements:* In each plot containing 30 trees ten trees were marked at 20 cm above the graft union (every other tree with 4 buffer trees on each end of the row). Diameter measurements were conducted on May 22, 2019 using a tree caliper (2 perpendicular measurements) to establish baseline size and again on October 7, 2019 to measure first year growth. *Root and Rhizosphere soil sampling:* Five of 10 trees were randomly designated for root and

rhizosphere soil sampling. Two to 4 sections of fine roots at a depth of 5-20 cm at a distance of 20 to 40 cm from the tree base were harvested with sanitized tools on October 7, 2019. Rhizosphere soil (approximately 4 to 6 grams) from immediately around roots sampled was gently removed from sampled root segments. Root samples were kept at 4 C until processing. Rhizosphere soil was kept at -20 C short term (extracted DNA, or long-term storage at -80 C).

Southern Site: Othello *Tree growth measurements:* A total of 36 trees were selected per plot in a checkerboard pattern in the central 2 rows of each acre block (15 inner trees in control plots). Diameter was measured at 20 cm above the graft union on October 25, 2018 and October 14 to 24, 2019. *Root and Rhizosphere soil sampling:* Five of 36 trees were randomly designated for root and rhizosphere soil sampling. Two to four sections of fine roots at a depth of 5-20 cm at a distance of 20 to 40 cm from the tree base were harvested with sanitized tools on October 20 to 25, 2018 and October 11 to 15, 2019. Rhizosphere soil (approximately 4 to 6 grams) from immediately around roots sampled was gently removed from sampled root segments. Root samples were kept at 4 C until processing. Rhizosphere soil was kept at -20 C.

Southern 2: Othello *Tree growth measurements:* Eight of thirteen trees per plot were selected for growth measurements (every other tree with two buffer trees). Diameter was measured at 20 cm above the graft union on June 5, 2019 and October 15, 2019. *Root and Rhizosphere soil sampling:* Five of eight trees were randomly designated for root and rhizosphere soil sampling. Two to 4 sections of fine roots at a depth of 5-20 cm at a distance of 20 to 40 cm from the tree base were harvested with sanitized tools on October 15, 2019. Rhizosphere soil (approximately 4 to 6 grams) from immediately around roots sampled was gently removed from sampled root segments. Root samples were kept at 4 C until processing. Rhizosphere soil was kept at -20 C.

Yield

Othello: Full plot yield was determined via commercial picking of 1 acre (6 row plots) where set of two rows were picked into labeled bins placed down the center of two rows. Bins per acre were calculated via bins per plot accounting for number of trees per plot. Twenty tree control plots were hand harvested and yield per plot calculated to yield per acre based on trees per acre and percent harvestable fruit (commercially unpickable sunburned fruit discarded).

Lab methods

Assessment of lesion nematode root populations: *Pratylenchus penetrans* root densities were determined in October of the year. Nematodes were extracted from a 0.5-g fine root sample obtained from each sampled tree. Root tissue was placed in a 150-ml flask containing 70 ml sterile distilled water and incubated on a rotary shaker at 140 rpm for 5 days. Nematodes were collected by passing the suspension twice through a 500 µm-mesh sieve and then backwashing into a counting dish. Number of *P. penetrans* were counted using a light microscope (×40 magnification).

T-RFLP analysis: Rhizosphere soil samples collected October of the year were removed from root samples and extraction of DNA was conducted using the MO BIO PowerMax Soil DNA Isolation Kit from 4 to 6 grams of soil. Terminal-restriction fragment length polymorphism (T-RFLP) analysis was utilized to profile bacterial and fungal communities in these soil samples. Fluorescently labeled PCR products were generated by using labeled ITS1F/ITS4 primers for amplifying the fungal ITS region, and 8f/907r primers for amplification of the bacterial 16S rDNA region. Amplification reactions and T-RFLP analysis was conducted following the protocol as previously described (Weerakoon et al., 2012).

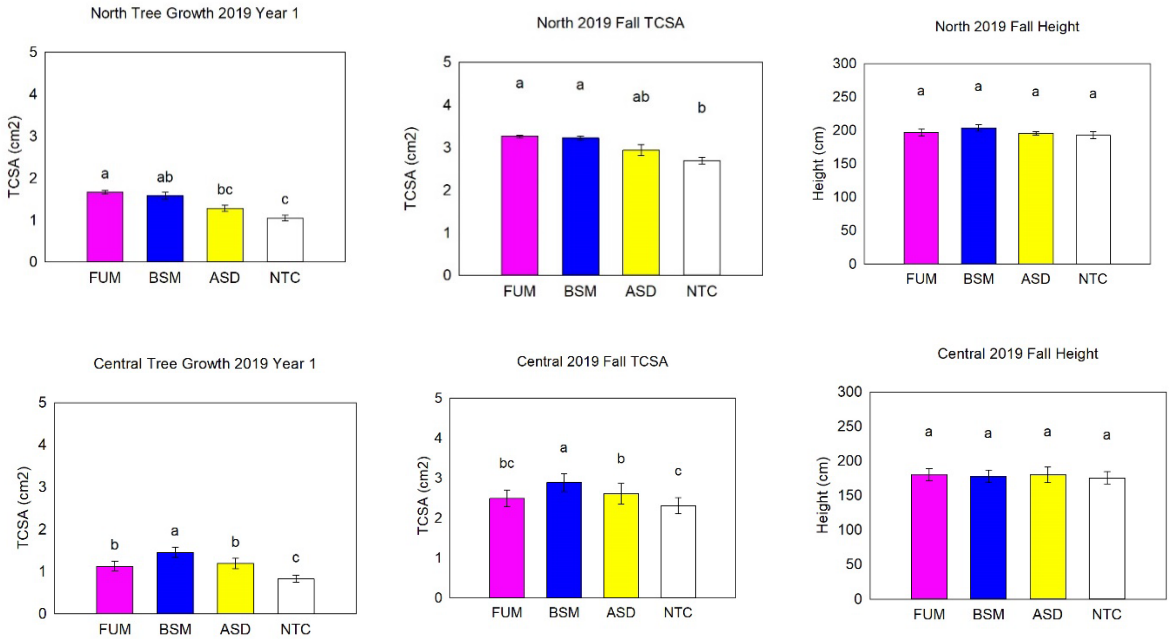
High throughput sequencing: Alternatively, the microbial profiles were generated using high-throughput DNA sequencing protocols. DNA was extracted from rhizosphere soil samples as noted above. PCR amplification, purification, library preparation and fungal/bacterial sequencing were conducted at an external facility (Molecular Research, Shallowater, TX) on a MiSeq platform. Post-processing statistical analysis of sequencing derived OTU data was conducted using Explicet software (Robertson et al., 2013) and visualization through ordination of microbiome data was conducted PAST software package ver 3.16 (Hammer et al., 2001).

Statistics: Tree height, tree cross sectional area and yield were analyzed using an analysis of variance, proc glm (SAS 9.4). Post hoc pairwise comparisons between treatments were determined using a *Tukey's* Honest Significant Difference *test***.**

RESULTS & DISCUSSION

Tree growth of brassica seedmeal treatments was not significantly different than or greater than the fumigated control across all sites as measured by the difference in tree cross sectional area (TCSA) between 2018 and 2019, 2019 TCSA and tree height. Brassica seedmeal treatments had larger tree growth than no treatment controls in North and Central sites but not South site as measured by the difference in tree cross sectional area (TCSA) between 2018 and 2019, 2019 TCSA and tree height (Fig. 1). Anaerobic soil disinfestation (ASD) tree growth was lower than fumigation and not different than no treatment control in Tonasket. In Rock Island ASD tree growth was not significantly different than fumigation and higher than no treatment control.

In the Southern site (Othello) the anaerobic treatment was initiated again in 2018 in order to attempt to successfully reach anaerobicity. 2017 treatments did not keep the soil as wet as the protocol required. In 2018 anaerobicity was successfully obtained. No significant differences were detected between ASD and control though the trend was towards larger growth in ASD.



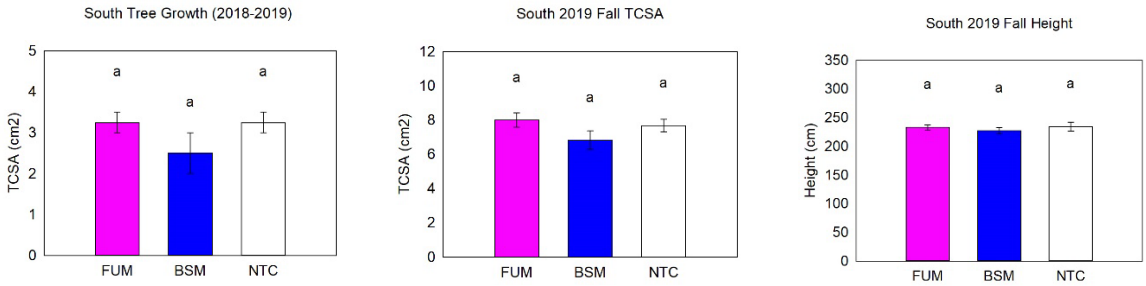


Figure 1. Growth measured in tree cross sectional area (TCSA) and height for three study locations North (Tonasket), Central (Rock Island) and South (Othello) for fumigated (FUM), brassica seedmeal (BSM), anaerobic soil disinfestation (ASD) and no treatment controls (NTC).

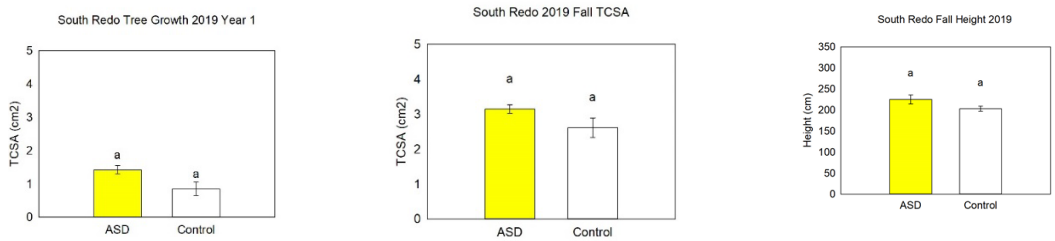


Figure 2. Growth measured in tree cross sectional area (TCSA) and height for plots initiated in 2018, Othello for fumigated (FUM), brassica seedmeal (BSM), anaerobic soil disinfestation (ASD) and no treatment controls (NTC).

In the Othello site planted in 2018 fruit was harvested from second leaf trees. Brassica seedmeal yield in bins per acre was significantly lower in brassica seedmeal and no treatment control compared to the fumigated control (*Figure 3*).

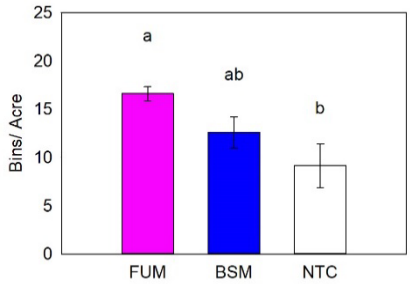


Figure 3 Yield in bins per acre, Othello for fumigated (FUM), brassica seedmeal (BSM), and no treatment controls (NTC).

Lesion nematode root density at the end of the initial growing season differed significantly among soil treatments at all three study sites. Pre-plant soil fumigation did not control lesion nematode populations at the South (Othello) site but did significantly suppression root populations at the North and Central sites (Figure 4). Seed meal treatment provided effective nematode control at all three sites and was superior to soil fumigation in lesion nematode suppression at the Central and South sites. ASD was as effective as soil fumigation at reducing lesion nematode root populations at the North and Central sites.

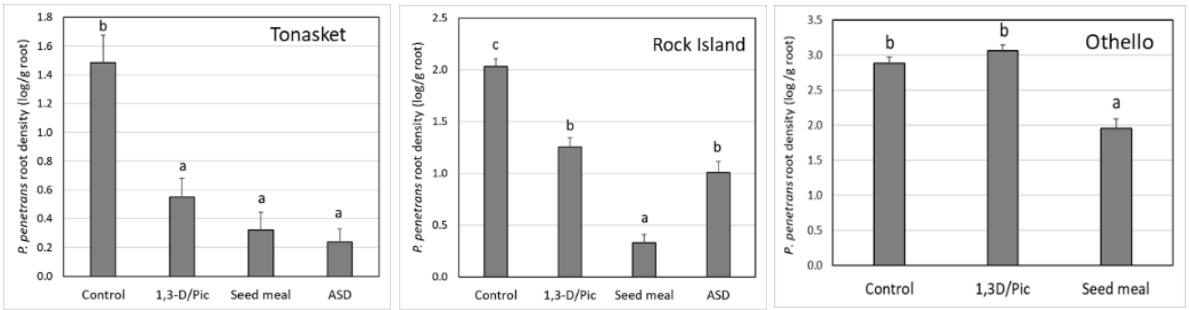


Figure 4. Lesion nematode (Pratylenchus penetrans) apple root density at the end of the initial growing season at the North (Tonasket), Central (Rock Island) and South (Othello) replant sites.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CP-19-101

Project Title: Outreach program for apple decays management in Washington

PI: Achour Amiri
Organization: Washington State Univ.
Telephone: 509-293-8752
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Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA, 98801

Co-PI (2): Tianna DuPont
Organization: Washington State Univ.
Telephone: 509-293-8758
Email: tianna.dupont@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA, 98801

Cooperators: Syngenta Crop Protection, Bernardita Sallato (WSU-Extension), Pace International.

Total Project Request: **Year 1:** $10,254 **Year 2: $10,425**

Other funding sources: None

Budget 1 : Amiri
Organization Name: WSU **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu/ shelli.tompkins@wsu.edu

Item	2019	**2020**
Salaries[1]	2,950	3,068
Benefits[1]	1,304	1,357
Wages		
Benefits		
Equipment		
Supplies[2]	5,000	5,000
Travel[3]	1,000	1,000
Miscellaneous		
Plot Fees		
Total	10,254	**10,425**

Footnotes:
[1] Salaries are for Research Assistant in Amiri Lab at $4,000/ month for 4 months at 10% FTE at 46.5% benefit rate and for a Research Assistant in Dupont Lab at $4,500/month for 3 months at 10% FTE at 41.5% benefit rate.
[2] Supplies include, room renting, printing material packets for participants in workshops and diagnostic tools and chemical reagents needed for LAMP training during the workshops.
[3] Funds to travel to different locations for workshop and travel fees for potential speakers.

OBJECTIVES

1- Conduct statewide workshops to provide up-to date knowledge about disease infection timing, management and fungicide resistance mitigation (Year 1).
2- Demonstrate visual approaches and portable field/packinghouses DNA-based devices for disease detection. Updates on organic management and disease management of 'WA 38' (Year 2).

SIGNIFICANT FINDINGS:

- A full day workshop is planned for March 4th 2020.
- The workshop will be organized at the Confluence Technology Center in Wenatchee.
- A press release has been sent on December 4th 2019.
- The registration for the workshop has been opened since.

METHODS

The flyer below summaries the presentations and activities planned for the workshop in 2020.

WASHINGTON STATE UNIVERSITY Plant Pathology

WSU TFREC Wenatchee • Washington

WASHINGTON STATE UNIVERSITY EXTENSION

Pre and Postharvest Disease Management Workshop

March 4, 2020 8:30 am – 3:00pm

Confluence Technology Center-CTC
285 Technology Center Way#102, Wenatchee

Who Should Attend

Pome fruit growers, packers, field and warehouse workers, extension specialists, industry representatives, consultants and others interested in learning about best management practices in orchards and packinghouses to reduce the impact of postharvest rots and increase pack-out.

What to Expect

Six hours of training and interactive activities:

- Introduction to pre and postharvest rot pathogens and their occurrence in the PNW;
- Timeline of infections from bloom to packing;
- Best management practices in conventional systems
- Cultural control.
- Fungicide resistance occurrence and mitigation.

Materials

Each participant will leave with digital and hard copies of presentations and new factsheets of the major postharvest rots: gray mold, blue mold, speck rot, bulls eye rot and Mucor.

Benefits of Attending

- Build a foundation for best management of major postharvest rots.
- Learn the latest research-based information for effective management.
- Understand how and when major pathogens infect flowers and fruit.
- Better understand the risks of fungicide resistance.
- Develop strategies to minimize resistance development.

Instructors

- Ashour Amiri, Postharvest Pathologist, Assistant Professor, WSU Plant Pathology
- Tianna DuPont, Tree Fruit Extension Specialist

WASHINGTON TREE FRUIT RESEARCH COMMISSION

Cost to Attend: $25

Please register at: http://treefruit.wsu.edu/event/postharvest-workshop/

Cost includes the training materials, lunch and refreshments.

Seats are limited. For questions, email Ashour Amiri at a.amiri@wsu.edu 509-293-8752 (primary contact) or Tianna DuPont at Tianna.dupont@wsu.edu

Agenda

08:30 am ARRIVAL AND REGISTRATION

09:00 am **Welcome:** Objectives and overview of the program

MODULE 1: Know your foes

09:10 am **Overview of the biology and occurrence of major pathogens**

09:30 am **Sources and timeline for infections: bloom to postharvest**

10:15 am BREAK

10: 30 am **Activity 1**: Assess the Risks and Anticipate the Problem

MODULE 2: Best Management Practices (11:00 am - 01: 30 pm)

11: 00 am **Current and novel disease management tactics**

12: 00 pm LUNCH BREAK

01:00 pm **Activity 2**: Know the Problem and Develop the Right Management

MODULE 3: Fungicide Resistance: Risks and Mitigation (01:30 - 02:45 pm)

01:30 pm **Fungicide resistance development and management**

02:10 pm **Activity 3:** Select an Optimal Fungicide Spray to Minimize Resistance

02: 45 pm Final thoughts, Survey, Pesticide credits, Adjourn

FUTURE WORK

In the spring to 2020, we will plan for the second year Extension Program that will be organized in 2021.

TIMELINE

		Year and months																										
		2019								**2020**												**2021**					PI involved	
	Activity type	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	Amiri	DuPont
Year 1 Meeting	**Disease Management in Apple: From Bloom to Packaging**																											
	Develop extension materials Year 1	■	■	■	■	■	■	■	■																		+	Review
	Year 1 Outreach & Education meeting											■															+	Support
	Report Year 1																			■	■	■	■				+	
Year 2 Meeting	**Disease Detection and Management in Organic Systems and 'WA 38'**																											
	Develop extension materials Year 2												■	■	■	■	■	■	■	■	■						+	Review
	Year 2 Training & Outreach																							■			+	Support
	Final Report																				■	■	■	■			+	+

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP-19-103A

Project Title: Pre and postharvest disease management in organic apple systems

PI: Achour Amiri
Organization: Washington State University
Telephone: 509-293-8752
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City/State/Zip: Wenatchee,WA,98801

Co-PI (2): Yanmin Zhu
Organization: USDA-ARS
Telephone: 509-664-2280 ext. 215
Email: yanmin.zhu@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee,WA,98801

Cooperators: Dr. Wojciech Janisiewicz (USDA-ARS, Kearneysville); Certis, Sym-Agro, Marrone Bio, Wilbur-Ellis, Several growers and packers in Washington State.

Total Project Request: **Year 1:** $67,715 **Year 2: $82,312** **Year 3:** $87,686

Other funding sources

Agency Name: WSDA-SCBG
Amt. requested: $150,000
Notes: A pre-application has been submitted to the above agency**.** Invitation for full proposal should be released in December 2019. If funded, this project will support a Research Associate and part of the expenses needed for the microbiome studies.

WTFRC Budget:

Item	2019	**2020**	2021
RCA Room Rental	6,300*	6,500	6,695
Total	0	**6,500**	6,695

Footnotes: RCA room(s) will be used to assess the efficacy of regular CA versus DCA for decay reduction.
*RCA rooms have not been used in 2019

Budget 1: Amiri
Organization Name: WSU **Contract Administrator:** Katy Roberts / Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu/ shelli.tompkins@wsu.edu

Item	2019	**2020**	2021
Salaries[1]	38,400	39,936	41,533
Benefits[1]	14,008	14,569	15,151
Wages			
Benefits			
Equipment[2]	10,000		
Supplies[3]	2,200	18,200	21,200
Travel[4]	1,007	1,007	1,007
Miscellaneous			
Plot Fees[5]	2,100	2,100	2,100
Total	67,715	**75,812**	80,991

Footnotes:
[1] Salary for a PostDoc at $4,000/ month for 12 months at 0.8 FTE and benefit rate of 36.5%. The PostDoc will work jointly between Amiri' and Zhu's labs as needed.
[2] Funds for Safepod **or** Harvestwatch system for the DCA study in the present project and will be used in planned future research. We expect to obtain additional funds for one of these systems from the pending WSDA-SCBG grant if funded.
[3] Supplies include biological and microbiological reagents for fungi and fungicide tests, manuscript publication fees. In Year 2 and 3, we budget funds for molecular reagents and microbiome analyses work.
[4] Domestic travel to orchards in WA for trials, sampling and outreach activity.
[5] Annual plot fees for an experimental block at Sunrise to be used for the work outlined in the proposal below.

OBJECTIVES

1- Evaluate the adequacy and efficacy of current and novel preharvest management organic strategies.
2- Evaluate the benefits of using dynamic control atmosphere (DCA), GRAS products and biocontrol agents to control rots in storage.
3- Acquire novel knowledge about the impact of different spray regimes and storage conditions on fruit microbiomes pre- and postharvest to enhance management in the future.

SIGNIFICANT FINDINGS:

- Trials outlined in Activity 1.1 to evaluate preharvest efficacy of several organic products have been conducted on Fuji at the Sunrise Orchard in spring-Summer of 2019. Fruit are stored at 34°F and will be evaluated in Spring of 2020 for decay development.
- Lab trials have been set in October 2019 to conduct the work planned in Activity 1.2 to study the efficacy of products listed in Table 1 on artificially inoculated fruit. Results will be available in Spring 2019.
- The efficacy of DCA and static CA (Activity 2.1) is being evaluated against 5 major postharvest pathogens using the Safepod and Labpod technologies.

METHODS

OBJECTIVE 1. Evaluate the adequacy and efficacy of current and novel preharvest management organic strategies (Year 1-3)

Activity 1.1. Efficacy of existing and potential new products in the orchard: The epidemiology of the main preharvest pathogens of pome fruit differs from one another. Therefore, management approaches should be adjusted accordingly to manage them effectively. In a first step, we aim to evaluate the efficacy of several treatments i.e. biocontrol agents, plant/microbial extracts, and bio-fungicides applied at a single time during the growing season against the most predominant pathogen in the PNW (Amiri and Ali, 2016). Trials will be conducted on Fuji apple at Sunrise experimental orchard for two successive seasons. Treatments outlined in Table 1 will be applied with a backpack sprayer at appropriate timing and rates indicated on the label in a randomized complete block design (RCB) with four replicate trees for each treatment. The ability of different treatments to cause phytotoxicity and russeting will be evaluated and recorded. Fruit will be picked (25 fruit/replicate tree for a total of 100 fruit/treatment) at commercial maturity and stored in separate crates at 34°F and 90% HR in a RA. Disease incidence and identification of pathogens will be carried out after 2, 4, and 6 months of storage or extended beyond as needed. For the treatments applied at bloom, flowers will be sampled 3 days before and 3 days after the treatment is applied and samples will be subjected to a quantitative PCR to detect and quantify the amount of *B. cinerea*, which is the most important pathogen expected to be found at this stage.

Activity 1.2: Efficacy of existing and potential new products on artificially-inoculated fruit: Because the disease pressure of different pathogens may not always be sufficient to evaluate the real efficacy of the different product, we propose to conduct detached fruit assay at harvest to obtain a more accurate knowledge about the efficacy of the treatments listed in Table 1. Fruits (Fuji), picked at commercial maturity, will be inoculated with spore suspensions (50,000 spores/ml) of *Botrytis cinerea*, *Neofabraea perennans*, or *Phacidiopycnis washingtonensis.* For *P. expansum*, fruit will be inoculated after wounds and treatments have been applied. Inoculated fruit will be stored at room temperature for 7 days, then treated with the treatments listed in Table 1, stored at 34°F, and decay incidence will be

recorded 1, 2, and 4 months post-treatment or extended beyond as needed. Results from this trial will be compared to those from the orchard trial to better assess the efficacy of the treatments.

Table 1. List of treatments labeled or pending for preharvest organic disease management to be tested in this study

#	Trade name	Hypothesized Mode of action	Product Type	Target		Application time		PHI
				Storage Pathogens	Mildew	Bloom/Pe	Preharves	
1	**Non-treated control**	-	-	-	-	-	-	NA
2	Pristine[a]	FRAC 7 + 11	Fungicide	+	+	-	+	0 d
3	Serenade OPTI[b]	Elicitor/Competitor	**Biological**	+	+	-	+	0 d
4	Serenade OPTI	Elicitor/Competitor	**Biological**	+	+	+	+	0 d
5	AVIV™	Elicitor/Competitor	**Biological**	+	+	+	+	0 d
6	Double Nickel	Competitor	**Biological**	+	+	+	+	0 d
7	Sonata	Competitor	**Biological**	+	+	+	+	4 hrs
8	Botector	Competitor	**Biological**	+	-	+	+	0 d
9	Blossom protect	Competitor	**Biological**	+	-	+	+	0 d
10	Actinovate®AG	Competitor	**Biological**	+	+	+	+	0 d
11	Regalia	Elicitor	**Extract**	+	+	-	+	0 d
12	Neem Concentrate	Broad spectrum	**Extract**	+	+	+	+	0 d
13	Kaligreen/Carb-o-Nator	Elicitor/Broad spectrum	**Salt**	+	+	+	+	4 hrs
14	Etidot-67	Elicitor/Broad spectrum	**Salt/acid**	+	+	+	+	4 hrs
15	Sil-Matrix	Elicitor/Broad spectrum	**Salts**	+	+	-	+	4 hrs
16	Cueva	Broad spectrum	**Salt/acid**	+	+	+	-	4 hrs
17	Cinnerate	Fungicide	**Extract**	+	NA	-	+	0 d
18	Jet-Ag	Fungicide	**Sanitizer**	+	+	+	+	4 hrs
19	OSO™ 5%SC[c]	FRAC 19	**Biofungicide**	+	+	+	+	0 d

[a] Synthetic conventional fungicide to control efficacy of other products. [b] Current Standard growers' organic treatment. [c] Organic label pending. PHI: Preharvest interval.

<u>Activity 1.3</u>: Develop effective spray programs based on optimal timing and number of applications of combined treatments: Based on the efficacy of different treatments from trials conducted as outlined in activities 1 and 2, the best treatments at bloom and preharvest will be combined for up to 5 applications from bloom to harvest. Aggressive (up to 5 applications/season) and conservative (1-2 applications/season) spray programs will be designed and tested for two successive seasons as described in Activities 1 & 2 above. Adjustments, in timing and numbers of sprays, may be made after Year 2 to enhance efficacy in Year 3.

OBJECTIVE 2. Evaluate the benefits of using dynamic control atmosphere (DCA), GRAS products and biocontrol agents to control rots in storage. (Year 1-3)

The work planned in Objective 2 will focus on enhancing the level of decay control during storage. Herein, we will focus on the effect of Dynamic Controlled Atmosphere in lowering the risks of decay development compared to regular CA or RA atmospheres. We will also evaluate the efficacy of some GRAS and biocontrol products.

<u>Activity 2.1</u>. Effect of Dynamic Controlled Atmosphere. While many benefits of DCA systems on the fruit quality and reduction of physiological disorders have been evidenced, the impact on disease reduction in such storage conditions is still unknown. The ability of fungi to survive in hypoxia varies but their metabolism is tremendously diminished. The DCA systems (O_2 <1 to 0.3%) will only be relevant to pome fruit packers if a significant reduction in decay rate compared to static controlled atmosphere (1.5 to 3% oxygen) is shown.

In Year 1 and 2, we will focus on 4 key postharvest pathogens, i.e. *Botrytis cinerea*, *Neofabraea perennans, Penicillium expansum* and *Mucor piriformis*. For the two first pathogens, Fuji apples will be inoculated with spore suspensions at 500,000 spores/ml on the trees 15 days prior to harvest to mimic pre-harvest conditions. For *P. expansum and M. piriformis*, fruit picked at commercial maturity, will be surface-disinfected in sodium hypochlorite, rinsed with sterile water, and inoculated with spore suspensions of each pathogen at 500,000 spores/ml. Fruit inoculated with each pathogen will be stored accordingly as shown in Table 2. Four replicates of 25 fruit each (total of 100 fruit/treatment) previously randomized using an RCB design will be used. Decay incidence and severity will be determined after 2, 4 and 6 months of storage. Additionally, 40 non-inoculated fruit (4 replicates of 10 fruit each), picked at commercial maturity, will be stored in the same atmospheres for the same storage periods and will be used to asses fruit quality parameters (firmness, solid content, sugar content, acidity). Fruit will be stored at Stemilt RCA rooms for regular (RA) and controlled (CA) atmosphere treatments and in Safepod or Harvestwatch containers for the DCA treatment.

In Year 2 and 3, we will assess the efficacy of RA, CA and DCA for the control of natural infections on Fuji apples harvested at commercial maturity for the experimental block at Sunrise orchard and will be stored as in the three different atmospheres. A total of 400 fruit will be used for each atmosphere and fruit will be stored as explained in the previous section. Disease incidence will be recorded after 3 and 6 months of storage or beyond if disease rate is low.

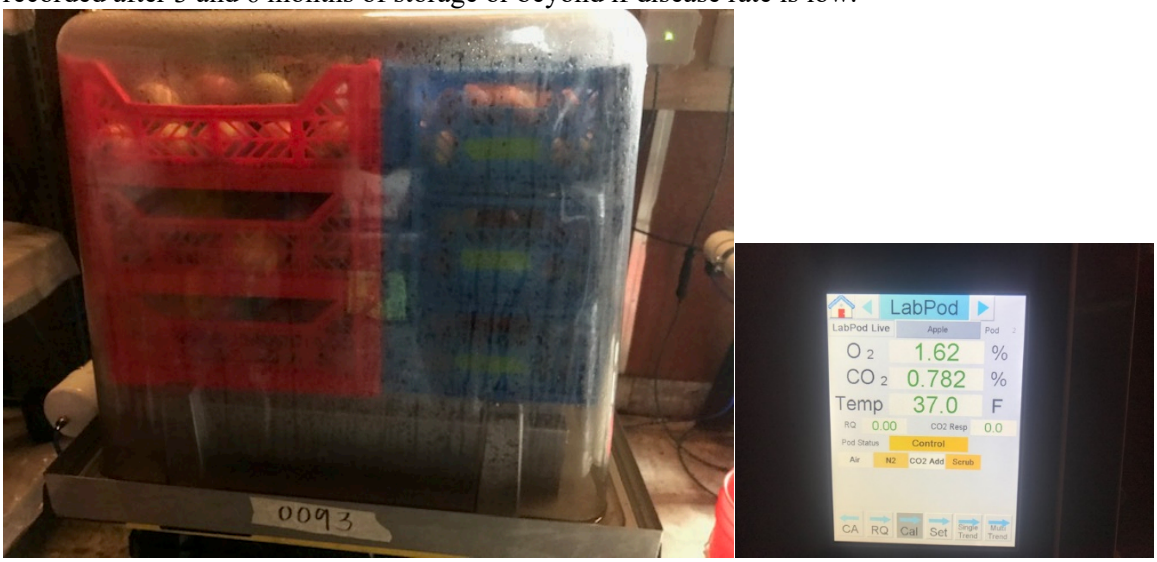


Figure 1. Trials set in Labpod at WSU-TFREC in Fall 2019 to evaluate the efficacy of CA and DCA on postharvest decay development.

Table 2. Suggested number of fruits, atmosphere types, and storage duration to test on four pome fruit pathogens

	Number of fruit, atmosphere type, and duration of storage (months)								
	RA[a]			CA			DCA		
Pathogen	**2 m[b]**	**4 m**	**6 m**	**2 m**	**4 m**	**6 m**	**2 m**	**4 m**	**6 m**
Penicillium expansum	100[c]	100	100	100	100	100	100	100	100
Botrytis cinerea	100	100	100	100	100	100	100	100	100
Neofabraea perennans	100	100	100	100	100	100	100	100	100
Mucor piriformis	100	100	100	100	100	100	100	100	100
# fruit/treatment	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**

[a] RA, CA and DCA indicate regular, controlled and dynamic controlled atmospheres, respectively. [b] m indicates months.

Activity 2.2. Efficacy of organic postharvest treatments. Currently, there are no known effective products that can provide an acceptable level of efficacy against major postharvest diseases in commercial packinghouses. Similar to the conventional systems, the possibility of adding a postharvest fungicide application would add to the level of efficacy provided by the preharvest treatments. Herein, we suggest to test some GRAS products and rare biopesticides (Table 3) that may be labeled in the future is their efficacy if proven. We will conduct trials using artificially-inoculated experiments, focused on 4 major pathogens *B. cinerea*, *N. perennans*, *P. expansum*, and *M. piriformis*, and also using naturally infected fruits. Experiments and disease evaluations will be conducted as explained in Activities 1.1 and 1.2.

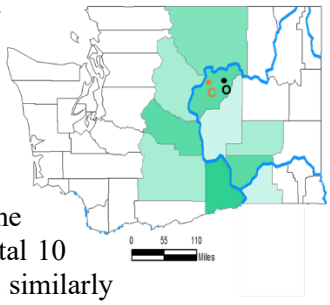
Table 3. Suggested number of fruits, atmosphere types, and storage duration to test on artificially and naturally-infected fruit

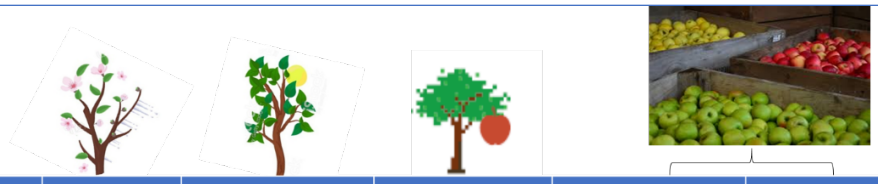
#	Trade name	Hypothesized Mode of action	Product Type
1	**Non-treated control**	**-**	**-**
2	Scholar SC[a]	FRAC 12	Fungicide
3	Bioferm	Competition	Biological
4	Cinnerate	Contact	Plant extract
5	SB. PSS 5.6	Sanitizer/Contact	Anions
6	Glyceryl palmitate(s)	Fruit Enhancer/Contact?	Inducer
7	Natamycin	Antibiotic	Biofungicide
8	OSO™ 5%SC[c]	FRAC 19	Biofungicide[b]

[a] is a conventional fungicide to be used for comparison with other treatments. [b] Organic label for pre and potentially postharvest application is pending for OSO™.

OBJECTIVE 3. Acquire novel knowledge about the impact of different spray regimes and storage conditions on fruit microbiomes pre- and postharvest (Year 2-3, Amiri & Zhu).

Activity 3.1. Sites and sampling: One conventional 'Fuji' (C) and another organic (O) orchard located in Chelan or Grant counties will be used for sampling (Figure on right). To avoid any interference of the rootstock, blocks established on the same rootstock will be used. In each orchard, 10 non-adjacent trees (with 10 buffer-rows) will be randomly selected and tagged before bloom. Samples will be collected as detailed in Figure 1 (below). At full bloom, 10 flowers/tree will be sampled from the circumference of the tree and pooled to make one biological replicate (total 10 biological replicates/orchard). The same number of fruits will be sampled similarly at each sampling time. All samples will be collected 3 days before and 3 days after pesticides treatments have been made. Samples will be transported in separate Ziploc bags to TFREC and will be immediately processed for DNA extractions or frozen at -80°C until further usage.





	Full bloom	Immature fruit	Mature fruit	1 month storage	6 months storage	Total
Organic	20 (10+10)[a]	20	20	20	20	**100**
Conventional	20	20	20	20	20	**100**

Figure2. Scheme of the sampling protocol to be used for microbial analyses. [a]10 samples will be collected 3 days before fungicide treatments and 10 others will be 3 days after the treatments.

Activity 3.2. DNA extraction, sequencing and analyses: Microbial and fungal DNA will be extracted from flowers and fruits immediately or soon after sampling using the MP-FastPrep DNA extraction kit following the manufacturer' protocol. The verification of DNA quality and dilution will be done as described in Objective 4. The ITS (ITS1 and ITS4) rDNA and 16S rRNA genes will be used to investigate the fungal and microbial communities, respectively, as described previously (Manter and Vivanco, 2007). The amplified products will be sequenced using an Illumina MiSeq instrument at the Institute of Biotechnology at the University of Idaho, United States.

Raw sequence reads will de-multiplexed, low quality read ends will be trimmed using Trimmomatic (Bolger et al. 2014), and low-quality sequences will be removed. QIIME2 (https://qiime2.org/) was used to perform the downstream diversity and taxonomy composition analysis. Corresponding paired end reads will be merged, and un-joined reads will be discarded. The remaining sequences will be used to determine differences in bacterial and fungal communities between flowers and fruit samples from the organic and conventional orchards at each sampling time, and to calculate the Shannon diversity index to obtain alpha and beta diversity statistics. Sequences will be grouped to obtain operational taxonomic units (OTUs) with 97% similarity. The resulting OTUs will be compared against the trained full-length Greengenes 13_8 OTUs database (http://greengenes.secondgenome.com/) for bacterial taxonomic classification. For ITS taxonomy analysis, a database will be trained using the fungus sequences in UNITE (Fungal ITS) (https://unite.ut.ee/). The output files will be visualized in QIIME2 and underlying data will be extracted to perform a principal component analysis and plotting in R (https://www.r-project.org/).

The hypotheses that microbiomes will differ (i) between organic and conventional orchards (ii) between growth stages and (iii) before and after fungicide treatments will be tested. Each sample's richness and diversity will be evaluated using the Chao1 and the Shannon indices (Chiu and Chao, 2016), respectively. To evaluate the effect of different environmental and management factors on microbiome composition, multivariate mixed regression models will be fitted to the normalized counts using the GLMM or GLMIMX (i.e., combination of general linear and mixed modes) approach. Non-Metric Multidimensional Scaling (NMDS) analysis will be performed to visualize the dissimilarity matrix based on the (i) presence of cultured pathogens and (ii) use of specific practices. To test the association of the composition of microbial community with these covariates of interest, we will use the Permutational Multivariate Analysis of Variance (PERMANOVA) and the recently developed PERMANOVA-S (Tang et al., 2016). Statistical modeling will be conducted in R and Statistical Analysis Software (SAS).

OUTREACH ACTIVITIES: (Amiri, Year 3)

Amiri will summarize the most important and major findings from the work accomplished in Years 1 & 2 to be presented at an extension event planned in early spring of 2021 (if the other extension proposal submitted is funded) and/or at regular meetings, such as Northcentral Apple days, organized by Extension Specialist Dr. DuPont. Additional work from Year 3 will be presented at other extension meetings occurring throughout the region. Data will also be summarized for publication in the WSU Fruit Matter newsletter.

FUTURE WORK

- We will reconduct work outlined in Activities 1.1. and 1.2 for Year 2 data
- Based on results from Year 1 of the above activities, we will set trials to conduct work outlined for Activity 3.1.
- We will reconduct work outlined in Activity 2.1 and combine with Activity 2.2.
- We will start the work outlined in Objective 3 in spring of 2020.

CROP YEAR: 2019

CONTINUING REPORT **PROJECT LENGTH (CROP YEARS)**: 2019-2021

Project Title: Pesticide Residues on WA Apples

PI: Tory Schmidt
Organization: WTFRC
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Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Gerardo Garcia, Sandy Stone, Pacific Agricultural Labs, Northwest Hort Council, Doug Stockwell, Doyle Smith, various ag chemical companies

Item	2019	2020 (est.)	2021 (est.)
Salaries			
Benefits			
Wages[1]	1350	1400	1450
Benefits[1]	700	725	750
RCA Room Rental			
Shipping			
Supplies/Chemicals	250	275	300
Travel[2]	1000	1000	1000
Plot Fees			
Analytical lab fees	3500	3750	4000
Total gross costs	6,800	7,150	7,500
Anticipated Income (contracts and gift grants)	0	0	0
Total net costs	6,800	**7,150**	7,500

Footnotes: Schmidt estimates 10% of his time is dedicated to this project on an annual basis
Most pesticides tested are donated by their registrants or an ag chemical supply company
1 Wages & benefits primarily for Garcia (spray applications), crew help for Garcia, and Stone (data entry & review)
2 Travel costs include hauling equipment to & from plots & delivery of samples to Sherwood, OR

2019 WTFRC APPLE PESTICIDE RESIDUE STUDY

Since 2011, the Washington Tree Fruit Research Commission (WTFRC) has conducted annual trials to evaluate pesticide residues on 'Gala' apples. This year, we applied ten insecticide/acaricides and five fungicides (seventeen total active ingredients) with a Rears Pak-Blast sprayer in two different scenarios. SCENARIO A simulates typical industry use patterns for these products applied at 100 gal water/acre. SCENARIO B reflects an aggressive protocol intended to simulate a worst-case scenario with the highest possible residues while observing label guidelines (maximum label rates at minimum retreatment and pre-harvest intervals) applied at 200 gal water/acre. We had intended to apply both standard and aggressive spray protocols at both carrier volumes, but sprayer error confounded the results. Fruit samples were collected at commercial maturity on August 28 and delivered the next day to Pacific Agricultural Labs (Sherwood, OR) for chemical residue analysis.

TRIAL DETAILS

- 12th leaf 'Pacific' Gala / M.9 Nic.29 trained to central leader/spindle on 3' x 10' spacing
- 2 x 25 gal Rears Pak-Blast sprayer calibrated to 100 or 200 gal / acre
- All pesticides applied with 8 oz Regulaid / 100 gal water / acre
- No measurable precipitation recorded during trial except 0.75" of rain on Aug 9 & 10 (19 & 18 days before harvest)

Measured residues vs. maximum residue levels (MRLs) for apple pesticide programs in SCENARIO A: typical industry rates, timings, and retreatment intervals applied in 100 gal water/acre. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2019.

Chemical name	Trade name	Application rate	Application timing(s)	Measured residue	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*
Flutianil	Gatten	8	35	<0.01	0.15	0.01 (many)
Isofetamid	Kenja 400SC	12.5	35	0.019	0.6	0.01 (India)
Spinetoram	Delegate WG	7	35 & 21	<0.01	0.2	0.01 (India)
Cyantraniliprole	Exirel	13.5	35 & 21	0.097	1.5	0.01 (IND,TAI)
Spinosad	Entrust	3	35 & 21	<0.01	0.2	0.01 (India)
Tolfenpyrad	Bexar	27	35 & 21	0.20	1.0	0.01 (many)
Myclobutanil	Rally 40WSP	10	35 & 21	0.12	0.5	0.01 (UAE)
Novaluron	Rimon	32	35 & 21	0.22	3.0	0.01 (India)
Fluxapyroxad	Merivon	5.5	28	0.045	0.8	0.8 (CAN,MEX)
Pyraclostrobin	Merivon	5.5	28	0.029	1.5	0.5 (many)
Etoxazole	Zeal	2	28	0.026	0.2	0.01 (India)
Difenoconazole	Inspire Super	12	28	0.027	5.0	0.01 (India)
Cyprodinil	Inspire Super	12	28	0.052	1.7	0.01 (India)
Diazinon	Diazinon 50WS	16	28	0.016	0.5	0.01 (India)
Bifenazate	Acramite 50WS	16	28	0.032	0.7	0.01 (India)
Phosmet*	Imidan 70-W*	92	14	3.4	10.0	0.01 (India)
Fenpropathrin	Danitol	18	14	0.20	5.0	0.01 (IND,SAU)

[1] Top markets for WA apples; 26 Sep 2019. http://nwhort.org/export-manual/comparisonmrls/apple-mrls/, https://bcglobal.bryantchristie.com
* Imidan 70-W applications included 8 fl oz Tech-Spray/100 gal water to acidify spray tank

For more information, contact Tory Schmidt (509) 669-3903 or email tory@treefruitresearch.com

Measured residues vs. maximum residue levels (MRLs) for apple pesticide programs in SCENARIO B: aggressive use patterns (maximum rates with minimum retreatment and preharvest intervals) applied in 200 gal water/acre. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2019.

Chemical name	Trade name	Application rate	Application timing(s)	Measured residue	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*
Isofetamid	Kenja 400SC	12.5	35 & 21	0.034	0.6	0.01 (India)
Diazinon	Diazinon 50WS	16	35 & 21	<0.01	0.5	0.01 (India)
Tolfenpyrad	Bexar	27	28 & 14	0.20	1.0	0.01 (many)
Novaluron	Rimon	32	28 & 14	0.20	3.0	0.01 (India)
Difenoconazole	Inspire Super	12	28 & 14	0.045	5.0	0.01 (India)
Cyprodinil	Inspire Super	12	28 & 14	0.081	1.7	0.01 (India)
Fenpropathrin	Danitol	18	28 & 14	0.26	5.0	0.01 (IND,SAU)
Myclobutanil	Rally 40WSP	10	21 & 14	0.065	0.5	0.01 (UAE)
Flutianil	Gatten	8	21 & 14	<0.01	0.15	0.01 (many)
Spinosad	Entrust	3	21 & 7	0.024	0.2	0.01 (India)
Phosmet*	Imidan 70-W*	92	21 & 7	3.7	10.0	0.01 (India)
Spinetoram	Delegate WG	7	14 & 7	0.014	0.2	0.01 (India)
Cyantraniliprole	Exirel	13.5	14 & 5	0.11	1.5	0.01 (IND,TAI)
Bifenazate	Acramite 50WS	16	7	0.027	0.2	0.01 (India)
Fluxapyroxad	Merivon	5.5	7 & 1	0.086	0.8	0.8 (CAN,MEX)
Pyraclostrobin	Merivon	5.5	7 & 1	0.072	1.5	0.5 (many)

[1] Top markets for WA apples; 26 Sep 2019. http://nwhort.org/export-manual/comparisonmrls/apple-mrls/, https://bcglobal.bryantchristie.com
* Imidan 70-W applications included 8 fl oz Tech-Spray/100 gal water to acidify spray tank

DISCUSSION

As with all previous WTFRC studies, no residue exceeded the US Environmental Protection Agency's tolerance, affirming that application of these materials following label guidelines produce residues determined to be safe for domestic US markets. Most of the products assayed in our 2019 study, however, generated residues which exceed MRLs for important export markets, particularly India. In most cases, those actual residue levels were relatively low, but could trigger potential problems because India has yet to publish MRLs on apples for most of these products; in the absence of a posted MRL, the *de facto* limit falls to the national default value, which is 0.01 ppm for India. Once India publishes more apple MRLs, most of those tolerances will be relaxed, allowing US growers a better chance of using a variety of pesticides while still meeting Indian standards.

Our intent this year was to apply both "standard" and "aggressive" spray programs at 100 and 200 gal water/acre, as we have done in the past. Unfortunately, application error confounded the results in two of our plots, leaving only proper application of the standard protocol at 100 gal/acre (Scenario A) and the aggressive protocol at 200 gal/acre (Scenario B). This error precludes valid comparison of spraying standard vs. aggressive protocols or concentrate vs. dilute applications. Nonetheless, the results reported here are valid and accurately mimic real-world spray programs for commercial Washington apple orchards.

Reports from previous pesticide residue studies on apple and cherry which provide a broader context for these results are available on the WTFRC website at www.treefruitresearch.com. We encourage growers and consultants to stay abreast of current information on MRLs, which often change in response to trade negotiations and/or political developments. For more information, visit the Northwest Horticultural Council website at www.nwhort.org.

WASHINGTON TREE FRUIT RESEARCH COMMISSION

Results of this lone unreplicated trial are shared for informational purposes only and should not be construed as endorsements of any product, reflections of their efficacy, or a guarantee of similar results regarding residues for any user. Apple growers should consult their extension team members, crop advisors, and warehouses to develop responsible pest control programs.

FINAL PROJECT REPORT

Project Title: Data to model apple airblast spraying drift exposure levels

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Cooperators: Dr. Milt Teske (Continuum Dynamics Inc., Princeton, NJ)

Budget: Year 1: $96,108 Year 2: $66,334

Other funding sources

Agency Name: U.S. Forest Service
Amt. requested/awarded: $39,315
Notes: equipment cost share

Agency Name: Washington State Specialty Crop Block Grant (SCBG) for FY2017-FY2019
Amt. requested/awarded: $120,000
Notes: To fund the modeling effort (see 'Justification'). Awarded to Washington Tree Fruit Research Commission (Lead: Mike Willett)

Agency Name: USDA Office of Pest Management Policy
Amt. requested/awarded: $187,000
Notes: To fund the modeling effort (see 'Justification').

Agency Name: U.S. Forest Service
Amt. requested/awarded: $23,000
Notes: To fund the modeling effort and canopy characterization (see 'Justification').

Total in-kind and other funding sources: $369,315

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

Item	2018	2019
Salaries	21,000	21,840
Benefits	11,668	12,134
Wages	14,880	15,475
Benefits		
Equipment	12,000	
Supplies	34,955	15,280
Travel	1,605	1,605
Miscellaneous		
Plot Fees		
Total	96,108	66,334

Footnotes: (Year 1) Salaries of $21,000 plus $8,364 benefits will support a postdoctoral researcher who will work closely with the PIs in planning and conducting field experiments, laboratory analysis, data analytics and reporting. $12,318 (including 22.2% benefit) requested to support field work pertinent to field deposition studies (20 trials/crop/season) and canopy characterization (8h/day/person x 7 personnel x 12 days). Additionally, $5,866 requested to support lab work (fluorometry analysis) pertinent to field deposition trials (8 h/day/person x 4-person x 10 days). $1,605 is requested to travel to field sites (100 mile return trip @ 0.535/mile x 15 trips with two vehicles). $34,955 is requested for material and supplies that include procurement of deposit samplers (flat cards, string samplers, artificial foliage samplers), fluorescent tracer, labels, gloves, zip ties, general field supplies, vials, ethanol, chem-wipes and general lab supplies as well as the tractor & sprayer rentals. The material cost also includes $12,675 procurement of 8-m string tower drift poles ($3975+250 S&H/set of 3 x 3 sets). Funds of $12,000 are requested to procure Plant Canopy Analyzer (LAI-2200C) from LI-COR Biosciences.

Year 2 cost includes all the above except the cost of equipment and material supplies available in year-1. Please note that salaries are inflated 4% rate in year-2.

1. OBJECTIVES

The primary objective of this project is to generate data for validation of ***a mechanistic airblast spray drift model*** currently being developed (see 'Justification' of original proposal) to estimate exposure values to assess risk from airblast spraying in 'central leader' apple. Such model would be more accurate than **'worst-case scenario'** estimates currently used by EPA. Overall, during the two year project, measurements were made up to 600 ft downwind from the apple block (central leader) to assess drift and relate it to key meteorological parameters. Studies were conducted at dormant (***obj. #1 year-1 efforts***) and in full canopy (***obj. #2 year-2 efforts***) growth stages. The fluorometry analysis-based deposition data along with the pertinent canopy and environmental conditions has been collected to validate the mechanistic model being developed to estimate airblast sprayer drift/exposure levels.

Table 1. Project activity schedule and quarterly benchmarks (*Planned activities; √ Completed activities).

Objectives	Year 1-2				Year 2-3			
	Q 3	Q 4	Q1	Q 2	Q 3	Q 4	Q 1	Q 2
1. Airblast sprayer drift/exposure levels assessment up to 600 ft downwind from 'central leader' apple blocks during dormant stage								
Task 1.1: Field block scouting and setting up of the field samplers and metrological stations	√	√	√					
Task 1.2: Canopy mapping via standard ground-reference methods, data processing		√	√					
Task 1.3: Conduct field trials (20 runs)		√						
Task 1.4: Fluorometry analysis, data digitization and statistical analysis		√	√					
2. Airblast sprayer drift/exposure levels assessment up to 600 ft downwind from 'central leader' apple blocks at full canopy stage								
Task 2.1: Use block from 1.1 and setting up of the field samplers and metrological stations				√	√			
Task 2.2: Canopy mapping via standard ground-reference methods, data processing					√	√		
Task 2.3: Conduct field trials (20 runs)					√			
Task 2.4: Fluorometry analysis, data digitization, processing and statistical analysis					√	√		
Data from obj. #1 & #2 for USDA-FHAAST and EPA team developed drift model validation			√	√		√	√, *	√,
Reports and publication			√	√	√	√	√, *	√, *

2. SIGNIFICANT FINDINGS

Year-1 (Dormant stage)

The field data collection for dormant stage was conducted in Winter (November 27-through December 2, 2018). Major findings from this trial are given below.

- Spraying was done in row-3 upwind the orchard edge row. There was considerable spray deposition on the within row ground deposit samplers located on either sides of the spray row, i.e., at row-2 and row-5. For example, the average spray deposition (of 17 trials) on deposit samplers [cards] located in row-5 (at 45' inside the orchard) and row-2 (at 18' inside the orchard) was 4204 ng cm^{-2} and 3463 ng cm^{-2}, respectively. However, spray deposition on the ground at the edge of the orchard was reduced down to 14% (average deposition = 548 ng cm^{-2}) compared to 45' inside of the orchard.
- The spray deposition decreased exponentially along the sampled downwind distances up to 600'. It was minimal (0.35 ng cm^{-2}) at 600' downwind from the orchard edge.

- The deposition on vertical strings showed exponentially decreasing trend with the increase in height above ground level (AGL). The average spray deposition (of 17 trials) at 0-12', 12'-18', and 18'-24' sections was 457, 290 and 186 ng cm^{-2}, respectively at 25' downwind from orchard edge. Similarly, the spray deposition at 75' away from the orchard edge and for above respective heights was 152, 104, and 81 ng cm^{-2}.
- Pertinent weather, canopy attributes and spray deposition data has been transferred to the modeler for development and validation of the 'mechanistic spray drift' model.

Year-2 (Full canopy stage)

The field data collected for full canopy growth stage (May 13-16, 2019) showed similar trends as in the dormant stage. Results are summarized below.

- The average spray deposition on ground deposit samplers [cards] at 45' and 18' inside the orchard was 3592 ng cm^{-2} and 1385 ng cm^{-2}, respectively. Data showed 91% reduction (deposition = 336 ng cm^{-2}) in ground spray deposition at the edge of the orchard.
- Overall, the spray deposition showed exponential decreasing trend with 0.48 ng cm^{-2} deposited at 600' downwind distance.
- The spray deposition on vertical strings located at 25' downwind showed exponentially decreasing trend with the increase of height AGL. The average deposition (of 17 trials) at 0-12', 12'-18', and 18'-24' vertical sections was 764, 698, and 576 ng cm^{-2}, respectively. At 75' ft downwind from the orchard edge, pertinent vertical string sections had deposition of 237, 206, and 164 ng cm^{-2}, respectively.
- Pertinent weather, canopy attributes and spray deposition data has been transferred to the modeler for development and validation of the 'mechanistic spray drift' model.

Overall, dormant stage and full canopy stage spray deposition data was significantly not different at 5 % level. However, the spray deposition on vertical strings was almost doubled in full canopy stage (year-2) compared to dormant stage (year-1). The year-1 study was conducted at winter season (no leaves) in lower ambient temperatures and high humidity conditions, whereas year-2 study was conducted in spring season, where the ambient temperatures were higher (60.6-75.0 °F) and humidity was lower (22.8-48.8%). Further details on the spray deposition trends at two growth canopy stages are given under the results & discussion section.

3. RESULTS and DISCUSSION

Sprayer characteristics

The spray trials were conducted using an airblast sprayer (Powerblast Pultank, Rears Mfg. OR, see Fig. 1). It was a PTO driven conventional airblast sprayer with 400 gal tank size. The axial fan on rear of the sprayer had six blades and was operated to supply air-assist velocity to the spray mix. The fan diameter was 2.8'. It rotates in anticlockwise direction (looking from rear end of the sprayer). At 540 rpm of PTO, the fan speed was 2074 rpm and resulting air flowrate was 25, 426 ft^3 min^{-1} (12 m^3 s^{-1}). The air outlet area per side was 1.94 ft^2 (0.18 m^2). During the trials, the sprayer was operated at 90 psi to have 100 GPA application rate and had 10 active nozzles on each side. Sprayer was equipped with hollow cone disc-core nozzles (TeeJet Technologies, USA) (see Table 1 for details)

Fig. 1. Airblast sprayer used in the study

Table 1. Sprayer nozzle attributes.

Nozzle number and type	Droplet classification*	Sprayer side[x]			
		Left		Right	
		Nozzle orientation/Exit angle (° w.r.t. ground)	Flow rate (L min^{-1})	Nozzle orientation/Exit angle (° w.r.t. ground)	Flow rate (L min^{-1})
1 – not used	-	-	-	-	-
2 – D4, DC 25	Medium	66.7	1.77	61.6	1.66
3 – D4, DC 25	Medium	58.4	1.75	54.4	1.62
4 – D3, DC 25	Very coarse	25.3	1.12	52.1	1.11
5 – D3, DC 25	Very coarse	48.1	1.09	46.2	1.12
6 – D3, DC 25	Very coarse	40.3	1.10	37.5	1.13
7 – D3, DC 25	Very coarse	39.8	1.11	30.2	1.03
8 – D3, DC 25	Very coarse	31.3	1.15	21.6	1.16
9 – D3, DC 25	Very coarse	19.9	1.08	11.4	1.13
10 – D3, DC 25	Very coarse	20.1	1.17	8.8	1.08
11 – D3, DC 25	Very coarse	7.0	1.21	4.8	1.11
12 – not used	-	-	-	-	-

* From manufacturer which color codes the discs per the ASABE Standard S572.1, actual droplet size may vary with pertinent disc-core combination; [x] looking from back of the sprayer.

Prior to field trails, the sprayer air-assist velocities and spray liquid delivery patterns were assessed using two calibration tools; the WSU team custom developed Smart Spray Analytical System – SSAS (Bahlol et al., 2019) and commercial vertical spray patternator (AAMS Salvarani BVBA, Maldegem, Belgium).

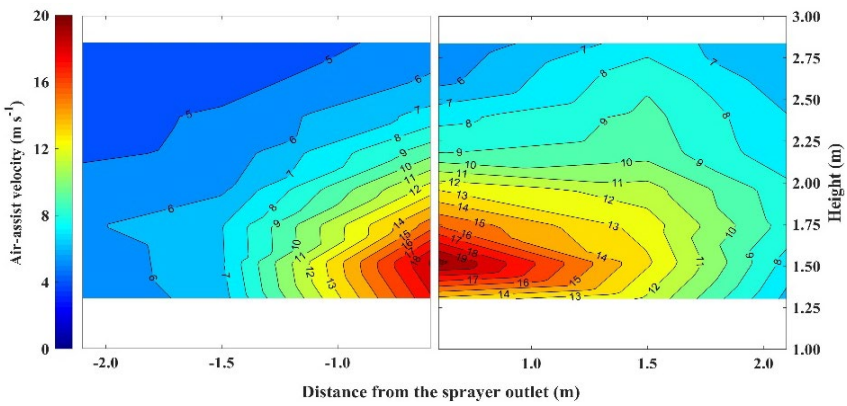

Fig. 2. Airblast sprayer air-assist velocity patterns derived from the SSAS data.

The air-assist velocity patterns derived from the data collected by SSAS are shown in Fig. 2. The spray liquid delivery patterns derived from the data collected by both the SSAS and commercial vertical spray patternator are shown in Figs. 3 and 4, respectively. Overall, sprayer had asymmetric air-assist velocity distribution with right side delivering higher velocities compared to the left. This can be attributed to the

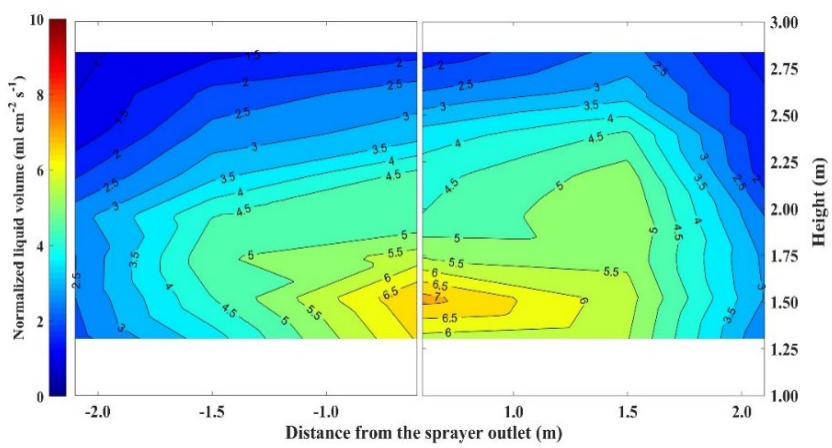

Fig. 3. Airblast sprayer spray liquid delivery patterns derived from the SSAS data.

counterclockwise rotation of axial-fan, despite the presence of an air straightener behind the rotating fan. Differences would have likely been greater without the straightener. Such effect was propagated in the spray liquid volume delivery patterns.

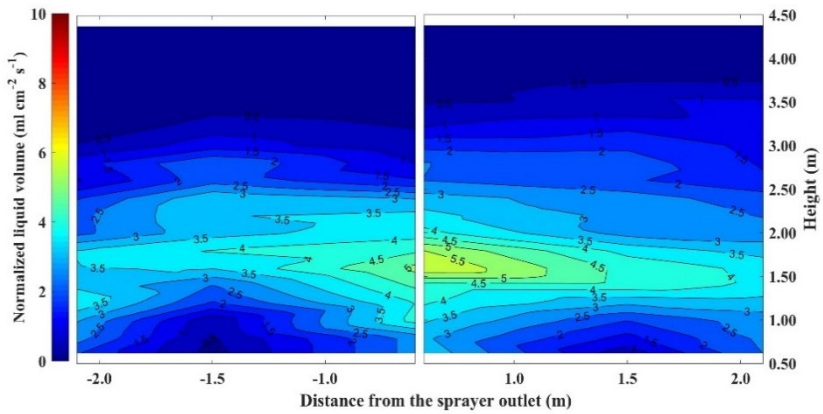

Fig. 4. Airblast sprayer spray liquid delivery patterns derived from the commercial vertical spray patternator tests.

Metrological condition

Field was instrumented with three main weather stations (MET) (see Fig. 5). MET 1 was inside the orchard at 60' from the spraying row at upwind direction. It consisted of 3D sonic anemometers at 3', 6', 12' and 24' AGL (One 3-axis 81000 from R.M. Young sonic and three 3-axis Vx probe sonics from Applied Technologies, Inc., CO). MET 2 was located downwind at 600' away from the orchard edge in an open field. It consisted of 2D sonic four anemometers at 3', 6', 12' and 24' AGL (ATMOS 41, Meter Group, WA). MET3 was located at the same row as MET1 but 120' further away to be closer to the end of orchard row. It was fixed with four anemometers (ATMOS 22, Meter Group, WA) similar heights of 3', 6', 12' and 24' AGL. Pertinent information recorded by a station at 600' downwind open field conditions during spray trails is summarized in table 1.

Fig. 5. Weather stations setup inside the field (left) MET 1 at 87' upwind from the edge of the orchard; (middle) MET 3 at 120' away towards the end of orchard from MET 1 in the same row; and (right) open field MET 2 at 600' downwind.

During the dormant stage spray trails, the mean wind speed was in the range of 0.87-8.08 mph (0.39-3.61 m s^{-1}) and wind direction was between 351° to 2.5° from the North, and within ± 30 degrees downwind direction. The average air temperature varied between 37.4 and 48.0 °F (i.e., about 3.0 to 8.9 °C). The relative humidity was in the range of 72.8 to100%. Overall, weather during trials can be characterized as 'unstable' (see table 1 stability class) with low ambient air temperatures and high relative humidity.

During full canopy stage spray trials, mean wind speed was in the ranges of 3.18-10.29 mph (1.42-4.60 m s^{-1}) and wind direction was between 347° to 17° from the North. The average air temperature was in the range of 60.6-75.0 °F (15.8-24.0 °C). The relative humidity varied between 22.8 and 48.8%. Overall, for 13 out of 17 trials, weather can be characterized as 'unstable' (see table 1 for details).

Table 2. Metrological data recorded at 600' downwind open field location

	Apple canopy Growth stage									
	Dormant					Full canopy				
Trial	**Stability Ratio**	**Stability Class[a]**	**Mean Wind Speed (m/s)**	**Average Temp. (°C)**	**Average RH (%)**	**Stability Ratio**	**Stability Class[a]**	**Mean Wind Speed (m/s)**	**Average Temp. (°C)**	**Average RH (%)**
1	-0.63	Unstable	2.78	7.7	95.1	-0.36	Unstable	2.63	16.5	40.9
2	-0.49	Unstable	3.61	7.3	94.3	-0.41	Unstable	3.15	18.3	37.6
3	-1.06	Unstable	2.79	8.9	92.1	-0.40	Unstable	2.84	19.6	32.9
4	-0.83	Unstable	2.74	7.8	93.1	-0.40	Unstable	2.43	20.4	29.4
5	-3.60	Unstable	1.18	8.1	86.0	-0.48	Unstable	2.17	21.1	30.2
6	4.23	Very stable	0.39	8.4	84.6	-0.74	Unstable	1.94	21.6	32.0
7	0.60	Stable	0.60	8.6	83.6	-0.48	Unstable	2.48	22.6	28.0
8	-19.0	Unstable	0.79	5.1	100.0	-0.68	Unstable	1.92	24.0	22.8
9	-2.41	Unstable	1.76	5.8	97.4	0.13	Stable	2.08	16.3	45.5
10	-2.94	Unstable	1.85	5.3	100.0	-0.06	Neutral	2.60	15.8	48.8
11	-1.48	Unstable	2.85	6.3	98.4	-0.23	Unstable	2.80	17.0	45.9
12	-1.23	Unstable	2.84	7.7	81.5	-0.11	Unstable	3.64	18.3	41.1
13	-0.06	Neutral	0.90	8.4	72.8	-0.11	Unstable	4.60	20.2	35.0
14	-8.16	Unstable	1.26	2.5	99.6	0.17	Stable	1.42	10.3	77.6
15	-7.25	Unstable	1.48	4.1	96.7	0.02	Neutral	2.34	10.0	80.7
16	-2.10	Unstable	1.91	3.0	93.4	-0.11	Unstable	3.04	9.83	85.7
17	-30.82	Unstable	0.55	3.0	97.6	-0.67	Unstable	1.71	10.1	84.1
18	-17.93	Unstable	0.52	3.5	95.1	-0.09	Neutral	4.10	13.2	66.3
19	-9.05	Unstable	1.00	3.4	100.0	-0.09	Neutral	3.55	14.0	63.0
20	-6.77	Unstable	1.03	3.9	99.1	0.09	Neutral	1.01	11.3	98.3

[a] Based on the stability classifications given in Fitz (2006) and Yates et al. (1974). Unstable: -1.7 to -0.1, Neutral: -0.1 to 0.1, Stable: 0.1 to 1.2, Very stable: 1.2 to 4.9.

Canopy characteristics

The field experiments were conducted at cooperating grower (Olsen Brothers) field site (46°18'57.6" N, 119°34'36.8" W) located near Benton City, WA. It is ~9.24 acre (760' x 530') 'Gala' (M9-337 rootstock) apple block planted in 2005. Rows were spaced at 9' and trees trained in 'central leader' canopy architecture were spaced at 3'. Downwind the orchard had 22.4 acre (780' x 1250') bare field (see Fig. 6) block making an ideal location for this study.

Fig. 6. Experimental field site.

At each growth stage (dormant and full canopy), total of 30 random trees along the spraying row were measured for determining the canopy characteristics. Average height of trees was 12' (3.66 m) and the average tree trunk diameter was 2.5" (0.064 m). The average width of a tree was about 4' (1.25 m). The leaf area index (LAI) as a function of tree height was measured in dormant and full canopy growth stages using PAR measurement sensor (AccuPAR LP-80, Meter Group, WA). Pertinent data is shown in Fig. 7.

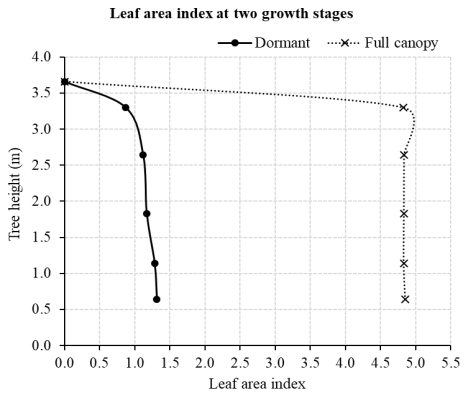

Fig. 7. Apple tree canopy attributes.

Fig. 8. Apple tree canopies at two growth stages (a) dormant and (b) full canopy.

The LAI at dormant growth stage varied from 0 (top of the canopy) to 1.32 (at the lowest trellis at 25” [0.64 m] AGL. The LAI at the topmost trellis (130” [3.3 m]) was 0.87. In full canopy growth stage, the LAI at topmost trellis was 4.82 while the LAI at the lowest trellis was 4.85. Overall, trees at full canopy stage were uniform and had dense foliage with LAI difference of about 0.03. The typical canopies at two growth stages are shown in the Fig. 8.

Spray deposition

There were four types of drift catching samplers; Mylar cards (2”x2”), Artificial foliage (Hedge slats of 1.5” length), Horizontal strings (1 m length), and Vertical strings (sectioned at 12’, 18’ and 24’). The string samplers were made from 1.8 mm dia. white color spear gun spectra cord (SGT Knots, Mooresville, NC). The arrangement of Mylar card, artificial foliage and the horizontal string samplers in the field is shown in Fig. 9.

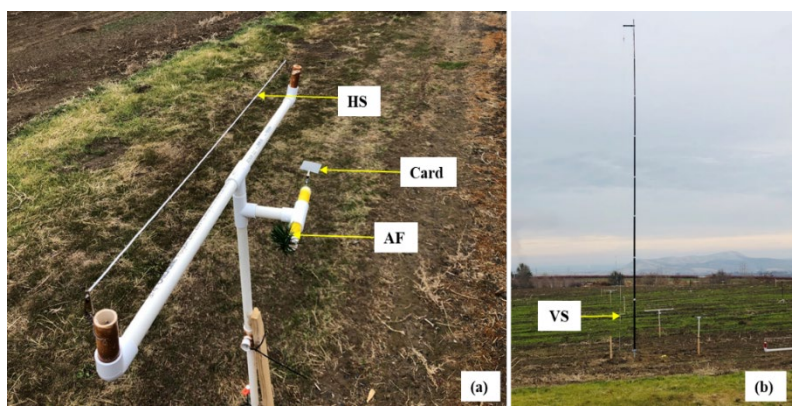

Fig. 9. Arrangement of different samplers at the field setting; (a) horizontal string-HS, artificial foliage-AF and the Mylar card (card); (b) vertical string-VS set-up with a telescopic pole.

The dormant (at leaf drop stage) and full canopy stage data collection was conducted per the experimental protocol given in the original proposal. There were three blank trails and 17 spray trials that were conducted at each of the growth stage (total of 40 trails). Each trial involved spraying four passes of spray mix that had fluorescent tracer dye at 2 g L^{-1} (Pyranine 10G, Keystone Inc.). Spraying was done in the third row from the edge.

Collected deposit samples, 2320 at each growth stage, were analyzed using the fluorometry analysis. The details of the fluorometry analysis has been reported in Salyani (2000) and Khot et al. (2012). Briefly, a known volume of deionized water will be added to the plastic bags containing the deposit samples. The sample bags were then shook for 1-min using a mechanical shaker, to thoroughly mix the tracer deposit into deionized water in the sampler bags. The rinsate was then transferred into two

10- ml matching cuvettes (Fisher Scientific, Hampton, NH). Each cuvette was analyzed twice for fluorescence intensity using the fluorometer (Model: 10AU, Turner Designs, San Jose, CA). The fluorometry analysis-based deposition results (as amount of tracer per sample in mass/area) were summarized and transferred to 'Spray Drift Task Force' to validate an orchard airblast 'spray drift model'.

The ground spray deposition during dormant and full canopy stages and on each type of sampler is shown in Figs. 10a-c. Overall, there was exponential decay in ground deposition on cards along the downwind distance at both dormant ($y=1191e^{-0.7x}$, $R^2=0.99$) and full canopy ($y=1063e^{-0.7x}$, $R^2=0.99$) growth stages (Fig. 10a). The average depostion at the edge of orchard (0') at dormant and full canopy growth stages was 591 ng cm^{-2} and 336 ng cm^{-2}, respectively. However, at 600' (183 m) downwind the orchard edge, the average depostion on cards was 0.42 ng cm^{-2} at dormant and 0.48 ng cm^{-2} at full canopy growth stage.

In dormant growth stage, the average deposition on artificial foliage samplers at 10' (3 m) from the orchard edge was 591 ng cm^{-2} and found decreased to 2.54 ng cm^{-2} at 600' downind. At the full canopy stage, the pertinent sampler had average deposition of 526 ng cm^{-2} at 10' and 1.60 ng cm^{-2} at 600' downwind from the orchard edge. At both stages, the deposition data showed exponentially decreasing trend (Dormant stage: $y=5748e^{-0.6x}$, $R^2=0.98$; Full canopy stage: $y=9405e^{-0.6x}$ $R^2=0.98$; Fig. 10b).

The horizontal strings were placed at five locations (50', 100', 200', 400', and 600') along the

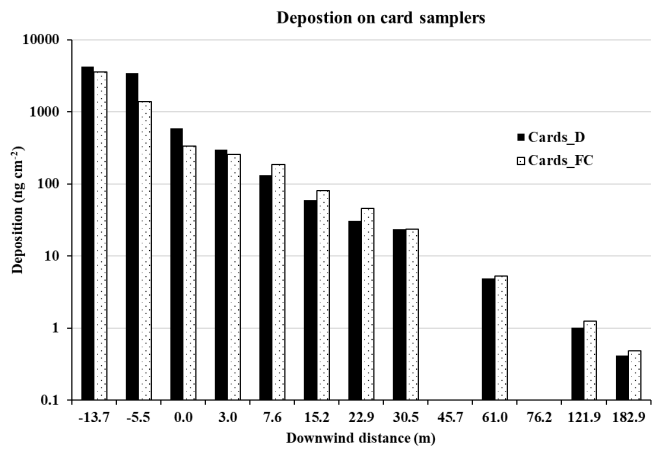

Fig. 10a) Average (*n*=17) deposition on Mylar card samplers along the downwind distance (D-dormant and FC-full canopy growth stage).

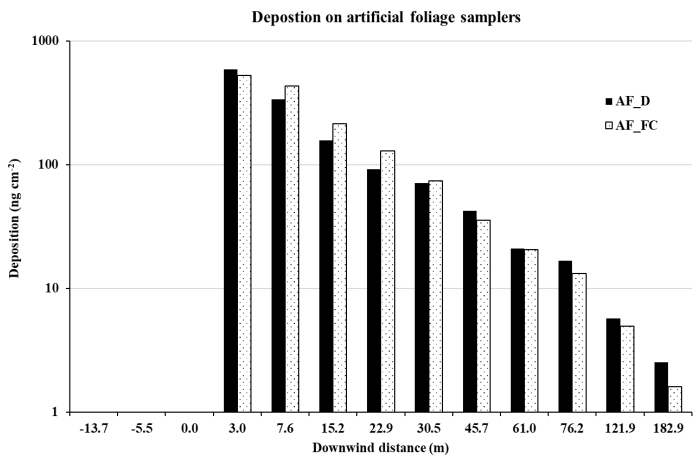

Fig. 10b) Average (*n*=17) deposition on artificial foliage samplers along the downwind distance (D-dormant and FC-full canopy stage).

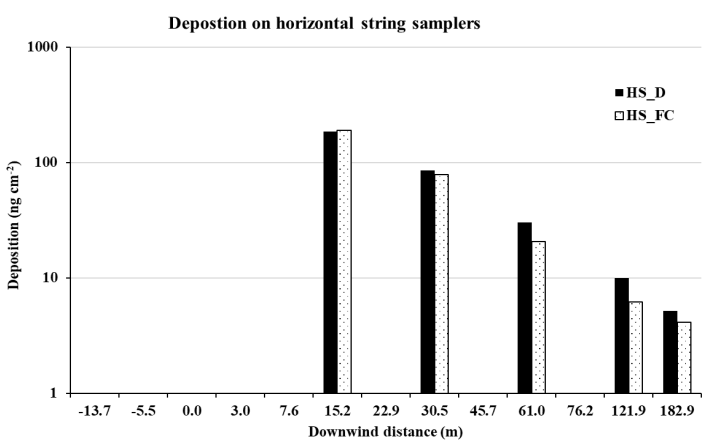

Fig. 10c) Average (*n*=17) deposition on horizontal string samplers along the downwind distance (D-dormant and FC-full canopy growth stage).

downwind distance. Similar to the Mylar cards and artificial foliage samplers, the depositions along the downwind distance decreased exponentially (Dormant stage: $y=4601e^{-0.5x}$, $R^2=0.93$; Full canopy stage: $y=6234e^{-0.6x}$, $R^2=0.99$; Fig. 10c). At dormant growth stage, the average deposition on strings at 50' and 600' downwind distances was 185 and 5.18 ng cm^{-2}, respectively. Similarly, at full canopy growth stage, the respective average depostion for pertinent downwind distances was 189 and 4.11 ng cm^{-2}.

The spray deposition on vertical string samplers at each growth stage (dormant and full canopy) is shown in Fig. 11.
On vertical strings, the spray deposition decreased with the increase of height AGL and downwind distances. At the dormant growth stage, the deposition decreased with the increase of height (0-12', 12'-18', and 18'-24') and downwind distance (from 25' to 75') ($y=585e^{-0.3x}$, $R^2=0.99$). Furthermore, at full canopy stage, reduction in deposition was much faster along the downwind distance ($y=1248e^{-0.4x}$, $R^2=0.92$).

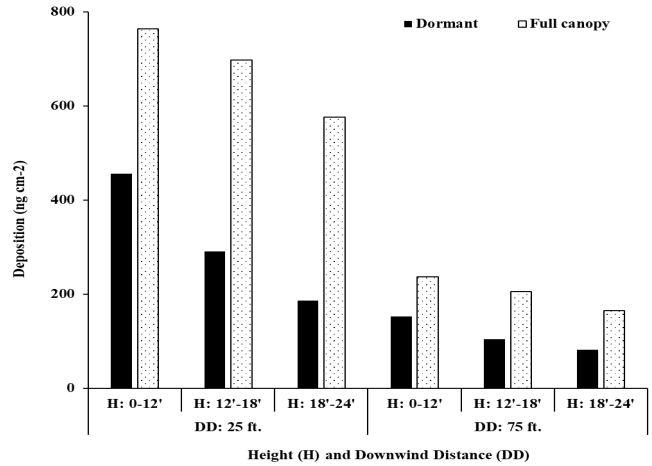

Fig. 11. Average (n=17) deposition on vertical string samplers (sectioned as 0-12'; 12'-18'; and 18'-24' heights at each sampling location, D-dormant and FC-full canopy stage).

During the study, three different types of samplers (Mylar cards, artificial foliage, and horizontal strings) were placed along each transects for ground deposition assessment. At five locations along each transect had all three types of samplers. Therefore, the deposition per unit area on each sampler would provide an indication on the collection efficiency of the pertinent sampler type. Fig. 12 shows the average deposition on each sampler along the downwind distance at dormant (left plot) and full canopy (right plot) growth stages.

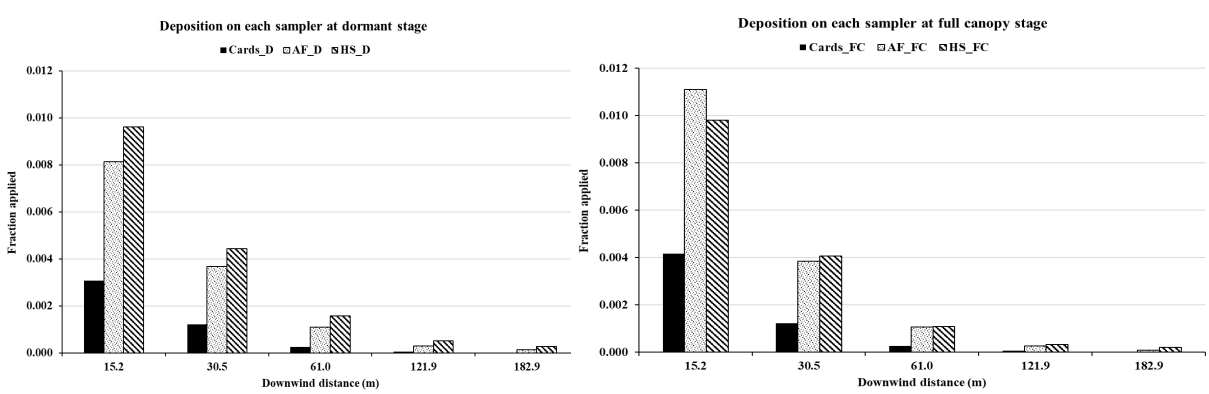

Fig. 12. Deposition as fraction applied on each sampler along the downwind distance at dormant (left) and full canopy (right) growth stages (D-Dormant, FC-Full canopy, Cards- Mylar cards, AF-Artificial foliage, HS-Horizontal strings).

The overall comparison suggested that the artificial foliage and horizontal string samplers have significantly higher collection efficiency, at 5% level, compared to the Mylar cards. In terms of field

data collection, the handling of strings would require extra caution due to longer length. Therefore, the artificial foliage samplers would be more effective as drift catching samplers compared to Mylar cards.

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EXECUTIVE SUMMARY

Project title: Data to Model Apple Airblast Spraying Drift Exposure Levels

Key words: Apple, airblast, deposition, spray drift, mechanistic model

Abstract: Study generated field data for validation of a mechanistic spray drift model to help assess pesticide drift and human exposure risks from airblast sprayer applications in 'central leader' apple. Such data driven models will help change regulatory rules that currently consider the worst-case scenario, resulting unnecessary label restrictions.

Summary:

This project was conducted to generate data for the validation of a mechanistic airblast spray drift model currently being developed to estimate exposure values and assess risk from airblast spraying in 'central leader' apple orchards. Spray drift measurements were collected up to 600 ft downwind from the apple orchard edge-row and relate it to pertinent meteorological parameters at both dormant and full canopy growth stages. The field data collection for the dormant and full canopy growth stages was conducted in winter 2018 and in spring 2019. Four types of drift catching samplers (Mylar cards, Artificial foliage, Horizontal strings, and Vertical strings) were used in the study. Three blank trials and 17 spray trials were conducted for each of the growth stages. Each trial involved spraying four passes of spray liquid containing 2 g L^{-1} fluorescent tracer dye. In total, 2320 samplers were collected for each growth stage. Collected deposit samplers were analyzed using the fluorometry analysis.

The dormant stage trial was conducted at winter season (no leaves) in lower ambient temperatures (37.4-48.0 °F) and higher humidity (72.8-100%) conditions; whereas the full canopy stage trial was conducted in spring season, where the ambient temperatures were higher (60.6-75.0 °F) and humidity was lower (22.8-48.8%). The comparison of all data (dormant stage and full canopy growth stage) showed that there was no significant difference in drift collected from various locations at 5% level at two growth stages. The analysis of card, artificial foliage, and horizontal string deposit samplers showed an exponential decay in ground deposition along the downwind distance at both dormant and full canopy growth stages. However, the spray deposition on vertical strings almost doubled in the full canopy stage compared to the dormant stage. The overall comparison on the different ground deposit samplers suggested that the artificial foliage and horizontal string samplers have significantly higher collection efficiency, at 5% level, compared to the Mylar cards. In terms of field data collection, the handling of strings required extra caution due to longer length. Therefore, the artificial foliage samplers would appear to be the most effective drift-catching samplers in this study.

In order to relate the drift data, the nature of the canopies at two growth stages was characterized by leaf area index measurements (LAI). The LAI at the dormant stage was in the range 0-1.32 (from the treetop to bottom trellis at 25" above ground level) with no leaves present, and that in full canopy growth stage varied from 4.85-4.82 with a highly dense foliage.

The Rears Powerblast Pultank 400 gal sprayer used for spray applications was assessed for air-assist velocities and spray liquid delivery patterns using commercial and custom developed vertical patternators. Overall, the sprayer had asymmetric air-assisted velocity distribution with right side delivering higher velocities compared to the left. This can be attributed to the counterclockwise rotation of axial-fan, despite the presence of an air straightener behind the rotating fan. Such effect was propagated in the spray liquid volume delivery patterns.

FINAL REPORT **YEAR**: 2 of 2

Project Title: Refinement of practical fire blight control: Non-antibiotic and SAR

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Total Project Request: Year 1: $39,980 **Year 2**: $40,779

Other funding sources: None

WTFRC Collaborative expenses: None

Budget

Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Russ Karow
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Item	**2018-19**	**2019-20**
Salaries Faculty Res. Assist. 3.5 mo	17,339	17,686
Benefits OPE 61%	10,577	10,788
Undergraduate labor (&OPE 12%)	1,064	1,085
Equipment	0	0
Materials and Supplies	1,500	1,530
Local Travel	1,500	1,530
Plot Fees	2,000	2,040
Medford russet trials (plot fees and labor)	6,000	6,120
Total	**$39,980**	**$40,779**

OBJECTIVES:

1) In integrated programs with Blossom Protect + Buffer Protect, evaluate EPA-registered, NOP-approved materials with demonstrated anti-microbial activity (e.g., Previsto, Jet Ag, and lime sulfur) for their ability to suppress fire blight and to induce fruit russeting on apple and pear trees.

2) In integrated programs with Blossom Protect + Buffer Protect, evaluate the mineral material, alum ($KAl(SO_4)_2$) and an alum-containing stone powder for their ability to suppress fire blight and to induce fruit russeting on apple and pear trees.

3) Evaluate alternative yeasts for their ability to suppress fire blight.

4) Evaluate amount and placement of Actigard trunk paints for the purpose of protection from fire blight during primary bloom.

SIGNIFICANT FINDINGS:

- Blossom Protect (yeast), Previsto (copper) at full bloom and lime sulfur 4% at petal fall provided outstanding fire blight control.
- Lime sulfur 4% at petal fall did not increase fruit russeting of apple compared to a water treated control
- Alternative yeast strains (with Buffer Protect) suppressed fire blight but to a degree less than observed with Blossom Protect.
- Population size of *E. amylovora* on pear and apple flowers continued to increase during the post-petal fall period except when treated with lime sulfur at petal fall.
- Lime sulfur or Jet Ag (H_2O_2 in peracetic acid) sprayed near petal fall suppressed yeast populations on flowers.
- Alum (potassium aluminum sulfate, 8 lb/100 gal), applied after Blossom Protect, provides excellent fire blight control.
- Alum, an effective fire blight control material, reduces the pH of floral surfaces; lime sulfur increases pH of floral surfaces.
- Alum increased fruit russeting in both pear and apple.
- Concentrated Actigard treatments applied to the trunks of apple trees at 10% bloom provided partial suppression fire blight but not to the outstanding level observed in 2017.
- Kudos (prohexidione-CA) and Regalia (giant knotweed extract) provided partial fire blight suppression when sprayed onto flower clusters at 10% bloom.

RESULTS

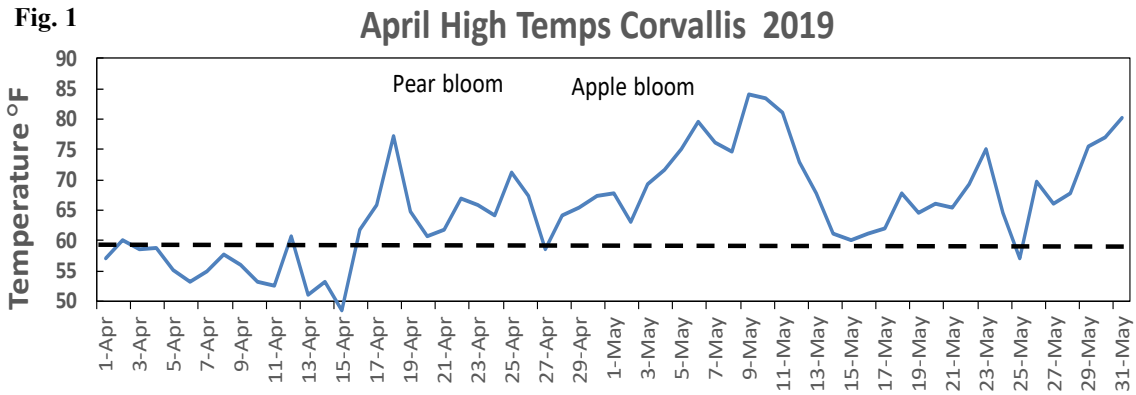
Experimental design. Objectives 1-3 were addressed in experimental orchards located at Oregon State University's Botany & Plant Pathology Field Laboratory near Corvallis (pathogen-inoculated), and at the OSU Southern Oregon Research and Extension Center near Medford, OR (fruit finish only). Experiments were arranged in a randomized complete block designs with 3 to 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 on each tree at 2- to 4-weeks after bloom. In Medford, in late August, the percent of the fruit surface with symptoms of russeting was scored with a modified Horsfall-Barratt rating scale.

Weather in spring 2018: See continuing report from last year for spring 2018 weather information. *Weather in spring 2019*: Fire blight risk as determined by the heat unit model,

COUGARBLIGHT, was moderate through the bloom periods of pear and apple. Epiphytic pathogen populations built up quickly on inoculated control trees and remained high (> 1 million cells per flower) through bloom. Nonetheless, in pear, fire blight incidence was lower than typical for an inoculated trials because of a light bloom (following heavy bloom in 2018). In apple, weather conditions during and after petal fall were very warm and dry, and therefore, all apple trees were misted with water near petal fall to promote infection.

For objectives 1-3, the water-controls averaged 25 infections per tree in Bartlett pear (9% of total clusters) and 93 infections per tree in Golden Delicious apple (38% of total clusters). For objective 4, water-treated control trees in the 6-yr-old Gala apple block averaged 22 strikes per tree (27% of total clusters); on non-treated trees averaged 11 strikes per tree (14% of total clusters).

Fig. 1



Objectives 1 and 2. Refer to the 2018 continuing report for data concerned with microbial populations on flowers and floral pH in that season. In this report, fire blight infection and fruit russeting data are shown for both 2018 and 2019 but the text mostly discusses refers to data from 2019. Also for 2019, non-antibiotic control focused on somewhat more complex spray programs to evaluate series of materials that could best meet these combined these attributes: i) outstanding infection suppression, ii) outstanding suppression of pathogen populations, iii) significant suppression of yeast populations at petal fall, and iv) negligible induction of fruit russeting. In general, these programs were initiated with the biological material Blossom Protect (+ Buffer Protect), followed by two <u>different</u> non-antibiotic chemical(s) (Table 1).

Infection suppression. In pear in 2019, outstanding suppression (> 70%) was observed with FireWall 50 (streptomycin sulfate), and Blossom Protect plus Buffer Protect (twice), Blossom Protect plus Buffer Protect (once) followed by alum (twice), and Blossom Protect plus Buffer Protect (once) then Previsto (once at full bloom) then 4% lime sulfur (once at petal fall) (Table 1). Other programs that included a treatment with a *Bacillus*-based material after Blossom Protect/Buffer Protect (e.g., Serenade Opti or Stargus) were less effective.

Pathogen populations in flowers. Population size of *E. amylovora* on pear flowers continued to increase during the post-petal fall period with the exception of integrated programs that included 4% lime sulfur at petal fall (Fig. 2). Treatment programs that included the *Bacillus*-based material, Serenade Opti, showed less suppression of pathogen populations than treatment programs that included Previsto copper or alum.

Yeast populations in flowers. Trees that received a non-antibiotic chemical(s) material after Blossom Protect had lower floral yeast populations than flowers from trees treated with Blossom Protect only (Fig. 3). Trees that received Previsto at full bloom and 4% lime sulfur at petal fall had the lowest yeast populations in the post-petal fall period.

*Floral pH***.** Relative to other treatments, lower floral pH measurements were associated with the treatment program that included alum, and higher floral pH measurements were associated with treatment programs that included lime sulfur (Fig. 4).

Table 1. Evaluluation of non-antibiotic materials for fire blight control in Bartlett pear and Golden Delicious apple, Corvallis, 2018 and 2019.

					PEAR 2018		APPLE 2018		PEAR 2019	
Treatment	Rate per 100 gallons water	70% bloom	Full bloom	Petal Fall	% blighted floral clusters **		% blighted floral clusters**		% blighted floral clusters**	
Water		**---** §	**X**	**X**	29.7	**a**	35.9	**a**	9.0	**a**
FireWall	8 oz.	---	X	---	4.5	ef	0.8	d	1.7	cd
Serenade	20 oz.	---	X	X	-		-		5.1	abc
Alum 1%	133.5 oz.	---	X	X	16.9	abc	1.7	cd	-	
VP20	144 oz.	---	X	X	16.2	bc	1.1	cd	-	
Blossom Protect	21.4 oz.	X	X	---	5.8	def	2.2	bcd	2.7	bcd
Buffer Protect	150 oz.	X	X	---						
Blossom Protect	21.4 oz.	X	---	---	9.9	cde	**0.5**	d	2.4	cd
Buffer Protect	150 oz.	X	---	---						
then Alum 1%	133.5 oz.	---	X	X						
Blossom Protect	21.4 oz.	X	---	---	11.1	cd	3.1	bcd	-	
Buffer Protect	150 oz.	X	---	---						
then VP20	144 oz.	---	X	X						
Blossom Protect	21.4 oz.	X	---	---	**4.2**	ef	**0.9**	d	-	
Buffer Protect	150 oz.	X	---	---						
then Previsto 1%	96 fl. oz.	---	X	X						
Blossom Protect	21.4 oz.	X	---	---	-		-		**1.5**	d
Buffer Protect	150 oz.	X	---	---						
then Previsto 1%	96 fl. oz.	---	X	---						
then Lime sulfur 4%	512 fl. oz	---	---	X						
Blossom Protect	21.4 oz.	X	---	---	22.6	ab	1.0	cd	3.6	bcd
Buffer Protect	150 oz.	X	---	---						
then Serenade Opti	20 oz.	---	X	---						
then Lime sulfur 4%	512 fl. oz	---	---	X						
Blossom Protect	21.4 oz.	X	---	---	-		-		2.9	bcd
Buffer Protect	150 oz.	X	---	---						
then Previsto 1%	96 fl. oz.	---	X	---						
then Serenade Opti	20 oz.	---	---	X						
Blossom Protect	21.4 oz.	X	---	---	13.0	bcd	2.5	bcd	-	
Buffer Protect	150 oz.	X	---	---						
then Serenade Opti	20 oz.	---	X	---						
then Jet Ag	167 fl. oz		---	X						
Blossom Protect	21.4 oz.	X	---	---	14.6	bc	-		-	
Buffer Protect	150 oz.	X	---	---						
then Serenade Opti	20 oz.	---	X	X						
Regalia (popcorn)	64 fl. oz. **X**	---	---	---	-		-		5.5	abc
Blossom Protect	21.4 oz.	X	---	---						
Buffer Protect	150 oz.	X	---	---						
then Stargus	64 fl. oz.	---	X	---						
then Regalia	64 fl. oz.	---	---	X						

*** Trees inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at 1 x 10^6 CFU/ml on 12 April (pear 2018), 25 April (apple 2018), and 18 April (pear 2019). ** Trees used in the experiments averaged 841, 256, and 266 flower clusters per tree for pear 2018, apple 2018, and pear 2019, 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.**

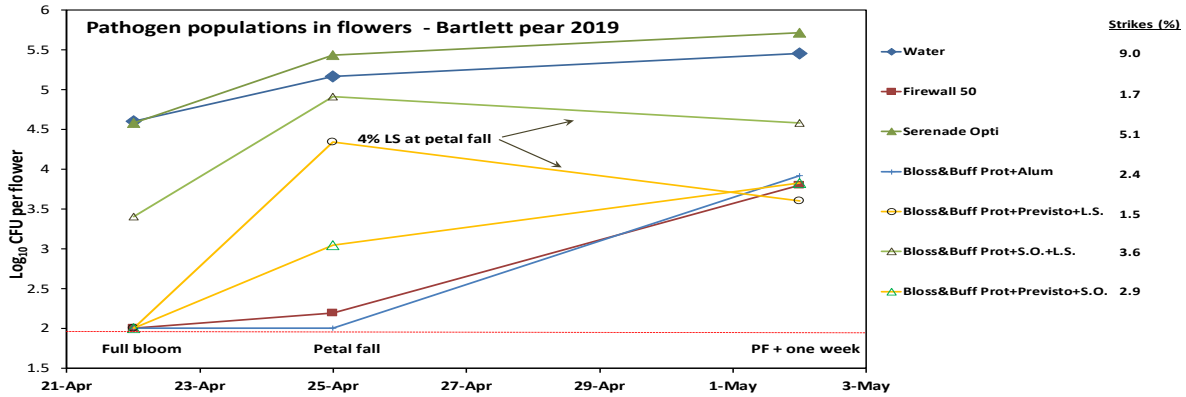


Fig. 2. Effect of treatments applied to A) Bartlett pear trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers during April and May 2019. Pathogen populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree, and plating the wash onto a selective culture medium. Log_{10} = 2.0 was the detection limit of the assay. Data depict mean of each treatment program on each sampling date.

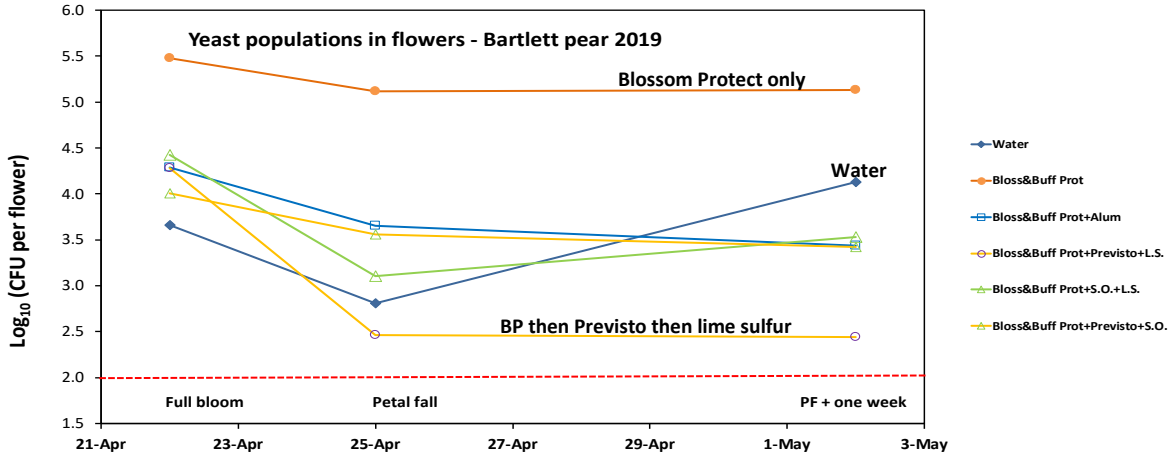


Fig. 3. Effect of treatments applied to Bartlett pear trees to suppress fire blight on the population size of *Aureobasidium pullulans* (the yeast in Blossom Protect) on flowers during April and May 2019. *A. pullulans* populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree and plating the wash onto a selective culture medium. Data depict mean of each treatment program on each sampling date.

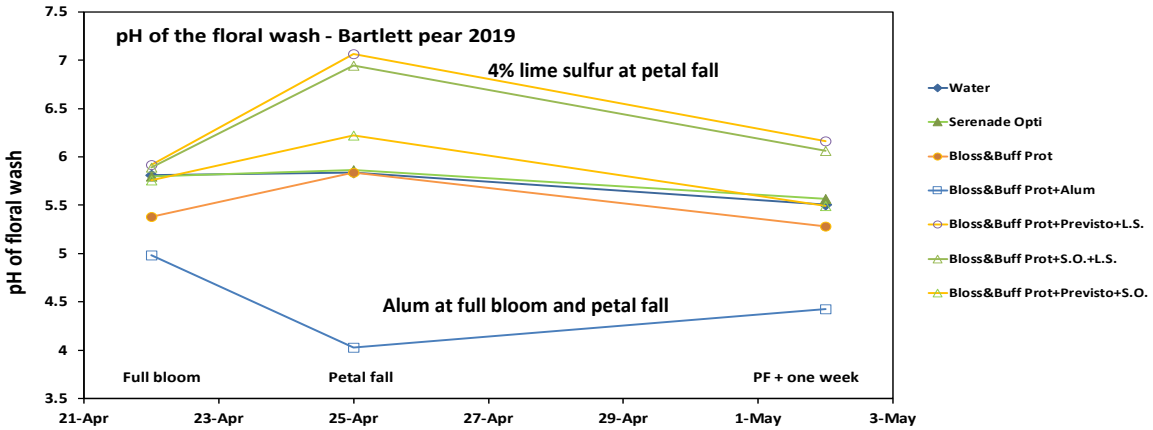


Fig. 4. Effect of treatments applied to Bartlett pear trees to suppress fire blight on the pH of floral surfaces during April and May 2019. A hand-held pH-probe was placed in a deionized-water wash of five flower clusters (~25 flowers, bulked in 25 ml of water) from each replicate tree. Data depict mean of each treatment program on each sampling date.

Fruit russeting. As in 2018, application of non-antibiotic fire blight materials to Comice pear and Granny Smith apple in 2019 resulted in significant differences ($P \leq 0.05$) in fruit russeting severity, although the specific material that caused the most russeting differed among crop species. For example, Serenade Opti or Previsto (full bloom) and then 4% lime sulfur (petal fall) was the most injurious treatment applied to Comice pear, but these same treatments resulted in the least amount of russeting on Granny Smith apple (less than the water control). Conversely, Blossom Protect and Buffer Protect was the least injurious to pear but the most injurious to apple. Noteworthy, and in contrast to 2018 results, integrated spray programs applied to apple in 2019 that contained Previsto copper (in 2019 this material was applied at full bloom only) did not increase fruit russeting relative to the water control. Also in 2019, as in the previous season, alum elevated russeting severity in both pear and apple compared to the water control.

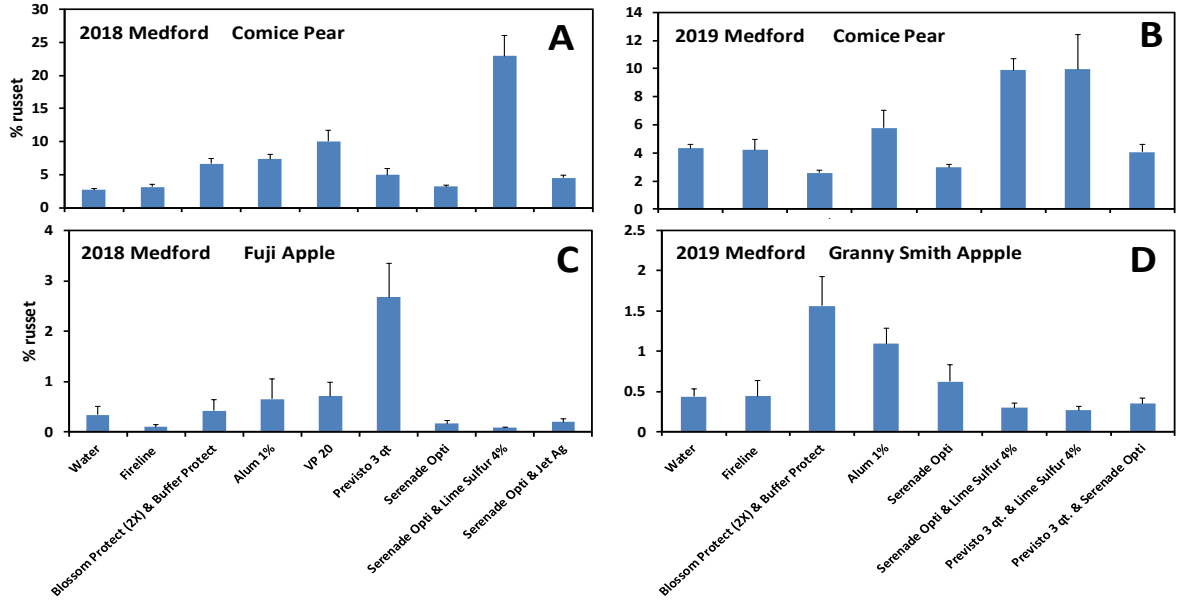


Fig. 5. Effect of non-antibiotic fire blight control materials applied to A,B) Comice pear and C) Fuji or D) Granny Smith apple trees on severity of russeting injury (%) of the fruit surface in the 2018 (A,C) and 2019 (B,D) seasons. Orchards were located near Medford, OR. Treatments were applied to trees 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.

Objective 3. Trials were established in Bartlett pear and Golden Delicious apple to evaluate the effectiveness of Blossom Protect for fire blight suppression relative to other yeasts. Treatments were Blossom Protect (1.6 g/liter, for which the titer was 1 x 10^7 CFU/ml of *A. pullulans*), a water-treated control and lab-grown yeast isolates: a postharvest biocontrol strain of *Cystofilobasidium infirmominiatum* strain YY6 (Spotts et al. 2009), two local field isolates of *A. pullulans* (strains AP3 (used in pear only in 2018) and AP6 (used in 2019)), and two local field isolates of *Cryptococcus neoformans* (strains C16 (used in 2018) and C9 (used in 2019)). Local strains of *A. pullulan*s and *C. neoformans* were isolated from flower washes during a 2016 experiment and identified by sequencing PCR-amplicons from primers ITS and ELO2. Yeast cultures were grown on PDA for 4 to 6 days (20°C), and then scraped from the media surface. Resulting cell suspensions were sprayed onto trees at 1 x 10^7 CFU/ml. Prior to spraying, Buffer Protect (11.2 g/liter) was added to each yeast isolate suspension. Experimental trees were inoculated with *Erwinia amylovora* strain 153N at 80% bloom.

Yeasts were readily recovered from flowers sampled from yeast-treated trees (Fig. 4) with the species that was applied to the trees being the dominant species recovered. Moreover, regardless of isolate, each yeast generally attained population sizes exceeding 1 x 10^4 CFU/ flower and these populations were significantly larger ($P \leq 0.05$) than the total yeast population size on the water-treated controls. Significant differences in yeast populations were observed among yeast treatments. For example, in both pear and apple in 2018, trees treated with C. *infirmominiatum* strain YY6 had significantly larger populations ($P \leq 0.05$) of this yeast on flowers compared to the *A. pullulans* populations measured on flowers treated with Blossom Protect (Fig. 4 A, C). For all trials, *A. pullulans* populations on Blossom Protect-treated trees were either statistically similar to or smaller than population sizes measured for the other yeast treatments (the exception was apple in 2019 where the *A. pullulans* population measured on Blossom Protect-treated trees was significantly greater ($P <$ 0.05) than the *A. pullulans* population on trees treated with local isolate AP6 of this fungus (Fig. 4D).

With regard to fire blight, all yeast treatments had a smaller incidence of infection than the water-treated control (Fig. 5). Fire blight suppression by Blossom Protect was always numerically superior to suppression by other yeast isolates and significantly superior ($P \leq 0.05$) to the other yeast isolates in two of the trials (Fig. 5A, C). Averaged over trials, Blossom Protect (plus Buffer Protect) applied twice provided 81 $\pm$ (s. e.) 5% control of fire blight. In contrast, the next most suppressive treatment, C. *infirmominiatum* strain YY6 (plus Buffer Protect) provided 58 $\pm$ 13% control.

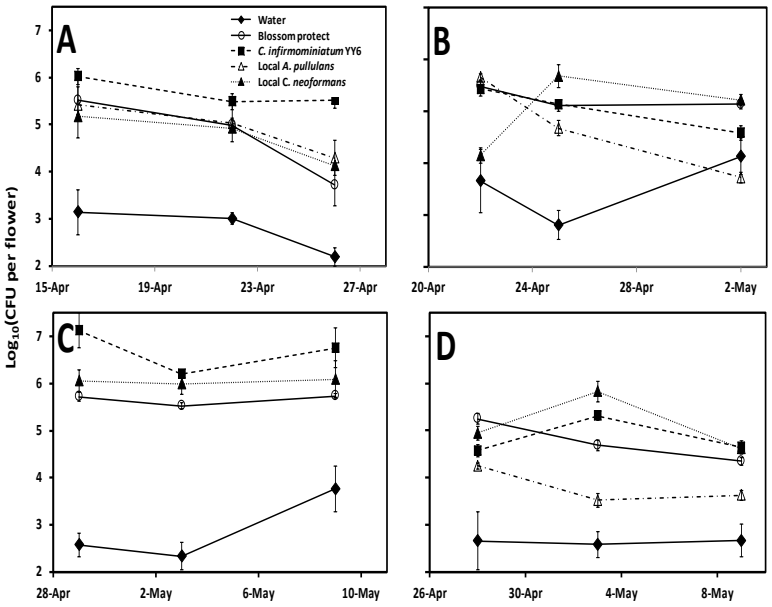


Fig. 4. Log_{10} (population size) of yeast isolates on A,B) Bartlett pear or C, D) Golden Delicious apple flowers sprayed at 70% and full bloom during spring seasons of 2018 (left) and 2019 (right) in orchards near Corvallis, OR. Treatments were water (black diamond), Blossom Protect (open circle), *C. infirmominiatum* YY6 (black square), two local field isolates of *A. pullulans*, AP3 (2018) and AP6 (2019) (open triangle), and two local field isolates of *C. neoformans*, C16 (2018) and C9 (2019) (black triangle); all yeast treatments were amended with Buffer Protect. Data depict mean and standard error of four treatment replicates on each sampling date.

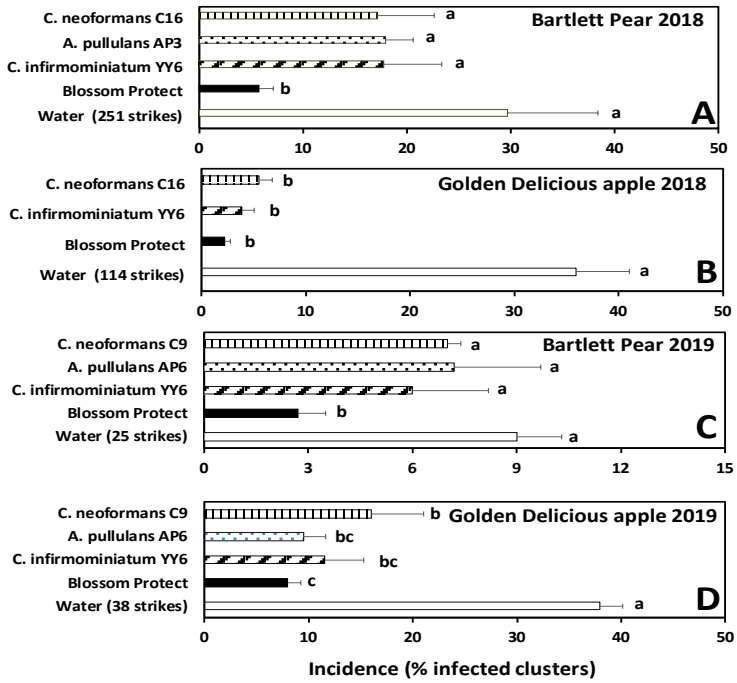


Fig. 5. Effect of yeast plus Buffer Protect treatments on incidence of fire blight infection in 'Bartlett' pear and 'Golden Delicious' apple orchards located near Corvallis, OR in April-May, 2018 and 2019. Yeast (or water) treatments were arranged in a RCB design with four replications and sprayed at 80% and full bloom. *E. amylovora* was inoculated onto trees on an evening between the two treatment dates. Incidence of infection = infected flower clusters/total flower clusters per tree. Absolute number of infections per tree (strikes) for the water controls are shown in parentheses. Within panel, bars labeled with a different small-case letter indicate a significant difference in disease incidence at *P* = 0.05; error bars represent one standard error of the mean.

Objective 4. Refer to the 2018 continuing report for fire blight incidence data concerned with prebloom application of SAR inducers to apple tree trunks.

In 2019, eight resistance inducing materials were evaluated: Actigard 50WG (acibenzolar-S-methyl, Syngenta Crop Protection, Greensboro, NC), Kudos (prohexidione calcium, Fine Americas, Walnut Creek, CA), Blush (prohydrojasmon, Fine Americas), Regalia (extract of *Reynoutria sachalinensis,* Marrone Bio, Davis, CA), Employ (harpin-αß protein, SymAgro, Visalia, CA), Ecoswing (extract of *Swinglea glutinosa,* Gowan, Yuma, AZ), Romeo (cell walls of *Saccharomyces cerevisiae* LAS117, Agrauxine, Beaucouzé, France), and proprietary material 'A'.

The experiment had 19 treatments with six replications but for analysis, treatments were categorized by how and when materials were placed onto each tree: a) concentrated material paints applied once to the tree trunk only, b) materials sprayed onto floral clusters once at 10% bloom, and c) materials sprayed three times onto flower clusters at 10% bloom, full bloom and petal fall. Control treatments were a non-treated control, and a water-treated control (sprayed with water at 10% bloom, full bloom and petal fall).

Concentrated materials were applied by spraying the tree's central leader with the material in a 1-liter, hand held pump sprayer (model 418, Solo Inc., Newport News, VA). The sprayer was equipped with a cone-shielded nozzle, and during application, the nozzle tip was positioned a distance of 1-cm from the trunk surface. All treatments except one were applied as a 'full' treatment, which meant spraying a 100-cm length of the central leader on two opposing sides of trunk; this treatment applied ~60 ml of spray suspension onto the tree. The exception, an Actigard '½' treatment, was applied to a 100-cm length of trunk to one side only. All materials were applied in combination with a surfactant to aid material absorption: 1% Break-Thru S 240 (polyether-modified polysiloxane, Evonik Corp., Richmond, VA) for Actigard and Blush, and 1% BioLink Spreader Sticker (organic soapbark spreader, Westbridge Agricultural Products, Vista, CA) for Regalia. For trees that received floral treatments, materials were sprayed to near runoff with 12-L backpack sprayers equipped with hand wands (0.5 liter/tree); amended surfactants are listed as footnotes to the data table.

Symptoms of fire blight were first observed on 15 May. Incidence of fire blight was determined by counting the number of blighted flower clusters (i.e. strikes) on each tree during three inspections on 20 and 30 May. Blighted clusters were removed immediately after counting. Incidence of disease (total strikes/total cluster number) was subjected to analysis of variance (Analyze-It Software v. 3.0, Leeds, UK).

Trees used in the study averaged 88 flower clusters per tree. For a pathogen-inoculated trial, disease intensity was moderate with fire blight infections on water-treated trees averaging 22 strikes per tree (27% of total clusters) and on non-treated trees averaging 11 strikes per tree (14% of total clusters). For concentrated material paints applied to the tree trunk only, Actigard (full trunk treatment) had an average infection incidence of 7%, which was 50% less infection than the non-treated control (14%) but this difference was not significant ($P = 0.08$). Other materials paints applied to the trunk only showed responses intermediate to Actigard (full trunk) and the non-treated control.

For materials sprayed onto floral clusters once at 10% bloom, infection incidence on trees that received Regalia and material A averaged 5 and 7%, respectively, which was significantly less ($P \leq 0.05$) than the nontreated control (14%). Similarly, for trees sprayed three times during the bloom period, incidences of infection (%) on trees treated with Actigard (4%), Regalia (4%), Employ (8%), material A (13%), and Regalia plus material A (16%) were significantly less ($P \leq 0.05$) than the water-treated control (27%).

Table 2. Evaluluation of SAR-inducer materials for fire blight control in Gala apple, Corvallis, 2019

Treatment	Rate	Date treatment applied* 23 Apr 10% bloom	26 Apr Full bloom	1 May Petal fall	Number of blighted clusters per tree**	Percent blighted floral clusters**
Trunk paint 1X	**per quart**					
Non-treated	-	---§	---	---	**11**	**14 a#**
Actigard - full	1 oz.	X	---	---	6	7 a
Actigard – ½ one side	1 oz.	X	---	---	10	10 a
Regalia	16 fl. oz.	X	---	---	7	9 a
Blush	16 fl. oz.	X	---	---	7	11 a
Flower clusters 1X	**per 100 gal**					
Water-treated	-	**X**	**X**	**X**	**22**	**27** a
Non-treated	-	---	---	---	**11**	**14** bc
Kudos [x,y]	3 oz.	X	---	---	15	17 ab
Kudos [x,y]	6 oz.	X	---	---	7	10 bcd
Actigard [y]	6 oz.	X	---	---	12	12 bcd
Kudos [x,y] Actigard	2 oz. 3.2 oz.	X	---	---	10	11 bcd
Regalia [z]	256 fl. oz.	X	---	---	3	5 d
Material A [z]	128 fl. oz.	X	---	---	7	7 cd
Bloom sprays 3X	**per 100 gal**					
Water-Treated	-	X	X	X	22	27 a
Actigard [z]	2 oz.	X	X	X	4	5 c
Employ [z]	2 oz.	X	X	X	8	10 bc
Regalia [z]	64 fl. oz	X	X	X	4	6 c
Material A [z]	16 fl. oz.	X	X	X	13	16 b
Material A [z] plus Regalia	16 fl. oz. 32 fl. oz.	X	X	X	11	13 bc
Ecoswing [z]	32 fl. oz.	X	X	X	14	17 ab
Romeo [z]	14.6 oz.	X	X	X	21	19 ab

* Trees mist inoculated on 24 April with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain).
** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown.
§ 'X' indicates material was sprayed on that specific date; '---' indicates material was not applied on that specific date.
# Means within a column and within a section followed by same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference.
[x] Amended 1:1 with ammonium sulfate.
[y] Amended with Regulaid: 16 fl. oz. per 100 gallons.
[z] Amended with BioLink Spreader-Sticker: 4 fl. oz. per 100 gallons.

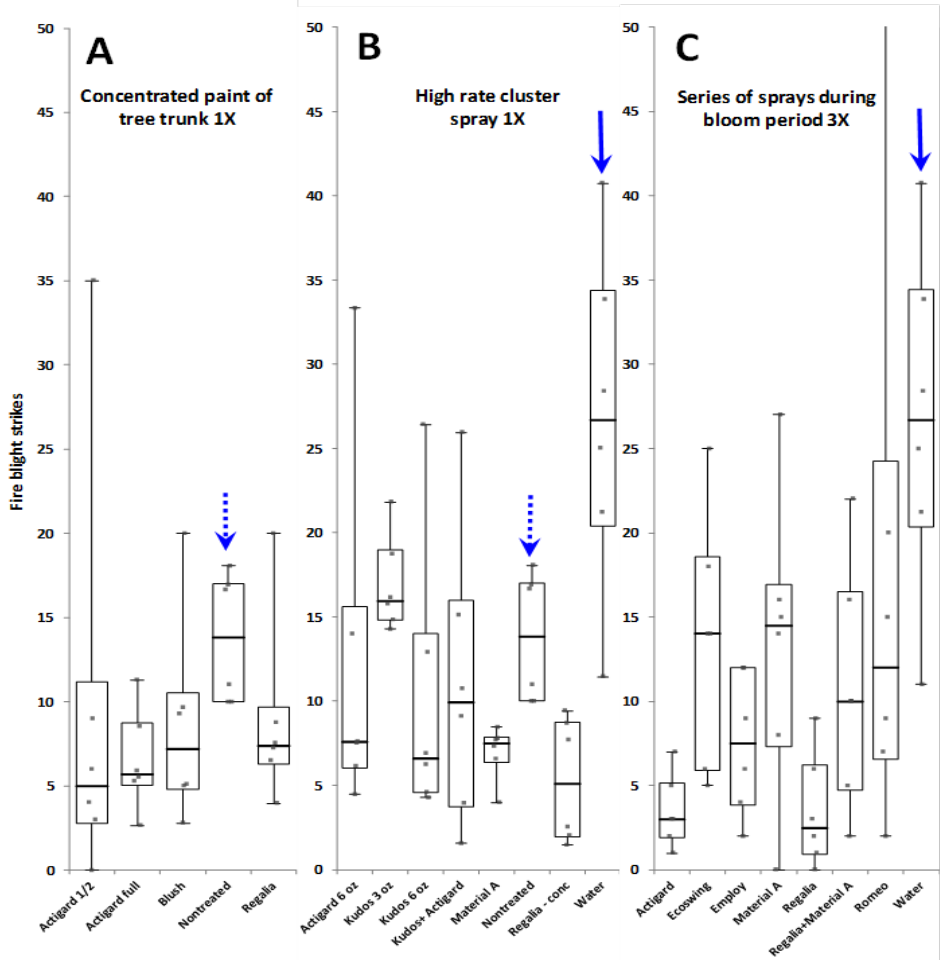


Fig 5. Box plots of strike counts in individual trees that received an SAR-inducer treatment. Panel A: concentrated paint treatment of tree trunk once; panel B: high-rate treatment of flower clusters once; and panel C: three spray treatments during bloom period. Blue arrows mark control treatments: water-treated = solid line, nontreated = dashed line.

DISCUSSION

In the last decade, there has been an increase in the number of biopesticide materials available for non-antibiotic fire blight control. Many of these material achieved EPA-registration with only a limited number of field trials that demonstrated efficacy, and most are approved for organic production. Since 2010, in inoculated orchard experiments we have sought to understand on a comparative scale the value of these materials on fire blight suppression (as well as many more materials not listed in this report). In 2015, we added additional insight to material efficacy by measuring floral populations of the fire blight pathogen and yeasts at the growth stages of full bloom, petal fall and a week post-petal fall. And in 2017, we added measurements of floral pH and fruit russeting severity to provide a more complete understating of the antimicrobial impacts of the control programs as well as risk of inducing phytotoxic injury to developing fruit.

Overall, our data indicate strongly that integrated programs that begin with the biological material Blossom Protect (+ Buffer Protect), and are followed by a non-antibiotic chemical(s) can provide: i) outstanding infection suppression, ii) outstanding suppression of pathogen populations, iii) significant suppression of yeast populations at petal fall, and iv) negligible induction of fruit russeting. This control program is summarized in Fig. 6. Over all the years of effort, we have concluded that Blossom Protect (and Buffer Protect) is essential to organic fire blight control, and under higher infection risk conditions, the 'harsher' chemical materials (e.g., Previsto copper at full bloom and lime sulfur at petal fall) provide better suppression than comparatively softer materials such as Serenade Opti (and other *Bacillus*-based products). Nonetheless, our trial data on these more complex treatment programs (e.g., soluble copper then lime sulfur) is still relatively limited. Consequently, we will continue to evaluate these control programs in 2020. Current data also shows that a soluble copper (e.g., Previsto) at full bloom will suppresses/eradicates pathogen populations to

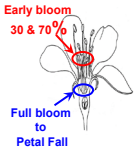
a greater degree than a *Bacillus*-based material (e.g., Serenade Opti), and that among the harsher chemical materials, lime sulfur at petal fall poses the least risk of fruit russeting compared the soluble coppers or alum. Frustratingly, this project has revealed that alum, which provides excellent fire blight control when used at 8 lb/100 gallons (where it strongly reduces floral pH), poses a relatively high potential to induce fruit russeting.

Fig. 6. Current recommendation for non-antibiotic fire blight control.

Integrated, non-antibiotic fire blight control:

Example PNW non-antibiotic spray program with considerations for fruit safety

1) Prebloom (just prior to green tip):
   Fixed copper sanitation if fire blight was in orchard last year (5 to 6 lb/A)



2) Early bloom apple: (crop load thinning)
   Lime sulfur (plus oil) early bloom at 20 and 70% bloom
   Reapply biological if lime sulfur goes on after biological
3) Early bloom apple and pear: Blossom Protect
   One full, or two half apps, or two full apps if blight in orchard last year – cover every row
   In apple, Blossom Protect immediately after 2nd lime sulfur.
   In smooth-skinned pears in wetter areas, russet risk might be unacceptably high
4) Full bloom to petal fall, depending on cultivar russet risk/CougarBlight model risk:
   Low to moderate risk(negligible russeting risk):
      Serenade Opti every 2 to 4 days
   Improved control under high and extreme risk conditions (increased russeting risk):
      mix Serenade Opti with Cueva (3 qts/A)
      Previsto (3 qts/A) or Cueva (4 qts/A) every 3 to 6 days
5) Apples at petal fall: lime sulfur (2 to 4%) to clean up bacteria, yeast, mildew and rot fungi

On Objective 3 (understanding yeast biocontrol), our initial hypothesis was that *A. pullulans*-based biocontrol would not be strain specific. We adopted this hypothesis because *A. pullulans* is very common on pome flowers and because the *A. pullulans* strains in the Blossom Protect product were selected originally to suppress postharvest fruit rots of pome fruit and not fire blight. Somewhat surprisingly, some of the alternative yeasts attained higher populations on flowers than the Blossom Protect strains of *A. pullulans*. These yeast strains also suppressed fire blight, but not to the same degree as the strains in Blossom Protect. Results from Obj. 3 have been published in a journal article along with other data to create a relatively comprehensive guide to the use of this material for fire blight control in semi-arid orchard production systems (Temple et al. 2020, apsjournals.apsnet.org/doi/10.1094/PDIS-09-18-1512-RE).

Future research efforts on control of fire blight with yeasts should ask the question 'how do the Blossom Protect strains of *A. pullulans* provide superior suppression compared to other yeasts sprayed for this purpose?' Related to this, we have observed that pome flowers colonized by Blossom Protect strains of *A. pullulans* are not greatly suppressive of epiphytic populations of *E. amylovora*, and yet, fire blight is controlled. This may indicate that the apparent mechanism of biocontrol possessed the Blossom Protect strains is more complex than a simple explanation of superior competitive exclusion.

Regarding Objective 4, after very positive results with a concentrated trunk treatment of Actigard in 2017, we observed less infection with the full Actigard trunk paint (30 g/L) in 2018 and 2019; i.e., responses were partial/intermediate to the original observation. Given that the rate of Actigard we applied to trunks is very costly if every tree in an orchard receives the treatment, it is unlikely that an expensive approach yielding a partial response can be practical for preventative fire blight control. Spraying of flowers cluster at 10% bloom, however, is an approach that warrants further investigation (i.e., perhaps a partial response but material costs are less inexpensive). Most SAR materials applied to clusters at this early timing had less infection than the non-treated control; although, variability in the data meant not all differences were statistically significant. With Kudos (prohexidione-CA), the level of suppression we observed was less than has been reported by Cox at Cornell University. Nonetheless, the data argue for further evaluation; it also argues for a higher number of experimental replications and the utilization of the use of proper experimental controls.

EXECUTIVE SUMMARY

Project title: Refinement of practical fire blight control: Non-antibiotic and SAR

Key words: fire blight, non-antibiotic control

Abstract: Suppression of fire blight (caused by *Erwinia amylovora*) with non-antibiotic methods was investigated. Integrated organic programs beginning with Blossom Protect (70% bloom), followed by Previsto (fb) and then 4% lime sulfur (pf) provided outstanding control with negligible risk of fruit russeting. SAR materials Actigard, Kudos and Regalia provided partial suppression responses.

SIGNIFICANT FINDINGS:

- Blossom Protect (yeast), Previsto (copper) at full bloom and lime sulfur 4% at petal fall provided outstanding fire blight control.
- Lime sulfur 4% at petal fall did not increase fruit russeting of apple compared to a water treated control
- Alternative yeast strains (with Buffer Protect) suppressed fire blight but to a degree less than observed with Blossom Protect.
- Population size of *E. amylovora* on pear and apple flowers continued to increase during the post-petal fall period except when treated with lime sulfur at petal fall.
- Lime sulfur or Jet Ag (H_2O_2 in peracetic acid) sprayed near petal fall suppressed yeast populations on flowers.
- Alum (potassium aluminum sulfate, 8 lb/100 gal), applied after Blossom Protect, provides excellent fire blight control.
- Alum, an effective fire blight control material, reduces the pH of floral surfaces; lime sulfur increases pH of floral surfaces.
- Alum increased fruit russeting in both pear and apple.
- Concentrated Actigard treatments applied to the trunks of apple trees at 10% bloom provided partial suppression fire blight but not to the outstanding level observed in 2017.
- Kudos (prohexidione-CA) and Regalia (giant knotweed extract) provided partial fire blight suppression when sprayed onto flower clusters at 10% bloom.

FUTURE DIRECTIONS

- Multi-material, integrated programs (e.g., Blossom Protect then soluble copper then lime sulfur) require additional evaluation to better document expected efficacy and risk of fruit russeting.
- Future efforts on understanding control of fire blight with yeasts could be concerned with how Blossom Protect strains of *A. pullulans* achieve superior blight suppression compared to other yeasts.
- Pre- and early bloom resistance induction directed at treatment of flower clusters requires additional evaluation in trials with a high number of experimental replications and appropriately designed experimental controls.

FINAL PROJECT REPORT
WTFRC Project Number: CP-17-102

Project Title: Optimizing control for leafrollers and Western tentiform leafminer

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Total Project Request: **Year 1:** $78,428 **Year 2:** $81,565 **Year 3:** $38,934

Other funding sources: None

Total Project Funding:

Item	2017	2018	2019
Salaries[1]	45,000	46,800	22,725
Benefits[2]	17,069	17,751	7,817
Wages	9,600	9,984	4,800
Benefits[3]	259	270	77
Equipment	0	0	0
Supplies[4]	2,500	2,600	1,352
Travel	4,000	4,160	2,163
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	78,428	81,565	**38,934**

Footnotes:
[1] new position
[2] 34.1%
[3] 2.7%
[4] includes lab and field supplies
[5] w/in state travel

Original Objectives:

1. Evaluate different timing strategies for leafroller management using *Bt* and/or Entrust in organic orchards

2. Evaluate control strategies for leafroller in conventional orchards

3. Determine the causes of western tentiform leafminer (WTLM) outbreaks and evaluate how to optimize management to reduce effects on parasitoids.

Significant Findings:

- For the first 2 years, we put out the treatments for OBLR laid out in objectives 1 & 2, but populations in previously infested areas did not developed. We decided to drop these two objectives (with approval of Dr. Hanrahan), reduced the budget in the final year, and focused on objective 3.
- Comparison of conventional and organic orchards in all three years showed that western tentiform leafminer (WTLM) pheromone trap catches were higher in the organic blocks, and leaf samples showed all the leafmining activity was restricted to the organic blocks.
- Organic orchards where Entrust® was used in the early-mid May time period typically showed elevated levels of WTLM, often above 10 mines per leaf.
- We found that a new parasitoid, *Pholetesor ornigis*, was much more common in our Quincy and Sunrise sites than *Pnigalio flavipes*. This parasitoid had previously been reported in mid-western and eastern orchards attacking spotted tentiform leafminer, but not in surveys done in the late 1980's in Washington.
- The timing of the Entrust® application would have affected most of the parasitoid adults and also killed a significant fraction of the sap feeding stages of WTLM which are the primary host feeding target for the parasitoid *P. flavipes* and oviposition target for the parasitoid *P. ornigis*.
- Our data collection and literature search allowed us to develop a phenology model that predicts all stages of WTLM that can be included in the WSU-Decision Aid System.
- White apple leafhopper was also affected by the Entrust® applications and reached extreme levels in some of the organic orchards compared to the conventional paired orchard.
- The WALH parasitoid *Anagrus epos* was found to coincide with the emergence of the first generation of WALH, however, the second generation of parasitoids only occurred after the peak population of second generation WALH. This asynchrony means that any disruption of the first generation of *Anagrus* would allow the second generation of WALH to increase without any significant population suppression.

Objectives 1 & 2: *(1) Evaluate different timing strategies for leafroller management using Bt and/or Entrust in organic orchards (2). Evaluate control strategies for leafroller in conventional orchards*

These two objectives were not met. During both 2017 and 2018, we set up and sprayed blocks that had previously had severe problems with OBLR, but populations never developed. In season, we attempted to change plots to evaluate the different timings needed but could not find plots that were suitable. In 2018, we decided (in consultation with Dr. Hanrahan) to drop these two objectives and cut out budget to just fund the third objective.

Objective 3. *Determine the causes of western tentiform leafminer (WTLM) outbreaks and evaluate how to optimize management to reduce effects on parasitoids.*

Methods: From 2017-2019, we placed pheromone traps into orchards that used at least a single treatment of Entrust® during the early to mid-May period for control of OBLR (note this would also provide some control of codling moth). In 2017, we monitored two orchards in the Quincy area: one was an organic orchard treated with Entrust®, the other a paired conventional block across the road. We used pheromone traps, yellow panels (for parasitoids), and leaf samples collected throughout the season at weekly intervals. In 2018, we expanded into six blocks: three sites were in Quincy, two were near Sunrise Research Orchard and one site was located near Brays Landing. Two of the three Quincy blocks were Honeycrisp apples that were planted adjacent to each other; one was farmed organically and the other was farmed conventionally. The third Quincy site was a block of organic Red Delicious. The two blocks near Sunrise Research Orchard were adjacent to each other consisted of Red and Golden Delicious. One block was organic, the other was transitioning to organic. The site north of Orondo was a block of organically farmed Gala. In 2019, we set up three pairs of orchards, two pairs in Quincy and one in Brays Landing. As in previous years, we used pheromone traps, yellow panels, and leaf samples to evaluate population dynamics of tentiform leafminers. We were also able to evaluate white apple leafhopper populations that reached outbreak status in the orchard blocks in 2018-2019.

Timing of Entrust® Treatments:

Location	Date	JD	WTLM DD
Brays Landing	5/11/18	131	725
Quincy B	5/12/18	132	845
	5/24/18	144	1185
	7/4/18	185	2180
	8/12/18	224	3490
Quincy A	5/9/18	129	780
Sunrise	5/14/18	134	1000
Sunrise B	6/19/18	170	1965
	8/3/18	215	3470
Brays Landing	5/9/19	129	650
Quincy B	5/16/19	136	825
	8/3/19	215	2985
Quincy A	5/9/19	129	685

Results and Discussion:

Population trends of different stages:

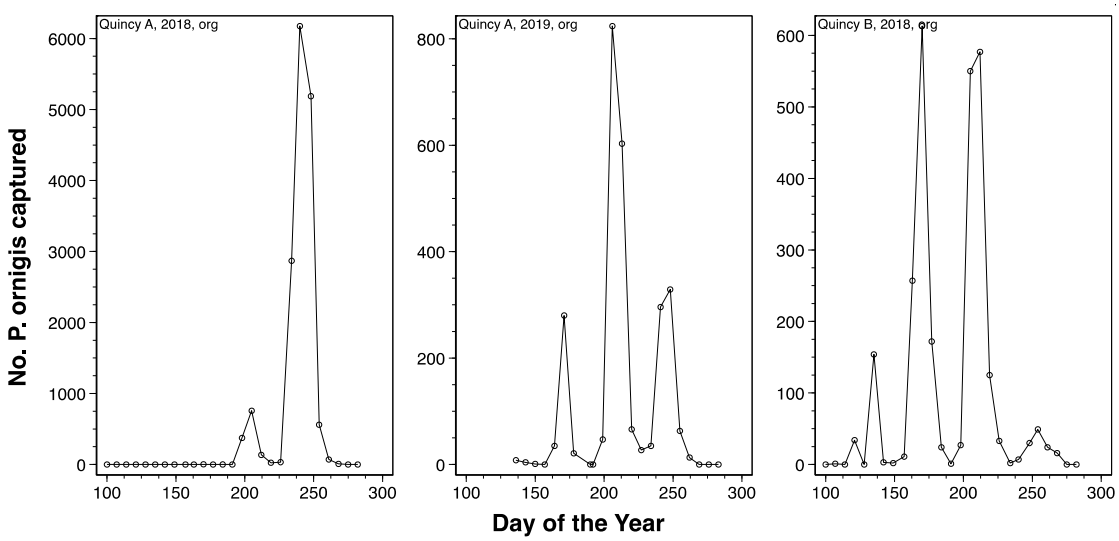
Parasitoids on yellow sticky cards. The number of parasitoids caught on yellow panels is important because it gives us not only the emergence patterns of the parasitoids, but also because it allows us to see which ones are dominant at the different sites. We expect that parasitoids will be more common in orchards where high density of leafmines are present, because the parasitoids both feed on the larval leafminers and because they are needed for maturation of the parasitoid immatures.

Bruce Barrett (a PhD student of Jay Brunner) worked with WTLM and the parasitoid *Pnigalio flavipes* in the late 1980's and showed the major parasitoid species in all the WTLM generations in Washington was *Pnigalio flavipes.* He found it constituted 85% of all parasitoid species reared.

Pnigalio flavipes prefers to lay its eggs in the tissue feeding stages (instars 4-5), and host feed primarily in the sap feeding stages (instars 1-3); the emergence of *P. flavipes* in the spring in Barrett's work was similar to the timing of WTLM adult emergence.

Our yellow panel studies in 2017-2019 showed that that *P. flavipes* was not the dominant leafminer parasitoid caught, with only 148 specimens caught at the six sites in 2019. We also evaluated how many were caught in before 1 June, which would be indicative of the potential impact of the parasitoid in early season. We found only 5 total *Pnigalio flavipes* were caught in the 11 orchards monitored in 2018 and 2019 (both conventional and organic orchards). Contrast this to 1985 and 1987 data collected by Bruce Barrett where 782 and 192 *P. flavipes* were caught before 1 June. In the entire seasons of 2018 and 2019, 1697 and 148 *P flavipes* were caught, mostly in the last generation and the majority in the organic orchard at Quincy A. The total numbers caught by Barrett in conventional orchards was 4686 and 3517, in 1985 and 1987, respectively. These statistics suggest that the suppressive effect of *P. flavipes* on WTLM currently was rather low to non-existent in these orchards compared to what historically was a major mortality source.

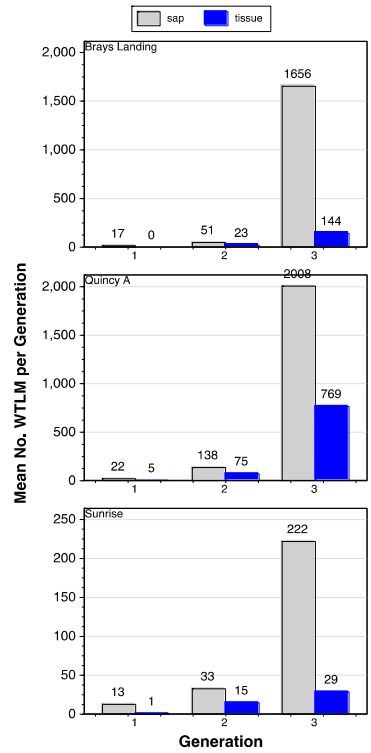
Fig. 1. Number of *Pholetesor ornigis* captured at the highest density sites in 2018-2019.



The most common parasitoid on our yellow panel traps was *Pholetesor ornigis* which is commonly found attacking the spotted tentiform leafminer in the eastern and mid-western US (STLM is not present in WA). This species was not reported by Barrett in his survey of parasitoids attacking WTLM in the late 1980's, so it is relatively new to the state. This species tends to emerge slightly later than *P. flavipes*, and we collected 224 parasitoids before 1 June, with the majority of them collected in a single site (194) when the sprays were on early (9 May). All season long, we collected 21,641 *P. ornigis* in our organic blocks (where leafmines were abundant) and only 615 in our adjacent conventional blocks (where leafmines were virtually non-existent, so we didn't expect to find many there). The vast majority were collected at the Quincy A site where we caught 16,624 in 2018 and 2,817 in 2019 (Fig. 1), but they were also captured at Quincy B and at the Sunrise orchard as well.

WTLM larvae and parasitoids: Leaf samples showed in all years that mines were restricted to the organic blocks – we only had 3 dates where we found any mined leaves in conventional blocks across all blocks and years. In 2018 and 2019, we found that the number of both tissue and sap feeders generally increased from generation to generation (Fig. 2). Early in the season, it is difficult to find mines, whereas during the 3rd summer generation, high levels of the two different stages of the larvae are common. Population increases from generation 2-3 averaged 17.5 fold (which is similar to lab studies show the reproductive rate/female WTLM), but varied from 6.7-32.5 fold. The number of tissue feeders per generation is always lower than the number of sap feeders as parasitism and host feeding both reduce the number of individuals that reach the tissue feeder stages (Fig. 2). The Quincy A sites (same sites used in 2018-2019) showed the populations were highest in 2018 and dropped sharply in 2019.

Fig. 2. Number of sap and tissue leafmines in 2018 by generation.



The leaf samples also allowed us to evaluate both parasitism and host feeding by the parasitoids at least later in the season when they become more common. In 2018, sap feeders were not parasitized to any significant degree all season long at any site (3.3%), but host feeding accounted for a significant amount of mortality (mean=53.1%) at all sites (Fig. 3). The tissue feeding larvae showed host feeding only 14.6% of the time, but 51.4% showed parasitism over the three sites (Fig. 4). Overall, these figures were similar in 2019 and parasitoid-caused mortality resulted in 60.5% reduction in WTLM larval survival. The parasitism of the larvae occurred primarily in the second and third generations of the tissue feeding stage.

Fig. 3. Evaluation of the season trends in parasitism and host feeding effects on WTLM sap feeding stage in 2018.

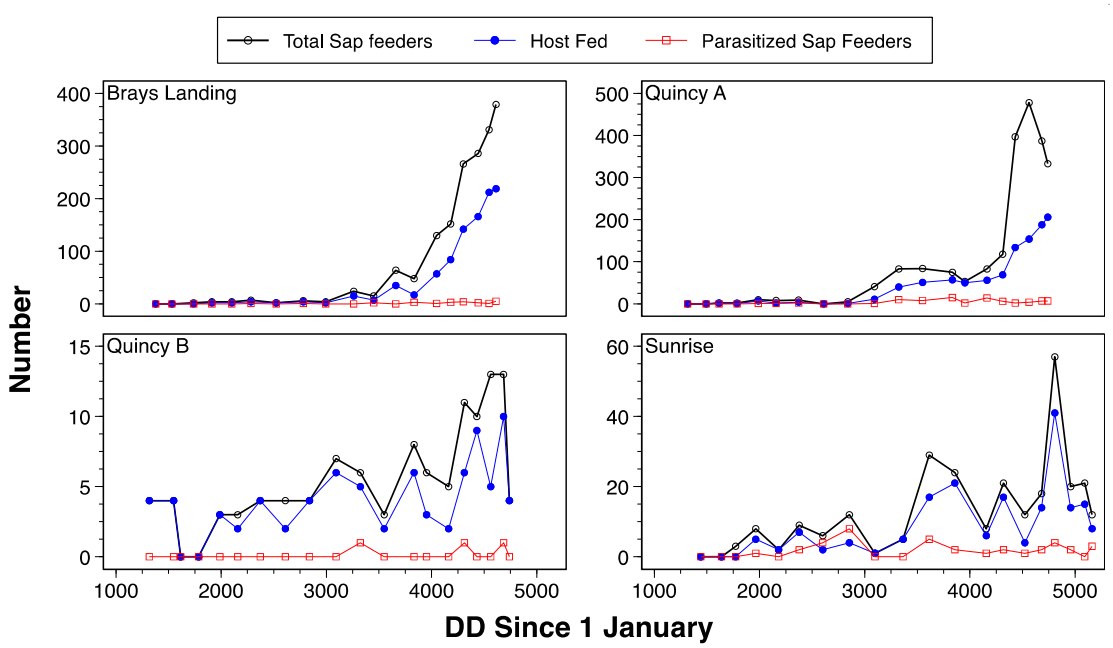
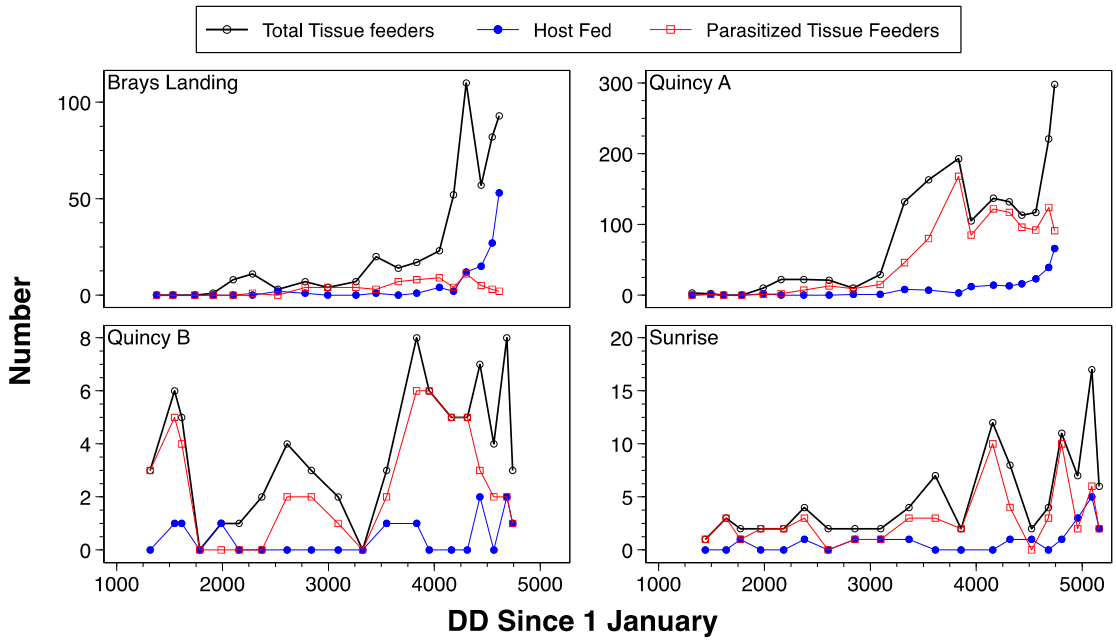


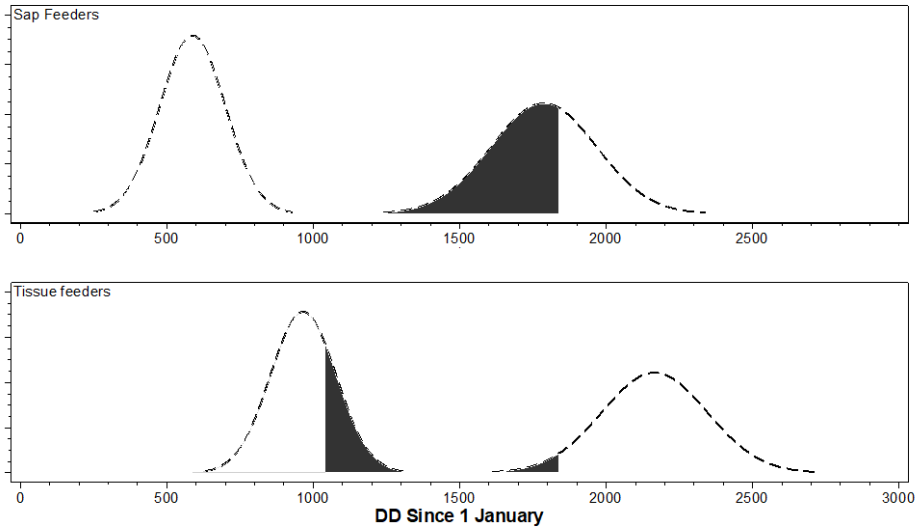
Fig. 4. Trends in parasitization and host feeding on the tissue-feeding stage of WTLM in 2018.



Summary: The effect of the early season Entrust applications happened in virtually all our orchards before the tissue feeding stages of WTLM were present. Both the adult WTLM and the adult *P flavipes* or *P. ornigris* would have been present at the times the sprays were applied and likely would have been affected by the spray program by coming into contact with the treated surfaces or potentially during the host feeding process which would have been common at that point in time. The low level of WTLM larvae of either stage at that point would require the parasitoid to be a very active searcher and thus would increase the probability that they pick up a lethal dose of the pesticide. *P. flavipes* is highly susceptible to Entrust® but Entrust® has been used to treat WTLM outbreaks, so it would be expected to provide at least short-term suppression of the larval stages of the leafminer. Likely, the early season application eliminated most of the sap-feeding larvae which would be used by *P. flavipes* for host feeding and by *P. ornigis* for oviposition and host-feeding. By reducing this resource early in the season, later generations of the two parasitoids would have to migrate into the treated orchard from surrounding blocks. It is interesting that two of the conventional orchards applied Delegate® (which is similar mode of action to Entrust®) at the same time as Entrust® in the paired organic block but did not experience outbreaks of WTLM. In both instances, roughly two weeks after the Delegate® application, the grower also applied Altacor® for codling moth control (the two sprays were applied 14 days apart). As WTLM is on the Altacor® label, it is likely that the outbreaks were suppressed by the Altacor® applications whose activity would have covered the first 62% of the second sap feeding generation and would have affected the latter part of the first tissue feeding generation and first part of the second tissue feeding generation (Fig. 5).

WTLM model: The pheromone traps allowed us to develop a phenology model that predicted emergence of the adult males in both conventional and organic orchards (Fig. 6). The model was developed using data from the conventional orchards in 2018 and 2019, and then validated using data from organic orchards in 2017 and 2019, as well as data from an attractant trap orchard in 2013, and data digitized from Bruce Barrett's dissertation (a PhD student of Jay Brunner's that worked on WTLM and *Pnigalio flavipes* in the late 1980's). The model fit well for the overwintering generation, and the 2nd and 3rd summer generation, but started slightly late in the first summer

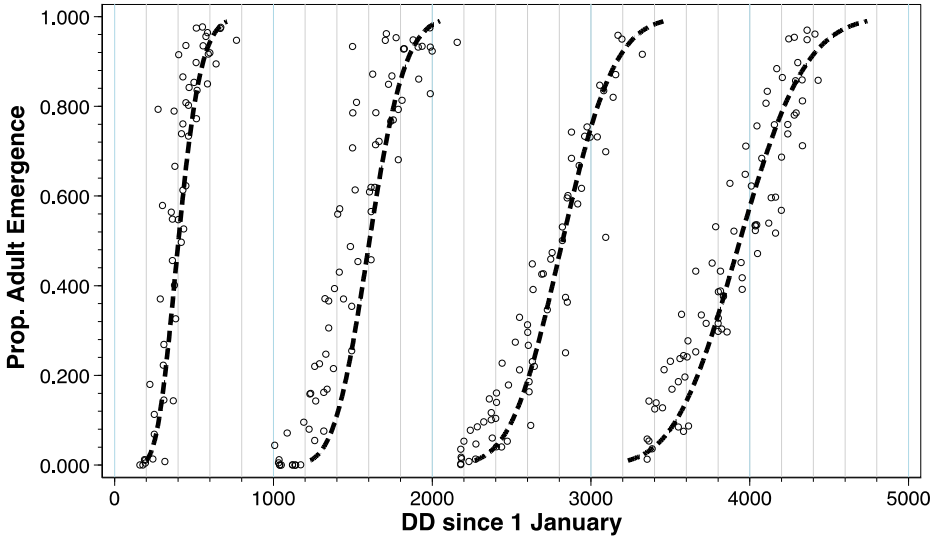
Fig. 5. The coverage of Delegate (gray area) and the two sprays of Altacor (black area) at the Quincy B conventional site in 2019. Either colored area would suppress WTLM occurring during those periods.



generation. We expect to implement this model next year on DAS.

We were also able to use some of the data in Barrett's dissertation to evaluate when the sap (early instars) and tissue (late instars) feeding larvae would be found. This data was used in conjunction with the adult catch to allow us to evaluate which stages of larvae would be found at any point in time. Our field data from the leafmines provided some data, but ultimately, we did not develop the model strictly from our sampling because there was virtually no data early in the year to evaluate the first several summer generations. Moreover, the data of Barrett added to our adult catch data predicted subsequent adult generations almost perfectly.

Fig. 6. Trap capture of male WTLM (open circles) versus model predictions (dashed lines). Model predictions generated from an independent set of data compared to the observed emergence.



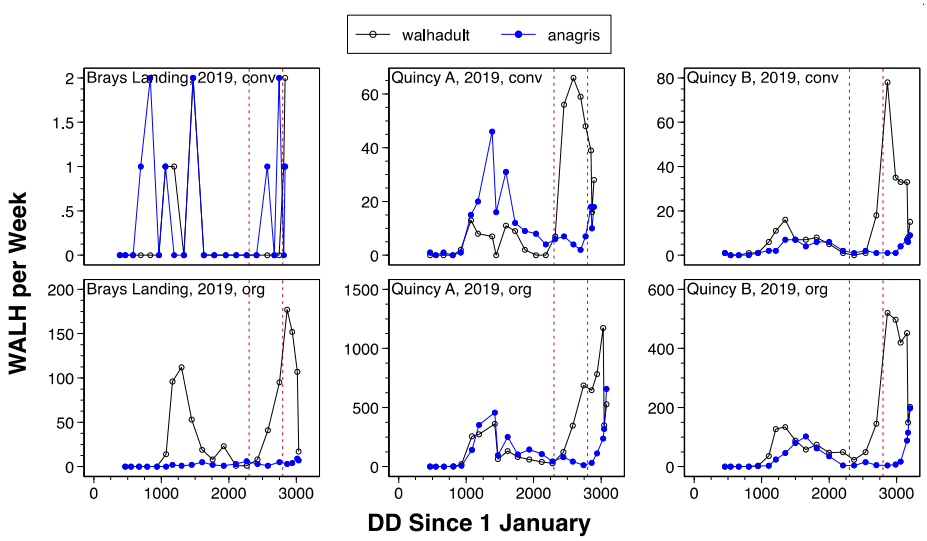
Effect of Entrust sprays on White Apple Leafhopper (WALH).

WALH population trends:

As with the WTLM, the organic orchards with Entrust applied in mid-May reached much higher levels than in the paired conventional orchards. If we just look at the 2019 data (Fig. 7), examination of just the scale of the y-axis, the organic orchards range from 7.5 to 100 times higher than paired conventional orchard. All the orchards (except Brays Landing conventional site) show a marked increase in the population level during the second generation, even though it appears that we did not complete the second generation at any of the sites.

Anagrus epos is the specific parasitoid of WALH. At the two paired Quincy sites, *Anagrus* responded to the first generation of WALH, but showed asynchrony in the second generation with population increases of *Anagrus* only occurring after the peak of the second generation WALH (around 2800 DD). The same asynchrony with *Anagrus* and WALH occurred in 2018 as well. The asynchrony in the second generation suggests that any disruption of *Anagris* in the first generation is the cause of the increase of the WALH which tends to happen right before harvest, because the second generation of parasitoids would not build up until after the WALH population had already peaked.

Fig. 7. WALH trap catches on yellow panels over the season at the three paired conventional and organic orchards in 2019.



Project Title: Optimizing control for leafrollers and Western tentiform leafminer

Keywords*:* Western tentiform leafminer, *Phyllonorycter elmaella*, WTLM phenology model, *Pholetesor ornigis, Pnigalio flavipes*, biological control, pesticide upset, *Typhlocyba pomeria*

Abstract. The biological control of western tentiform leafminer (*Pyllonorycter elmaella*) was found to be susceptible to disruption in organic orchards when Entrust was sprayed in early-mid May. These treatments resulted in very high levels of damage and also affected biological control of the white apple leafhopper, *Typhlocyba pomeria*.

Executive Summary.

Sprays of Entrust in early-mid May resulted in increased population levels at harvest of both the Western Tentiform Leafminer (WTLM – *Phyllonorycter elmaella*) and the white apple leafhopper (WALH- *Typhlocyba pomeria*). In the case of WTLM, we found very low populations of the parasitoid *Pnigalio flavipes*, which had been reported in the 1980's to be the most important natural enemy controlling WTLM. The timings of the sprays for oblique-banded leafroller, *Choristoneura rosaceana* occurred when *P. flavipes* adults had emerged from overwintering and coincided with the majority of the larval population being in the sap feeding stage. This is a critical period because the parasitoid adult was exposed to the pesticide residue, which lab studies have shown are very toxic. The pesticide also suppresses the WTLM sap-feeding larvae (instars 1-3), which restricted the ability of the parasitoid to host-feed, which is important for parasitoid females to achieve full reproductive potential. We also found that a new parasitoid, *Pholetesor ornigis*, was at very high levels at some of the Quincy area orchards. This parasitoid is best known from the mid-western and eastern apple orchards where it attacks the spotted tentiform leafminer (*Phyllonorycter blancardella*). This parasitoid was also affected because it tends to attack the sap feeding stages and parasitizes them (as well as host-feeding on the same stages). If this species has established in a wide area of Washington state, it could provide more stability in the control of WTLM, but it is also affected by the same timing as *P. flavipes*.

Our data also allowed us to develop a phenology model for all stages of WTLM and that model will be implemented on the WSU-DAS system in the next year.

The increased populations of the WALH also seem to be a result of pesticide-induced interference with its primary parasitoid, *Anagrus epos*. *Anagrus* normally has good synchrony with the first generation of WALH, however, the second generation of *Anagrus* starts to build well after the peak population of the second generation WALH population. Thus, any disruption of *Anagrus* during the first generation reduces its ability to regulate WALH populations by season's end. This results in high populations at harvest, when it becomes a nuisance pest for pickers.

FINAL PROJECT REPORT

Project Title: Evaluating and improving biological control of WAA

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Cooperators: None

Total Project Request: **Year 1:** $54,301 **Year 2:** $87,233 **Year 3:** $84,878

Other funding sources

Agency Name: WSDA-USDA SCRI Block Grant
Amt. awarded: $194,910
Notes: (expired early 2017)

WTFRC Collaborative Expenses: None

Budget 1

Item	2017	2018	2019
Salaries[1]	31,146	51,612	48,041
Benefits[2]	7,439	17,368143	17,167
Wages	8,000	8,320	8,653
Benefits[3]	216	225	234
Equipment	0	0	0
Supplies[4]	3,500	3,640	3,786
Travel[5]	4,000	4,160	4,326
Miscellaneous	0	0	0
Plot Fees	0	2,184	2,271
Total	54,301	85,049	82,207

Footnotes:
[1] Project Assistant (Y1 -12 months, Y2 – 3 months); Y2-3 Matt Jones 50% FTE; Tawnee Melton 30% FTE Y1-Y3.
[2] Project Assistant 11.7%, U Chambers (32.1%), T Melton (47.5%)
[3] 2.7%
[4] includes lab and field supplies
[5] w/in state travel

Objectives

1. Evaluate the effect of augmenting/reducing earwigs on woolly apple aphid population levels and earwig-related fruit damage
2. Use molecular methods to evaluate the gut contents of earwigs to assess feeding habits
3. Use HD video monitoring to observe natural enemy attack rates on WAA in a field situation
4. Evaluate changes in biological control of WAA when natural enemy lures are placed in the field

Significant findings

- We found clear evidence that earwigs suppress woolly apple aphids at our four study orchards.
- There was no evidence that earwigs-initiated fruit damage in any of our four study orchards (one Gala orchard and three Fuji)
- Molecular gut content analysis shows that earwigs eat a variety of foods in apple orchards including fungi, plants, and numerous arthropods, not just aphid pests.
- Video analysis showed that earwigs were the most common predator near WAA colonies throughout the season. Lacewings were also commonly found, but occurred in more restricted time periods than the earwigs.
- We did not find that use of natural enemy lures season-long increased population suppression of WAA. However, they still might be useful for helping manage WAA populations in hot spot areas.
- A model developed under a technology grant suggests that there are two different types of locations based on the number of hours per day over 92°F in mid-summer and the length of that period. Warmer sites tend to have lower populations that drop sharply in July and peak in the fall in September. Cooler locations have higher population levels that slow in mid-summer (but don't crash) and rely more on biological control.

Objective 1. Evaluate the effect of augmenting/reducing earwigs on woolly apple aphid (WAA) population levels and earwig-related fruit damage

Methods

Study sites. We worked at four orchard blocks described in Table 1. In 2017, sites M, T, and O were each divided into 12 sections consisting of two adjacent rows of 8 trees. All sections were at least 30 meters apart from each other and the edges of the block. Each of the 12 sections was assigned to an earwig treatment, either 'control', 'augmentation', or 'removal' (explained below). Site W was set up in 2016 and differed in that there were only 10 sections and two treatments ('augmentation' and 'control') and each section consisted of three adjacent rows of 14 trees. This was because two years of previous monitoring data suggested that there was no (or a very small) natural earwig population, so removal and control treatments would be redundant.

Table 1. Information about study sites

Block name	Nearest town	Variety	Spacing (trees x rows)
M	Quincy	Fuji	3.5' x 12'
T	Quincy	Fuji	7' x 15'
O	Orondo	Fuji	5' x 13'
W	Winchester	Gala	3' x 10'

Insect monitoring and earwig manipulations. WAA and earwigs were sampled at roughly weekly intervals from April to November. We sampled all trees in study sections of sites M, T, and O, and

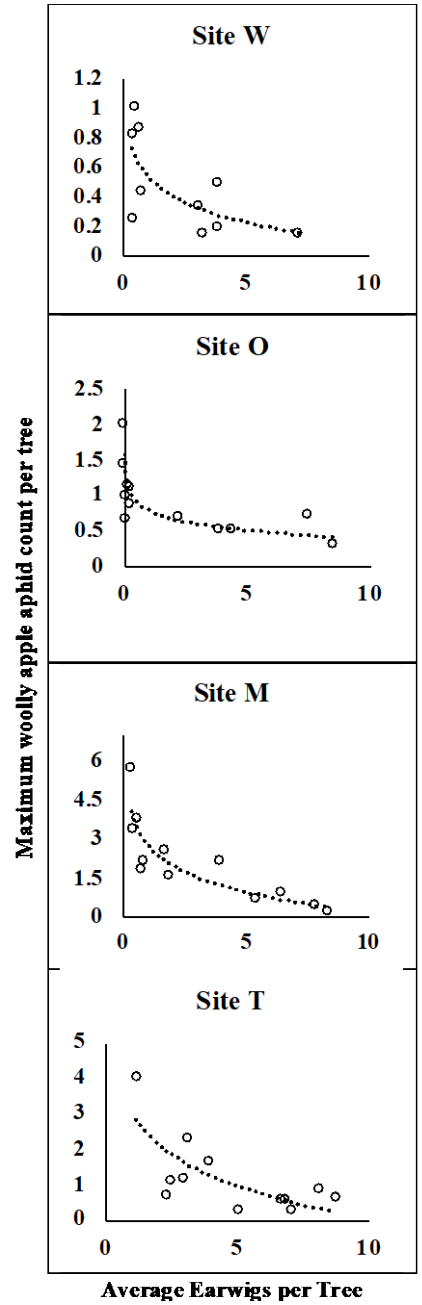
every other tree at site W. WAA colonies were recorded as the number of infested axils on a survey of ten ~1' long twigs plus all colonies on pruning cuts and trunks. Earwigs were monitored by counting the number found in rolled tubes of corrugated cardboard placed in each tree.

In control and augmentation treatment areas, all earwigs found were counted and released. In the removal areas, earwigs were counted and collected into a plastic bag. In addition, earwigs were collected by the thousands at an orchard near Quincy, counted, and released into augmentation areas from May to July. In total, 120 earwigs per tree were released in augmentation areas of site M, 350 per tree at site T, and 175 per tree at site O. At site W, 38 earwigs were released per tree in 2016 in augmentation areas. There was no further manipulation of site W in 2017 because earwigs established at the augmentation areas and were more abundant there than in the control areas.

The number of earwigs released per tree varied between sites depending on the amount we found during monitoring. If less than ten earwigs were found per tree during monitoring, or if there was no significant difference between earwig counts in augmentation vs. control areas, we released more earwigs on the next visit. The number of earwigs released per tree may seem like a very large amount, but our trap counts were not extremely high compared to monitoring data collected from commercial orchards in 2014 and 2015.

Fruit damage survey. Within 5 days of harvest, we inspected up to 30 apples on each study tree in earwig augmentation and removal areas at sites M, O, and W (total fruit examined over 3 sites = 11,950). Site T was not evaluated because earwigs remained prevalent in the removal treatment areas. Each inspected apple was scored as 'good' (having no visible defects) or categorized by defects.

Fig. 1. Correlation between woolly apple aphids and earwigs at four sites in 2017.



Data analysis. To quantify the relationship between earwig and woolly apple aphid abundance, we summarized each study section of each site into two numbers: 1) the maximum count of woolly apple aphid colonies per tree (to represent 'how bad the problem got') and 2) the average earwig count during the observation period. To correct for variation in the number of days between observations, the average earwig count was calculated as $\sum (T_{i+1} - T_i)[(Y_i + Y_{i+1})/2]$ divided by total days of observation, where T is the day of an observation, and Y is the number of earwigs found per tree during an observation.

Results and discussion

Woolly apple aphid suppression. At all four orchard blocks, locations with fewer earwigs had a higher risk of WAA outbreaks, while at locations with higher levels of earwigs, WAA populations remained consistently low (Fig. 1), strongly suggesting that earwigs suppress WAA populations.

At sections where the average number of earwigs per tree was >5 earwigs over the season, WAA counts never exceeded 1 colony per tree. In sections with <5 earwigs per tree, the maximum WAA colonies per tree were 1–6 fold higher.

Earwig damage to fruit. We found no evidence that earwigs caused increased fruit damage. There was evidence that rounded and expanded stem splits were more common in earwig augmentation areas at site W, and when all sites were pooled, but the overall occurrence of any type stem bowl splitting was not significantly greater in earwig augmentation areas at any site or overall (Table 2). Strangely, stem bowl splitting was more prevalent at Site M in earwig removal areas, but when all sites were pooled, augmentation and removal areas were not significantly different in total stem bowl split occurrence. Overall, the results suggest that while earwigs can attack damaged apples, they do not initiate damage frequently enough to be detectable. In addition, the frequency of apples with putative earwig exacerbation of stem bowl damage was very low: 0.3% in augmentation areas and 0.1% in removal areas.

Table 2. Apple damage survey. Chi-squared tests were conducted to assess the chance of finding apples belonging to each category of damage in earwig augmentation (Aug) vs. removal (Rem) areas. Tests were conducted within each study site and for total apples pooled across sites.

Site	Trt.	Number of apples								
		Total	Good	Round hole	'Bird' hole	Depression	Side crack	Stem bowl split		
								Normal	Expanded	Total
W	Aug.	2692	2585	2	0	14	1	81	8	89
	Rem.	2632	2536	0	0	9	2	81	0	81
	Chi-square P:		*0.53*	*0.16*	*NA*	*0.32*	*0.55*	*0.90*	***0.005***	*0.63*
O	Aug.	1478	1394	3	3	20	7	11	3	14
	Rem.	1482	1393	2	6	10	8	11	4	15
	Chi-square P:		*0.71*	*0.65*	*0.32*	*0.07*	*0.80*	*0.99*	*0.71*	*0.86*
M	Aug.	1835	1778	2	1	18	12	13	6	19
	Rem.	1831	1758	3	0	16	12	35	1	36
	Chi-square P:		*0.15*	*0.65*	*0.32*	*0.74*	*0.99*	***0.001***	*0.06*	***0.02***
Total	Aug.	6005	5757	7	4	52	20	105	17	122
	Rem.	5945	5687	5	6	35	22	127	5	132
	Chi-square P:		*0.57*	*0.58*	*0.52*	*0.07*	*0.73*	*0.12*	***0.01***	*0.47*

Objective 2. Molecular analysis of earwig diet in an apple orchard

Methods: During previous experimentation at site W in 2016, samples of 20 earwigs were collected on 6 visits between June 17 and September 21. Collections were made within an hour of sunrise into

plastic Ziploc bags stored in a cooler with ice and transported to a -20°C laboratory freezer. Later, each earwig's stomach was dissected the DNA extracted using QIAGEN's DNeasy spin column kit. Each set of extractions included a negative control with no earwig stomach to check for DNA contamination.

Extractions from the 20 earwigs from each day were pooled to yield one sample representing earwig diet for each of the 6 collection days. These samples, along with a pooled sample of negative control extractions were sent to RTL Genomics in Lubbock, TX, for sequencing on Illumina MiSeq platform. Sequencing involved different sets of 'universal primers' designed to amplify DNA from the COI region for arthropods, trnL for plants, and ITS for fungi. RTL Genomics also performs analysis and identification of DNA sequences.

Results
The RTL Genomics commercial laboratory analysis identified in total 441 'operational taxonomic units' from animals, 120 from fungi, and 16 from plants (Table 3). It is important to note that when databases do not contain sequence data for the species, or there was too much uncertainty in which species a sequence may belong to, OTUs cannot be identified. Some of the taxa identified were odd, such as a spider endemic to Australia and a cactus thought to occur only in the Southern Hemisphere, which may be indicative of some closely related taxa present in Washington, but not currently in the gene sequence databases. Many of the insects identified by the databases were expected in apple orchards and the earwig stomachs contained DNA sequences from both pest insects and beneficial insects. One caveat is that this analysis did not address the quantity of any food eaten and whether the food was killed by the earwig or already dead and scavenged.

Objective 3. Use HD video monitoring to observe natural enemy attack rates on WAA in a field situation

Methods. Four video cameras were set up at Sunrise and focused on WAA colonies to record natural enemy activity around the WAA colonies; two cameras were in trees with lures (squalene and a composite lure of acetic acid + methyl salicylate+2-phenylethanol) and two were set up in trees with lures, but without any chemical in the bags. In 2018, recordings were done daily between 4 a.m. and 11 p.m. The cameras were moved when any of the WAA colonies disappeared (2,800 hrs). In 2019, we ran the video cameras 24 hours a day over the period of 31 May to 21 October (3456 hrs).

Results. In 2018, video recording of WAA colonies where lures were nearby showed a total of only 58 lacewings in ≈2,800 hours of recording between 7 June and 5 September. Those low counts were likely related to low counts of WAA (it was very difficult to find colonies to video) and high populations of ladybird beetles bolstered by a massive outbreak of rosy apple aphid and parasitism by the WAA parasitoid, *A. mali*. The problems were exacerbated by issues with the video system that prevented recording in July. During the July and August periods, we attempted to transfer WAA colonies to build the population in the block, but they were unsuccessful.

Table 3. List of taxa found in earwig stomachs according to DNA analysis. OTUs represent genetic diversity, not abundance in the stomach.

Kingdom	Order	Species name, explanation	OTUs	Ecological relevance
Animal	Capitellida	Polychaete worm	4	
	Araneae	*Anames sp* spider endemic to Australia	1	
	Opiliones	*Phalangium opilio* harvestman	1	Predator
	Prostigmata	*Abacarus lolii* grass mite	1	
	Lithobiomorpha	*Lamyctes africanus* centipede	1	
	Entomobryomorpha	*Entomobrya unostrigata* 'slender springtail'	1	
	Coleoptera	*Stethoris punctillum* spider mite destroyer	1	Predator
		Carpophilus sp sap beetle	1	
	Dermaptera	*Forficula auricularia* European earwig	18	
		Unclassified or unknown	14	
	Diptera	*Pollenia rudis* calliphorid fly	1	
		Drosophila melanogaster vinegar fly	1	
		Symplecta sp crane fly	1	
	Hemiptera	*Macrosiphum euphorbiae* potato aphid	1	
		Dikrella californica Blackberry leafhopper	2	
		Zonocyba pomaria white apple leafhopper	2	Pest
		Campylomma verbasci campylomma bug	1	Pest and predator
		Daraeocoris brevis predatory bug	1	Predator
		Eriosoma lanigerum woolly apple aphid	1	Pest
		Pemphigus sp aphid	2	
	Hymenoptera	*Aphelinus varipes* wasp	1	Parasitoid
		Aphidius ervi wasp	1	Parasitoid
	Neuroptera	*Micromus sp* brown lacewing	1	Predator
	Thysanoptera	*Frankliniella occidentalis* Western flower thrips	1	Pest
	Passeriformes	*Cardellina pusilla* Wilson's warbler bird	1	
	Rhabdita	Unclassified roundworms	3	
	Tylenchida	*Bursaphelenchus mucronatus nematode*	1	
Fungi	31 Orders		142	
Plant	Bryales	*Bryum sp* moss	1	
	Dicranales	Unknown moss	1	
	Poales	Unknown grass	2	
	Brassicales	Unknown mustard	1	
	Caryophyllales	Unknown cactus	1	
		Polygonum sp buckwheat	1	
	Fabales	*Medicago sativa* alfalfa	1	
	Oxalidales	Cunoniaceae, a family from the S. Hemisphere	1	
	Pinales	Conifer	3	
	Rosales	Oleaster	1	
	Solanales	Unknown potato family plant	1	

'Operational taxonomic units' (OTUs) are groupings of very similar DNA sequences. A unique OTU usually corresponds to a unique species, but one species can also have multiple OTUs due to genetic variation in the species.

In 2019, the most common groups we found were earwigs, lacewing adults and nymphs, and syrphid larvae, with 45.3, 25,3, 11.7 and 7.4% of the total observations, respectively. Earwigs in the lured areas were not only more common (71.3% of all earwigs observed) but also stayed around the WAA colonies 2.6-fold longer. Moreover, the number of days through the season was also much longer in the lured areas (Fig. 2). This seems to confirm that the lures can change predation rates in small localized areas.

Fig. 2. Number of minutes that earwigs were observed around WAA colonies in the lured and unlured areas throughout the season in 2019.

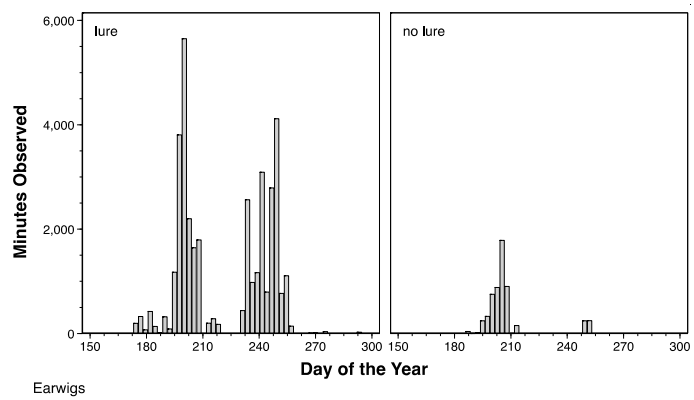
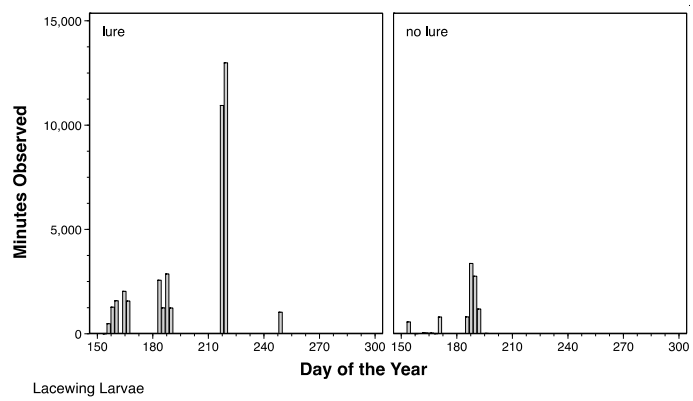


Fig. 3. Number of minutes that lacewing larvae were observed around WAA colonies in the lured and unlured areas throughout the season in 2019.



Lacewing larvae were found roughly the same number of times during the season in the lured and unlured areas (25 versus 21 times), but larvae tended to stay around the WAA colonies 3.5-fold longer in the lured areas. In addition, lacewing larvae in the lured areas were observed at least 3 times throughout the season after mid-June, whereas they were not found in the unlured area after that time (Fig. 3.). Lacewing adults were found in roughly the same number of times in the two different areas but remained around the WAA colonies in the unlured area about 1.5-fold longer than in the lured area. The distribution of adults being observed throughout the season was unaffected.

The syrphid larvae were only found in the lured are twice versus 27 times in the unlured areas. These occurred primarily in mid-July and again in late-September to the end of the season.

Objective 4. Evaluate changes in biological control of WAA when natural enemy lures are placed in field situations.

*Methods***.** Trials to study the effect of lures on the biological control performance of lacewings on WAA colonies were set up in four orchards in 2018. Trials were set up at Sunrise and near Quincy on 27 June to coincide with the second lacewing generation. However, by 3 July, it became obvious that most of the colonies at both sites were heavily parasitized and not suitable for the experiment. Even netting WAA colonies to exclude predators and parasitoids as well as attempts at transplanting WAA colonies did not improve the infestation levels in the experimental plot at Sunrise.

An additional orchard near Orondo with high WAA infestation was then included in the study, and 30 plots were set up on 12 July: 10 for the untreated control, 10 for treatment with acetic acid + methyl salicylate + 2-phenylethanol (AMP) lures, and 10 with squalene (SQ). The order of the plots within the orchard block was randomized. Each plot consisted of a 15-feet section of a tree row, and the plots were approximately 60 feet apart to reduce interference of the lures. In each treatment plot, 6 lures were placed 3 feet apart and between 3-6 feet above the ground near WAA colonies. The number of WAA colonies was recorded once per week within a 1.5-foot radius around each lure. The monitoring included the number of WAA colonies, classified into colony length categories, the approximate percentage parasitism, the number of single (*C. plorabunda*) or clustered lacewing eggs (*C. nigricornis*) was recorded. The color of the lacewing eggs was noted as that indicates the age and hatch status of the eggs (green – new eggs, darker-grey – near hatching, white – hatched). The lacewing eggs were then marked to avoid recount in the following weeks. The presence of any other natural enemies was also recorded. The same parameters were monitored in the control plots, where no lures were placed, in the 1.5-foot radius around random 6 locations with WAA colonies.

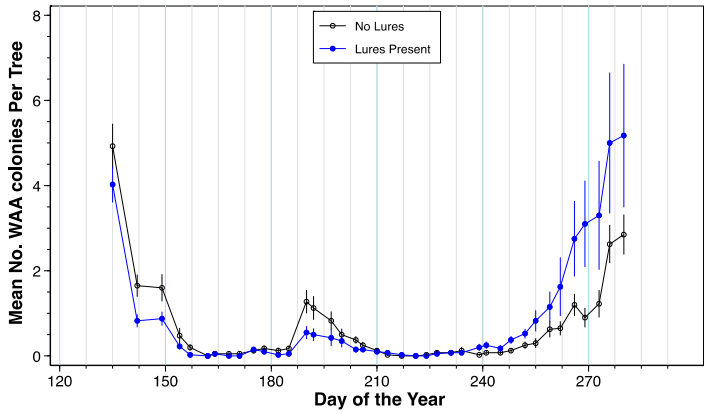
In 2019, we set up a block in Quincy with 20 plots, each consisting of four trees. The plots were randomly assigned to either a no lure or lure treatment. The lures consisted of squalene + an AMP lure as described above. Lures were placed in the orchard on 15 May and then replaced on 25 July. Each lure lasts approximately 6 weeks, so the lures would have run out on 2 September in the fall. Plots were sampled for WAA twice a week from 15 May until 7 October.

Results. In 2018, even though the experiments were well designed, the unpredictable nature of WAA and hindered our best efforts to evaluate the effect of lacewing lures on lacewing oviposition and WAA predation. WAA infestation had been sufficient at our study sites in the past years. However, an unprecedented population explosion of ladybeetles at Sunrise, likely due to the extremely high infestation with rosy apple aphid, as well as high parasitism rates early in the season prevented the resident WAA population from ever reaching sufficient levels. WAA numbers in two other grower orchards also did not increase as expected from previous years. Therefore, the experiment was relocated in the second half of July, when lacewings were still active, to a block in Orondo that had a very high infestation level where the consultant reported that it was due for a pesticide application to get the WAA under control.

At the site near Orondo, WAA colonies disappeared after only 3 weeks into the trial. With only two dates of data no statistical analysis is possible as to the effects of lures on the number of lacewing egg clusters or number of WAA colonies, and the data showed no differences in the number of egg clusters or WAA colonies. No lacewing eggs were found during that time.

In 2019, our experiments were designed to see if we could suppress the populations of WAA in the early spring before high summer heat reduced populations and reduce population buildup in the fall when temperatures cooled. We found that the populations in the untreated and treated areas were similar in the spring, with the plots with lures present having slightly lower number of WAA colonies compared to the untreated areas. However, in the fall populations of WAA in one plot of the lured treatment jumped up rapidly to very high levels (Fig. 4). Analysis of variance showed that the number of colonies was not significantly different over the entire season, with the spring performance

Fig. 4. Comparison of the number of WAA colonies on trees with lures versus no-lure treatment. Dashed vertical line indicates where the lures ran out in early September. Entire increase came from a single plot (of 10) in the lured treatment.

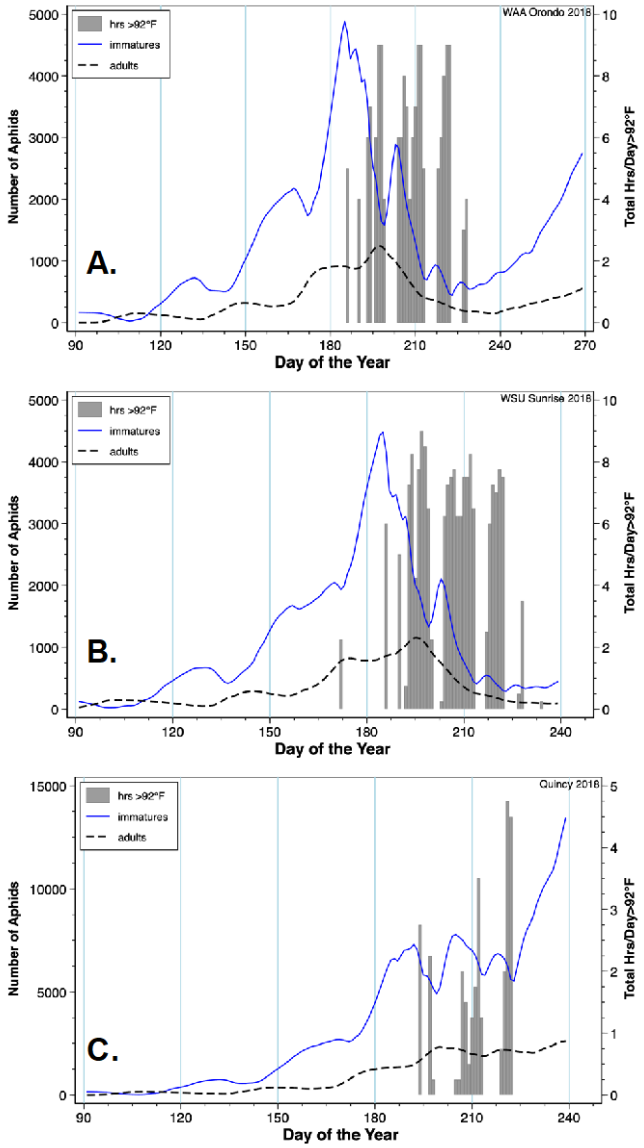


cancelling out the fall performance. If we evaluate the data before the lure ran out, the lure did significantly reduce the number of colonies, but biologically, it was of pretty dubious importance (the mean difference was only 0.36 colonies/tree). Elimination of that one plot showed the control and treated areas were otherwise similar in the fall.

Regardless of the lures running out, it does not appear that putting lures on every tree would be a good control tactic – essentially, the costs would be significantly higher and not have as large an impact as we had hoped. There is still the possibility of putting lures on trees in problem areas to lure lacewings to those areas to jump-start predation there, but season-long lures are unlikely to be a reasonable management tactic.

Model Evaluation. A model for WAA based solely on temperature effects on reproduction, survival, developmental times was completed in late September 2018. The model is based on a synthesis of studies going back to the 1930's. The model updates every 5 DD throughout the year using the average temperatures observed in the field during each 5 DD period and tracks the age and abundance of individuals in the immature and adult stages. The model is not intended to predict exact numbers seen in the field, but instead to provide us with an understanding of how temperature affects population growth. The model demonstrates that much of the population dynamics of this insect is driven by the temperature and are rendered more unstable by predation (which is not tracked by the model). Temperatures above 92°F are especially telling on the WAA abundance and these factors show that in 2018 the sites where we were attempting the studies were going to crash significantly based solely on the temperatures (Fig. 5). However, it is mentioned here because evaluating the temperature profiles at the sites of Sunrise and Orondo, the population would have crashed regardless of the presence of natural enemies. The Quincy location had much less temperature-driven mortality and the peak population size was much higher than at the other two sites. Quincy would exemplify a location where biological control is much more important even during mid-season where the population is suppressed during the heat, but the heat is not enough to crash the population on its own.

Fig. 5. WAA model runs based on temperatures in 2018 at: **A**. Orondo grower's field **B.** WSU Sunrise, and **C**. Quincy. Notice the difference in y-axis scales and the number of hours per day when temperatures were >92°F.



Executive summary

Title: Evaluating and improving biological control of WAA

Keywords: Woolly apple aphid, *Eriosoma lanigerum*, biological control, natural enemy lures, HIPV, green lacewings, earwigs, *Forficula auricularia*.

Abstract. Earwigs (*Forficula auricularia*) were found to moderate population levels of the woolly apple aphid (*Eriosoma lanigerum)*, but did not contribute to fruit damage in samples from 4 orchards comprising >12,000 fruit. Using natural enemy lures in season-long trials did not result in biologically significant reductions in WAA.

Summary. Our data showed that season-long average earwig (*Forficula auricularia*) densities of >5 per tree resulted in significantly lower woolly apple aphid (WAA- *Eriosoma lanigerum)* populations compared to areas where earwigs were removed or unmanipulated. We also found no significant differences in damage between areas where earwig levels were augmented or where earwigs were removed. Molecular data did show that earwigs can feed on both apple and other natural enemies although the techniques do not allow us to determine whether it was direct attack or whether the natural enemies were already dead and scavenged at that time.

Video analysis showed that earwigs attacked WAA colonies in our orchard at a greater frequency than other predators and throughout much of the season. Lacewings spent more total time around WAA colonies, primarily because their attacks averaged nearly 8-fold longer duration, but their seasonal presence was quite restricted in comparison to the earwigs. Antagonistic interactions between predators was rare, and earwigs did not antagonize other predators. Ant-earwig interactions were common and greatly reduced earwig-WAA attack rates.

The idea of using natural enemy lures was tested in both 2018 and 2019. In 2018, studies were incomplete because of high levels of parasitism and predation in our blocks destroyed WAA populations within a few weeks of our setting up the experiments. We attempted to move to other areas, but the high temperatures in those areas knocked populations down to near zero. In 2019, we attempted to use the lures to keep populations low in the spring and then hoped to see population levels suppressed in the fall after high temperatures had passed. Our studies showed a knock down in the spring, but not in the fall. The amount of suppression was relatively low and suggests that the lures would not be a good fit for management on an orchard-wide basis. However, the lures still might be useful in treating hot spots within an orchard to jump-start the establishment of lacewings populations.

A temperature driven model for WAA population growth showed that temperatures above 92°F greatly reduced population growth of the WAA populations and tended to crash populations at locations with high temperatures occur over a significant period of the summer but allow continued summer population growth rates at areas with milder temperatures.

FINAL PROJECT REPORT

Project Title: Brown marmorated stink bug control in Washington

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Other funding sources

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Notes:

Total Project Funding: $254,793

Budget History:

Item	Year 1: 2016	Year 2: 2017	Year 3: 2018	Year 4: 2010
WTFRC expenses				
Salaries	44,564	59,716	62,104	0
Benefits	9,435	14,973	15,572	0
Wages	8,042	8,364	8,699	0
Benefits	431	448	467	0
Equipment	0	0	0	0
Supplies	3,000	1,500	1,500	0
Travel	3,326	3,326	3,326	0
Plot Fees	2,000	2,000	2,000	0
Miscellaneous				
Total	70,798	90,327	93,668	0

Objectives:

1. *Determine distribution of Trissolcus japonicus in Washington*. The samurai wasp, *Trissolcus japonicus*, was discovered in Vancouver, Washington in 2015. This exotic parasitoid is the most promising biological control agent for brown marmorated stink bug (BMSB). In preparation for re-distribution within Washington, a survey of its distribution in the state was necessary.
2. *Maintain a laboratory culture of T. japonicus in preparation for release.* We started a laboratory culture from the original find in Vancouver and refined the rearing methods. As we determined that re-distribution would be allowable/advisable, this objective was re-directed to redistribution within the state and measuring the success of such releases.
3. *Evaluate IPM-friendly management strategies for BMSB.* As it became clearer that BMSB would be established and become a pest requiring management in Washington tree fruits, we began preparing for implementation of integrated pest management (IPM) control tactics versus broad-spectrum pesticide use.
4. *Document the spread of BMSB within the state.* This pest was first documented in the state in 2010 (Vancouver), and is slowly spreading into the agriculturally intensive area of eastern Washington. Tracking its spread provides an unparalleled opportunity to look at patterns and speed of invasion of an exotic species.
5. *Determine suitability of native shrub-steppe plants as hosts for BMSB.* The semi-arid shrub-steppe presents a novel environment for BMSB, and its ability to successfully establish and reproduce on native flora is unknown. Proactively investigating this will help determine landscape risk of pest outbreaks.

Significant Findings

- BMSB has been detected in 27 counties in Washington State as of December 2018, with the highest numbers of reports from the urbanized areas of western Washington. All of the major fruit-growing counties have reported BMSB finds.
- *T. japonicus,* an exotic parasitoid of BMSB, was first found in Washington in 2015 in the Vancouver area; since then, all Vancouver sites surveyed have been positive for *T. japonicus*, with high levels of parasitism.
- *T. japonicus* from a laboratory colony was released at 8 sites in eastern Washington (2 sites each in Prosser, Walla Walla, White Salmon, and Yakima); it was recaptured at both Walla Walla sites and one of the Yakima sites.
- Cages made from shade net reduced the amount of stink bug damage inside the cage compared to a spray-only treatment. Codling moth numbers and damage were also reduced.
- Woolly apple aphid densities were consistently higher inside cages, likely due to exclusion of macropredators such as syrphids and lacewings; releasing insectary lacewings did not correct the problem.
- Stink bugs migrate in and out of orchards over an extended period. There is a consistent trend for barriers to reduce immigration into orchards.
- The majority of stink bugs fly into orchards between 4 and 9 ft, showing potential for exclusion using single-wall net barriers.
- There is preliminary evidence that BMSB can complete development on an assemblage of native host plants; preliminary results of gut content analysis indicate a signal persistence of at least 2 weeks, setting the stage for future experiments.

Objective 1. Determine distribution of *Trissolcus japonicus* in Washington.

The first adventive *Trissolcus japonicus* (Fig. 1) population on the west coast was discovered in Vancouver, WA in 2015. These detections prompted a more extensive survey in both eastern and western Washington. Surveys were conducted in 2016-2018 in the Vancouver area (in the same general region as the original find) and four regions in eastern Washington. The 2018 SEM survey also served as an indicator of the success of releases made in 2017 and 2018 (see Objective 2).

Fig. 1. *Trissolcus japonicus*, an Asian parasitoid of BMSB.

Egg masses for the survey were taken from a colony of BMSB maintained in small insect cages on a diet of sunflower seeds, peanuts, carrots, and potted bean plants. Egg masses were removed daily, as previous research has shown that they become less attractive to parasitoids as they age. The egg masses were cut from the leaf, leaving a small portion of leaf tissue, and were glued to card stock (Fig. 2). The card stock was labeled and transported to the survey sites the same day. The pieces of card stock were pinned to the lower surface of known BMSB host plants (deciduous trees). The masses were retrieved 3-4 days after deployment, returned to the laboratory and held at 22 °C (72 °F) until host or parasitoid emergence was complete.

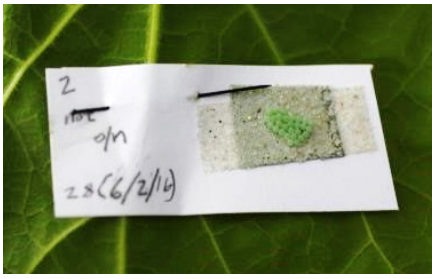

Fig. 2. Sentinel BMSB egg mass.

2016 SEM: A total of 134 sentinel egg masses were deployed in the field from 1 June to 19 August at six sites around Vancouver, WA. *Trissolcus japonicus* was found at five out of six sites surveyed (26 egg parasitized egg masses); only Site 2 was negative. Site 6 was particularly productive, with over half of the 13 egg masses yielding *T. japonicus*. A total of 451 adult wasps were recovered from the 26 egg masses, of which 86% were females. This survey confirms the widespread presence of *T. japonicus* in the Vancouver area. The 2015 find, confined to a single site (Site 3) may have been indicative of only low levels of this parasitoid. To date, this represents one of the more evident *T. japonicus* populations in the nation.

2017 SEM: A total of 173 egg masses from the BMSB colony were deployed and monitored in 2017, or a total of 4,435 eggs. Of the egg masses deployed, 16 were attacked by *T. japonicus*; the majority (14) of positive finds were from the Vancouver area (Sites 3 and 6), which were also positive in 2016. The other positive 2 egg masses were deployed on the same card in Pioneer Park, Walla Walla, and yielded 6 males and 36 females. **This collection of *T. japonicus* constitutes the first detection of this species in eastern Washington**. The location of this find is about 7 miles from a release site in Milton-Freewater, OR, made by David Lowenstein and Nik Wiman in 2016, raising the question of whether the Walla Walla population arose from the nearby release or by natural spread with the host.

In addition to the sentinel egg masses from the colony. a single wild type BMSB egg mass was collected on 4 August on the same *Paulownia tomentosa* tree used for most of the SEM deployments at Site 6 (Vancouver). From the 26 eggs, 25 *T. japonicus* emerged (data not shown).

One encouraging aspect of these results is the relatively high rate of attack in the Vancouver sites, where *T. japonicus* appears to be established. In Site 3, 6 of 32 egg masses (19%) were parasitized, and at the Site 6, 8 of 15 egg masses (53%) were parasitized. In contrast, a single group of 2 egg masses was attacked in Pioneer Park (Walla Walla) out of the 71 deployed. Further survey efforts may reveal whether establishment is in its early phases, or *T. japonicus* is less well adapted to the climate of eastern Washington.

Other parasitoids. Two of the sites (Pioneer Park, Walla Walla and Site 3, Vancouver) yielded *Trissolcus euschisti,* with a total of 10 egg masses attacked and 34 adults produced. *Anastatus reduvii* was found only at a single site (Site 3, Vancouver), with 3 egg masses attacked and 17 adults produced. While the overall number of egg masses (13) attacked by other parasitoids was similar to that attacked by *T. japonicus* (16), emergence of adults was much lower. The egg masses parasitized by *T. euschisti* averaged 13.9% successful adult emergence, and the egg masses parasitized by *A. reduvii* averaged 22.2%. In contrast, the successful rate of SEM attack by *T. japonicus* was 79.5% (17.9 – 100%) and 96.2% for the wild egg mass.

2018 SEM: A total of 232 BMSB sentinel egg masses plus two wild egg masses, were deployed/found in in 2018. *T. japonicus* was found at two locations in Walla Walla (one a repeat find from 2017), and a single location in Yakima (the site of the sole 2017 release). This shows the likely persistence of the population in Walla Walla, and the success of release efforts made in Yakima in 2017. The BMSB egg masses deployed in Vancouver yielded *T. japonicus*, bringing it to four consecutive years being detected in Vancouver. Other parasitoids detected by the survey were *T. brochymenae*, *T. euschisti*, and *Trissolcus* sp.

Objective 2. Maintain a laboratory culture of *T. japonicus* in preparation for release. Re-distribute this species in eastern Washington and perform follow up monitoring of the success of establishment.
Adult *T. japonicus* found in the Vancouver sites were returned to the laboratory to rear for release. Adults were kept in Petri dishes with honey water. When BMSB egg masses were available, a pair of *T. japonicus* was transferred to a small plastic cup containing the egg mass, and the female allowed to oviposit. After oviposition was complete, the egg mass was removed and incubated at 20 °C (72 °F) for three weeks until new adults emerged. Adults held with only honey-water were quite long-lived, and the colony could be perpetuated whenever egg masses were available.

In 2017, *T. japonicus* was released at a single site (Franklin Park, Yakima) in mid-October. A total of 21 parasitized egg masses (507 eggs) on card stock were pinned to host trees. In 2018, two parasitized egg masses were placed in a small closed container with honey-water until all adults had emerged. They were transported to the selected release sites, and the lid removed to allow adults to escape (Fig. 3). Releases were repeated 4-5 times during the growing season (late June-early October). A total of 1,827 adults were released (112 males, 1,715 females) in eight sites (two sites each in Prosser, Walla Walla, White Salmon, and Yakima).

Fig. 3. Releasing *T. japonicus.*

Objective 3. *Evaluate IPM-friendly management strategies for BMSB.*
We examined physical exclusion as an alternative, non-insecticidal means of suppression of BMSB. The outbreak of BMSB in the mid-Atlantic area in 2010 caused a widespread increase in broad-

spectrum insecticide use, which provided only mediocre control of the target pest and flare-ups of secondary pests from disruption of biological control. While some level of insecticide use may be inevitable in the control of BMSB, all available tactics that minimize the unwanted side effects should be explored. Washington State fruit growers are making a substantial investment in overhead nets for sunburn control, and these structures provide an opportunity to enclose orchards against pests (both vertebrate and invertebrate). We used native stink bugs as a proxy for BMSB to determine the efficacy of nets, in preparation for the future when BMSB populations expand throughout the state. We also examined the micro-ecosystem changes that occur due to the use of nets as barriers to pest entry into the orchard, including the effect on other apple pests and non-target effects on natural enemies. We used two sizes of complete cages, and for orchards where building a cage is not feasible, we examined the efficacy of a single-wall net barrier as an alternative.

Obj. 3a. Large-cage exclusion tests (2016-2017). The plots used in this experiment were built over mature trees in a 1.2-acre apple orchard at the WSU Sunrise Orchard (Fig. 4). Cages were built over mature trellised apple trees planted at 3 x 10 ft spacing. Cage frames were built from dimensional lumber supported with posts and guy wires and covered on all sides and top by white shade net (pearl leno 20% shade, Green-Tek West, Dinuba, CA). Each cage was 40 x 50 ft and enclosed 4 rows x 12 trees (total 48 trees/cage). Each row was a different apple cultivar (Jonagold, Gala, Granny Smith, and Golden Delicious).

Fig. 4. Large exclusion cages, Sunrise Orchard.

Three treatments were tested: 1) cages made from shade netting (plus supplemental sprays), 2) uncaged, conventional management (routine airblast sprays), and 3) an uncaged, unsprayed check. Treatment 2 received routine sprays for codling moth, but none specific to secondary pests (mites, aphids). All plots received routine applications of herbicides, fungicides and fertilizer. With the exception of stink bugs, all pest and beneficial populations were naturally occurring in the block. Because the orchard had no history of stink bug damage, artificial pressure was created by collecting consperse stink bug, *Euschistus conspersus*, and releasing them in the block. The ability of pests and natural enemies to penetrate the cage barrier was measured with visual observations (timed counts), traps baited with pheromones or kairomones, or behavioral traps (yellow sticky cards, earwig shelters). Counts were made every 2 weeks throughout the season, and a single index of the seasonal counts for each insect was calculated (cumulative insect days, or CID). This index is the average of two successive counts multiplied by the number of intervening days and summed over the season. The CID were analyzed using analysis of variance (SAS 2017, PROC GLIMMIX). Fruit damage was sampled in mid-summer and again just prior to harvest and analyzed using logistic regression with a binomial distribution (PROC GLIMMIX).

Very few stink bugs were recaptured in pheromone traps, and stink bug fruit damage was correspondingly low in both years (Fig. 5); however, the damage was lowest in the cage treatment in both 2016 and 2017. Other direct pests such as codling moth and leafrollers (data not shown), were also excluded to a marked degree by the cages. Woolly apple aphid reached outbreak levels inside the cages (Fig. 6A) but were present in very low numbers outside the cages. The specialist parasitoid of woolly apple aphid, *Aphelinus mali*, was similarly high inside the cages (Fig. 6B), likely in response to the high aphid populations. It is probable that the cage netting is permeable to this tiny wasp, but also possible that the parasitized aphids present when the trees were caged simply continue to reproduce inside the cages. Despite the high numbers of *A. mali*, the populations of woolly apple aphid were not sufficiently suppressed. Unlike the parasitoid, the macropredators of woolly apple aphid (lacewings and syrphids) were much lower inside the cages, indicating the winged adults were prevented from entering the cage. Earwigs, another woolly apple aphid predator, tended to be lower inside the cages (2016), but this effect was less pronounced than with the winged predators.

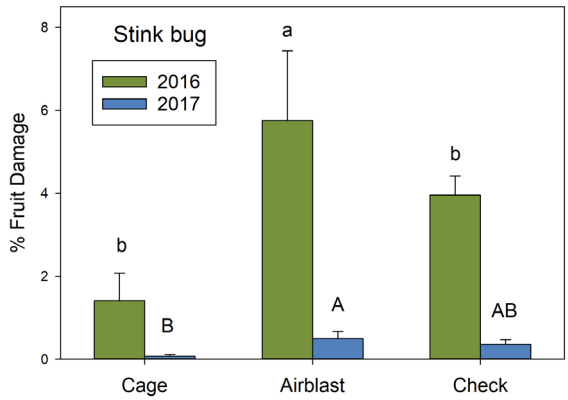

Fig. 5. Stink bug damage inside and outside large exclusion cages, 2016-2017.

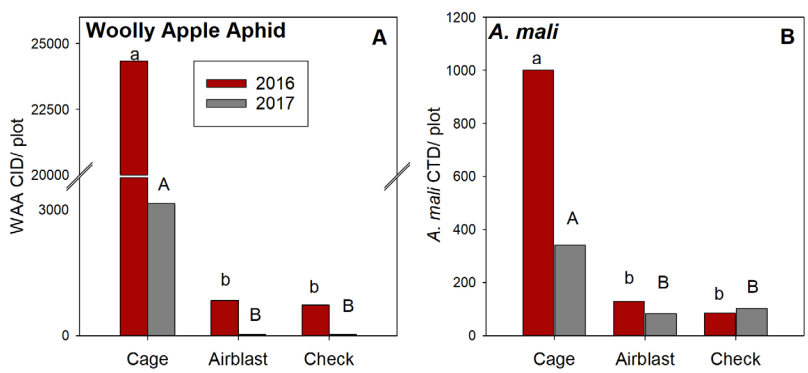

Fig. 6. Woolly apple aphid and *Aphelinus mali* densities inside and outside cages, 2016-2017.

Obj. 3b. Small-cage exclusion tests. The experimental design of the small cage (Fig. 7) experiment was similar to the large one, except that the plots were three 'Golden Delicious' trees (single row), and the cages were 10 x 10 x 5 ft. The same treatments were used, but each had 10 replicates in a randomized complete block design. All cages had a pheromone trap for the three tortricids, but the other two treatments were sampled with 2 traps/ species placed in buffer rows to avoid inter-trap competition. Sampling and analysis were done as in the large cage experiment, except that stink bug releases were not made (on the assumption that the large cages represented a more realistic commercial scale).

Fig. 7. Small exclusion cage, Sunrise Orchard.

Woolly apple aphid densities were 100- to 400-fold higher inside the cages than in the airblast and check treatments, respectively. Spider mites were significantly higher inside the cages (2016 only); 95% of the mites found were brown mite. Earwigs were not significantly different between the treatments. Lacewing and syrphid adults were effectively excluded by the cages. Codling moth pheromone trap captures were greatly reduced inside the cages (1.8-15 moths/trap) vs outside (123-240 moths/trap). Likewise, fruit damage by codling moth was 2.0-8.4% inside the cages, vs. 15.9-19.4% (airblast) and 58.2-59.8% (untreated). Sunburn was significantly reduced inside the cages; however, as in the large cages, the airblast treatment also reduced sunburn relative to the check.

In 2018, we tested augmentative release of a woolly apple aphid predator to determine if we could correct the macropredator deficiency inside the cages. We purchased green lacewing, *Chrysoperla rufilabris* (Fig. 8) from Rincon-Vitova Insectaries (Ventura, CA) in the adult stage. The adult of this species is not predacious, thus only eggs and resulting larvae were assessed. We divided the 10 cages into two groups of five each, counted the woolly apple aphid colonies, and used that as a blocking factor to assign treatments (1 – Lacewings released; 2 – check, no release). Ten adults were placed into cups and released into the small cages on 31 May. Lacewing eggs and woolly apple aphid densities were counted weekly for 3 weeks. A second release at a higher predator density was performed on 25 July, using 100 adults/cage. After the first (low density) release, no lacewing eggs were found in the release or check cages. After the second (high density) release, 3.6 eggs/cage were found in the release cages. Woolly apple aphid densities did not differ between the two treatments, indicating the predator release had no measurable effect.

Fig. 8. Lacewing release in small cages.

Obj. 3c. Physical exclusion, single-wall barriers. For the single-wall exclusion study, three commercial apple orchards in the Manson, WA area with a history of stink bug damage were used. We constructed net barriers in 2016 and assessed the results in 2016-2018. The barriers (150 ft long x 15 ft high) were made of commercial shade netting (20% pearl leno [white] net; Green-Tek West, Dinuba, CA) fastened to 16 ft posts at the edge of the sagebrush/native vegetation that bordered the orchards. The nets had 6-inch flaps of net sewn at three heights facing the native vegetation. In 2018, approx. one-half the length of the three flaps (75 ft) was retro-fitted with deltamethrin-infused netting (ZeroFly netting, Vestergaard-Frandsen, Washington, DC) (Fig. 9). This resulted in three treatments replicated at each site: 1) net barrier with deltamethrin flaps; 2) net barrier with shade net flaps; and 3) a no-barrier check.

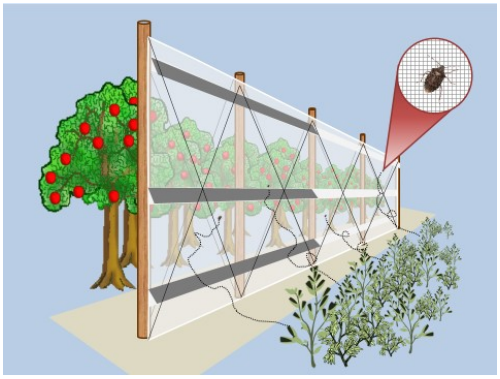

Fig. 9. Schematic drawing of net barrier with deltamethrin-infused flaps.

Stink bug populations were assessed with a beating tray in the natural vegetation and in the orchard throughout the field season. Fruit damage was determined through visual inspection in late August/early September. Stink bug damage levels were extremely low with none of the treatments reaching levels higher than 0.2%. Beat tray samples of the surrounding vegetation behind both net treatments resulted in substantially higher counts than samples in the check, and there was no

significant difference in the amount of stink bugs found in the orchard between the three treatments (Fig. 10). While the orchard counts were not significantly different, the netted plots received a much higher pressure of stink bugs migrating from the natural vegetation. There is a greater difference in the amount of stink bugs in the orchard compared to the vegetation in the netted plots (>80% reduction) than in the control plot (40% reduction), indicating the barrier prevented most of the stink bugs from reaching the orchard.

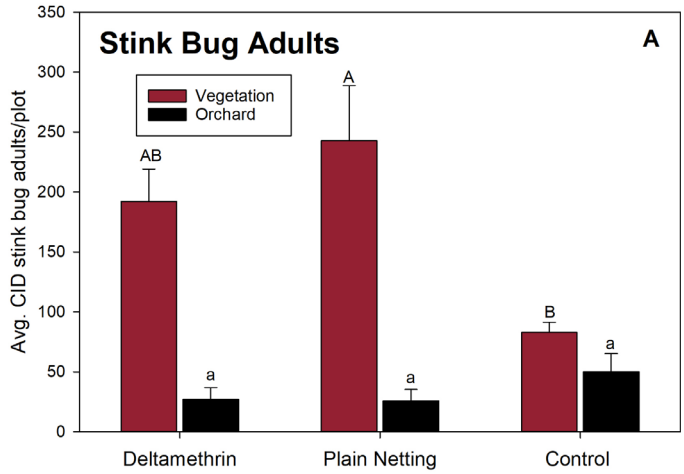

Fig. 10. Reduction in stink bug adult behind single wall barriers with and without deltamethrin-infused netting.

A complimentary study was conducted from June - September to determine the height at which stink bugs migrate into orchards. A sticky barrier (13 ft high x 6 ft wide; Fig. 11) was constructed using dimensional lumber and clear sticky panels, 1 x 6 ft (Alpha Scents, West Linn OR), at the orchard boarder in five locations in Manson. Stink bugs on the sticky panels were removed and their height of interception was recorded every week. A single index of the seasonal counts for adult counts was calculated (CID) as the average of two successive counts multiplied by the number of intervening days and summed over the season, and a cumulative trap-day CTD index was used for the sticky panel traps. All data were analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS 2018).

Fig. 11. Sticky barriers for immigration studies.

Height of immigration/emigration. An additional question for the single-wall barrier concept is how tall the barriers needed to be to intercept stink bugs. Observations in the mid-Atlantic states indicated that barriers were more effective if they were interposed between the orchard and a field crop (corn, ca 6 ft high) versus a deciduous woodlot (ca. 40 ft high). Vegetation bordering eastern Washington orchards (with the exception of riparian zones) is composed of shrubs, forbs, and small trees, with heights ranging from 1-10 ft. We hypothesized that height of immigration would be related to the height of the surrounding donor vegetation. To test this, we built wooden frames onto which double-sided sticky panels (AlphaScents, West Linn, OR) were attached at 8 (2017) or 11 (2018) heights.

In 2017, there were no statistical differences in stink bug capture among heights over 2 ft; very few stink bugs were caught below this level. The maximum height in 2017 was 8 ft, and it was apparent that much of the immigration was at that height (or higher), so the maximum height was increased 12.5 ft in 2018. In the latter test, there were no statistical differences in interception height for the sticky barriers for stink bugs moving *out* of the orchard (data not shown). However, there was a significant difference in height of movement *into* the orchard (from of the surrounding vegetation). The highest counts were at 4.5 ft (7.20 stink bugs) and the lowest at 12.5 ft (1.20 stink bugs), with the majority captured between 4 and 9 ft (Fig. 12). Similarly, a significantly higher number of stink bugs were caught in the middle flap (6 ft) of the

single-wall barriers than in the lower (1 ft) or upper (9 ft) flaps, a further indication that the majority of stink bugs migrate into the orchard ~6 ft.

Obj. 3 Conclusions. The preliminary information from both the large and small cage experiments indicates there is potential for a substantial degree of exclusion of codling moth. The wild moth pressure in the large cage research blocks was considerable, and late season pheromone trap captures indicate a high degree of success in excluding adults. The small cages trials show that shade netting can be an effective barrier to external moth populations Interestingly, a larger proportion of moths were able to escape the cages than enter them.

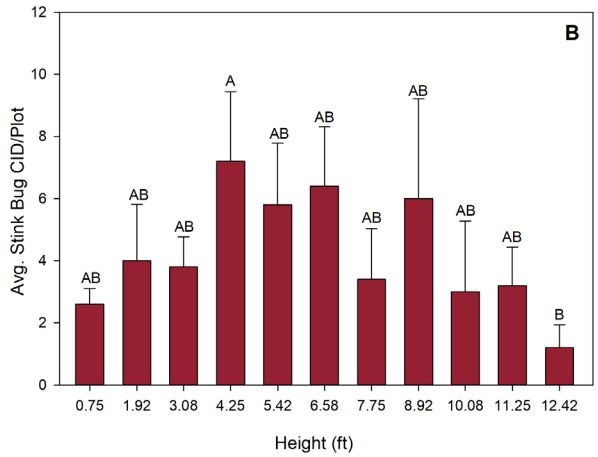

Fig. 12. Height of stink bug immigration into orchards from native vegetation.

Sampling stink bugs captured on the net barriers, in the orchard, and in the surrounding vegetation in the Manson trials provided insight into stink bug seasonal migration habits. Stink bugs densities were higher in the surrounding vegetation for both net treatments than the check, yet numerically fewer stink bugs were found in the orchard behind the net treatments than the check. This indicates that the net barrier may be preventing a large portion of the migrating stink bugs from reaching the orchard. The sticky barrier trial further confirms that the majority of adult stink bugs fly into orchards below 12 ft, which implies a barrier may only need to be 12 ft to provide successful control.

Objective 4. Document the spread of BMSB within the state.

As BMSB continues to spread, efforts in 2018 focused on sampling in location gaps between known populations and in new cities within important fruit growing regions. We used direct surveys (beating trays, pheromone traps) to determine the presence and relative abundance of BMSB in the state. We also solicited input from homeowners and Master Gardeners. Verified finds were recorded in a database available to BMSB researchers, and the results available in map form (http://tfrec.cahnrs.wsu.edu/beers-tfentomology/bmsb/bmsb-wa/).

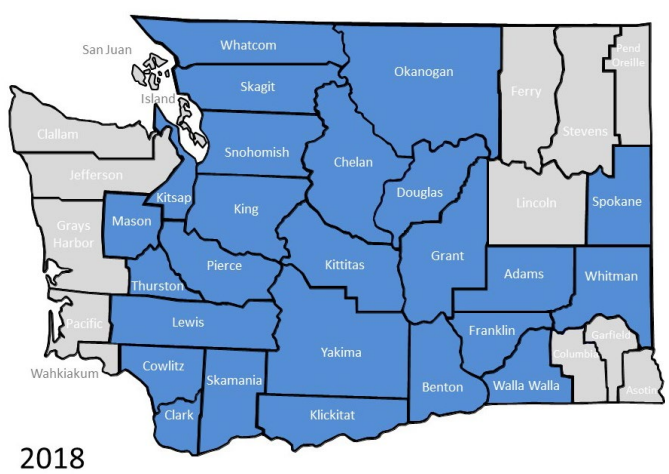

Fig. 13. BMSB distribution, December 2018.

Obj. 4. Results and Discussion. A total of 27 counties have reported detections of BMSB (Fig. 13). The highest numbers of BMSB reports came from Clark, King, Pierce, Snohomish, and Thurston counties in 2018 likely reflecting the large amount of vehicular traffic that could help spread this invasive species. Conversely, Chelan county has a (relatively) smaller human population and vehicular traffic, but a keen degree of awareness and interest in this species. Trapping studies and citizen reports discovered

the first BMSB in both Okanogan county, and the city of Chelan in early October of this year. This is a major concern, as those are both major fruit growing areas. In general, it appears that the arid climate of eastern Washington will not effectively limit the establishment of BMSB, given the growing number of reports in this part of the state, and establishment to the north in the Okanagan Valley of British Columbia.

Objective 5. Determine suitability of native shrub-steppe plants as hosts for BMSB
BMSB has usually been associated with humid temperate environments such as the deciduous hardwood forests in northeast Asia, the Mid-Atlantic States in the American Northeast, and the Pacific Northwest west of the Cascades. However, BMSB has shown itself to be remarkably adaptable. It rapidly colonized many areas of Washington State, including population centers in the arid Columbia Plateau. As its range continues to expand and its populations grow, there is legitimate concern that this highly mobile landscape-level pest will be able to build up in the shrub-steppe habitats that border Washington's tree fruit orchards. Knowledge of the landscape ecology of BMSB in the arid PNW is critical for effective scouting, risk assessment, and area-wide IPM efforts.

Obj. 5a. Laboratory feeding studies. In a preliminary experiment, we noted that BMSB could develop from egg to adult on an assemblage of plants native to eastern Washington (Fig. 14). In 2018, we evaluated more fully the relative suitability of two common shrub-steppe plants for BMSB feeding and oviposition in comparison to Lima bean seedlings (a standard BMSB colony diet), which served as the check. Cuttings of sagebrush and bitterbrush (or bean seedling in potting soil) were placed in acetate cages over 6-in pots. Using a randomized complete block design (3 treatments, 10 reps), we placed a male-female BMSB pair in each arena. Insects were weighed at Day 0, 7, 15, and 22 to monitor weight loss or gain as a metric of host plant suitability. At the same intervals, adult mortality was assessed, eggs were counted, and plant material was replaced. Due to high mortality in our check treatment, no significant differences were observed in any fitness parameters measured. This mortality is likely due to insufficient ventilation in the arenas and the stress experienced by the insects during handling from the frequent weight measurements. In addition, future studies will use an assemblage of native plants, which is consistent with its known near-obligate polyphagy and the conditions it will likely experience in the field.

Fig. 14. BMSB adult feeding on serviceberry.

Obj. 5b. Gut content analysis. Gut content analysis has been used extensively in biological control studies to determine which prey are being used by predators, by screening gut contents with various prey primers. Screening with primers asks the question '*Did you eat species X*?', with the understanding the species screened would likely be pests of interest. This concept may be extended to

examining the feeding habits of highly mobile and polyphagous pests (such as BMSB) to better understand their ecology at the landscape level.

Recent developments in "deep sequencing" technology for genetic analysis enables detailed, plant species-level feeding histories of individual insects to be determined by extracting and sequencing plant genetic material from their gut. This method effectively asks the question '*What did you eat*?', and as such, is a more comprehensive look at feeding history. This approach has been successfully used on various psylla species by Dr Rodney Cooper (USDA-ARS, Wapato, WA) who demonstrated its use for the first time for Hemiptera. We are refining the technique for BMSB in collaboration with Dr. Cooper.

The first step in assessing this methodology is to determine signal persistence, or how long plant DNA is detectable in the gut after feeding has ceased. We used two known hosts of BMSB, Lima bean plants and carrots. We allowed BMSB to feed on Lima bean for 7 days, then switched them to carrots. Insects were killed and their gut dissected (Fig. 15) at 5 time points (0, 1, 3, 7, and 14 days) post-host switch. DNA was extracted using kits (DNeasy, Qiagen, Hilden, Germany) and amplified with two universal plant primers (*trnF* and *ITS*). Amplified plant DNA was quantified with a NanoDrop and submitted for deep sequencing on the PacBio platform at WSU's Core Facility. Each sample included a barcoded primer to identify it post-sequencing. Sequence data from the Core Facility was sorted and compared to known plant sequences (GenBank) using the Geneious Prime software.

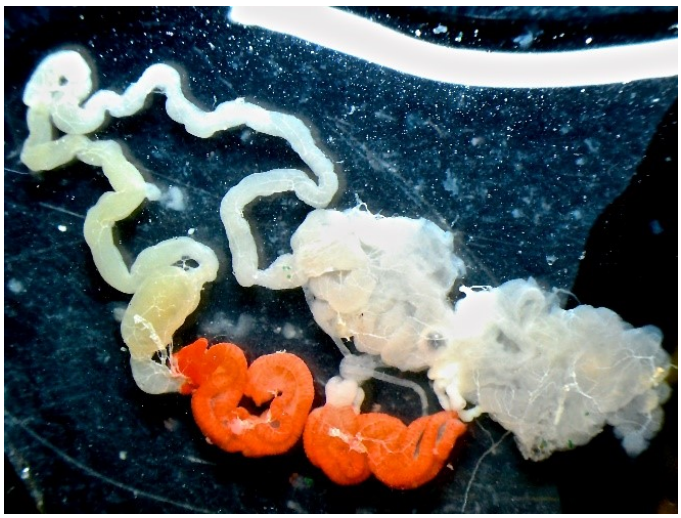

Fig. 15. Dissected BMSB guts (note orange coloration due to feeding on carrot.

The *trnF* primer yielded satisfactory results; however, the *ITS* primer failed to yield sufficient sequences for host plant identifications. The original host plant (Lima bean) was recovered from BMSB guts at all time points post switch, indicating a signal persistence of at least 2 weeks. The sequence composition remained predominantly that of Lima bean at 0 days (pre-switch) and shifted to carrot at all subsequent time points. However, Lima bean DNA was still detected at all post-switch time points, but in decreasing amounts. Sequences identified as plants not included in this study and/or not present locally suggest that this method is sensitive to contamination and that ground truthing is necessary to confirm the presence of identified putative hosts at the site of insect collection. A third universal primer, *trnL*, will be tested in the future to verify the results of *trnF*.

Executive Summary

Project Title: Brown marmorated stink bug control in Washington

Keywords: invasive pest, biological control, parasitoid, host plant suitability

Abstract: Brown marmorated stink bug (BMSB), an invasive pentatomid from Asia, continues to expand its range in Washington State. Its ability to use native host plants may be a factor in its success in the arid interior. An exotic parasitoid, *Trissolcus japonicus*, was found in Vancouver and may help regulate populations.

BMSB has continued to expand it range in Washington State since it was first found in 2010. Homeowner complaints (and likely densities) are higher in the populous urban/suburban areas of western Washington, but there has been substantial incursion into the arid areas east of the Cascades. As these populations continue to spread and grow, they threaten the intensive agricultural production in this region, with tree fruits being among the most vulnerable crops. If broad-spectrum pesticides are the only remedy to prevent damage, we can expect widespread disruption of integrated pest management (IPM) programs resulting in the reduction of natural enemies and outbreaks of secondary pests. To mitigate this outcome, we need to explore non-insecticidal tactics and gain a better understanding of landscape ecology as it relates to risk.

Initial assessments of native natural enemy impact were disappointing, with generally very low levels of predation and parasitism. The native species that attacked native stink bugs were apparently poorly adapted to use BMSB as a host and may have played a role in outbreaks in the eastern US. The discovery of adventive populations of an Asian parasitoid, *Trissolcus japonicus*, provided an opportunity to exploit biological control more fully. One such population was found in Vancouver, WA in 2015, which allowed the re-distribution within the state's borders (inter-state movement is regulated). These efforts were initiated, along with monitoring establishment in the release sites. Due diligence also demands that the non-target effects of this exotic parasitoid on native stink bugs be assessed; to date, there appears to be a small to moderate impact on native pest stink bugs, as they are poor hosts for *T. japonicus*. While not species-specific, *T. japonicus* has promise to moderate BMSB densities, especially in unmanaged habitats.

While biological control will always be helpful, it is likely that direct protection of orchard crops will be necessary at some point. To this end, we have begun exploring two promising tactics: physical exclusion and attract-and-kill. Our physical exclusion experiments have taken two forms: a full orchard enclosure made of shade netting, and a single-wall barrier between the native vegetation harboring stink bugs and the orchard, where topography, orchard architecture, or expense does not permit full enclosure. Both approaches have shown some promise of reducing pest pressure, although to a higher degree with full enclosure. Full enclosure has multiple benefits, including sunburn control, shading orchard workers, and minimizing multiple vertebrate and invertebrate pests. However, they are not without non-target effects, as they appear to exclude natural enemies and promote certain secondary pests. Similarly, single-wall barriers, especially those that incorporate insecticide-infused netting (IIN) may kill some beneficial insects along with the target stink bug pests.

Lastly, understanding the landscape ecology of BMSB in the shrub-steppe will help inform risk analyses for fruit damage. It has been speculated that arid growing regions will not support BMSB, but its rapid establishment in eastern Washington belies this. BMSB appears to be sufficiently adaptable to a wide range of suboptimal environmental conditions, and we have preliminary evidence that it can complete development on (at least) the same host plants that support native stink bugs. Its ability to expand and pose a threat beyond these habitats remains to be determined.

FINAL PROJECT REPORT

Project Title: Assessment of apple immune responses to wooly apple aphid saliva

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Other funding sources

Agency Name: Dovetail Genomics LLC & University of California, Riverside, Office of Research and Economic Development
Amount awarded: $13,550
Notes: Dovetail Genomics LLC and the UCR ORED provided funds for sequencing the WAA genome

Total Project Funding: **$**164,987 **Year 1**: 58,710 **Year 2**: 49,079 **Year 3**: 57,198

Budget History:

Item	2016	2017	2018
Salaries	$32,836	$20,646	$32654
Benefits	$3,424	$16,463	$22044
Wages			
Benefits			
Equipment			
Supplies	$22,450	$11,970	$2,000
Travel			$500
Plot Fees			
Miscellaneous			
Total	$58,710	$49,079	$57,198

OBJECTIVES

All plants share networks of related genes and proteins that work together to generate immune responses to both insects and pathogens. The main goal of our project was to identify these networks in apple as they relate to aphid feeding, although resolving these immune networks will inform upon any biotic stress response imposed on apple in the future. A complementary goal was to examine how the aphids trigger these networks by characterizing insect genes. For both of these goals, we trained a MS student in current bioinformatics techniques and with expertise in apple-aphid interactions. Our approach combined transcriptomic information on the apple genes induced by aphid colonization with the genes active in aphids as a first hurdle in linking insects to plants. This fundamental research will help us to better understand resistance mechanisms in apple and how insect populations vary across growing regions. Our specific objectives were to:

1. Identify the WAA salivary proteins that alter plant form and function in roots and shoots.
When feeding, WAA discharges salivary proteins into plant tissues. These proteins play critical roles in reprogramming the physiological processes of infested plant tissues, i.e., roots and shoots. Because salivary proteins are secreted by salivary glands, we used a transcriptomic assessment of extracted salivary glands to identify all the genes that encode secretory proteins in WAA. We compared this to whole body extractions to rule out transcripts expressed in dissected tissue but not associated with salivary glands. To verify the gene products, we also collected salivary proteins for proteomic analysis. Initial proteome screens (2016-2017) revealed more insect material (within replicates and total number of samples) was necessary to increase replicates and detection given many of these proteins are low in abundance. However, recently published studies indicate the number of proteins found in insect saliva is much less than what should be produced from protein-encoding genes annotated in the transcriptome (Thorpe et al. 2016, Boulain et al. 2018). *Thus, transcriptome profiling of salivary glands was selected as a better approach to identify insect secretory proteins that antagonize plants compared to proteome collections*. Because of this we did not pursue more proteome studies. <u>We secured extra funding to create a WAA genome to increase the ability to detect genes and their products related to colonization</u>. This genome is assembled with annotation ongoing. Once completed, these data will represent the most comprehensive database of WAA genetics that will be publicly available at http://bipaa.genouest.org/is/.

2. Characterize the plant immune response in resistant and susceptible rootstocks.
Apple resistance to aphids is known to depend on at least four genes (Er 1-4). By assessing transcriptomes of apples that differ in susceptibility (Er-2 background) to aphid attack we can identify how genes interact to protect against aphids. We may also identify WAA-specific processes unrelated to typical resistance signaling to increase candidates or markers for resistance breeding. Sample collection was completed in Fall 2017 using the susceptible genotype G.935 and two commonly used genotypes with greater resistance G.16 and G.87 determined by performance trials. Sequencing data was returned Spring 2018 and analyses completed 2019. We also screened novel rootstocks from a resistance mapping population for performance with WAA in collaboration with G. Fazio during Summer and Fall 2017.

3. Identify functional plant traits that confer immunity to WAA.
Preliminary screens of commercial and unreleased rootstocks with known resistance genes showed variable colonization by WAA. We originally planned to phenotype the underlying biochemistry related to resistance using the transcriptome as a guide for which processes to assess. Given the analyses (objective 2) indicated RNA signaling, transcription, and post translational processing such as ubiquitination (degradation) of proteins were strongly upregulated, we chose not to screen for biochemical changes in phenolics, callose, or reactive oxygen species known to alter colonization (e.g., Zhou et al. 2013). Rather we diverted resources to annotate and compare effector genes in WAA populations from CA and WA, and evaluate apple response to the different populations. We

hypothesize that effector proteins from the aphid are targeting upstream genetic processes that regulate transcription and translation to ensure colonization. These pathways also regulate resistance in plants, and are co-expressed with nucleotide-binding site leucine-rich repeats (NBS-LRR) proteins that act to monitor effector targeting of plant processes by pathogens (McHale et al. 2006).

4. Map these traits to genes in apple to facilitate marker-assisted breeding.
Breeding-program specific DNA tests for high impact attributes are required to streamline cultivar development. Objectives 2 and 3 showed numerous genes across chromosomes play a role in resistance, making it difficult to identify single markers. However, several genes induced by aphid feeding showed both general regulation under aphid attack, and unique genotype and population-specific patterns. Motifs containing LRR, NB-ARC, TRR, R and other domains with known roles in resistance to disease were identified and linked to their chromosomal location for future marker development.

SIGNIFICANT FINDINGS
Woolly apple aphid

- >390 genes were identified as putative effectors from the de novo transcriptome. These include enzymes that detoxify compounds or otherwise mobilize nutrients for feeding, and protein-binding molecules that regulate protein signaling in apple.
- >60% of effector genes are unique to the WAA and do not occur in other insects, but some known to enhance insect performance do occur in WAA (Fig 1).
- At least one protein mimics a transmission protein necessary for successful infection of two families of plant viruses (the caulimoviruses and the potyviruses).
- CA and WA aphid populations may differ little in their genes (reanalysis with the complete genome will confirm this) but each population differentially alters apple gene expression.
- A high-quality genome assembly will serve the global apple community in understanding local and rootstock-specific resistance. With this information, virulence potential of aphid populations can be identified at local and regional levels, with regard to management (organic vs conventional), and specific to rootstocks.

Apple

- 10 unreleased genotypes were screened for resistance. One genotype prevented colonization that led to aphid dispersal/death in 5 days (Fig 6). Nine genotypes showed a range of survival between 15–40% (five shown in Fig 1). This evaluation identified rootstocks for aphid-apple transcriptomic evaluations in the future.
- Rescreening aphid performance on resistant (G.87/5087) and susceptible Geneva (16/G.16, 935/G.935) rootstocks showed similar survival (50%), indicating resistance exists beyond Er-2 for select aphid populations.
- Apple genotypes vary in constitutive expression of defense and immune-related genes, but other processes (e.g., photosynthesis, RNA processing, protein) emerge as determinants of successful colonization. Thus, a lack of Er resistance may still provide tolerance to aphids if other processes in the genotype function in an enhanced manner.
- In G.87, seven immune/effector recognition genes found on several chromosomes may contribute to aphid resistance because of elevated expression without aphids.
- Aphid feeding on apple plants remodeled the apple transcriptome more than other aphid studies at a similar time point.
- G.16 responded the greatest whereas G.935 and G.87 responded less to aphids. Comparisons among treatments revealed effector targets (genes suppressed by aphids) and effective immune response (genes induced by aphids).
- Immune signaling was differentially induced depending on the aphid population and overall CA aphids altered rootstocks more than WA aphids (Fig 5).

- Immune genes altered by aphids were identified specific to three genotypes, Er-2 resistance, aphid populations, and shared among all comparisons.

RESULTS & DISCUSSION

The woolly apple aphid has numerous plant-manipulating genes. Using a linked read technology (10x Genomics) we assembled large genomic DNA fragments from a lane of Hiseq 3000/4000, 150bp paired end reads at a coverage of 80x (Supernova 2.1.1). Reducing the total number of input reads produced an assembly of 300.82 Mbp with scaffold N50 of 3.37 Mbp. The analysis of gene content (BUSCO) resulted in 1580 complete genes, 1551 single copy, and 64 missing. This represented a high-quality assembly as a good starting point; however, we improved this genome using Dovetail Genomics, LLC and their Chicago-HiRise sequencing method. Here they constructed another short-read Chicago library of 150bp paired end reads from Illumina Miseq at a read coverage of ~80x. Using HiRise software the 10x assembly was scaffolded, increasing the N50 to 29.96kbp, and a slightly improved BUSCO with 1 more gene. This genome is currently being annotated using the Dovetail topologically associated domains method with other well annotated aphid genomes as reference. These final data will become available to the public in 2020.

Because effector characterization is becoming critical for understanding insect-induced plant responses, we performed differential expression analysis between salivary glands (SG) and whole-body samples and assessed SG-specific genes for potential to act as plant-manipulating genes (effectors). For this we de novo assembled a genome from a pool of all transcripts that showed similar statistics as the genome (e.g., 1598 complete genes by BUSCO) but will be improved once annotation is complete. Our analysis revealed 5,377 transcripts upregulated in SG at the 'isoform' level but only 390 genes that encode for secretory proteins. Known aphid effectors were found at both the isoform and gene level, indicating WAA interacts with plant signaling through processes similar to other aphids (**Fig. 1**), but 250 genes were found to be unique to WAA. Our experience annotating another aphid-like galling insect genome (grape phylloxera), leads us to predict the percentage of genes unique to WAA will remain high (~60%) and the overall effector count will increase. For example, the pea aphid encodes 3600 candidate effector genes, but only 740 are up regulated in salivary glands (Boulain et al., 2018).

We assigned tentative functions to 95 candidate effectors including various enzymes such as glycoside hydrolases (GHs) (8), peptidases (6), peroxidases (5), lipases (4), and several other enzymes. These enzymes are important to plant cell wall development, occur in other aphids, and may enable stylet penetration (Calderón-Cortés et al. 2012; Eyun et al., 2014; Wybouw et al. 2016). (Harmel et al., 2008; Miles 1999; Rao et al., 2013). Analogous to other organism antioxidant defenses, the salivary peroxidases we identified in WAA may function to counter ROS burst by scavenging H_2O_2. Differential expression analysis of WAA across different host genotypes resulted in few genes altered (26, 17 and 4 DE genes for the contrasts G.935 vs G.16, G.87 vs G.16, and G.935 vs G.87, respectively); however, we expect this

Gene	*A. pisum* gene (BIPAA)	WAA Trinity ID	Secretory	LogFC in salivary gland	Aphid Performance
C002	ACYPI008617	DN4506_c0_g1_i1	**Yes**	8.3	Increases fecundity & enables phloem feeding
Me23	ACYPI002439	DN20770_c0_g2_i4	**Yes**	NS	Increases fecundity
		DN20770_c0_g2_i2	No	NS	
Shp	ACYPI009881	DN6812_c0_g1_i16	No	5.9	Increases fecundity
		DN6812_c0_g1_i21	No	7.4	
		DN6812_c0_g1_i3	**Yes**	6.5	
		DN6812_c0_g1_i7	No	6	
		DN6812_c0_g1_i13	No	6.4	
Mp10	ACYPI000097	DN4653_c0_g3_i2	**Yes**	-2.86	Increases fecundity
		DN11304_c0_g1_i1	**Yes**	NS	
		DN4660_c0_g2_i3	**Yes**	NS	
		DN3464_c1_g1_i1	**Yes**	NS	
Mif1	ACYPI002465	DN2575_c7_g1_i1	No	-3.27	Increases fecundity
		DN2761_c0_g1_i3	**Yes**	-2.32	
		DN2761_c0_g1_i5	No	-2.31	
Armet	ACYPI008001	DN5107_c1_g1_i1	**Yes**	1.7	Increases survival

Fig. 1. Effectors known from other aphids to alter plants are present in WAA. At least 390 more effectors were detected with the majority (250) found only in WAA and not in other aphids.

number to increase with the completed annotation and re-analysis. Based on other studies, transcriptional plasticity largely determines host-specific performance of aphids (Boulain et al., 2019) but broader population assessment may reveal genotype/biotype specific genes retained or lost in distinct geographic regions.

INDUSTRY BENEFIT

- High quality genome for future WAA population and genotype-specific assessment
- Gene expression profiles for populations that vary in plant resistance responses
- Markers (effector genes) for understanding rootstock x aphid interactions

FUTURE DIRECTIONS

- Investigate population genetics of WAA to better link genotypes to growing regions and virulence on rootstocks, especially in areas where rootstock resistance failed/is failing or management costs/methods are increasing.
- Assess plant phenotypes/responses in native WAA-host (elm, hawthorn, cotoneaster) interactions to understand mechanisms for tolerance.
- Develop a genome-based genotyping protocol for virulence (effector) prediction of new genotypes that arise/invade.

Aphids used in omics analyses perform along expectations for rootstocks G.935, G.16, G.87, and G.202. A log-rank Kaplan-Meier survival analysis revealed aphids declined initially on G.935 then remained constant, indicating tolerance to or a lack of inducible defenses. Aphids declined to ~50% survival on G.16 and G.87, indicating a stronger defense response (**Fig. 2**). For all four genotypes, there were no visible signs of a hypersensitive response (i.e. necrosis), and no aphid mortality was observed. This suggests antixenotic factors determine early defense responses for these genotypes. Previous characterizations of WAA performance on apple genotypes derived from 'Robusta 5' and *M. floribunda* genetic backgrounds showed similar WAA performance/feeding behaviors (Sandanayaka et al., 2003; Sandanayaka et al., 2005).

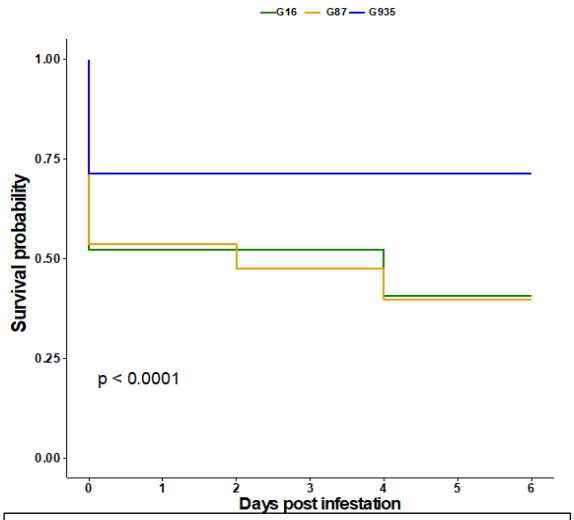

Fig. 2: Aphids showed high survivorship on susceptible G.935 but equivalent survivorship on Er2-resistant G.87 and susceptible G.16. No insects survived on G.202 (data not shown).

G.87 and G.935 are from crosses between 'Ottawa 3' and 'Robusta 5', and thus share a similar genetic background compared to G.16 ('Ottawa 3' x *M. floribunda*), yet G.87 is characterized as resistant whereas G.16 and G.935 are not. Because we found aphids performed similarly on genotypes differing in characterized resistance, we profiled their transcriptomes to understand what contributes to aphid survival in the first 2-3d of feeding.

Apple genotypes vary in constitutive expression of defense-related genes. Contrasts between **uninfested** G.935 vs G.16, G.935 vs G.87, and G.16 vs G.87 resulted in 2294, 178, and 2005 uniquely expressed genes, respectively. Notably, the expression profile for G.16 is highly dissimilar to G.935 and G.87, whereas G.87 and G.935 expression patterns are similar: a confirmation of their genetic backgrounds. Of the genes different between G.935 and G.87, there was 1 enriched bin (external stimulus response). Of the genes different between G.16 and G.87 or G.16 and G.935, bins

enriched with greater expression included photosynthesis, protein biosynthesis, RNA processing, and coenzyme modification whereas bins enriched with lesser expression were in cell wall organization and protein modification. **These profiles (Fig 3) indicate G.16 has significant gene activity relative to G.935 and G.87, which likely contributes to the reduced aphid performance**.

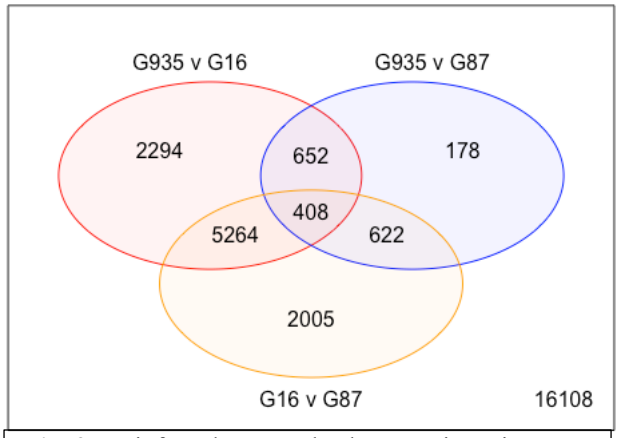

Fig. 3: Uninfested rootstocks that vary in resistance show different expression levels of many genes. Notably Er-based resistance (G.87) and enhanced gene activity (G.16) independently contribute to aphid resistance in the first days of colonization.

Of the 178 (91:up, 87:down) genes that are unique in G.87, seven immune/effector recognition (LRR/disease resistance proteins) are up and found on several chromosomes (3, 11, 2 adjacent on 13 and 3 on 15). Of the 5264 unique to G.16, **14 immune related genes are expressed more than other genotypes and further induced by aphids.** These include two disease resistance (LRR and NB-ARC proteins) found on different chromosomes (1 and 3), indicating potential loci for ER independent resistance. Furthermore, of the 210 genes that are up in G.16 but *suppressed* by aphids, 8 are related to stress response and function in protein-protein interactions. These genes may be targeted by aphid effectors to enable colonization, given how the proteasome is emerging as a novel target of galling and non-galling insects to manipulate plant function (Nabity 2016, Miao et al., 2018, Zhao et al., 2019).

G.935 and G.87 are relatively similar in gene expression, but G.935 lacks resistance and G.87 shows the Er-2 signature (Fazio & Beers 2010). This suggests the Er-based resistance may be linked to relatively few genes working together. Without these genes, however, a baseline of gene expression such as was found in G.16 is enough to reduce aphid colonization success.

Apple transcriptome undergoes remodeling shortly after colonization. Aphid feeding on apple plants remodeled the host transcriptome with a total of 1474 genes differentially expressed between all infested and control plants. This is nearly double other studies that found 637 altered genes in tomato after potato aphid feeding (Coppola et al. 2013), and ~650 DE genes in maize after corn leaf aphid feeding (Tzin et al. 2015) after 48h. In our study enriched bins included photosynthesis (85 genes), cell wall organization (85 genes), and cytoskeleton organization (23 genes), indicating these processes are perturbed more than expected compared to other processes during the first phase of colonization.

For individual genotype comparisons, G.16 responded the greatest (1858:up, 1685:down) whereas G.935 (2:up, 1:down) and G.87 (103:up, 41:down) responded less to aphids. A closer look at

the two genotypes that reduced aphid survival showed 15 genes were expressed similarly when aphids attacked (**Fig. 4**). Three of the seven suppressed by aphids were upregulated in G.16 and G.87 uninfested compared to G.935, indicating the insect may suppress these to enable feeding. These included an unknown protein, a detoxifying enzyme (cytochrome p450), and a developmental (MADs-Box) transcription factor. Five of the eight induced by aphids were suppressed in uninfested plants, indicating feeding triggered a strong induction of gene expression. These included 2 LRR disease proteins, an auxin transport P-glycoprotein, stress response ethylene forming enzyme, and a membrane stabilizing protein. Of note, the LRR genes were induced when the CA population fed but not when the WA population fed. Both LRR genes were located on chromosome 5.

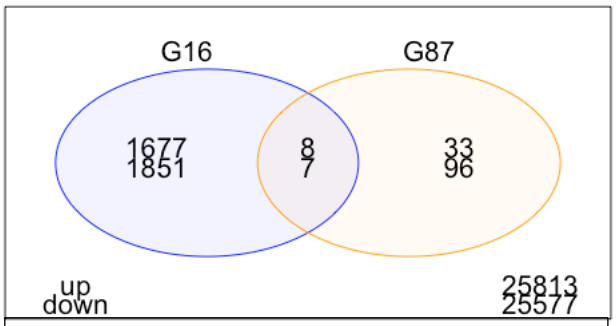

Fig. 4: Aphids feeding on G.16 induced many more genes than when feeding on G.87 where Er2 CA and WA induce different responses in the same rootstocks. 20 genes shared among rootstocks are expressed in opposite directions in CA versus WA relative to controls.

Of note, CA aphids altered rootstocks more than WA aphids but they also shared a set of genes (**Fig. 5**). Plant processes altered similarly among populations included enhanced basic stress response, suppressed photosynthesis, and enhanced cell wall organization. **Six immune related genes found across several chromosomes (1, 2, 4, 5, and 15) were up and may serve as targets to enhance apple resistance to WAA, especially for select aphid genotypes.**

Of the regionally-specific genes, WA aphids triggered two immune genes including one LRR on Chromosome 10 and increased three laccases linked to plant defense hormone signaling (Hu et al., 2018). CA aphids triggered 13 wound and immune related genes including seven LRR on chromosome 5, an R-gene on chromosome 15, and 3 disease resistance proteins on 3 different chromosomes, but only 1 laccase. Because CA aphids also suppressed a JAZ domain gene, five laccases, and seven effector-associated LRR genes, **plant defense hormone signaling may play a stronger role in plants encountering CA aphids than WA aphids**. Additional screening of aphid genotypes and different rootstocks across growing regions will help refine this hypothesis.

Twenty genes shared among rootstocks are expressed in opposite directions in CA versus WA relative to controls. These genes include one disease resistance protein that is up in CA. This differential expression confirms a role for genotype-specific secretions in altering plant response.

Several biological processes not directly functioning in immune or defense responses were altered by aphids, but why are these important? Photosynthetic downregulation is a common plant response to diverse forms of biotic stress ranging from viruses, bacteria, fungi, and arthropods (Bilgin et al. 2010), and WAA suppresses numerous photosynthesis genes. This is important because new evidence on plant perception of stress (PTI; Nomura et al., 2012, ETI; Su et al., 2018) indicates defense genes are induced by the chloroplast (through retrograde signaling) and photosynthetic inhibition is required to recognize effectors. Because WAA alters apple reactive oxygen species (ROS) profiles (Zhou et al., 2013), and breakdown in light harvesting/photosynthesis creates ROS, we predict a link between photosynthesis and ROS gene regulation during aphid attack. In support

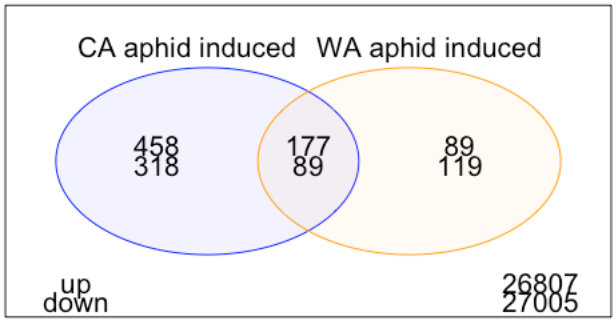

Fig. 5: 177 genes are up and 89 down in response to aphid feeding but the populations differed in how plants responded.

of this we find G.16 has more photosynthesis genes active (and up regulated) compared to G.87 or G.935 when no aphids are present and the majority of these genes are suppressed when aphids attack. This pattern indicates a role for photosynthetic inhibition in mediating WAA resistance. In contrast, G.87 had fewer photosynthesis genes (7 up and 5 down) compared to G.935, and when aphids attacked only 2 in G.87 were down. Additional study on the role of photosynthetic proteins in aphid interactions will help identify how primary growth responses like photosynthesis may provide aphid tolerance through both sustained growth and ROS-mediated protection.

Suberin is a waxy polymer that forms a barrier between the environment and living plant tissue, and functions to prevent desiccation and protect against biotic attack (Graça 2015). We identified the upregulation of 12 genes necessary for suberin synthesis and deposition after aphid infestation. Although suberization of the cell wall has been shown to be a plant response to aphid feeding (Tzin et al. 2015), and may prevent further stylet penetration, it is unclear if suberin deposition is part of the general wound healing response caused by stylet piercing, or is elicited by to benefit the galling habit of WAA. Numerous other cell wall remodeling genes are differentially expressed in a manner consistent with the construction of a new plant phenotype.

INDUSTRY BENEFIT

- Profiling constitutive expression of existing genotypes revealed disease resistance genes differentially active among rootstocks that provide similar aphid performance phenotypes.
- Additional loci outside Er genes exist that provide aphid tolerance (increased aphid mortality)
- Several immune/effector recognition genes found on several chromosomes may contribute to aphid resistance because of elevated expression without aphids.
- Comparisons among treatments revealed effector targets (genes suppressed by aphids), globally effective immune response (genes induced by aphids), and population x genotype-specific gene regulation of apple immune processes.

FUTURE DIRECTIONS

- Expand combined transcriptomic approach to more genotypes (including other species) with greater variation in resistance to identify novel genes active with and without aphids. This may best focus on where resistance is currently failing.
- Apple genotypes vary in constitutive expression of defense and immune-related genes, but other processes (e.g., photosynthesis, RNA processing, protein) have emerged as determinants of successful colonization. Thus, assessment of immune genotypes for similar profiles in non-immune, non-defense processes that indirectly provide immunity will broaden selection for tolerant genotypes.
- Continue genetic mapping to refine gene structure and sequence organization around immune genes of interest. This will aid in additional marker development.
- *New genotypes with variation in resistance await profiling*. In another survival assay, 10 genotypes were scored for performance over 6 days (**Fig. 6**). Six genotypes containing the Er-2 gene but with unknown resistance phenotypes were found to reduce survival below 40% in 4 days, with one genotype preventing colonization entirely, leading to aphid dispersal and eventual death. These new genotypes can now be revisited for further analysis to identify the traits underlying the deterrence and death of WAA.

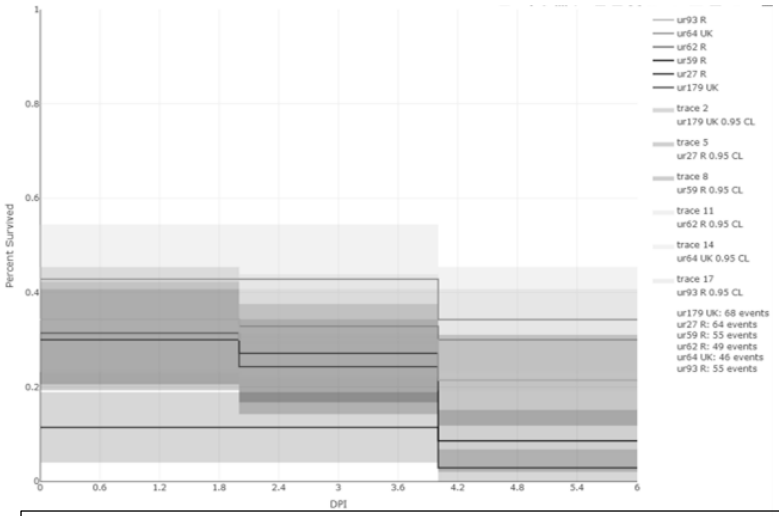

Fig. 6: Unreleased genotypes with known resistance genes were screened for aphid survival over 6 days to assess immediate immune functions. Each line represents a genotype within a confidence interval. All lines indicate low colonization (<50%) and reduced survival through time.

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EXECUTIVE SUMMARY
Project Title: Assessment of apple immune responses to wooly apple aphid saliva
KEY WORDS: insect effector, transcriptome, resistance, genome

ABSTRACT: We identified how *Eriosoma lanigerum* (WAA) colonizes apple using genome and transcriptome profiling. We found effectors enable colonization, differ among populations, and that genotype and population specific responses in apple exist. These data help track WAA resistance at local, regional, and global levels, and reveal processes underlying rootstock performance.

All plants share networks of related genes and proteins that work together to generate immune responses to both insects and pathogens. The main goal of our project was to identify these networks in apple as they relate to aphid feeding, although resolving these immune networks will inform upon any biotic stress response imposed on apple in the future. A complementary goal was to examine how the aphids trigger these networks by characterizing insect genes. For both of these goals, we trained a MS student in current bioinformatics techniques and with expertise in apple-aphid interactions. Our approach combined transcriptomic information on the apple genes induced by aphid colonization with the genes active in aphids as a first hurdle in linking insects to plants. This fundamental research will help us to better understand resistance mechanisms in apple and how insect populations vary across growing regions.

We used a robust sequencing approach to generate a high-quality genome assembly for the WAA, and predict >390 secretory effectors are used to evade immune detection and induce morphological change in apple hosts. Aphid populations from CA and WA differ in their effectors both by having different genes and also by expressing similar genes differently. Altogether, these effectors provide markers for population and genotype specific characterization of aphid performance to identify genes directly involved in colonization and better predict how future rootstocks will perform across insect populations. These effectors also provide a means to track aphid genotypes that overcome resistance and identify how this occurred.

We also profiled genotypes that varied in susceptibility/resistance and the activity of the Er-2 gene. We found that the degree of host response depends on the genotype attacked and the population attacking, but found a core set of immune genes linked to reduced aphid performance. We identify several more genes across chromosomes that are strongly upregulated during aphid attack, thus contribute to resistance, or are suppressed by aphids, thus likely are targeted by aphid effectors. We found more immune genes regulated when Er resistance was not active and also found constitutive and altered expression of non-immune and non-defense processes that can indirectly reduce aphid performance. This provides a source of non-Er-gene tolerance to aphids and a background to screen against when examining how new genotypes may perform. Altogether, these data provide a means to identify and track WAA resistance at local, regional, and global levels, and characterize why rootstocks perform the way they do given where they are grown.

Publications in preparation

- Wemmer J, Zhao C, Borowsky A, Fazio G, Nabity PD et al. Transcriptional remodeling of apple, *Malus domestica* (Borkh.) across a host resistance spectrum upon colonization by a gall-inducing aphid, *Eriosoma lanigerum* (Hausmann).
- Nabity et al. Genome sequence of the woolly apple aphid, *Eriosoma lanigerum*

MS Thesis: Wemmer, JD. 2019. Characterizing the Dual Transcriptomes of Woolly Apple Aphid, *Eriosoma lanigerum* (Hausmann), and its Host, *Malus domestica* (Borkh.), Across a Host Resistance Spectrum. University of California.

FINAL PROJECT REPORT

Project Title: Improved monitoring and lure and kill for codling moth management

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Other funding sources: None

Total Project Funding: $21,000

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

Item	2018	2019
Equipment	-	
Supplies	12,000	
Travel	3,000	
Miscellaneous	-	
Plot Fees	-	
Total	$15,000	$0

Budget 2
Organization Name: Universidad Austral de Chile
Contract Administrator: Ricardo Leal **Telephone:** +56 63 222 1778
Email address: rleal@uach.cl

Item	2018	2019
Travel (visit USDA lab June)	6,000	
Total	$6,000	$0

RECAP ORIGINAL OBJECTIVES:

1. Develop two newly discovered attractant blends that can significantly increase catch of both sexes of codling moth to improve monitoring and increase the effectiveness of lure and kill technologies for this pest.
2. Field test and fine-tune the components of mass trapping for codling moth using a *hands-free approach* during the season.
3. Develop and evaluate *Zerofly* netting for use against codling moth.

SIGNIFICANT FINDINGS

- The ***4-way K*** lure was developed through extensive testing of host plant volatiles. This non-pheromone lure outperformed the standard pheromone lure used by the industry and caught 60-70% females
- The ***4-way K*** lure caught 4-fold more females than the previous best female attractant (pear ester plus acetic acid) and was effective in variable apple orchards with different cultivars, fruit loads, and throughout the season (green fruits, mature fruits, injured fruits, and fruits rotting on the ground).
- Lure development is continuing in South America.
- Mass trapping of codling moth using 24 combo plus acetic acid-baited bucket traps per acre were used to reduce levels of fruit injury 71% at harvest across four organic apple orchards. Traps were only serviced once at mid-season to replace the lures.
- The *Zero-fly* deltamethrin-impregnated netting was developed into a ***Grey Ghost*** and evaluated with moths in laboratory experiments. These tests demonstrated that the netting killed moths within 24 h with contacts as brief as 5 s. A strong sublethal effect on mating and egg laying occurred in moths that did not die within 24 h.
- The ***Grey Ghost*** remains toxic throughout the season and may last for more than one year.
- ***Grey ghost***-treated plots had reduced moth catch in monitoring traps and 50% less fruit injury at midseason.
- Lures used with the ***Grey Ghost*** were not replaced and both the lure attractiveness and fruit protection declined after mid-season.

RESULTS & DISCUSSION

A series of studies were conducted during the 2018 season in a heavily infested set of apple orchards situated near Wapato, WA. Several host plant volatiles were evaluated in delta traps to see if they were attractive to adult codling moth. Individual compounds, binary, ternary, and quandary blends were compared. All of these compounds and blends were compared to the effectiveness of pear ester plus acetic acid lures as the industry best lure for female codling moth. Only the quandary blend outperformed the PEAA lure and caught nearly 4-fold more total moths and females with levels of females comprising as much as 80% of all moths caught (Figs. 1 and 2). This blend was coined ***4-way K*** because there are four kairomone compounds. Late in the season various modifications of this blend were evaluated with various substitutions of compounds. Just prior to the end of the season several additional volatiles were identified that could also be used in various substitutions. However, this work was incomplete and ongoing studies have been established in Uruguay and Chile to continue these studies.

The significance to the WA tree fruit industry of this discovery is tremendous. Identification of an attractant that is more powerful than sex pheromones and catches a very high proportion of female moths can benefit growers in several ways. The lure is very effective in orchards treated with sex pheromones for mating disruption as the traps are not disrupted. Enhanced capability to track female moths can allow significant improvements in timing sprays to target egg hatch. The lure does not draw moths into orchards and effective thresholds based on moth catch could be established. Data

from 2018 suggested the ***4-way K*** lure worked well in several cultivars, throughout the season, and in blocks with few fruit and heavy crop loads. Also, it worked whether the crop was injured, moderately injured, heavily injured and even when the orchard floor was littered with damage fruit. Trap height does not seem to be as important compared with sex pheromone lures, so traps could be placed at a lower and more convenient height in the orchard. The dogma in insect behavior research is that the best attractant makes the best disruptant of sexual behavior. Thus, it may be possible to develop improved mating disruption dispenser systems using one or more of these compounds. Experimental dispensers have already been formulated and will be tested in 2019. Finally, the tremendous increase in female catch afforded using the ***4-way K*** lure could make mass trapping extremely effective. Our results from last season using just 24 bucket traps baited with the Combo plus acetic acid lures (71% less injury) for an organic grower was exciting to both him and us, and perhaps these good results could be greatly improved using the 4X more potent ***4-way K*** lure.

The ***4-way K*** lure may also facilitate the eventual use of an attract-and-kill concept we explored in 2018. The *Zero-fly* netting was developed for battling malaria in third world countries and has been used effectively in research to manage the brown marmorated stink bug in Pennsylvania orchards. We developed the ***Grey Ghost*** which is baited with codling moth lures and hung in the canopy (Fig. 3). Our laboratory studies demonstrated that moths are not repelled and land on the netting in response to the lures. Moths typically walk on the netting for 1-60 s and often rest on the netting for longer periods. Forced touch tests demonstrated that as little as a 5 sec contact with the netting kills moths within 2 h and most moths by 24 h after contact ended. Sublethal effects are also pronounced with survivors unable to mate or to lay a full complement of eggs. A small field research study was established in 2018 using replicated 1-acre plots. We had hoped that the lures would last all season so once the ***Grey Ghosts*** were applied, we did nothing all season except check traps and sample fruit injury. Lures at mid-season were still effective, and moth counts, and levels of fruit injury were about 50% lower. However, by the end of the season no difference in fruit injury between the treated and untreated plots were seen and no difference in moth catches occurred in the two treatments. Lures near the end of the season only caught 30% as many moths as new lures. We consider these results to be interesting and will develop a method to attach a lure holder to the ***Grey Ghost*** that will allow lures to be more easily replaced at mid-season. We have a 3D printer at the laboratory to develop this device. We demonstrate that the netting does not lose any toxicity over the course of the season and could perhaps last more than one year.

Figure 1. Comparison of the 4-way K lure with industry standard lures.

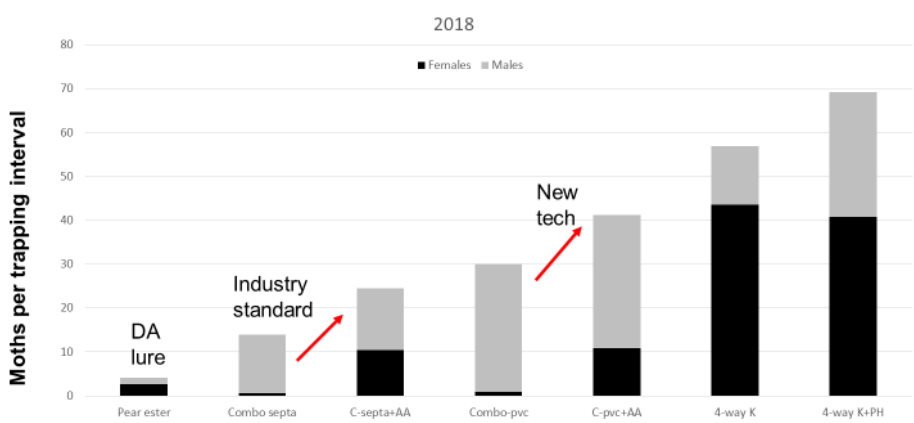
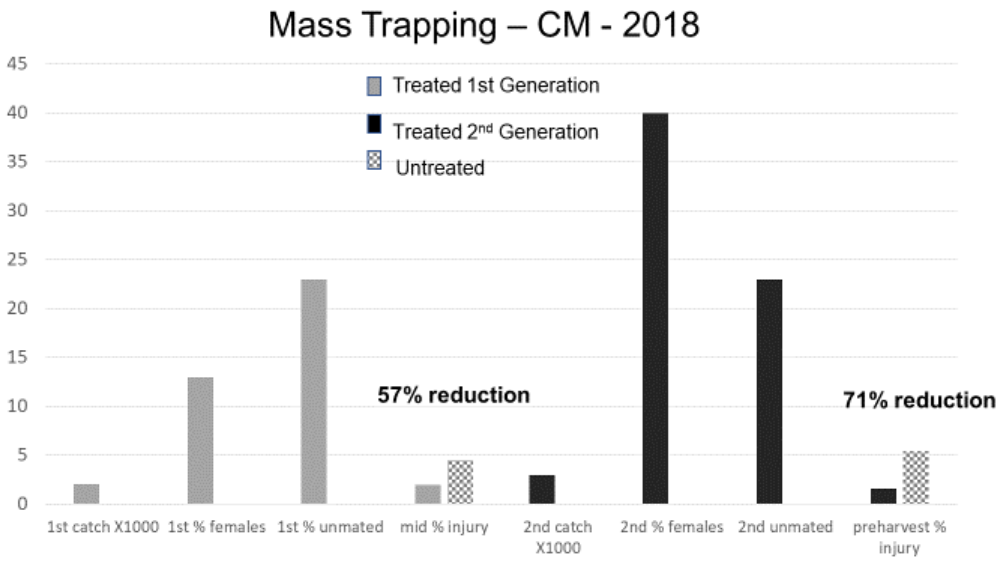


Figure 2. All the codling moths (males at top, females below) sorted and removed from the one liner on the right after only one night (21 July 2018) using the ***4-way K*** lure in combination with sex pheromone. *Note*: scale-less areas on liner are where the lures were placed.

Figure 3. Photograph of the ***Grey Ghost*** developed with *Zero-fly* netting for attract and kill of codling moth, 2018.

Figure 4. Summary of mass trapping experiments N = 4, using 24 bucket traps per acre baited with Combo plus acetic acid lures, Tieton, 2018.



KEYWORDS: codling moth, mass trapping, monitoring

ABSTRACT: A volatile blend was discovered that is effective for both male and female codling moth, *Cydia pomonella.* This blend catches more moths than the standard sex pheromone lure. Initial field trials demonstrated that this lure can be used to effectively reduce fruit injury through female removal.

EXECUTIVE SUMMARY: The ***4-way K*** lure was developed through extensive testing of host plant volatiles. This non-pheromone lure outperformed the standard pheromone lure used by the industry and caught 60-70% females. The ***4-way K*** lure caught 4-fold more females than the previous best female attractant (pear ester plus acetic acid) and was effective in variable apple orchards with different cultivars, fruit loads, and throughout the season (green fruits, mature fruits, injured fruits, and fruits rotting on the ground). Lure development continued throughout 2019 including evaluations of the effectiveness of this approach in grower's orchards, and further refinement of lures. Mass trapping of codling moth using 24 combo plus acetic acid-baited bucket traps per acre were used to reduce levels of fruit injury 71% at harvest across four organic apple orchards. Traps were only serviced once at mid-season to replace the lures. The *Zero-fly* deltamethrin-impregnated netting was developed into a ***Grey Ghost*** and evaluated with moths in laboratory experiments. These tests demonstrated that the netting killed moths within 24 h with contacts as brief as 5 s. A strong sublethal effect on mating and egg laying occurred in moths that did not die within 24 h. The ***Grey Ghost*** remains toxic throughout the season and may last for more than one year. ***Grey ghost***-treated plots had reduced moth catch in monitoring traps and 50% less fruit injury at midseason. Lures used with the ***Grey Ghost*** were not replaced and both the lure attractiveness and fruit protection declined after mid-season. Unfortunately, this netting has not been registered by the EPA, and future work with this material is on hold until the distributor is ready to move forward.