Apple Crop Protection Research Review

Thursday, January 28, 2021

Time	Page	Presenter	Project Title	Yrs
8:30		Smith	**Welcome**	
8:40		Hanrahan	Meeting etiquette	
			Continuing Projects (10 minutes)	
8:50	1	Beers	Integrated control of BMSB	19-21
9:00	8	Johnson	Pre-bloom defense induction in young trees for fire blight control	20-21
9:10	15	Amiri	Pre- and postharvest disease management in organic apple systems	19-21
9:20	22	DuPont	Integrated fire blight management: ***NCE***	19-20
9:30	30	Amiri	Understand the epidemiology of Botrytis to curb gray mold postharvest: ***NCE***	18-20
9:40	35	Gut	Optimization of release strategies for sterile codling moths: ***NCE***	19-20
9:50	41	Walker	Can we get codling moth females to stop laying eggs on apple?	18-22
	48	Amiri	Outreach program for apple decay mgt. in WA**:** ***NCE written report only***	19-20
			Final Reports: No-Cost Extensions (10 minutes)	
10:00	54	Amiri	Rapid lab and field detection of two major apple quarantine pathogens	17-19
10:10	60	Hopkins	Using cold storage to increase the stability of honey bee supply	18-19
10:20	65	DuPont	Implementation of alternative methods to control replant disease	17-19
			Break	
11:00	79	Schmidt	Pesticide residue on WA apples	18-20
11:10	82	Doty	Development of new biocontrol strains from WA native trees: ***NCE***	20
			Final Reports (15 minutes)	
11:20	88	Beers	Optimizing sterile insect release of codling moth in Washington	18-20
11:35	102	Knight	New attractants for monitoring MD and mass trapping of codling moth	19-20

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

Project Title: Integrated control of brown marmorated stink bug

PI: Elizabeth H. Beers
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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: Grant total (5-years): $9,164,908; years 3-5 (current): $5,477,389; WSU/Beers 2019-2021 total direct budget: $156,047
Agency Name: USDA-NIFA-SCRI

Other funding sources: **Awarded**
Amount: $16,505
Agency Name: Washington State Commission on Pesticide Registration

WTFRC Budget: None

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu
Station manager: Chad Kruger **Email address:** cekruger@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.* Captures of BMSB in interior traps in blocks protected by A&K traps were consistently lower than in block not protected by traps. Fruit damage in the protected blocks was 50% lower than the unprotected blocks.
2. *Redistribute Trissolcus japonicus (the samurai wasp) where established BMSB populations are identified, and monitor its establishment and non-target effects.* Efforts in 2020 focused on release of *T. japonicus* (>7,000 released) in 3 urban and 2 agricultural areas. Low incidence of *T. japonicus* in the Vancouver site made non-target effect studies impossible; limited staff hindered plans to re-survey the 2018 release sites. Instead, we pursued the development of a new technique using PCR of individual eggs to investigate non-target effects.
3. *Determine development of BMSB on shrub-steppe plants.* A second year's study of development on shrub-steppe plants indicated clearly there is a dietary penalty for BMSB development on eastern Washington's native plants. This studied was followed by an additional study on the adult reproductive stage to verify if the lower adult weight correlated with poorer fecundity. We will continue these studies in 2021 using native stink bugs.
4. *Track the invasion of BMSB in Washington State*. The online reporting system for BMSB finds in the state recorded 61 finds in the state of Washington in 2020. The targeted sampling performed in previous years was greatly curtailed due to low staffing levels (CoVID-19 related) and the wildfires and poor air quality during late summer/early fall of 2020. We plan to expand the targeted sampling in 2021 if these two impediments are absent.

Significant Findings

- We released 7,005 adult *T. japonicus* in three urban areas (Seattle, Spokane, Tri-Cities) and two agricultural areas (Douglas and Klickitat counties).
- Using PCR and morphological methods, we found that *T. japonicus* had higher total impact (reproductive and non-reproductive) on the native spined soldier bug (a predator) than on BMSB.
- Deployment of insecticide-infused netting attract-and-kill (A&K) traps resulted in a 50% reduction in BMSB fruit damage in protected blocks.
- The addition of lights to pheromone traps did not increase trap capture.
- BMSB fed on native shrub-steppe plants from the egg to adult stage were smaller, slower to develop, and had poorer survivorship when compared to BMSB fed on plants typical of the mid-Atlantic. In addition, the resulting western-diet adults were shorter-lived and laid fewer eggs.
- Only 61 reports were made from citizens around the state in 2020, compared to 146 in 2019; 3 of the 2020 reports were from on the houses of TFREC entomologists. A total of 29 Washington counties have now reported BMSB (no change from 2019). Trap capture from blocks surveyed in 2019 was up to 95% lower in 2020.

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 for control of BMSB. Much of the initial research on BMSB in the mid-Atlantic area focused on determining the efficacy of various insecticides; this research was critical for enabling growers to prevent crop damage in the short term. Since that time, research efforts have transitioned to exploring longer-term solutions, especially biological control. Although biological control is expected to provide some overall population suppression, it is likely that vulnerable crops

will still need a more direct form of protection. Most of the insecticide options will be highly toxic to the samurai wasp and limit its impact in orchards; the primary impact will be in unmanaged habitats. Thus, development of tactics that are compatible with biological control are the highest priority for BMSB.

Behavioral controls have been the most intensively researched alternatives for BMSB control in the past 10 years. The most prevalent of these has been variations on a technique known as attract-and-kill (A&K). The attraction component has been the dual BMSB lure (currently available from Trécé); the means of killing them can be more variable, but often centers on an insecticide component. Initial experiments tested spraying baited trees at frequent intervals, but more recent efforts have focused on the use of long-lasting insecticide nets (LLIN) to cause mortality. This avoids the necessity of weekly sprays; in fact, the toxicity of the netting is projected to last several years.

Methods: We tested a perimeter of A&K traps using 3 pheromone lures and LLIN to protect an orchard from BMSB fruit damage. Traps were deployed every 50 m (164 ft) on the orchard border next to wooded areas (the latter is presumed to be a major source of BMSB). The traps were deployed in early July and checked every other week until late October. In addition, 3 sticky traps were placed near the center of the orchard to determine penetration of BMSB into the orchard interior. The A&K plots were compared to untreated sections of the same block, separated by a 55 to 756 ft buffer zone (sticky traps only). Adults and nymphs of BMSB retained by the traps were recorded, and a preharvest fruit damage sample (80 fruit/plot) was taken in early August and assessed after ca. 12 weeks of cold storage.

Results: Captures in A&K border traps were 93 to 99% lower (1 to 3 BMSB/traps/season) than the same blocks in 2019 (44 to 104 BMSB/trap/season), and interior sticky trap catches in 2020 were 61% of those in 2019, indicating lower overall bug pressure in 2020 in this orchard.

The A&K traps caught <1 BMSB/trap through most of the season, with no consistent seasonal trend. Surprisingly, the A&K traps caught less than the interior traps throughout the season. The interior sticky traps behind the protective perimeter of A&K traps caught consistently fewer BMSB (Fig. 1), resulting in significantly lower fruit damage at harvest (Fig. 2).

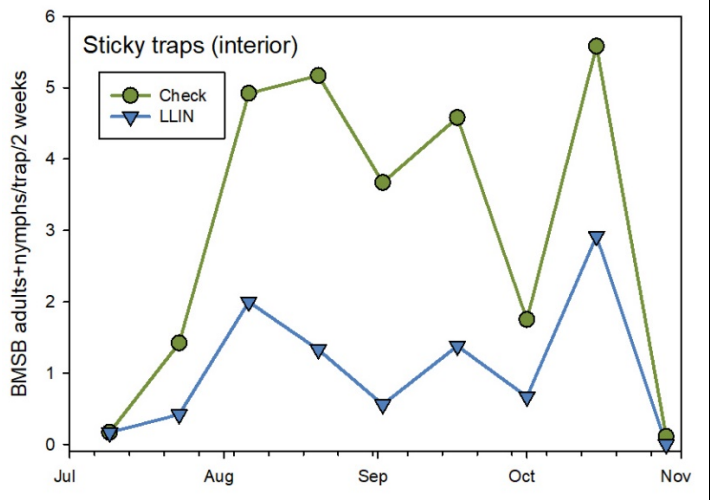


Fig. 1. BMSB captured in the interior of plots protected by A&K traps (LLIN) versus the unprotected (check) plots.

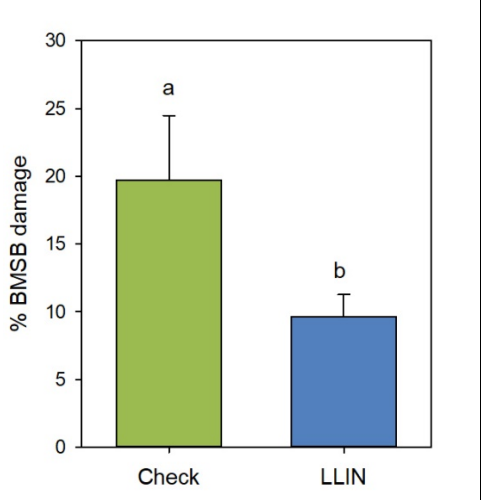


Fig. 2. Fruit damage caused by BMSB in LLIN versus check plots.

1b. Physical exclusion, net barriers. This objective was completed in 2019.

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.
Methods: We used information collected in Obj. 4 to target release areas in the state. BMSB has been reported from Washington's three large urban areas, viz., Seattle, Spokane, and the Tri-Cities. Our goal for release in those sites is that when BMSB spread from urban to agricultural areas (the pattern observed in other parts of the nation), their parasitoid will move with them. This may effectively constitute a *pro-active* release for agriculture. In addition, we released *T. japonicus* in two agricultural areas where is has been detected for one or more years: Douglas County (near Rock Island) and Klickitat County (near White Salmon). Due to limited staffing during the COVID-19 pandemic, we were unable to re-survey for *T. japonicus* in the sites of the 2018-19 releases.

Plate 1. Release of the samurai wasp in Washington

Results: We released a total of **7,005** adult *T. japonicus* in Aug/Sept of 2020, in the Seattle area (2,288), Spokane (740), Tri-Cities (1,239), Douglas county (1,950), and Klickitat county (788) (Plate 1, Fig. 1). The numbers released were dependent on the availability of egg masses from the BMSB colony. We also re-collected *T. japonicus* from the original Vancouver site to ensure wild-type viability in our colony; the 2020 releases were a mixture of the old and new colonies.

Plans for 2021: We plan to release *T. japonicus* in slightly different locations in the three major urban areas in 2021, and in smaller towns in agricultural areas.

Fig. 1. Release sites of the samurai wasp in Washington, 2020.

2b. Determine permeability of net enclosures to *T. japonicus*. This objective was deferred due to COVID-19 staffing/travel restrictions.

2c. Determine the effects of host plant and canopy height by *T. japonicus*. This objective was deferred due to COVID-19 staffing/travel restrictions.

2d. Determine the non-target effects of the samurai wasp on native stink bugs.
Methods: In the summer of 2019 we deployed sentinel stink bug egg masses of three native stink bugs (*Euschistus conspersus Chinavia hilaris*, and *Podisus maculiventris*) and compared them to BMSB to determine attack rate of *T. japonicus*. After allowing completion of egg hatch or development of a parasitoids, we characterized the eggs individually using a combination of morphological and PCR methods. The morphological methods used a classification scheme based on appearance where a normally hatched egg and one producing an adult parasitoid were assessed; unhatched eggs were classed based on appearance and subjected to PCR with the new *T. japonicus* primer developed by my lab (Dr. Kacie Athey). The combination of these methods allowed us to evaluate both reproductive (emerged adult parasitoid) and non-reproductive (egg is killed by the parasitoid, but no adult is produced) impacts. The latter can be a hidden, but potentially very important non-target effect of a parasitoid, and is an emerging criterium in evaluating natural enemies for classical biological control programs.

Results. Euschistus conspersus was not successfully attacked by *T. japonicus* during the course of this study (no adult parasitoids produced); however, it suffered a fairly high rate of non-reproductive effects (22.7%). *Chinavia hilaris*, another pest species, was attacked the least often (7.1% of eggs), with most of the effects being non-reproductive (5.4%). BMSB eggs suffered much higher total levels of impact (31%), with most of that successfully producing an adult parasitoid (25.5%). However, the highest level of attack was experienced by the native predator, *Podisus maculiventris* (67.2%), with higher levels of non-reproductive impacts (43.2%) versus reproductive (24.0%) (Fig. 2).

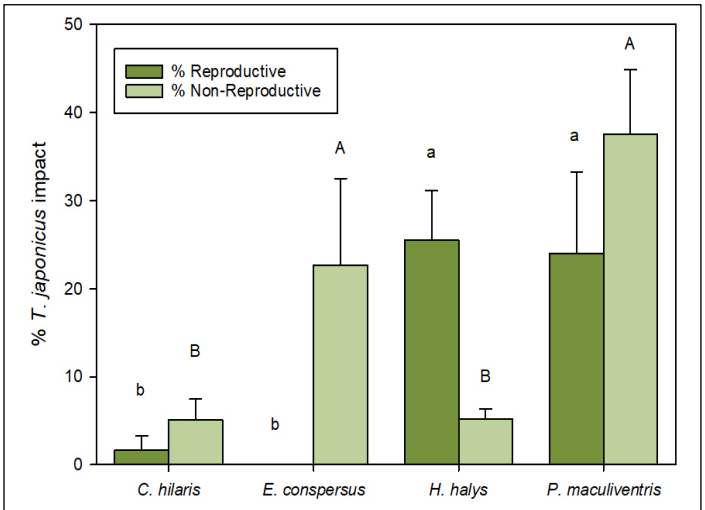


Fig. 2. Total parasitoid impact of *T. japonicus* on BMSB and 3 native stink bugs.

Objective 3: Determine development of BMSB on shrub-steppe plants
Methods: In 2019, we compared BMSB raised from the egg to the adult stage on either a typical colony diet (carrots, sunflower seeds, peanuts, bean plants) or plants native to Eastern Washington's sagebrush steppe habitat. Understanding the dietary limitations for development in different regions of the country should help us predict the relative risk of population buildup. The 2019 results indicated clearly that nymphs were slower to develop to the adult stage when fed on native plants, and that survivorship was significantly reduced. However, BMSB is unlikely to encounter a typical 'colony' diet in the wild (they do not have access to carrots and peanuts), so we followed up in 2020 with similar study using plants typical of the mid-Atlantic region compared to our native plants. The mid-Atlantic (or 'Eastern' plants) were cuttings taken from residential areas of Wenatchee, while the native or 'Western' plants were cuttings taken from unmanaged habitats (No. 2 Canyon, Horse Lake Preserve). The assemblage of Eastern plants was fairly consistent throughout the study (maple, tree of heaven, catalpa), while the Western plant assemblage changed as the various species bore fruit.

The Western diet was more varied, and included serviceberry, chokecherry, bitterbrush, currant, Oregon grape, elderberry, snowberry, and wild rose at various points during the season. In all cases, both foliage and fruit structures were included in the cuttings; the latter is believed to be essential for the development of BMSB. We followed the nymphal development from 1st instars through adults, noting developmental time, adult weight, and survivorship. Adult weight is believed to associated with reproductive success of the adult; to test this, we took 10 male/female pairs from each of the two diet regimes, and allowed them to mate and lay eggs until the death of the female. This gave us the important measures of fecundity (eggs/female) and longevity.

Results. The survivorship of the Western nymphs to the adult stage was about half the survivorship of those fed the Eastern diet (Fig. 3). The Eastern nymphs reached the adult stage in 42 to43 days, while the Western nymphs required 49 to 50 days, and their adult weight was 13-15% lower (Fig. 4). The same trends continued for the adults reared on these two diets; the Eastern females laid over twice as many eggs as the Western females (Fig. 5) and lived 63 vs 37 days[1] (Fig. 6). The longevity of the males was 36 to 37 days and was not affected by diet.

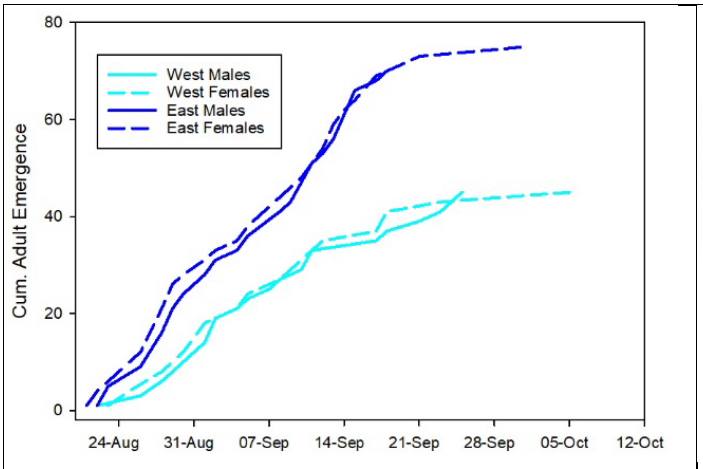


Fig. 3. Survivorship of nymphs fed on host plants from the eastern US vs the arid western US.

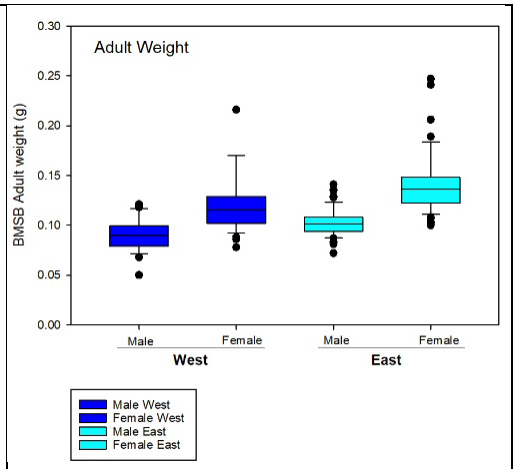


Fig. 4. Weight of surviving adults fed Eastern and Western plant diets.

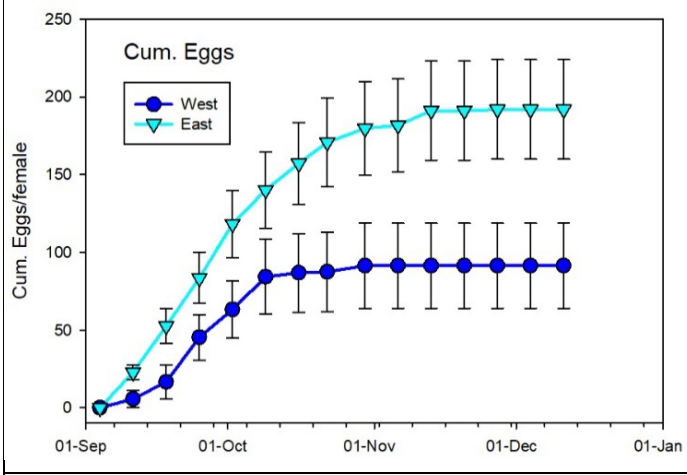


Fig. 5. Fecundity (cumulative eggs/female) from BMSB adults fed on either Eastern or Western plant diet.

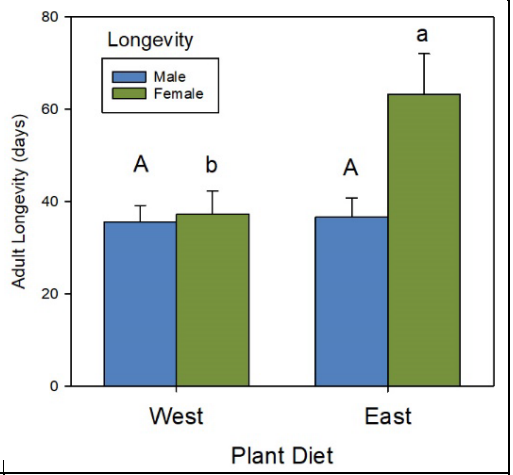


Fig. 6. Adult longevity of BMSB fed on either Eastern or Western plant diet.

[1] As of 14 December 2020; experiment still in progress.

Objective 4. Track the invasion of BMSB in Washington State

Methods: Trapping the same site in 2019 and 2020 allowed us to make comparisons of annual population differences. This site had both pheromone+LLIN traps and the pheromone+sticky card traps in both years. Captures in the LLIN traps were 93 to 99% lower in 2020 (1 to 3 BMSB/traps/season) when compared to 2019 (44 to 104 BMSB/trap/season) (Fig. 7). Sticky trap catches in 2020 were 39% lower than in 2019. These data indicate that BMSB populations (and possibly damage potential) will likely vary from year to year in Washington. This is consistent with observations in the mid-Atlantic area, which has also noted yearly fluctuation in insect pressure from this pest.

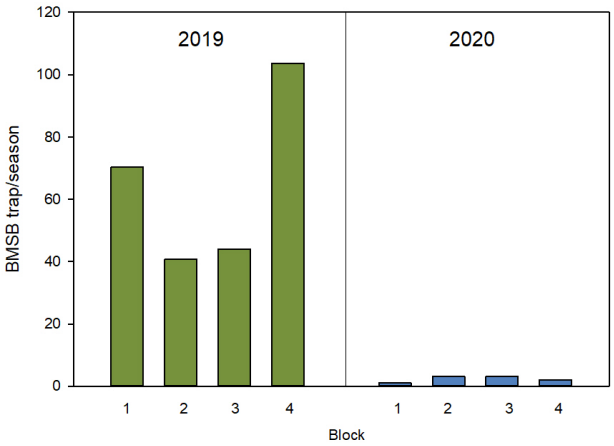


Fig. 7. Trap captures per block, 2019 vs 2020.

Results: The 2020 BMSB database also supports a reduction in population in 2020; only 61 reports were recorded in 2020 in comparison to 146 reports in 2019. However, the reporting database may also reflect greater familiarity on the part of homeowners with this pest, reducing their tendency to email a report. Most of the 2020 reports (52) came from counties west of the Cascades (King, Kitsap, Mason, Pierce, Snohomish, Thurston, Whatcom), with the remaining 9 reports from Chelan, Yakima, and Spokane counties (Fig. 8). Three of the BMSB reports from Chelan county were recorded from the residences of WSU employees associated with the entomology program. At low densities, those persons more aware of BMSB are more likely to find and report specimens.

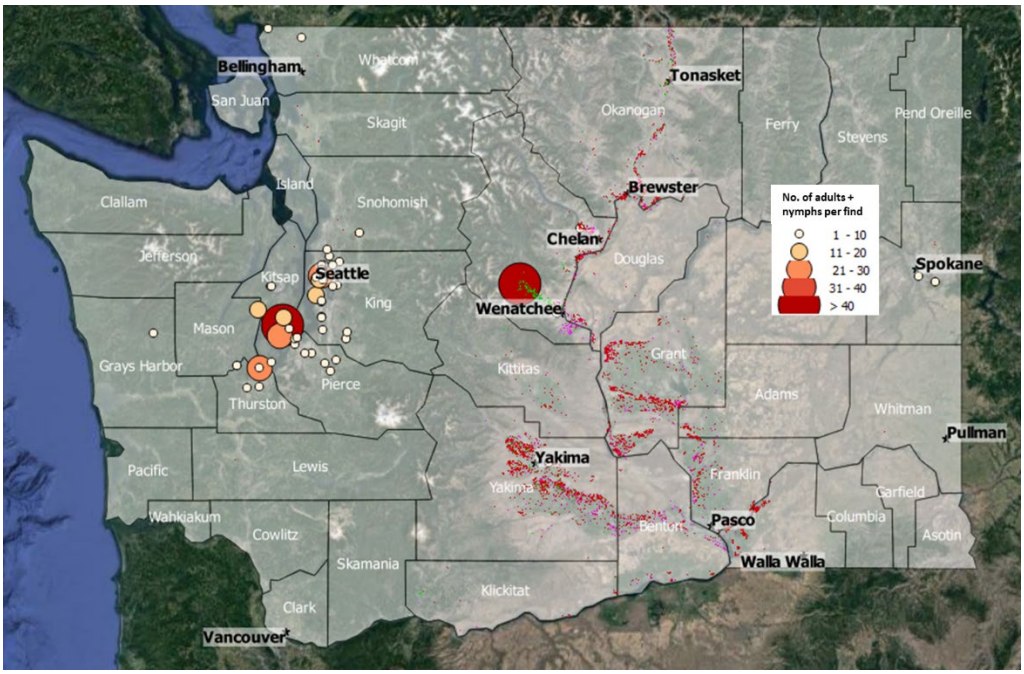


Fig. 8. BMSB reports in 2020; 61 individual reports, ranging from 1 to 100 BMSB (adults+nymphs).

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

Project Title: Pre-bloom resistance induction for fire blight suppression

PI: Kenneth B. Johnson
Organization: Oregon State University
Telephone: (541) 737-5249
Email: johnsonk@science.oregonstate.edu
Address: Dept. Botany & Plant Pathology
Address 2: Cordley Hall 2082
City/State/Zip: Corvallis, OR 97331-2902

Total Project Request: **Year 1:** $34,455 **Year 2: $35,488**

Other funding sources: **None**

Budget

Organization Name: **OSU Ag. Res. Foundation** **Contract Administrator:** **Dan Arp**

Telephone: (541) 737-3228 **Email address:** **Charlene.Wilkinson@oregonstate.edu**

Item	**2020**	**2021**
Salaries Faculty Res. Assist. 3.5 mo	17,860	18,396
Benefits OPE 61%	10,895	11,221
Wages undergraduate $12.50/hr	1,111	1,143
Benefits OPE 8%	89	93
Equipment		
Supplies	1,000	1,030
Travel	1,500	1,545
Miscellaneous		
Plot Fees	2,000	2,060
Total	$34,455	**$35,488**

Footnotes: 3% inflation in year 2

OBJECTIVES

Obj. 1a: Determine if prohexidione-Ca or acibenzolar-S-methyl applied prior to bloom achieves blossom blight control,

1b. Determine if prebloom PH-Ca enhances effectiveness of ASM treatments during bloom.

Obj. 2. Determine if Regalia applied prebloom influences blossom blight suppression.

Obj. 3. In greenhouse, investigate synergy between Ph-Ca and ASM for fire blight suppression, and resistance induction by Regalia for fire blight control.

SIGNIFICANT FINDINGS:

- Kudos, Actigard, or Regalia applied at prebloom timings did not provide significant suppression of fire blight in flowers compared to a non-treated control.
- Prohexidione-Ca (Kudos) applied as a solitary treatment at timings of first pink, full pink and 10% bloom, did not reveal a pattern related to time of treatment and level of suppression.
- A combination program of Kudos (prebloom) and Actigard (during bloom) reduced the incidence of infection compared the water-treated control (55 and 65 % reduction in pear and apple, respectively). In contrast, in the same trials, little to no control was obtained from Kudos (prebloom) or Actigard (during bloom) by themselves.
- Proposed greenhouse experiments were delayed to 2021 because of closure of the OSU greenhouse facility during the COVID-19 pandemic lockdown.

METHODS:

Field sites. Experiments were conducted in a 6-yr-old Gala and a 2-yr-old Gala block located at the Oregon State University, Botany and Plant Pathology Field Laboratory near Corvallis. Additional treatments were evaluated in older (20-yr-old) blocks of Gala apple and Bartlett pear.

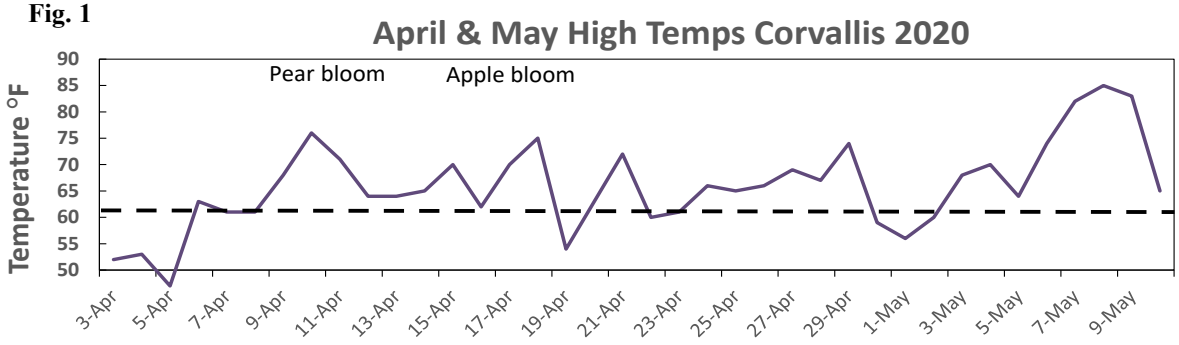
Experimental design for orchard trials. Experiments were arranged in randomized complete block designs with 9 replicates of single-tree plots in young tree blocks, and with 4 replicates of single-tree plots in older tree blocks. Prior to bloom, flower cluster density on individual trees was counted. These counts along with tree location in orchard were considered in the assignment of trees to blocks in the plot design. On the date of treatment, suspensions of each product in water were prepared at the specified rate and applied to near run-off with backpack sprayers in early morning under calm wind conditions. The amount of material suspension sprayed was 0.5 to 1.0 liter/tree in young tree trials, and 3 liters/tree in the older tree trials. On an evening near full bloom, re-suspended freeze-dried inoculum of pathogenic *E. amylovora* 153N (streptomycin and oxytetracycline sensitive pathogen strain) prepared at a concentration of 1 x 10^6 CFU/ml was fogged onto the trees. In the young trees trials, because of asynchronous bloom, the inoculation was repeated to ensure adequate disease pressure.

Disease assessment and data analysis. During bloom, treated trees were evaluated for phytotoxic responses from sprayed treatments. Beginning 2 to 3 weeks after petal fall, fire blight incidence was assessed by counting and removing blighted flower clusters from each tree. The number of blighted flower clusters was divided by the total number of clusters on each tree. Incidence of fire blight was subjected to analysis of variance (Analyze-It, v. 3.0, Leeds, UK).

RESULTS

Weather in spring 2020. Temperatures during primary bloom of both pear and apple were moderately favorable for fire blight development. Six days between 9 and 21 April had a maximum daily temperature > 70°F. On inoculated control trees, epiphytic pathogen populations were high with measured sizes exceeding 1 x 10^6 colony forming units (viable cells) on flowers sampled near petal fall. These high populations on non-treated or water-treated control trees resulted in infection incidences that averaged 31% of total clusters (the range was 14 to 41% among the four trials mentioned in this report).

Fig. 1



Objective 1a: Determine if Ph-Ca or ASM applied prior to bloom achieves blossom blight control, and **Objective 2:** Investigate if Regalia applied prebloom influences blossom blight suppression.

Rationale. Wallis and Cox (2020) recently achieved very good suppression of fire blight from a single application of prohexidione-Ca (Ph-Ca, 6 oz. /100 gal.) prior to bloom. Questions that arose from their study include: 1) can this repeated under western conditions?, 2) what is the optimal timing of Ph-Ca treatment?, and 3) could other resistance-inducing materials (Actigard (acibenzolar-S-methyl, ASM) or Regalia (extract of *Reynoutria sachalinensis*)) achieve this suppression response.

Approach. In young tree trials, the timing of Kudos (prohexadione calcium, 6 oz. /100 gallons, Fine Americas, Walnut Creek, CA) was varied from first pink to 10% bloom. In addition, Actigard 50WG (acibenzolar-S-methyl, 3.2 oz/100 gallons, Syngenta Crop Protection, Greensboro, NC), and Regalia (extract of *Reynoutria sachalinensis, 256 fl. oz/100 gallons,* Marrone **Bio, Davis, CA**) were each evaluated at the timing of full pink.

Observations. Trees used in the study averaged 82 flower clusters per tree in the 6-yr-old block and 57 flower clusters per tree in the 2-yr-old block. None of the materials (Kudos, Actigard, and Regalia) applied at prebloom timings produced symptoms of phytoxicity. Symptoms of fire blight were first observed on 5 May. For a pathogen-inoculated trial, incidence of infection was moderately high with non-treated control trees in the 6-yr-old block averaging 31 fire blight strikes per tree (41% of total clusters); in the 2-yr-old block, non-treated control trees averaged 23 strikes per tree (39% of total clusters). In both orchard blocks, while all treatments had fewer infections per tree than the non-treated controls, the overall suppression of infection from sprayed materials was only fair (17 to 39% in the 6-yr-old block) to poor (6 to 17% in the 2-yr-old block). For both trials, based on incidence of infected flower clusters and compared to the non-treated control, analysis of variance did not reveal any statistical differences ($P > 0.05$) resulting from the spray treatments. Kudos, which was applied as a solitary treatment at timings of first pink, full pink and 10% bloom, did not reveal a pattern related to time of treatment and level of suppression.

Table 1. Evaluation of prebloom treatments of resistance inducers for fire blight control in 6-year-old and 2-year-old Gala apple, Corvallis, 2020.

Treatment	Rate per 100 gal	Date treatment applied* 8 April First pink	11 April Full pink	14 April 10% bloom	6-yr-old trees % blighted floral clusters**	2-yr-old trees % blighted floral clusters**
Non-treated	-	--- [§]	---	---	**41** [#] (31 strikes)	**39** [#] (23 strikes)
Kudos [y]	6 oz.	X	---	---	25	34
Kudos [y]	6 oz.	---	X	---	34	36
Kudos [y]	6 oz.	---	---	X	28	26
Kudos [y] Actigard	2 oz. 3.2 oz.	---	X	---	31	32
Regalia [z]	256 fl. oz.	---	X	---	33	37

***On the evenings of 15 and 19 April, a motorized 25-gallon tank sprayer equipped with a hand wand was used to lightly fog a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1 x 10^6 CFU per ml (0.1 to 0.2 liters per tree). ** 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 did not differ significantly ($P > 0.05$) based on analysis of variance ($F > 0.05$). [y] Amended 1:1 with ammonium sulfate and Regulaid (16 fl. oz. per 100 gallons). [z] Amended with BioLink Spreader-Sticker: 4 fl. oz. per 100 gallons.**

Discussion. We rate the overall quality of both young-tree experiments reported under this sub-objective as 'excellent trials' based on the number of replications (9 per trial), bloom density on individual trees (very good), weather for pathogen growth in flowers and level of infection (not too much, not too little). Consequently, we were disappointed our inability to repeat results obtained in New York. Based on data of Wallis and Cox (2020), for at least the 'full pink' spray timing, we expected Ph-Ca (6 oz./100 gal.) to significantly reduce the incidence of fire blight compared to the non-treated control. In 6-yr-old Gala, box plots of the raw data perhaps hint at an effect of the prebloom Ph-Ca treatments but not in the 2-yr-old trees (Fig. 2). After a couple years of non-significant effects of prebloom Ph-Ca as a solitary treatment, we intend to shift towards evaluation of Ph-Ca as part of a broader, multi-material spray program (discussed below).

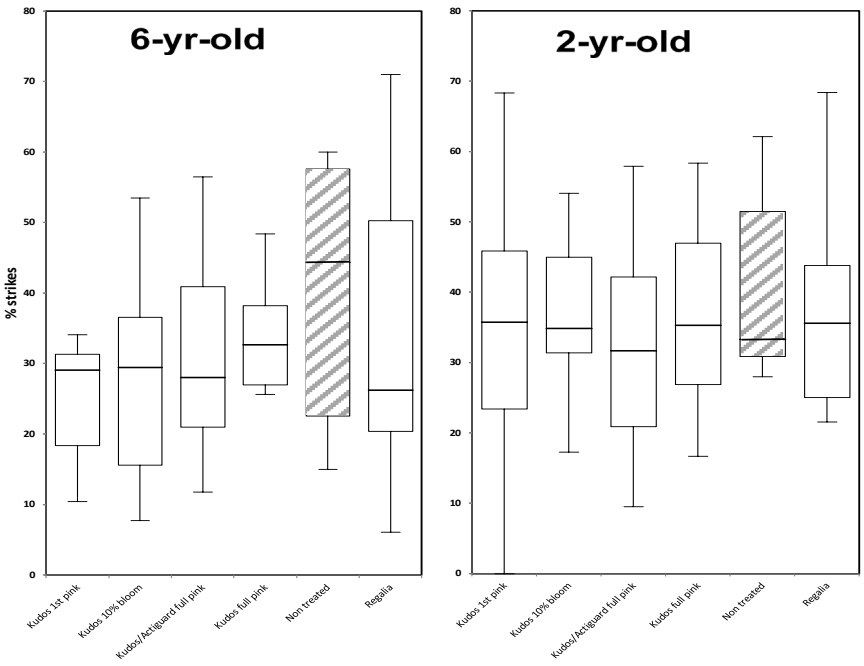


Fig 2. Box plots of percent infected flower clusters in Gala apple trees treated prebloom with potential resistance-inducing materials. The 6-yr-old (panel A) and 2-yr-old (panel B) orchards were located near Corvallis, OR with each treatment applied to nine replicate trees during April 2020. Within each panel, the non-treated control treatment is highlighted with a 'striped' fill pattern; within each box, horizontal line is the treatment median.

Objective 1b: Determine if prebloom PH-Ca enhances effectiveness of ASM treatments during bloom.

Rationale. Researchers in eastern U.S. (Sundin, Michigan St. U., and Acimovic, Cornell U. Hudson Valley) have begun recommending mixtures of Ph-Ca ((Kudos or Apogee) and acibenzolar-S-methyl (ASM, Actigard) for shoot blight suppression. Because we have shown previously that bloom sprays of ASM (in combination with antibiotics) contribute to blossom blight suppression, we sought to determine if prebloom treatment with Ph-Ca (as reported by Wallis and Cox 2020) would improve the efficacy of ASM treatments during bloom (without antibiotics).

Approach. Kudos (prohexadione calcium, 6 oz. /100 gallons) was applied at full pink; Actigard 50W (ASM, 2 oz. /100 gallons) was applied at 70% bloom, full bloom, and petal fall. An additional treatment added Stimplex (0.01% cytokinin, 128 fl. oz. /100 gallons, Acadian Seaplants Ltd., Dartmouth, Nova Scotia) at timings of prebloom (with Kudos) and petal fall (with Actigard). Regalia (extract of *Reynoutria sachalinensis, 128 fl. oz/100 gallons,* Marrone **Bio, Davis, CA**) was evaluated as a solitary treatment at the timings of full pink and petal fall.

Observations. The 20-yr-old Bartlett pear trees averaged 455 flower clusters per tree, and the 20-yr-old Gala apple trees averaged 430 flower clusters per tree. In pear, infection incidence was moderate with fire blight infections on water-treated trees averaging 67 strikes per tree (15% of total clusters); in apple, infection incidence was moderately high with water-treated trees averaging 126 strikes per tree (31% of total clusters). Based on percent blighted flower clusters and compared to the water-treated control, the solitary treatments of Kudos, Actigard or Regalia treatments did not reduce infection incidence significantly ($P < 0.05$). In contrast, in both trials, the combination program of Kudos (prebloom) and Actigard (during bloom) reduced significantly the incidence of infection compared the water-treated control (55 and 65% reductions in pear and apple, respectively). Regalia (1 gallon per 100 gallons of water) at the timings of pink and petal fall reduced incidence of infection (% blighted flower clusters) by 50% in the apple trial ($P< 0.05$).

Table 2. Evaluluation of prebloom PH-Ca and within-bloom ASM treatments for fire blight control in 20-year-old Bartlett pear and Gala apple, Corvallis, 2020.

20-year-old Bartlett pear

Treatment	Rate per 100 gallons water	Date treatment applied*: 3 Apr Full white bud	7 Apr Mid-bloom	10 Apr Full bloom	14 Apr Petal Fall	Number of blighted clusters per tree**	Percent blighted floral clusters***
Water	---	---	---	**X**[§]	**X**	**67 a** [#]	**14.5 a**
Actigard	2 oz.	---	X	X	X	52 a	11.8 ab
Kudos	6 oz.	X	---	---	---	74 a	19.1 a
Kudos	6 oz.	X	---	---	---	33 b	7.9 b
Actigard	2 oz.	---	X	X	X		
Kudos	6 oz.	X	---	---	---	32 b	8.7 b
Actigard	2 oz.	---	X	X	X		
Stimplex	128 fl. oz.	X	---	---	X		
Regalia	128 fl. oz	X	---	---	X	57 a	12.9 ab

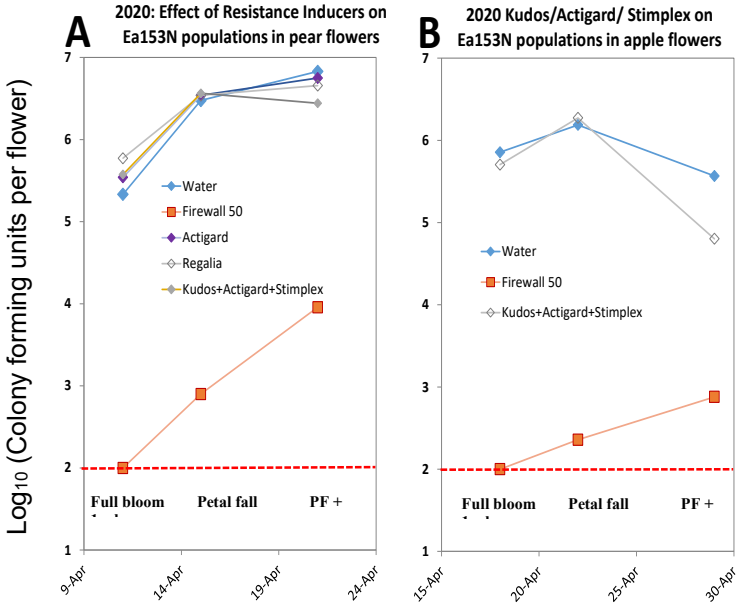
20-year-old Gala apple

Treatment	Rate per 100 gallons water	*Date treatment applied**: 10 Apr Full-pink	14 Apr Mid-bloom	17 Apr Full bloom	21 Apr Petal Fall	Number of blighted clusters per tree**	Percent blighted flora clusters***
Water	---	---	---	**X**[§]	**X**	**126 a** [#]	**30.7 a**
Kudos	6 oz.	X	---	---	---	137 a	35.5 a
Kudos	6 oz.	X	---	---	---	46 b	10.8 b
Actigard	2 oz.	---	X	X	X		
Kudos	6 oz.	X	---	---	---	57 ab	14.7 b
Actigard	2 oz.	---	X	X	X		
Stimplex	128 fl. oz.	X	---	---	X		
Regalia	128 fl. oz	X	---	---	X	66 ab	14.7 b

*** Trees inoculated on 8 (pear) or 15 (apple) April with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain). ** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown. *** 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 followed by same letter do not differ significantly (P = 0.05) based on Fischer's protected least significance difference.**

Discussion. In contrast to Table 1 (prebloom treatment only trials), some treatments under this sub-objective showed significant disease suppression. Importantly, in pear, fire blight suppression from Actigard sprays during bloom were improved when preceded by a prebloom treatment of prohexidione-Ca. A possible explanation for this result is that inhibition of gibberellin synthesis by Ph-Ca primes trees for a larger defense induction by ASM. Stimplex was added Ph-Ca/ASM program because some literature reports indicate that exogenous cytokinin enhances resistance induction from exogenous salicylic acid (which ASM mimics), but in these trials no enhancement was observed. It should be noted that neither Ph-Ca nor ASM have any effect on epiphytic populations of the fire blight pathogen in flowers (Fig. 3). Consequently, in conventional orchards, the approaches being evaluated here would need to be complemented by antibiotic treatments. In this context, the induced-resistance from a Ph-Ca/ASM treatment program could be expected to lessen variability in antibiotic suppression of fire blight (which can result from less than perfect coverage, and pathogen resistance to and/or the very short effective residuals of antibiotic materials). With regard to the organic material, Regalia, applying it twice to the older apple trees resulted in a better performance than its application as solitary prebloom treatment in the younger trees. In 2021, we intend to evaluate Regalia in an 'organic induced resistance program' that includes another organic material.

Fig. 3. Effect of resistance inducers applied to A) Bartlett pear and B) Gala apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers. Pathogen populations were measured by sampling five flower clusters (~25 flowers, bulked) from each replicate tree. Each sample was washed in 25-ml of sterile, de-ionized water followed by dilution plating onto nutrient agar plus nalidixic acid. Data for Firewall 50 (streptomycin) is shown for comparison. $Log_{10} = 2.0$ was the detection limit of the assay. Data depict mean of each treatment program on each sampling date.



Objective 3: In greenhouse, investigate further the potential synergy between Ph-Ca and ASM for fire blight control.

Planned greenhouse experiments proposed under Objective 3 did not occur owing to closure of the OSU greenhouse facility during the early months of the COVID-19 pandemic. These experiments will be done in 2021 and 2022 (under a no cost extension).

Rational and approach. We will conduct greenhouse experiments to investigate a potential disease suppression interaction between the host-resistance inducing materials, Ph-Ca and ASM, and to evaluate resistance induction by an *R. sachalinensis* (Regalia) extract. A large number of treated, uniform plants in a controlled environment can provide insight in induced resistance responses beyond that obtained from more variable orchard experiments.

Plant material. 400 trees of ELMA.26 for 2020 experiments were overwintered and will be used for 2021 experiments.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CP-19-103A

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

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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:** $74,015 **Year 2:** $82,112 **Year 3: $87,291**

Other funding sources

Agency Name: WSDA-Western SARE
Amt. requested: **$**190,000
Notes: A proposal is pending at the above agency**.** If funded, this project will allow us to continue research to develop more effective organic management practices

WTFRC Budget:

Item	2019	2020	2021
RCA Room Rental	6,300*	6,300	6,300
Total	6,300	6,300	**6.300**

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, so this amount will be used in 2020

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
Supervisor or Station Manager name and email address: Chad Kruger, cekruger@wsu.edu

Item	2019	2020	2021
Salaries[1]	38,400	39,936	41,533
Benefits[1]	14,008	14,569	15,151
Wages	0	0	0
Benefits	0	0	0
Equipment[2]	10,000	0	0
Supplies[3]	2,200	18,200	21,200
Travel[4]	1,007	1,007	1,007
Miscellaneous	0	0	0
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:

- ❖ The efficacy of 7 organic preharvest materials has been tested in 2019 and four to five of them show very good efficacy. In 2020, 15 materials have bee tested preharvest. Results will be available in 2021.
- ❖ The efficacy of these products was confirmed using artificial inoculations (Activity 1.2).
- ❖ Four most effective materials from 2019 trials were selected and tested in 2020 to develop a seasonal spray program (Activity 1.3) to enhance decay management.
- ❖ DCA and static CA (Activity 2.1) showed variability in reducing the incidence and severity of blue mold, gray mold, Mucor rot, Speck rot, and bull's eye rot. Trial are be redone in 2020 to fine-tune the DCA components (O_2 and CO_2 concentrations)
- ❖ Some of the preliminary data have been provided to the stakeholders via meetings in 2020.

METHODS

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

Activity 1.3: 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.

Activity 2.1. Efficacy 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.

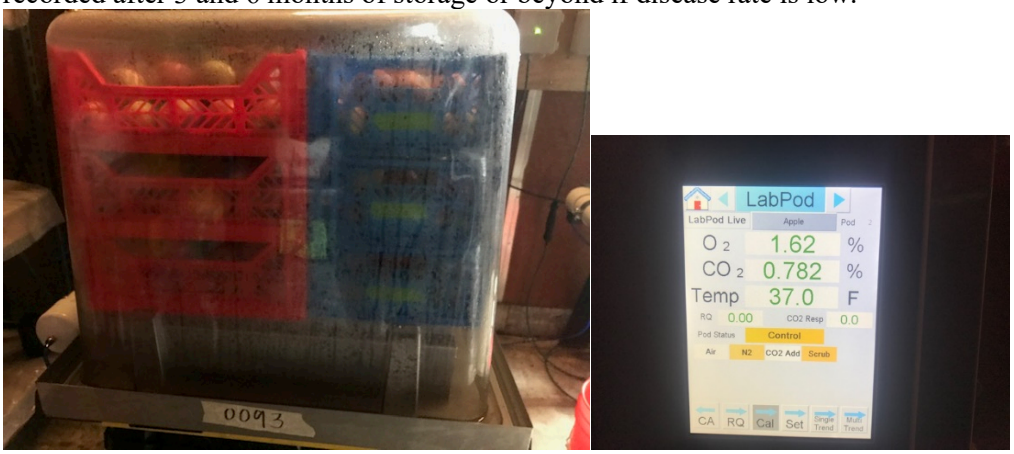


Figure 1. Trials set in Labpod at WSU-TFREC in Fall 2019 to evaluate the efficacy of CA and DCA on postharvest decay development.

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. Like 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 testing 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,

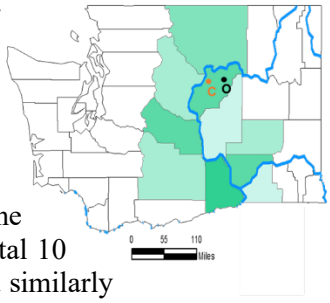
focused on 4 major pathogens *B. cinerea*, *N. perennans*, *P. expansum*, and *M. piriformis*, and also using naturally infected fruits. Experiments and disease evaluations will be conducted as explained in Activities 1.1 and 1.2.

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

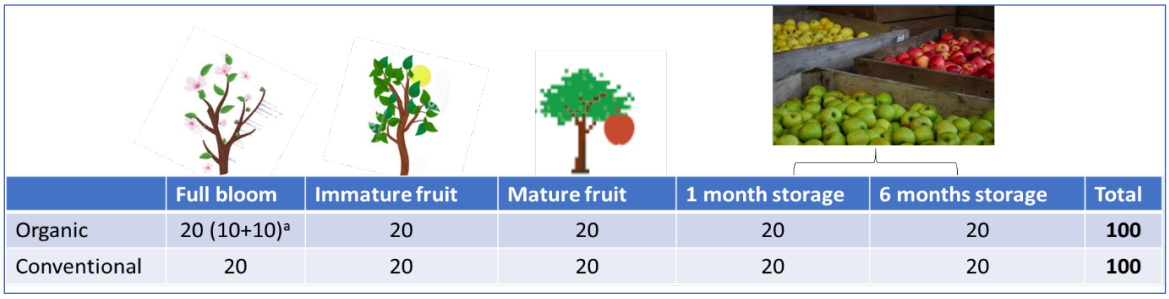
#	Trade name	Hypothesized Mode of action	Product Type
1	**Non-treated control**	-	-
2	Scholar SC[a]	FRAC 12	Fungicide
3	Bioferm	Competition	Biological
4	Cinnerate	Contact	Plant extract
5	SB. PSS 5.6	Sanitizer/Contact	Anions
6	Glyceryl palmitate(s)	Fruit Enhancer/Contact?	Inducer
7	Natamycin	Antibiotic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

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.

Results and Discussion

Activity 1.1. Efficacy of preharvest materials in reducing postharvest decays

Nine treatments, including a control (non-treated), a conventional fungicide (Merivon) and seven organic materials sprayed 7 days preharvest on a Fuji block in East Wenatchee in 2019.
After 8 months of storage at 34F in a regular atmosphere, overall decay (all decay types) incidence was 99% in the control and was reduced to below 50% by 5 organic products with Serenade Pro, Double Nickel and Regalia being the most effective (Figure 3). While OSO, provided a good efficacy against gray mold and blue mold when data were analyzed for each pathogen (Data not shown), it reduced overall decay incidence by 30%.

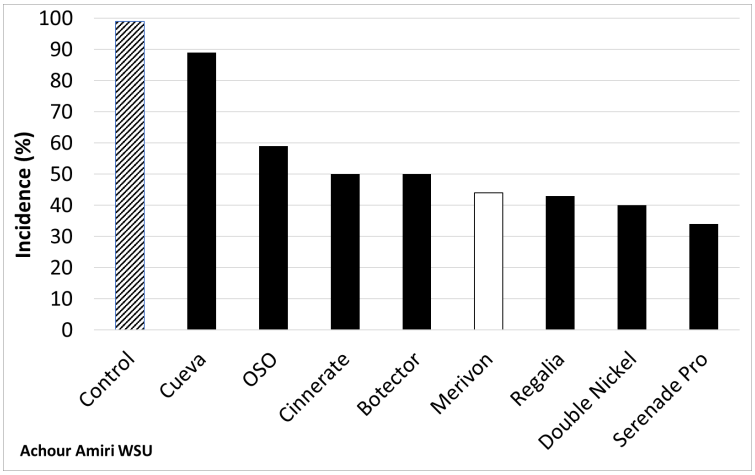
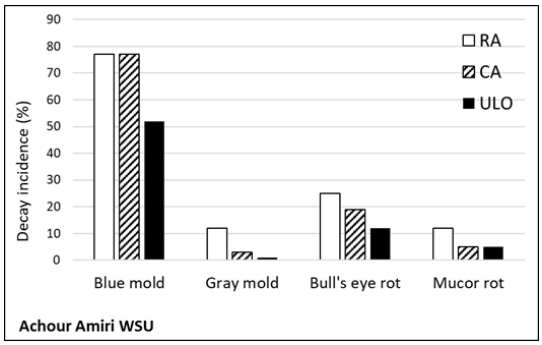


Figure 3. Overall decay (all decays combined) incidence on Fuji treated with the materials 7 days preharvest and stored at 34°F in a regular atmosphere for 8 months.

<u>Activity 2.1</u>. Efficacy of ultra-low oxygen (ULO) on decay development



The trials were conducted on Fuji artificially inoculated with spore suspensions of *P. expansum* (blue mold), B. cinerea (gray mold), *N. perennans* (bull's eye rot) and *M. piriformis* (Mucor rot) and stored in 3 different atmospheres, regular (RA), CA (O_2 = 4% and CO_2 = 0.8%), and ULO (O_2 = 1.5% and CO_2 = 0.8%) and stored at 34°F for 5 months. Figure on right shows that ULO program tested this year had a slight reduction of incidence of blue mold, gray mold, and bull's eye rot but not mucor. Much additional research with different atmospheres and duration will be needed before making overall conclusions on the ULO/DCA benefits in fighting decays.

FUTURE WORK

- Complete work in Objectives 1 and 2 by 2021. Results from some Activities will only be available in 2020.
- Work on Objective 3 will start only in 2021 because we only were able to hire someone with expertise in microbiome analysis in September 2020.

CONTINUING PROJECT REPORT **YEAR: No-Cost Extension**

Project Title: Integrated Fire Blight Management

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

Other funding sources

Agency Name: Applications made to SCRI
Amt. awarded: $418,722 to WA state

Budget 1
Organization Name: WSU **Contract Administrator:** Shelli Tompkins/Kate Roberts
Telephone: 509.335.2885 **Email address:** shelli.tompkins@wsu.edu/arcgrants@wsu.edu

Item	2019	2020	2021
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	$0

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	2021
Salaries	$8,000	$8,320	
Benefits	$5,200	$5,408	
Supplies	$2,000	$2,000	
Plot Fees	$1,700	$1,700	
Total	$16,900	$17,428	$0

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	2021
Salaries FRA 3.5 mo	$5,827	$8,765	
Benefits OPE 61%	$3,554	$5,347	
Supplies	$7,154	$2,500	
Travel	$1,365	$1,000	
Plot Fees	$2,100	$2,100	
Total	$20,000	$19,712	$0

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	2021
Salaries	$7,358[1]	$11,370[1]	
Benefits	$2,867[2]	$4,430[2]	
Supplies	$7,275[3]	$1,700[4]	
Travel	$1,000	$1,000	
Plot Fees	$1,500	$1,500	
Miscellaneous			
Total	$20,000	$20,000	$0

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

- Alternative organic program Blossom Protect+ Buffer Protect (70% bloom), Previsto (100% bloom), Thyme Gard (petal fall) performed as well as standard organic program Blossom Protect+ Buffer Protect (70% bloom), Previsto (100% bloom, petal fall) in one trial.
- Thyme oil (23%) product Thyme Gard treated trees had blossom infections lower but not statistically different than water treated checks in two trials.
- Cinnerate treated trees had significantly lower numbers of blossom infections than water treated checks in one of four trials.
- In young tree trials cluster removal performed best in Pennsylvania and 3 copper applications (Previsto NY, Basic Copper PA) performed best of spray applications to keep numbers of blossom infections low.
- Prohexodine Calcium 12 oz at tight cluster and petal fall reduced the numbers of blossom infections in New York and Pennsylvania young tree trials by 70-87% but provided no significant effect in Oregon.
- In 14th leaf Pink lady apple trees in Washington where fire blight cutting began when fruit were at 4 cm few new strikes (<1 avg) occurred after initial cutting. In 3rd leaf Gala in Oregon all cutting treatments significantly reduced the number of new strikes compared to the no-treatment control where aggressive, BMP and BMP+ Actigard treatments had zero additional strikes.
- In 14th leaf Pink Lady in WA leaving a long stub (approx. 4 in.) significantly reduced the number of cankers to progress through this year's growth onto structural wood compared to BMP and short stub cutting treatments. In 3rd leaf Gala in OR all cutting treatments used significantly reduced the number of cankers progressing to structural wood.
- In 3rd leaf Gala trees in OR 17% of no-treatment control and trees receiving breaking treatments had rootstock blight occur compared to zero in Aggressive, BMP, BMP No-Sanitize, and BMP+Actigard treatments.
- Breaking treatments left significantly more canker in the tree than other cutting treatments in OR leaving a source of infection for the subsequent year.

METHODS

Objective 1: Test materials to prevent bloom infections. This objective took place at research farms in Orondo, Washington (40-yr-old 'Red Delicious' apple); Corvallis, Oregon (60-yr-old 'Bartlett' pear and 5-yr-old 'Gala' apple); Geneva, New York (18-yr-old 'Gala' apple on B.9 rootstock), and Biglerville, Pennsylvania (12-year-old 'Cameo' apple on B.9 rootstocks). Experiments were arranged in a randomized complete block with 4 to 6 replications of single tree plots. Products were applied to the area of the tree to be inoculated 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. 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 18, 2020 Washington, April 15 or 19, 2020 Oregon (Gala and Golden Delicious apple, respectively), April 22, 2020, Pennsylvania Gala apple, May 21, 2020 New York Gala apple. Whole trees (OR, NY), or the bottom 8 feet (WA, PA) were inoculated. Trees were

visually evaluated for flower cluster infection every week following treatment and infection counts summed across all dates. Fruit was evaluated for russet fruit skin marking before fruit colored over. Statistical analysis was performed using mixed models, analysis of variance ANOVA, and multiple means comparison T test (LSD) SAS v 9.4.

Objective 2: Young Tree Trials. Young tree trials were performed in Orondo, WA (2-yr-old WA38 apple on M9.337 and G.41 rootstock); Geneva, NY (2-yr-old Gala apple on G.935 rootstock); Biglerville, PA (3-yr-old Gala apple on M.9 337 rootstock), and Corvallis, OR (6-yr-old Gala apple on M9.Nic 29 and 2-yr-old Gala apple on M9.337 rootstock). Experiments were arranged in a randomized complete block of four replicates with four trees per treated replicate in WA, NY, PA and 9 single tree replicates in each block in OR. Products were applied to wet, near dripping previously calibrated to equal 100 gal/A with a Stihl SR420 or Solo 451 mist blower backpack sprayer. At 100% bloom (of the king blooms) *Erwinia amylovora* was applied at $1x10^6$ CFU ml^{-1} dilution to lightly wet each cluster on April 23, 2020 Washington; April 15 or 19, 2020 Oregon; May 5, 2020 Pennsylvania; May 21, 2020 New York.

Objective 3: Test strategies to manage blocks once they are infected. Cutting trials were performed in Benton City, WA (14-yr-old cv. Pink Lady, rootstock M9.337); Biglerville, PA (3-yr-old cv. Gala, rootstock M9.337); Geneva, NY (15-yr-old cv. Idared, rootstock B.9) and Corvallis, OR (3-yr-old cv. Gala, rootstock M9.337). The experiment was arranged in a randomized, complete block design with 6-15 replications (15 WA, 10 PA, 6 OR, 6 NY) of 6-7 treatments applied to single tree plots where each tree had 1 to 15 WA (average 2.5), 4 to 23 (average 8.8) OR strikes per tree. No strikes developed in either NY or PA. Infections consisted of natural infection WA; inoculation April 24, 2020 OR ($9 x10^8$ CFU/ml); inoculation June 12, 2020 NY ($1 x10^6$ CFU/ml NY); May 5, 2020 PA ($1 x10^6$). Initial cutting was performed on June 2, 2020 WA, May 22, 2020 OR, July 5, 2020 NY, July 13, 2020 PA. Four and eight weeks after treatment additional cuts were made where new strikes were found. Trees were evaluated for the number of additional strikes, the length of infected area from new strikes, tree death, canker formation on structural wood and remaining canker size.

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.**

RESULTS

No noticeable fruit marking occurred with any treatments. The effect of materials on blossom infections is outlined in Table 1.

Table 1 Effect of Fire Blight Materials for Prevention of Blossom Blight[**]

Treatment	Rate per 100 gal	Timing	strikes per 100 clusters Washington*[‡]	Oregon[§]	Oregon[¥]	New York[#]	Pennsylvania[†]
Streptomycin standard[y] (Firewall 17)[x] (Firewall 50)[v]	28.8 oz[x] 2.7 oz[v]	100% bloom	2.8 ± 1.2 a	3.8 ± 1.5 a	1.5 ± 0.4 a	12.0 ± 2.2 bc	4.6 ± 7.5 c
Oxytetracycline standard[y] (Fireline 17)	28.8 oz[x] 16 oz[v]	50% bloom, 100% bloom, petal fall	8.2 ± 2 b	±	4.1 ± 0.6 b	27.5 ± 9.4 b	10.1 ± 9.4 a-c
Organic Standard Blossom Protect/Buffer + Soluble Copper (Previsto)	1.24 lb 8.75 lb 3 qt	50% bloom, 80% bloom, 100% bloom, petal fall	9.5 ± 1.3 bc	1.8 ± 0.4 a	---	7.0 ± 2.3 c	6.8 ± 6.2 a-c
Organic Alternative Blossom Protect/Buffer + Soluble Copper (Previsto) Thymegard	1.24 lb 8.8 lb 3 qt 2 qt	80% bloom, 100% bloom, petal fall	---	2.1 ± 0.8 a	---	---	---
Thyme Gard (0.5%)	2 qrt	80% bloom, 100% bloom +1 day, petal fall	17 ± 2.3 cd	---	---	---	4.9 ± 5.5 a-c
Alum[y]	8 lb	100% bloom, petal fall	22 ± 4.2 d		4.2 ± 1.6	28.0 ± 16.3 b	11.5 ± 6.2 ab
Cinnerate	1 qt	50% bloom, morning after inoc, petal fall	19 ± 3.5 d	---	---	24.0 ± 8.7 b	15.4 ± 26.6 a
Cinnerate	1 qt	100% bloom, petal fall	---	---	28 ± 1.7 c	---	---
Water-treated check	NA	100% bloom[x,v], +1 day[x], petal fall[x,v]	31 ± 7.1 d	24 ± 5 b	31 ± 1.7 c	80.1 ± 6.5 a	7.2 ± 3.4 a-c

[y] Amended with Regulaid: 30 fl. oz. per 100 gallons.

[x]Washington. Washington had additional 50% and petal fall applications. [v]Oregon.

* Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.

**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.

[‡]Washington application dates were: April 14 (20% bloom), April 16 (50% bloom), April 17 (80% bloom) and April 18 (full bloom), April 19 (full bloom plus 1 day), April 22 (petal fall). Inoculation was conducted on the evening of April 18, 2020 at full bloom (of king blooms) using a suspension of 50% freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain) and 50% live cells, which was prepared at 24 x 10^6 CFU per ml.

[§] Oregon Golden delicious apple, application dates were 17 April and 21 April, 2020 (petal fall). On the evening of 19 April, a motorized 25-gallon tank sprayer equipped with a hand wand was used to fog a suspension (~2 liters per tree) of freeze-dried cells of *Erwinia amylovora* strain 153N (1 x 10^6 CFU per ml).

[¥] Oregon Gala apple, application dates were 17 April and 21 April, 2020 (petal fall). Inoculation was done on the evening of 15 April.

[#]New York 2020 application dates were 29 April (tight cluster), 7 May (pink), 16 May (40% bloom), 20 May-(80% bloom), 22 May(100% bloom/petal fall), 29 May (petal fall/early terminal shoot growth).

[†]Pennsylvania application dates were: 4 April (tight cluster); 13 April (pink); 20 (20% bloom); 22 April (50% bloom; first inoculation); 23 April (+12 h post inoculation); 27 April (100% bloom, second inoculation); 28 April (+12 h post inoculation); 4 May (Petal fall). A frost occurred on 17 April, damaging a significant number of blossoms, thereby affecting results. In addition, the average temperature during the trial period was 49°F and no fire blight infection periods occurred.

Table 3. Effect of Products Applied for Prevention of Blossom and Shoot Blight in Young Trees on Blossom Blight.

Treatment	Rate per 100 gallons	*Timing*	Strikes per 100 clusters					
			Washington‡ 2nd leaf WA38	Oregon§ 6-yr-old Gala	Oregon§ 2-yr-old Gala	New York# 2nd leaf Gala	Pennsylvania† 3rd leaf Gala	
Inoculated Check	water	100% bloom, +1 day, petal fall	0 ± 0	41 ± 6 a	39 ± 7 a	77.2 ± 4.4 a	71 ± 20.1	a
Flower removal	NA	Pink	0 ± 0	---	---	---	0 ± 0	d
Basic Copper	1.5 lb	3 applications	5 ± 0	---	---	27.3 ± 3.3 b	8.3 ± 12.1	c
Previsto Or Cueva	3 qt 4 qt	3 applications	0 ± 0	---	---	5.5 ± 2.1 c	17.3 ± 17.2	c
PhCa[y z]	6 oz	tight cluster, petal fall	0 ± 0	---	---	6.5 ± 1.7 c	42.4 ± 24.0	b
PhCa[y z]	6 oz	full pink	---	34 ± 3 a	36 ± 4 a	29.5 ± 9.7 b	---	
PhCa[y z]	12 oz	tight cluster, petal fall	0 ± 0	---	---	10.5 ± 1.0 c	21.8 ± 23.5	c
Actigard	2 oz	10% bloom, 80% bloom, petal fall	0 ± 0	---	---	17.8 ± 2.3 bc	14.4 ± 16.1	c
PhCa[z y] Actigard	6 oz 2 oz	full pink	---	31 ± 5 a	32 ± 5 a	20.8 ± 3.9 bc	---	
Regalia	64 oz	10% bloom (pink), 80% bloom, petal fall	0 ± 0	33 ± 7 a	37 ± 5 a	26.5 ± 1.7 b	---	
Employ	2 oz	10% bloom, full bloom, petal fall	0 ± 0	---	---	23.5 ± 2.9 b	---	
Fireline 17 (standard oxytet)	28 oz	50% bloom, 100% bloom, PF	---	---	---	10.0 ± 1.3 c	---	

[y] Amended with surfactant (Regulaid) at 16 fl oz per 100 (Oregon) 32 oz per 100 gal (Washington).

[z] Kudos amended with 1 lb of ammonium sulfate per 100 gal (Washington), 6 oz. ammonium sulfate (Oregon).

‡ Washington application dates were: April 15, pink, April 19 (20% bloom), April 21 (50% bloom), April 23 (full bloom), April 24 (full bloom plus 1 day), April 28 (petal fall). Inoculation was conducted on the evening of April 23, 2020 at full bloom (of king blooms) using a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1.3 x10^6 CFU per ml. **Only 3 cluster infections occurred in the block.**

§Oregon application dates were: 11 April full pink). Inoculation was conducted on the evening of April 23. On the evenings of 15 and 19 April, a motorized 25-gallon tank sprayer equipped with a hand wand was used to lightly fog a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin and oxytetracycline sensitive pathogen strain), which was prepared at 1 x 10^6 CFU per ml (0.1 to 0.2 liters per tree).

#New York application dates were New York application dates were 29 Apr "tight cluster", 7 May "pink", 16 May-40% bloom, 20 May- 80% bloom, 22 May-100% bloom/petal fall, 29 May- petal fall/early terminal shoot growth.

†Pennsylvania application dates were: 6 Apr (tight cluster); 20 Apr (pink); 27 Apr (20% bloom); 1 May (50-80% bloom); 8 May (Petal fall). Frost occurred on 17 Apr and a freeze occurred on 9 May. There were no days indicating an infection period for fire blight during our trial. The average temperature was ~50°F during the test period.

**Values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to analysis of variance (F>0.05).

In 14th leaf Pink lady apple trees in Washington where fire blight cutting occurred when fruit were at 4 cm few new strikes (<1) occurred after initial cutting. In 3rd leaf Gala in Oregon all cutting treatments significantly reduced the number of new strikes compared to the no-treatment control where aggressive, BMP and BMP+ Actigard treatments had zero additional strikes. In 14th leaf Pink Lady in WA leaving a long stub (approx. 4 in.) significantly reduced the number of cankers to progress through this year's growth onto structural wood compared to BMP and short stub cutting treatments. In 3rd leaf Gala in OR all cutting treatments used significantly reduced the number of cankers progressing to structural wood. In 3rd leaf Gala trees in OR 17% of no-treatment control and trees receiving breaking treatments had rootstock blight occur compared to zero in Aggressive, BMP, BMP No-Sanitize, and BMP+Actigard treatments. Breaking treatments left significantly more canker in the tree than other cutting treatments in OR which signifies a source of infection in future years.

In Pennsylvania and New York cutting trials were performed but no infections occurred and thus cutting treatments were not conclusive.

Table 4. Effect of treatment on the number of new strikes after initial cutting of fire blight infections.

	Washington Pink lady 14th leaf		Oregon Gala 3rd leaf		New York* Idared 15th leaf	Pennsylvania Gala 3rd leaf
BMP	0.7 ± 0.30	ab	0 ± 0	a	---	---
Aggressive	0 ± 0	a	0 ± 0	a	---	---
BMP NO-sanitize	0.3 ± 0.19	ab	1.2 ± 0.5	a	---	---
Short Stub	0.4 ± 0.21	ab	---		---	---
Long Stub	0.3 ± 0.19	ab	---		---	---
Breaking	0.9 ± 0.66	b	1.3 ± 0.3	a	---	---
NTC	0.5 ± 0.27	ab	11.3 ± 1.5	b	---	---
BMP+Actigard	---		0 0	a	---	---

Table 5. Effect of treatment on the average cumulative length of cankers left in trees at the end of the season (cm).

	Washington Pink lady 14th leaf		Oregon Gala 3rd leaf		New York Idared 15th leaf	Pennsylvania Gala 3rd leaf
BMP	1.1 ± 0.12	a	0 ± 0	a	---	---
Aggressive	0 ± 0.03	a	0 ± 0	a	---	---
BMP NO-sanitize	1.1 ± 0.14	a	3 ± 3	ab	---	---
Short Stub	0.8 ± 0.04	a	---		---	---
Long Stub	1.0 ± 0.16	a	---		---	---
Breaking	3.2 ± 0.21	a	5 ± 2	b	---	---
NTC	29.1 ± 4.25	b	155 ± 31	c	---	---
BMP+Actigard	---		0 ± 0	a	---	---

Table 6. Effect of treatment on the percentage of strikes progressing to structural wood.

	Washington Pink lady 14th leaf		Oregon Gala 3rd leaf		New York Idared 15th leaf	Pennsylvania Gala 3rd leaf
BMP	11.9 ± 6.6	ab	0 ± 0	a	---	---
Aggressive	0 ± 0	a	0 ± 0	a	---	---
BMP NO-sanitize	12.4 ± 7.0	b	19 ± 9	b	---	---
Short Stub	14.4 ± 7.3	b	---		---	---
Long Stub	0 ± 0	a	---		---	---
Breaking	18.7 ± 5.5	b	12 ± 3	ab	---	---
NTC	10.5 ± 3.8	ab	64 ± 6	c	---	---
BMP+Actigard	---		0 ± 0	a	---	---

Table 7. Effect of treatment on the number of infected trees which develop rootstock blight (fall observation).

	Washington Pink lady 14th leaf	Oregon Gala 3rd leaf	New York Idared 15th leaf	Pennsylvania Gala 3rd leaf
BMP	0 ± 0	0 ± 0	---	---
Aggressive	0 ± 0	0 ± 0	---	---
BMP NO-sanitize	0 ± 0	0 ± 0	---	---
Short Stub	0 ± 0	---	---	---
Long Stub	0 ± 0	---	---	---
Breaking	0 ± 0	1 of 6	---	---
NTC	0 ± 0	1 of 6	---	---
BMP+Actigard	---	0 ± 0	---	---

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension
WTFRC Project Number: CP-18-102

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

PI: Achour Amiri
Organization: WSU-TFREC
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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:** Shelli Tompkins/Katy Roberts
Telephone: 509-293-8803 **Email address:** shelli.tompkins@wsu.edu/arcgrant@wsu.edu
Supervisor or Station Manager name and email address: Chad Kruger, cekruger@wsu.edu

Item	2018	2019	2020	2021
Salaries[1]	14,400	14,976	15,575	0
Benefits[1]	6,385	6,640	6,906	0
Wages[2]	5,760	5,990	6,230	0
Benefits[2]	545	567	590	0
Equipment	0	0	0	0
Supplies[3]	1,500	3,000	3,000	0
Travel[4]	1,070	1,070	1,070	0
Miscellaneous	0	0	0	0
Plot Fees[5]	2,700	2,700	0	0
Total	32,360	34,943	33,371	0

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.

Reseason for extension: Fruit from this trial were harvested in October 2020 and are stored in cold room to assess decay incidence and types. The results will be available in April-May 2021.

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).

Reseason for extension: Decay incidence results are needed from Objective 1 to finalize analyses of impact of weather conditions on Botrytis infections and gray mold development in cold storage. The analysis is expected to be completed by June 2021.

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-May 2021**.

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).

Reseason for extension: This genetic investigation include Botrytis isolates collected on dacyed apples after several months of storage. The fruit, from the 2020 season, are currently in cold storage and depending on gray mold development, this study is expected to be concluded by June 2021.

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.

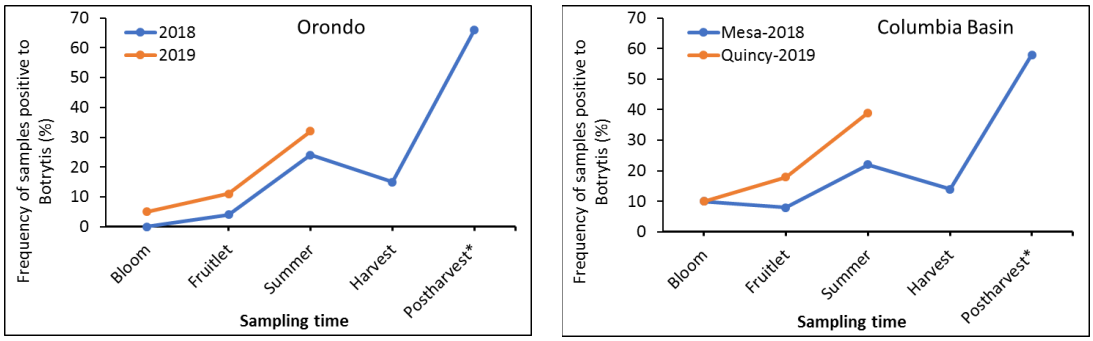

Figure 1. Evolution of *Botrytis* incidence throughout the preharvest growing season as detected by qPCR. 2019 Sampling after harvest are being analyzed.

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.

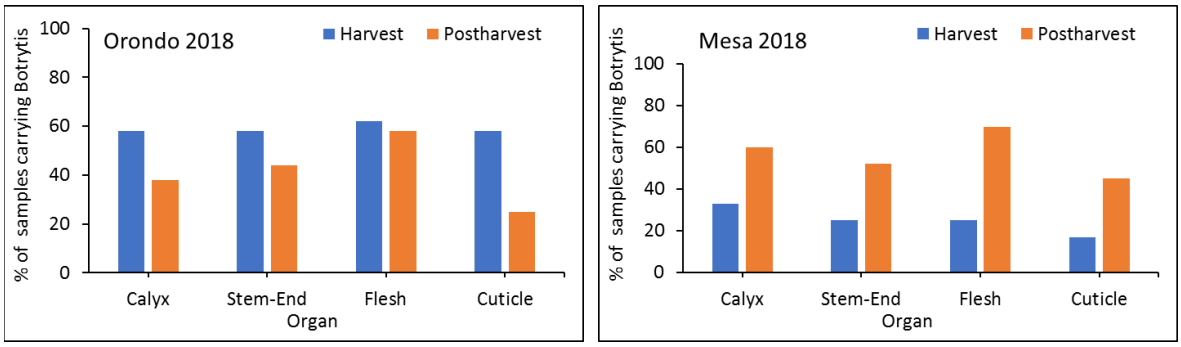

Figure 2. Incidence of *Botrytis cinerea* on different organs of the fruit at commercial maturity (harvest time) in organic and conventional orchards in 2018. Samples from 2019 Are being analyzed currently.

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: 2021

- Collect final data from gray mold incidence from cold storage (objective 1) by May 2021
- Conclude analyze of impact of weather data on Botrytis infections and gray mold development in cold storage by June 2021
- Conclude the genetic analyses of *Botrytis* collected in the two previous seasons.

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Optimization of release strategies for sterile codling moth

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Total Project Request: Year 1: $125,000 Year 2: $125,000 Year 3:

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

WTFRC Budget: ***(If no WTFRC expenses are anticipated, type none and delete table)***

Item	2019	2020	2021
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	0

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) to be matched 1:1 by FFAR Fellowship. [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.

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 – **Partially 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.

2021 Project Goals and Activities
Apple orchards in Brewster, WA will be used as sites for releases of sterilized codling moths from the Osoyoos-Kootenay Sterile Insect Release (OKSIR) facility. 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) Determine the Optimal Time of Sterile CM Release

Time of release:

Preliminary data suggests that sterile CM dispersion may be impacted by the time of day they are released. Additional data are needed to determine the optimal time to release sterile CM. Details of this experiment are outlined in the project proposal

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

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

Objective 2 – Release Factors 2020:

1. Release altitude (20 trap layout, central release), 8 replicates
   - No significant difference in recapture by release altitude
   - No significant difference in aggregation among the four release altitudes
   - Recapture at the closest traps significantly decreased with increasing altitude
2. Distributed or point releases (20 trap layout, central release), 14 replicates
   - Significantly more CM were recaptured when released at the center
   - Aggregation is significantly greater when moths are released at the center
   - More CM were captured at traps far from the center when they were not center released
3. Unmanned Aircraft System (UAS) release or hand release
   - Recapture was not significantly different for hand or UAS released moths
   - Aggregation was not different between moths released by hand or UAS

METHODS: Outline the methods to be employed.

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

Source of codling moths
Recently eclosed, mixed-sex, internally-marked sterile CM were obtained from the OKSIR facility in Osoyoos, British Columbia, Canada.

Sterile codling moth dispersion experiments
Handling of Codling Moths: Upon arrival at field sites, moths were 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 locations in the blocks.

Assessing treatments: Marked moths were 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 placed in a 20-trap grid pattern, approximately 30 meters apart, in order to measure dispersion of moths released from the center of the block.

Data Analysis: Average sterile moth recapture was reported as a percent of total released for each treatment and significant differences were determined. Morisita's index of dispersion was used to measure degree of evenness or aggregation of sterile moth dispersion for each replicate. An analysis of variance was conducted on Morisita's indices and % recapture calculated for replicates to compare treatments and determine if there were significant treatment differences.

RESULTS:

Experiment 1: Release Location – UAS vs. Hand
RECAPTURE

There were significant differences in the recapture of moths released by the four methods ($F=3.769$, $df=3$, $P=0.016$). Those differences were found to be between the hand-nine even points method (0.798%±0.324) and both UAS methods: center (2.478%±0.636) ($t=2.353$, $df=19$, $P=0.030$), and evenly spread (3.573%±0.737) ($t=3.447$, $df=18$, $P=0.003$). There were no significant recapture differences between the hand-center release (1.617%±0.669) and hand-nine even points release, nor between the hand-center release and either UAS release.

AGGREGATION
Absolute Capture:

There were significantly different Iδ indices calculated for the four treatments ($F=2.928$, $df=3$, $P=0.044$) for the absolute number recaptured in traps. Differences were however only found between hand-center (mean Iδ=2.633±0.475) and UAS-even (mean Iδ=1.427±0.138) releases ($t=2.438$, $df=13$, $P=0.030$), indicating that sterile moths released at the center of the orchard were significantly more aggregated about the point of release than those released in a more spread out pattern. Moths released by hand-at nine evenly spaced points (mean Iδ= 1.680±0.221) and UAS-center (mean Iδ=1.427±0.138) were not significantly more or less aggregated from each other or the other two treatments.

Percent of Replication's Recapture:

There were significant treatment differences found between the four release methods' (F=4.021, df=3, P=0.012) calculated Iδ by trap percent of total recaptured sterile C. pomonella. Moths released by hand at the center of blocks were highly aggregated (average Iδ=3.251±0.556), those released by hand at nine even points were less aggregated (average Iδ=2.359±0.442), followed by releases by UAS at the center (average Iδ=1.943±0.276), and least aggregated were the moths released by UAS spread throughout the orchard (average Iδ=1.403±0.168) approximating the hand release at nine evenly spaced points. However, only moths released by hand at the center were significantly more aggregated on average than those released by UAS at the center (t=2.107, df=19, P=0.049), and UAS approximating nine even points (t=3.810, df=15, P=0.006). There were no significant differences found between the two hand releases, nor between the two UAS releases.

DISTANCE OF RECAPTURE

Within Treatments

1) Release by hand at one central location

There were significant differences in recapture by distance from center (F=2.784, df=5, P=0.180) for moths released by hand at a single central location. More moths released at the center or test orchards were captured at the closest traps (15 meters) more than at traps 62 meters (t=2.154, df=32, P=0.017), and 75 meters (t=2.715, df=34, P=0.010) away . In addition, moths were significantly more likely to be captured in traps 33.5 meters from the release than those 75 meters from the center (t=2.077, df=109, P=0.020).

2) Release by hand at nine evenly spaced locations

Although average recapture was low at all trap distances from the center , there were no significant differences in capture by distance (F=0.849, df=5, P=0.516) when moths were released in an evenly spaced pattern in orchard blocks.

3) Release by UAS at 35 meters above the center of the orchard

Sterile moths released by UAS at the center of the orchards were significantly more likely to be captured in traps 15 meters from the center than those 45 meters (t=2.395, df=41, P=0.021), 54 meters (t=2.568, df=36, P=0.015), and 75 meters (t=3.110, df=31, P=0.004) away . In addition, they were less likely to be recaptured in traps 45 meters (t=2.099, df=81, P=0.039), 54 meters (t=2.370, df=89, P=0.020), and 75 meters (t=3.179, df=73, P=0.002) from the center than in traps 33.5 meters away from the point of release.

4) Release by UAS at 35 meters above nine evenly-spaced locations

There were no significant recapture differences by distance from center of block when moths were released by UAS approximating nine evenly spaced points in the orchards (f=0.263, df=5, P=0.933); recapture was highest at all distances for this treatment .

Between Treatments

1) Distance of 15 meters from the center of the orchard

There were significantly different treatment impacts on moths captured at this distance (F=3.934, df=3, P=0.010). Fewer moths were captured at 15 meters from the center of the orchard when they were released by hand at nine locations than by hand at the center (t=2.361, df=33, P=0.024), UAS at the center (t=3.317, df=33, P=0.002), and UAS approximating the nine locations (t=4.134, df=41, P<<0.001).

2) Distance of 33.5 meters from the center of the orchard

There were significant treatment differences on recapture of moths at 33.5 meters from the center of the orchard (F=11.407, df=3, P<<0.001). More were captured when they were released by hand at the center (t=2.473, df=80, P=0.015), by UAS at the center (t=4.191, df=63, P<<0.001), and by UAS approximating nine evenly spaced points (t=6.524, df=72, P<<0.001) than when they were released by hand at nine evenly spaced locations. There were also significantly fewer recaptured when they were released by hand at the center than UAS

at the center (t=2.446, df=86, P=0.016), or UAS at nine evenly spaced locations (t=3.720, df=105, P<<0.001).

3) Distance of 45 meters from the center of the orchard

At this distance, there were significant differences in recapture of moths by treatment (F=3.027, df=3, P=0.033): fewer moths were recaptured when released by hand at nine evenly spaced points than when they were released by UAS at nine evenly spaced points (t=4.360, df=44, P<<0.001), and fewer were recaptured when released at the center by UAS than by UAS approximating the nine evenly spaced points (t=2.133, df=54, P=0.038).

4) Distance of 54 meters from the center of the orchard

The results of an analysis of variance showed that there were recapture differences among the treatments (F=9.783, df=3, P<<0.001). Traps at 54 meters from the center recaptured significantly more sterile codling moths when moths were released by UAV at nine points than by hand at the center (t=3.022, df=110, P=0.003), and by hand at nine points (t=5.888, df=70, P<<0.001). Also, moths released by hand at nine evenly spaced locations were significantly less recaptured than those released by UAS at the center of the orchard (t=2.751, df=79, P=0.007), and fewer of the moths released by UAS at the center were recaptured than those by UAS approximating nine evenly spaced points (t=3.088, df=104, P=0.002).

5) Distance of 62 meters from the center of the orchard

An ANOVA indicated that there were treatment differences (F=7.958, df=3, P<<0.001) for recapture of moths at this distance. Fewer moths released by hand at the center of the orchard were recaptured than moths released by either UAS at the center (t=2.255, df=78, P=0.027) or UAS approximating the nine evenly spaced points (t=3.700, df=76, P<0.001). Likewise, moths released by hand at nine evenly spaced points were recaptured less than both UAS at center (t=2.786, df=70, P=0.007) and UAS approximating the none evenly spaced points (t=4.255, df=69, P<<0.001) released moths.

6) Distance of 75 meters from the center of the orchard

Significantly more moths that were released by the UAS at nine evenly spaced points were recaptured at this distance than those released by hand at the center (t=4.306, df=95, P<<0.001), hand at nine evenly spaced points (t=5.447, df=67, P<<0.001), and UAS at the center of the orchard (t=3.481, df=84, P<0.001). Also, significantly fewer moths released by hand at nine evenly spaced were recaptured than those released by UAS at the center of the block (t=2.857, df=92, P=0.005).

	Distance from center	*15 meters*	*33.5 meters*	*45 meters*	*54 meters*	*62 meters*	*75 meters*
Average # recaptured (mean±SEM) by release method	**Hand-center**	13.429±3.488	7.268±1.395	8.321±3.637	6.535±1.967	4.304±1.002	3.357±1.265
	Hand-9 points	4.714±1.209	3.429±0.680	3.5±1.184	2.964±0.696	3.268±0.803	2.196±0.646
	UAS-center	17.607±3.694	14.25±2.491	7.571±1.980	7.411±1.459	9.625±2.136	5.679±1.033
	UAS-~9 points	15.536±2.322	15.518±1.724	13.536±1.974	14.75±1.877	13.339±2.227	13.321±1.938

Table 1. Average recapture of sterile codling moths released by four methods at fixed trap distances.

Experiment 2: Release Altitude

RECAPTURE

Among the four treatments, 0-5 meters altitude release (2.355%±0.623), 10-15 meters altitude release (2.055%±0.696), 20-25 meters altitude release (1.116%±0.385), and 30-35 meters altitude release (1.469%±0.597), overall percent of recapture of released sterile codling moths was not significantly different (f=0.910, df=3, P=0.449).

AGGREGATION

Absolute Capture:

Moths released at the center of the orchard at four different altitudes, 0-5 meters altitude release (mean Iδ=2.267±0.336), 10-15 meters altitude release (mean Iδ=2.144±0.244), 20-25 meters altitude release (mean Iδ=2.898±0.478), and 30-35 meters altitude release (mean Iδ=2.490±0.649), overall percent of recapture of released sterile codling moths was not significantly different (f=0.573, df=3, P=0.638).

Percent of Replication's Recapture:

There were not significant differences in the aggregation index for percent recapture (f=0.815, df=3, P=0.496) of moths released at four different altitudes. The mean±SEM Morisita's index for moths released at 0-5 meters by hand was 2.139±0.363, at 10-15 meters by UAS was 2.183±0.280, 20-25 meters by UAS was 3.198±0.581, and at 30-35 meters by UAS was 2.657±0.674.

DISTANCE OF RECAPTURE

Within Treatments

1) 0-5 meters altitude release by hand

There were significant differences in recapture by distance from center (F=11.150, df=5, P<<0.001) for moths released by hand at 0-5 meters. More moths released at the center of test orchards were captured at the closest traps (15 meters) than at traps 33.5 meters (t=2.455, df=21, P=0.023), 45 meters (t=2.434, df=28, P=0.022), 54 meters (t=3.953, df=16, P=0.001), 62 meters (t=3.915, df=17, P=0.001), and 75 meters (4.164, df=16, P<0.001) away . In addition, moths were significantly more likely to be captured in traps 33.5 meters from the release than those 54 meters (t=, 2.836, df=44, P=0.007), 62 meters (t=2.734, df=47, P=0.009), and 75 meters (t=3.286, df=44, P=0.002) from the center.

2) 10-15 meters altitude release by UAS

There were significantly more moths recaptured at the center of the block than at the edges when they were released from this altitude (f=4.858, df=3, P<0.001). At a release altitude of 10-15 meters, more moths were recaptured at 15 meters than at 33.5 meters (t=2.538, df=22, P=0.019), 54 meters (t=2.415, df=22, P=0.024), 62 meters (t=3.113, df=20, P=0.005), and 75 meters (t=3.799, df=16, P=0.002). Also, moths were significantly more likely to be recaptured at 33.5 meters than at 75 meters (t=2.109, df=44, P=0.041).

3) 20-25 meters altitude release by UAS

There were no significant differences in recapture at any distance from the center when moths were released at 20-25 meters (F=1.559, df=3, P=0.175). Recapture at all distances was generally low for moths released at this altitude .

4) 30-35 meters altitude release by UAS

As distance from the center increased, significantly fewer sterile C. pomonella were recaptured (F=4.253, df=3, P=0.001) from releases at this altitude. More moths were recaptured at 15 meters from the center of the orchard than at 45 meters (t=2.476, df=17, P=0.024), 54 meters (t=2.267, df=17, P=0.037), and 75 meters (t=2.484, df=16, P=0.024) away . Also, recapture was higher at 33.5 meters from the center of the orchard than in traps 45 meters (t=2.526, df=43, P=0.015), 54 meters (t=2.165, df=43, P=0.036), and 75 meters (t=2.631, df=36, P=0.012) away.

Between Treatments

1) Distance of 15 meters from the center of the orchard

There were significant treatment differences in recapture at the closest distance from the release (f=3.668, df=3, P=0.017). More were captured at this distance from the 0-5 meter release than the 20-25 meter release (t=3.118, df=22, P=0.005) . Likewise, more were recaptured from the 10-15 meter release than the 20-25 meter release (t=2.255, df=27, P=0.032) recaptured.

2) Distance of 33.5 meter from the center of the orchard

There were no differences in recapture at this distance (f=2.314, df=3, P=0.079) .

3) Distance of 45 meters from the center of the orchard

There were no differences in recapture at this distance (f=1.693, df=3, P=0.178) .

4) Distance of 54 meters from the center of the orchard

At this distance from the center of the orchard there were significant differences (f=3.394, df=3, P=0.020). More moths released at 10-15 meters altitude were recaptured than those released at 20-25 meters altitude (t=2.431, df=46, P=0.019), and 30-35 meters (t=2.332, df=47, P=0.024).

5) Distance of 62 meters from the center of the orchard

There were no differences in recapture at this distance (f=0.280, df=3, P=0.840) .

6) Distance of 75 meters from the center of the orchard

There were no differences in recapture at this distance (f=1.129, df=3, P=0.340) .

		Distance from center					
		15 meters	***33.5 meters***	***45 meters***	***54 meters***	***62 meters***	***75 meters***
Average # recaptured (mean±SEM) by release altitude	**0-5 m (hand)**	27.250±5.408	12.781±2.346	10.875±4.004	5.438±1.098	5.531±1.237	4.281±1.090
	10-15 m (UAS)	19.000±3.848	8.219±1.798	9.313±3.556	8.688±1.851	6.000±1.623	4.031±0.844
	20-25 m (UAS)	8.375±2.719	5.250±1.756	5.313±1.477	3.656±0.925	4.125±1.411	2.4375±0.599
	30-35 m (UAS)	13.875±4.332	9.000±2.190	2.813±1.096	3.813±1.096	5.219±1.704	3.000±0.637

Table 2. Average recapture of sterile codling moths released at four altitudes at fixed trap distances.

DISCUSSION

To study the dispersion and recapture of released sterile codling moths, we tested the release variables of where in the orchard block, and how high above the canopy moths were released. Unmanned aerial systems, paired with the sterile insect technique, have become a novel new technology used in the centuries long struggle against the codling moth, and they are being used commercially and experimentally in orchards in many places, though to fully maximize the impact of this technology a thorough understanding of where best to deliver sterile codling moths is needed. Likewise, we need to understand if there are any negative impacts associated with being released by UAS when compared to the most basic method available: a release by hand from the ground. We found that CM released by hand at evenly spaced locations were recaptured significantly less than when released by UAS at 35 meters altitude on a flight path approximating the same locations. There was no difference in the degree of aggregation/dispersion found by either release method (Hand or UAS), and we also did not find altitude to be a significant factor in recapture or dispersion. Practical and economic considerations must be considered by individual farmers when they employ SIT codling moths as a commercial product at their own cost on an orchard scale – i.e. if they do their own releases it may be less expensive and equally effective to release by hand in one to a few locations when their orchards are small. In our 10-acre test blocks, after moths were marked for recapture, we could walk from the truck to the pre-marked center of the orchard and back for a hand release in less than five minutes, and walk to the nine evenly spaced locations in 10-15 minutes; both UAS releases covered the same ground in less than five minutes from the time the flight began to when it returned. Large commercial orchards may find that the labor costs of hand release are higher than the cost of hiring a service to release by UAS. Considering the orchard owner that will be conducting their own releases, as well as those that plan to release by UAS, it is important to understand where to release moths to maximize their effectiveness. Our data demonstrate that a single release at the center of 10 acre orchard blocks either manually or by UAV at any altitude is sufficient to allow moths to disperse independently to the edges of the block while retaining the maximum number of moths within the targeted treatment area while avoiding the need to travel to multiple sites for manual release. A UAV does however have some disadvantages, such as an inability to fly in high wind, rain, or in proximity to airports. In these cases, those wishing to release sterile codling moths will be heartened to know that a release by hand at the center of the orchard will still deliver sufficient moths throughout a 10-acre block.

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

Project Title: Can we get codling moth females to stop laying eggs on apple?

PI: William B. Walker III
Organization: USDA-ARS
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Email: william.walker@usda.gov
Address: Temperate Tree Fruit and Vegetable Research Laboratory
Address 2: 5230 Konnowac Pass Rd
City/State/Zip: Wapato, WA 98951

Cooperators: Dr. Man-Yeon Choi, ARS Corvallis, OR

Total Project Request: **Year 1:** $56,110 **Year 2: $58,817** **Year 3:** $61,610

Other funding sources

Agency Name: USDA-ARS, Pacific West Area Office
Amt. awarded: $35,000
Notes: Area Office awarded money to purchase a flight tunnel and Track3D system. The Track3D system is comprised of cameras and software to monitor insect behavioral responses in a flight tunnel.

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

Item	Year 1 - 2018	Year 2 -2021	Year 3 - 2022
Salaries	$37,306	$39,282	$41,321
Benefits	$13,804	$14,535	$15,288
Wages			
Benefits			
Equipment			
Supplies	$5,000	$5,000	$5,000
Travel			
Miscellaneous			
Plot Fees			
Total	$56,110	**$58,817**	$61,610

Footnotes: Salaries and benefits are requested for a full-time GS-6 Lab Technician. Supplies are for cloning ORs, oligonucleotide primer synthesis, Cas9 mRNA and kits for making CRISPR guide RNAs.

AMENDMENT

This section serves notification that there has been a change in PI for this project. After completion of the first year of the project, the lead PI, Dr. Stephen F. Garczynski passed away in February 2019. Dr. Garczynski had drafted a continuation report for this proposal. There was an agreement between USDA-ARS and WTFRC that this project could be paused while Dr. Garczynski's Research Geneticist position was vacant. New PI William B. Walker had been indicated on the continuation report as a "Cooperator" and was hired in October 2020 to fill the vacant Research Geneticist position. Dr. Walker intends to complete the project as planned and was advised to submit the continuation report as drafted by Dr. Garczynski. During the time this project was paused, no further project work was performed. The equipment items mentioned in "other funding sources" were obtained by Dr. Garczynski and are now available to facilitate completion of the project.

OBJECTIVES

1) Identify and clone Odorant Receptors expressed in the abdomen tip of codling moth females. More than twenty odorant receptors (OR) were identified in a transcriptome generated from abdomen tips of codling moth females. Ten of these receptors have been cloned this past year. In year two, the remaining ORs will be cloned. The cloned ORs will be used in expression assays (not funded by this proposal) to determine activating plant compounds.

2) Knock-out OR genes using CRISPR/Cas9 genome editing. CRISPR/Cas9 genome editing is fully developed for codling moth and will be used to knock-out genes expressed in the female abdomen tip. In the past year, materials to knock-out ORs using CRISPR have been generated or obtained from commercial sources. Codling moth eggs have been injected with different guide RNAs targeting five ORs and are currently being analyzed for effectiveness. This year, laboratory populations of codling moth with inactive OR genes will be generated for use in oviposition assays.

3) Determine which genes are essential for oviposition site selection. The hypothesis tested here will be that inactive OR genes for specific plant volatiles will alter female codling moth oviposition site selection. An oviposition assay for codling moth, developed by researchers in Sweden, will be used to assess the effects of OR gene knock-out populations. In year one, initiation of testing and adapting the oviposition assay was done. Because of the unsatisfactory results, we will continue to develop bioassays to assess the behavioral effects of CRISPR gene knock-outs. In September, ARS Pacific Area Office provided $35,000 for a flight tunnel system that will monitor behaviors using cameras and specialized behavioral analysis software. That system will be set up in January 2019.

SIGNIFICANT FINDINGS

- Transcripts for three of the 10 OR genes cloned are produced by alternative splicing (multiple transcripts/proteins produced by a single gene).
- In collaboration with Dr. William Walker (SLU Alnarp, Sweden), a female expressed OR that is activated by apple odorants or a male produced pheromone or has been identified.

METHODS

The CRISPR (clustered regularly interspaced palindromic repeats)/Cas9 (CRISPR associated protein 9) genome editing system has been used extensively in the past few years to study protein function in many organisms. Interest in the CRISPR/Cas 9 system to control insects or to overcome insecticide resistance is now coming of age with substantial investments in this technology by Bayer Crop Sciences. A CRISPR/Cas 9 genome editing system for use in codling moth (refer to Figure 1 for a summary) is fully developed and has been successfully used to knock-out an OR gene thought to be involved in codlemone detection (Garczynski et al., 2017). These methods will be used to knock-out OR genes expressed in codling moth females to evaluate protein function and determine which genes are critical for oviposition site selection by codling moth females.

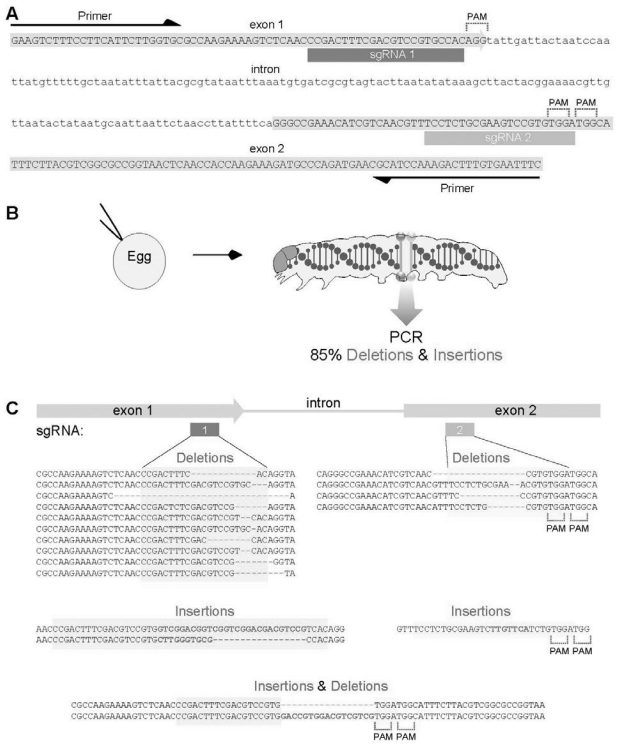

Figure 1. CRISPR/Cas9 genome editing of the *maleless* gene in codling moth. **A)** The region of the codling moth *maleless* gene (parts of exons 1 and 2 and the intron that connects them) targeted for genome editing. Primer sequences are denoted by single-headed arrows, regions targeted by single guide RNAs are denoted by dark grey (exon 1) and light grey (exon 2) boxes and coding sequences are highlighted in light grey. **B)** Pictorial representation of CRISPR/Cas9 injection and analysis. Eggs are injected using a microinjection needle containing Cas9 mRNA and sgRNAs, which are targeting exons 1 and 2 (saline used as control). Emerging neonate larvae are collected, their DNA is extracted and amplified using PCR with primers flanking the targeted exons. PCR products are cloned and 10 representative clones are sequenced to identify insertions and deletions in the targeted region generated by CRISPR/Cas9. **C)** Pictorial representation of the codling moth *maleless* gene and examples of insertions and deletions generated from CRISPR/Cas9 genome editing. Targeted regions of the *maleless* gene are denoted by dark grey (exon 1) and light grey (exon 2) boxes. The edited sequences are expanded and deletions are denoted by hyphens (-) and insertions are denoted by letters above or below hyphens.

1) Identification and cloning ORs expressed in the abdomen tips of codling moth females.

Gene transcripts encoding ORs expressed in antennae of codling moth males and females have been identified in transcriptomes (Walker et al., 2016). Recently, it was discovered that an OR was expressed in the abdomen tip of codling moth females (Garczynski et al., 2017). To determine the extent of OR expression, a female abdominal tip transcriptome was prepared late in 2017. Initial analysis of the transcriptome revealed that 38 potential OR transcripts are present, 28 of which are also found in the antennae (Walker et al., 2016, Garczynski and Walker, Unpublished data). Many of the ORs expressed in the codling moth abdomen tip are related to those activated by plant volatiles in other moths (de Fouchier et al., 2017). To confirm the initial identifications, PCR will be performed with oligonucleotide primers designed to amplify each individual OR found in the transcriptome. Once confirmed, the full-length transcript sequence will be determined using a PCR amplification technique. DNA sequence information gained from this step will be used in subsequent steps to design guide RNAs (see below).

2) Design and production of single guide RNAs.

Single guide RNAs (sgRNA) contain a target-specific nucleotide sequence of the gene of interest along with nucleotide sequence necessary for Cas9 (a protein that cleaves DNA) binding. Using the DNA sequence information above, the genome region containing target sites of the codling

moth OR genes of interest will be identified. With the gene sequence information, a minimum of three regions containing the features needed for CRISPR/Cas9 genome editing will be targeted for sgRNA design and production. Working in collaboration with a Chinese research group that has recently completed sequencing the codling moth genome, the genes for all the ORs expressed in the abdomen tip transcriptome have been identified. Knowing OR gene structure from the codling moth sequenced genome makes designing sgRNAs a relatively easy task. To generate sgRNAs specific to the selected codling moth OR genes, overlapping oligonucleotide primers containing the appropriate nucleotide sequence features will be designed and synthesized. These oligonucleotide primers will then be amplified in PCR reactions to generate a DNA template that will be used to produce sgRNAs in a test tube reaction. The sgRNAs are generated from the DNA template with an *in vitro* transcription kit which makes RNA from a DNA template. Once the sgRNAs are produced they are ready for use in genome editing experiments. The procedures for generating sgRNAs are already developed in the laboratory (Garczynski et al., 2017).

3) CRISPR/Cas9 genome editing of codling moth OR genes.

CRISPR/Cas9 genome editing takes place when an appropriate sgRNA and Cas9 protein are present in the same cell. For this to occur, sgRNA and Cas9 mRNA are co-injected into early stage eggs, and the sgRNAs are transported to the cell nucleus by the Cas9 protein. Once in the cell nucleus, the Cas9 protein/sgRNA complex binds to the target gene and the double-stranded nuclease of Cas9 cleaves the gene creating a mutation that can ultimately result in the loss of protein function. Procedures for performing CRISPR/Cas9 genome editing in codling moth have already been developed (Garczynski et al., 2017). To knock-out our selected OR genes, target specific sgRNAs along with Cas9 mRNA will be co-injected into freshly laid eggs. The genome editing takes place shortly after injection. At this stage, DNA will be extracted from neonate larvae to analyze the effectiveness of the genome editing (see below). It is important to note, that this initial analysis step is necessary because the efficiency of sgRNAs are not equal, and why three sgRNAs are designed for each target gene. The results of this initial analysis will identify the best sgRNAs for large scale knock-out experiments.

4) Analyzing the effectiveness of CRISPR/Cas9 genome editing using molecular techniques.

Two molecular techniques, DNA sequencing and high-resolution melt (HRM) analysis, are used to determine the effectiveness and extent of CRISPR/Cas9 genome editing, and to verify that the mutations made will knock out protein production (Garczynski et al., 2017). For each molecular technique, oligonucleotide primers surrounding the region of the gene being targeted are designed for PCR amplification. These oligonucleotide primers are first used in PCR reactions to amplify genomic DNA of treated and untreated insects, then the PCR products are cloned and sequenced to verify CRISPR/Cas9 generated mutations. Once success of the CRISPR/Cas9 genome editing technique is verified by cloning and sequencing, DNA from larger numbers of injected insects are analyzed with HRM analysis. To perform this assay, PCR amplifications of the targeted gene are done using a real-time PCR machine and the resultant PCR products are subjected to HRM analysis. HRM analysis is a high-throughput technique that is used to detect minor changes in the mutated gene when compared to its unaltered counterpart. Once CRISPR/Cas9 genome editing is verified molecularly, analyses of the effects of these mutations will be assessed using bioassays.

5) Assays to determine the effects of CRISPR/Cas9 genome editing on egg laying.

Oviposition in moths is a two-step process; females must first find their host plant, and after host recognition, oviposition sites need to be identified (Honda 1995). Ovipositing females use plant volatiles to locate the host plants and then use contact evaluation of plants to detect less or non-volatile chemical compounds to determine suitability for egg laying (Honda 1995). For codling moth, oviposition is stimulated by apple odor (Wearing 2016), and several volatiles in apple odors including α-farnesene, β-farnesene and nonanal have been specifically identified as stimulants (Sutherland et al.

1977, Witzgall et al. 2005). ORs expressed in the female antennae are thought to play important roles in host plant finding, bringing the moth in proximity of a suitable oviposition site. The hypothesis to be tested in this project is specific ORs expressed in the abdomen tip are important for contact evaluation in determining plant suitability for egg laying.

To determine if an OR gene knocked out by CRISPR/Cas9 genome editing has an effect on codling moth oviposition, a bioassay will be used. The assay uses jars or cups in which 10 mated females are placed in the presence or absence of varying concentrations of a volatile compound (see below for compounds to be tested). After one hour, females are removed from their container and eggs are counted. It is expected that oviposition stimulants will result in a greater amount of eggs laid vs controls. To determine which ORs play key roles in detecting oviposition stimulants, CRISPR/Cas9 edited females will be placed in the cups in the presence or absence of varying concentrations of volatile compounds and a positive result would be fewer eggs laid compared to unedited females. To determine statistical significance, full factorial analysis with concentration, treatment and their interaction as the dependent variables. All statistical analyses will be done using SAS 9.4 with the GLIMIX procedure.

There are at least 64 volatile compounds found in headspace collections from apple at different phenological stages (Bengtsson et al 2001). Using the assay above, apple volatile compounds that produce antennal activity in females will be tested (Bengtsson et al 2001). These compounds are (Z)3-hexenol, butyl butanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate, hexyl butanoate, hexyl 2-methyl-butanoate, hexyl hexanoate, methyl salicylate, benzyl alcohol, 4,8-dimethyl-1,3,(E)7-nonatriene, β-linalool, β-caryophyllene, (E)-β-farnesene, germacrene D, (Z,E)-α-farnesene and (E,E)-α-farnesene, all of which are commercially available. In addition, compounds found in apple leaves will be also be tested, including a mixture of Theaspirane, (2R, 5R) and (2S, 5R), and geraniol, which acts as a codling moth repellant (Wei et al 2004). Initially, volatiles previously identified as oviposition stimulants (α-farnesene, β-farnesene and nonanal) will be tested and candidate ORs for these compounds will be edited.

6) Expected Outcomes.

Completion of this project will identify ORs expressed in the abdomen tips of female codling moth that play key roles in oviposition site selection. This information, along with identification of ORs expressed in female antennae that have key roles in attracting females to oviposition sites, will provide targets that may be manipulated for codling moth control. Examples can include: 1) development of new compounds that are more potent attractants which can be used to trap females; 2) development of compounds that block receptor activity to prevent females from finding oviposition sites; and 3) development of a system that attracts females (using a more potent attractant from example 1) to a platform that contains oviposition stimulants enticing the females to lay eggs away from apple. In the long term and when socially acceptable, these targets can be knocked out in wild populations using CRISPR/Cas9 technology are currently being commercially developed (Bayer Crop Sciences and DuPont are working on this technology).

RESULTS AND DISCUSSION

The central role of olfaction (sense of smell) in the life of insects has been well documented. Olfactory cues from insects of the same species and environmental/ecological sources mediate most vital behaviors, including mate and host seeking, oviposition and predator avoidance. Extensive research efforts have characterized the role of odorant receptor (OR) proteins in the detection of odorants, where ORs provide the interface for insects with the environment and serve as the molecular gateway to olfactory centers in the brain and downstream behavior. Traditionally, ORs are thought to be localized to insect antennae. For codling moth, we have determined that ORs are also expressed in the female abdomen tip. The function of these ORs is not known, but it is hypothesized that they are present to identify males of their species, or environmental odors to find oviposition sites.

Identification and cloning odorant receptors expressed in female codling moth

We have prepared a transcriptome (a compilation of all genes expressed in a particular tissue) from abdomen tips dissected from codling moth females. When examined, 38 transcripts encoding ORs were discovered. In year one of this project, 10 OR transcripts were cloned to verify expression and DNA sequence for the design of guide RNAs for use in future CRISPR genome editing experiments to determine function. When analyzed, three of the 10 cloned OR transcripts showed evidence that they were products of a phenomena know as alternative splicing. Alternative splicing allows for multiple proteins to be produced from a single gene. Because insects have a reduced number of odorant receptor genes compared to vertebrates, alternative splicing provides a mechanism that allows for the production of more functional proteins than the number of genes present in their genome. For insect odorant receptor genes, alternative splicing has not been fully explored and a manuscript has been accepted describing this phenomena in codling moth (Garczynski et al. accepted by Journal of Economic Entomology citing this project as the primary funding source).

In collaboration with Dr. William Walker, a female antennae-biased OR transcript was expressed in a functional assay system to determine the odorant that activates that protein. From a panel of plant and fruit volatiles, Dr. Walker has determined that this OR responds to odorants that may serve as a male produced pheromone. These odorants are structurally related to one identified in the spruce budworm, *Choristoneura fumiferana*, which is in the same family as the codling moth. The pheromone produced by the spruce budworm has been shown to be important for mating courtship behaviors. Currently, codling moth male sex pheromones are being extracted so that we can identify the native compound that activates the female OR. This gene is also being targeted for CRISPR genome editing to determine its role in mating and if disruption of this gene provides a target for the development of new compounds that can be used to disrupt mating in codling moth control programs.

Additional receptors identified in the female abdomen tip transcriptome

The goal of this project is to identify proteins expressed in codling moth females that when disrupted cause females to stop laying eggs on apple. We proposed looking at the ORs expressed in the female abdomen tip, but in searching the transcripts in our transcriptome we have identified additional proteins that may also be valuable targets for control. The most interesting of the targets are neuropeptide receptors that are involved in letting the female know when she has been mated and play key roles in egg production. A collaboration has been initiated with Dr. Man-Yeon Choi at the ARS facility in Corvallis, OR. Dr. Choi will be cloning and expressing these receptors in his cell-based assay system to identify the native peptide hormones that activate these proteins. Dr. Choi holds a number of patents on compounds that disrupt neuropeptide receptor function in moths with the goal of developing new chemicals for insect control.

Conclusion

The progress made on this project is in line with the timeline provided in the initial proposal. In year 2 of this project we will complete the cloning of additional ORs expressed in the abdomen tip and design guide RNAs that target these receptors for CRISPR genome editing experiments. We have completed initial CRISPR genome editing experiments on four ORs and in year 2 we will be generating stable populations to use in behavior bioassays. We have received funding from Pacific West Area Office for a flight tunnel and a camera/software system that analyzes behavior in real time. This system will be used to identify codling moth behaviors modified by knocking out OR genes. The system is much more accurate than manual behavior monitoring in that it can detect variations not apparent to the naked eye. The success that we have had in the past year has attracted collaboration of additional researchers whose expertise's will allow for the development and testing of novel semiochemicals and chemical compounds that may be useful to control codling moth in the orchard.

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension
WTFRC Project Number: CP-19-101

Project Title: Outreach Program for Apple Decays Management in Washington

PI: **Achour Amiri**
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Co-PI (2): **Tianna DuPont**
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Telephone: 509-293-8758
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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:** Shelli Tompkins/Katy Roberts
Telephone: 509-293-8803 **Email address:** shelli.tompkins@wsu.edu/ arcgrant@wsu.edu
Supervisor or Station Manager name and email address: Chad Kruger, cekruger@wsu.edu

Item	2019	2020	2021
Salaries[1]	2,950	3,068	0
Benefits[1]	1,304	1,357	0
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[2]	5,000	5,000	0
Travel[3]	1,000	1,000	0
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	**10,254**	**10,425**	**0**

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 workshop was organized on March 4th, 2020 at the CTC in Wenatchee
- The workshop was attended by 110 stakeholders
- The participants were: packers (38%), growers/packers (29%), consultants (22%), industry reps (11%)
- 85 participants (77%) answered a survey at the end of the workshop
- 85% of surveyed participants said they learned a great/good deal
- The top areas the participants said they will do differently after the workshop are 1) pre and postharvest sanitation, 2) Re-evaluate fungicide programs/timing/, 3) fungicide rotation and 4) bin cleaning/sanitation
- The three top things liked by participants were: activities/hands-on (25%), presentations/flow (18%), and presentation on timeline/pre and postharvest management tactics (13%)
- 98% of participants increased their knowledge in infection timings, 84% increased their knowledge about most effective pre and postharvest fungicides, and 98% increased their knowledge about fungicide resistance risks and importance of rotating different FRAC groups.

METHODS

YEAR 2- 2021-22: Demonstrate Easy Pathogen Detection and Disease Management in Organic Systems

- WHEN: Meetings will occur in early to fall 2021 or winter (Feb 2021) (tentatively).
- Additional training meetings (like those provided in Year 2) may be held in orchards and packinghouses across other regions of the state if greater interest is shown after the two planned meetings.

Learning Objectives from meeting and training in Year 2:

- Use traditional and advanced methods for diseases and decays detection
- Use morphological and olfactive traits to detect them
- Use portable DNA-based methods for field/packinghouse detection

Additionally, we will update participants on new knowledge and approaches for organic disease management pre and postharvest

Audience:
Growers, packers, QC managers, packinghouse staff, field-staff, Extension Specialists, APHIS staff i.e. staff at export/entry ports, other stakeholders.

SUGGESTED TENTATIVE PROGRAM FOR THE MEETING IN YEAR 2

08:30 am ARRIVAL AND REGISTRATION
09:00 am **Welcome:** Objectives and overview of the workshop

MODULE 1: Easy Pathogen Detection: From Lab to the Field (9:10 am - 12:00 pm)
09:10 am **Morphological identification of 10 major diseases**

- Review of major pre and postharvest diseases
- Timing of infection and symptom appearance
- Lesion color, growth pattern, and sporulation
- Smell
- Microscopic traits

09: 50 am **Activity 1: Observe and Identify the problem**

- Break into groups
- Observe symptomatic fruits
- Observe different pathogens growing on artificial media on plates
- Make correlation between plates and diseases on fruit
- Make microscopy slides and observe differences

10:30 am BREAK
10: 45 am **Introduction into portable DNA-based methods for field detection**

- Definitions and Principles
- What is LAMP and how it works?
- Where can I use it and can I do it myself?
- How much will it cost?

11:15 am **Activity 2: Hands-on the portable LAMP detection**

- Presentation of different devices available
- Prepare material for detection
- Run the reaction, read the results and make a diagnostic

12:00 pm LUNCH BREAK
MODULE 2: Disease Management in Organic Systems (1:00 – 2:30 pm)
01: 00 pm **Disease management** in **organic** systems

- Updates on most effective material available for organic disease management
- How to optimize sanitation and sprays in the orchard to reduce disease in storage

02:20 pm Final thoughts, Survey, Pesticide credits, and Adjourn

Materials and documents to be provided to the attendees: Each participant will receive a full packet (binder) containing:

- Meeting program
- Slide-presentations of all talks
- Flyers & scenarios
- Evaluations and other relevant documents.

- Participants with break into groups and supplies will be provided for the hands-on activity on LAMP detection.

Expected Outcomes:

The overall expectation from this two-years activities is to increase knowledge and awareness about major aspect pertaining to diseases of pome fruit from orchard to packing and ways to recognize them. The mid- and long-term goals are to alleviate disease rates once recommendations are implemented and improved in the future. Additionally, participants will:

1. Have increased knowledge about emerging quarantined pathogens
2. Acquire new knowledge on accurate detection to improve management
3. Acquire knowledge about existing and easy to use methods for detection
4. Acquire knowledge about regulations pertaining to quarantine pathogens
5. Have increased knowledge about best management practices in organic systems.
6. This meeting will help foster close collaborations between scientists, industries, and stakeholders and
7. Identify needs for future research and education efforts.

RESULTS AND DISCUSSION

We present some statistics from the workshop and results from the survey conducted at the end of the workshop

1-Participants: 110 stakeholders from Washington and Oregon attended the full day workshop. The majority were identified as either packers (35%) or grower/packer (33%), whereas 20% and 13% of participants were consultants and industry representatives, respectively (**Figure 1**).

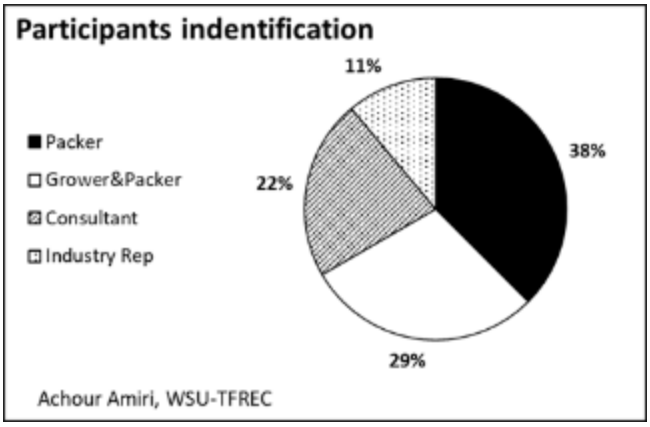

Figure 1. Percentages of different stakeholder categories who attended the 2020 disease workshop in Wenatchee, WA.

2- Practices the participants listed they will do differently as a result of the workshop. 85 participants (77%) filled a survey at the end of the workshop.

A majority of 34% of surveyed participants mentioned they would approach pre and postharvest sanitation differently after the workshop to enhance disease management (**Figure 2**). The three other

top things that the participants said they would differently are: re-evaluate their fungicide program and timing (21%), rotate fungicide (15%) and clean/sanitize bins (10%).

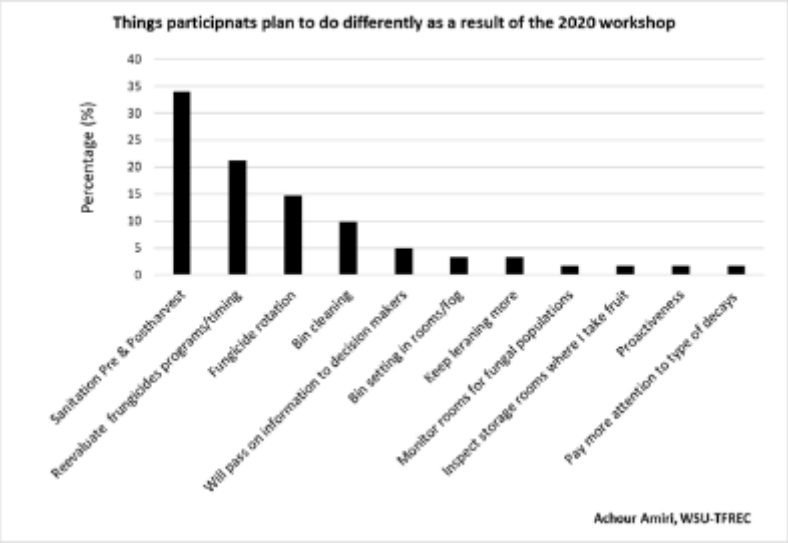

Figure 2. Practices that the 85 surveyed stakeholders would do differently as the result of the 2020 disease workshop in Wenatchee, WA.

3. Overall and specific knowledge learned by participants in the 2020 disease workshop

Overall knowledge: in all categories, a majority of participant learned a "great/good deal" as a result from the workshop (**Figure 3**). A majority of 81, 86, 87 and 87% of packers, packers/growers, consultants, and industry representatives, learned "great and good deal". None of the surveyed participant mentioned he learned "little" from this workshop

Figure 3. Overall knowledge acquired by different stakeholder categories (85 participants surveyed) who attended the 2020 disease workshop in Wenatchee, WA.

Specific knowledge about important practices learned after the workshop.

The participants were surveyed for their knowledge in five major topics before and after the workshop. The topics were 1) infections risks and timing in the orchard, 2) Effective preharvest materials, 3) most effective postharvest materials, 4) fungicide resistance risks/mitigation, and 5) importance of fungicide alternation. The % of participants with minimal knowledge before the workshop decreased very significantly after the workshop (Figure 4a). The % of participants with considerable knowledge before the workshop increased very significantly after the workshop in all topics (Figure 4B).

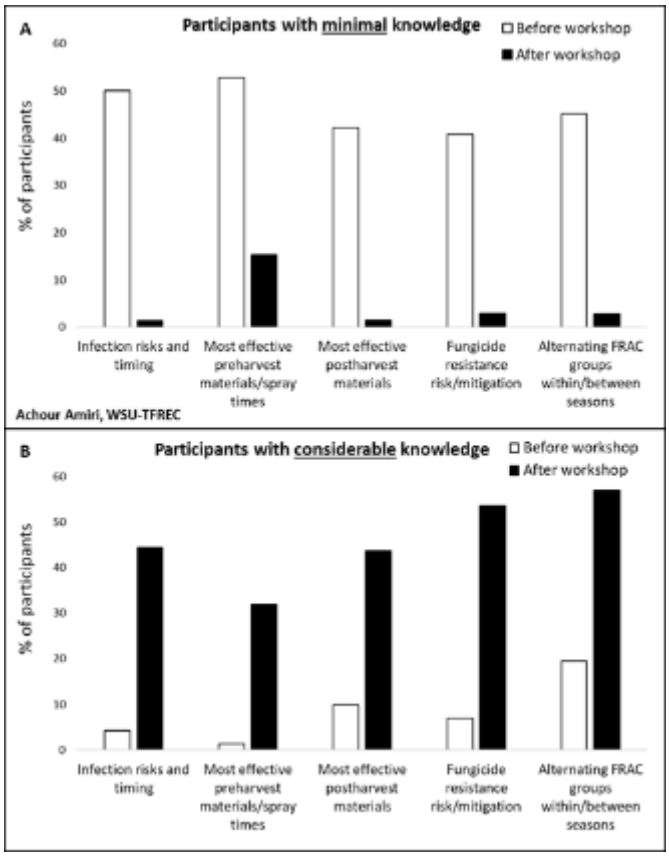

Figure 4. Percentage of surveyed participants with minimal (A) and considerable (B) knowledge on major disease management topics **before** and **AFTER** the 2020 disease workshop

Photographs from attendance and activities conducted during the disease workshop at CTC, Wenatchee, March 4th, 2020. Photo credit: Achour Amiri, WSU-FREC.

FINAL PROJECT REPORT
WTFRC Project Number: CP-17-100

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

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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 (Achour Amiri)
Organization Name: WSU **Contract Administrator:** Katy Roberts/Shelli Tompkins
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shelli.tompkins@wsu.edu

Item	2017	2018	2019	2020
Salaries[1]	12,635	9,371	0	0
Benefits	4,449	3,358	0	0
Wages	0	0	0	0
Benefits	0	0	0	0
Equipment[2]	13,735	0	0	0
Supplies[3]	4,000	2,000	3,000	0
Travel[4]	392	700	800	0
Miscellaneous	0	0	0	0
Plot Fees	0	0	0	0
Total	35,211	15,429	3,800	0

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.

- Six primer sets were developed for the detection of *Neofabraea perennans*.
- One 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.

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*

Specificity of LAMP primers to detect N. perennans only: 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).

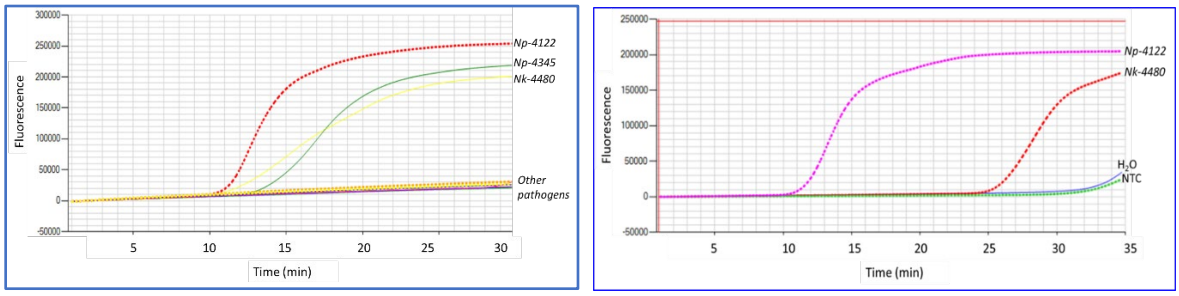


Figure 2. Initial specificity LAMP assessment using the portable LAMP Genie® II instrument to amplify *N. perennans* and other pathogens (left) and in comparison, with *N. kienholzii* (Nk.4480) (right). All reactions were run at 65°C and a DNA concentration of 1 ng/µl for each pathogen. Np and Nk indicate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

Sensitivity of LAMP to detect different DNA concentrations of N. perennans: 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.

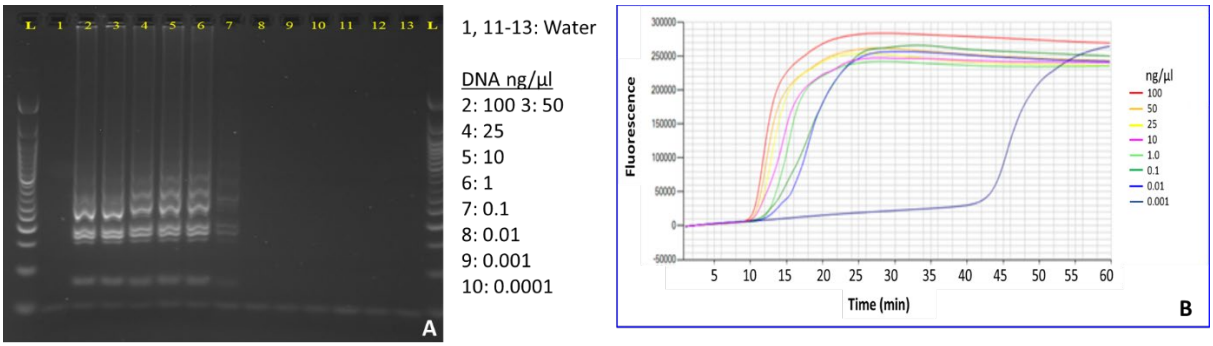


Figure 3. (**A**) Image of 1% agarose gel demonstrating the sensitivity of LAMP assay using the primer set 22 at 65°C to detect different DNA concentrations of *N. perennans*. (**B**) LAMP sensitivity in Genie® II portable instrument at 65°C for detection of *N. perennans* at different DNA concentrations 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).

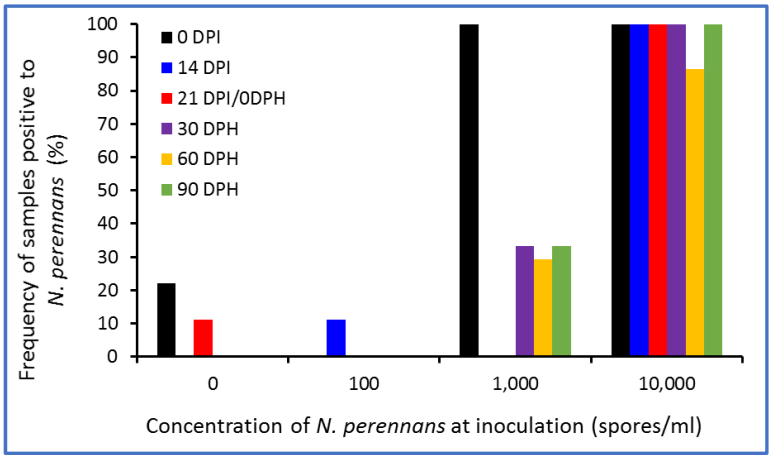
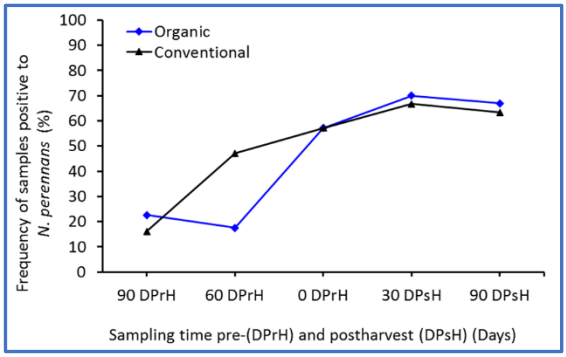


Figure 4. Mean frequency (%) of Golden Delicious samples positive to *N. perennans* detected by LAMP in Genie® II from fruit inoculated with different spore concentrations of the pathogen. DPI and DPH indicate day post-inoculation and day postharvest, respectively.

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).



ACKNOWLEDGMENTS

We thank the WTRC for funding this crucial project. We also thank the participating growers and packers for allowing us to use access their fields and rooms to conduct the work planned.

OTHER OUTCOMES

Manuscripts and Abstracts

1. Enicks D.A., Bomberger R.A., Amiri A. 2020. Development of a portable lamp assay for detection of *Neofabraea perennans* in commercial apple fruit. *Plant Disease* **104**:2346-2353.
2. Enicks D.A., Amiri A. 2019. LAMP detection of *Neofabraea perennans*, the causal agent of Bull's eye rot of pome fruit. *Phytopathology* **109**:11-S3.9.
3. WSU-developed field test could detect fruit-rotting diseases months before harvest. *CAHNRS News*. Dec. 2020
4. An early peek at pathogens. *Good Fruit Grower*. Oct. 2020

Talks

1. Amiri A., Enicks D. Development of a LAMP portable assay to detect two quarantined pathogens of apple fruit. *Annual Phytopathological Society-Pacific Division meeting, Fort Collins, CO*, June, 2019.
2. Enicks D., Amiri A. Development of a LAMP portable assay to detect two quarantined pathogens of apple fruit. *WA Tree Fruit Association Annual meeting*, Yakima, December 4th, 2018.

EXECUTIVE SUMMARY

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

Key words: Bull's eye rot, speck rot, early field detection, DNA, quarantine

Abstract: Most pathogens that cause decay in storage start fruit infections in the field during the growing season. Decay symptoms only develop after several months in cold storage and early detections of latent (dormant) infections is critical for decay management pre and postharvest and for fruit shipping in case of quarantine pathogens such as Neofabraea and Phacidiopycnis which cause bull's eye rot and speck rot, respectively. Herein, we developed a sensitive DNA-based assay to detect these two pathogens before decay symptoms become visible. This assay can provide an accurate diagnostic in less than 30 min and can be used by stakeholders at their facilities given that they have a received a minimum training. We were able to detect the bull's eye rot pathogen and the speck rot pathogen 3 and 2 months before harvest, respectively, in commercial apples which will be very useful to understand the epidemiology of the diseases and make timely sprays to reduce infections well before fruit are harvested and stored. We were also able detect the pathogens at harvest and follow their dynamics for up to 3 months in storage. We hope this will help packers decide about the best storage conditions and time based on infection levels to avoid consequential fruit loss in storage. We also hope this assay will ease restriction on fruit shipping about detecting quarantine pathogens as this assay can be used by packer of phytosanitary staff at ports of entry of exports to make final decision and avoid extra costs for shipped in case fruit are highly infected with latent pathogens. We plan to organize a workshop funded by the WTFRC in 2021 to provide a training to interested stakeholders and will continue top provide trainings in the future to interested growers or packers. We plan to extend this assay to detect the other major pathogens to improve overall decay management. Ultimately, we will develop a video to help interested stakeholder carry-out the assay independently or with minimum supervision.

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

Other funding sources

Agency Name: Project Apis M
Amt awarded $60,000
Notes: Awarded becaue we have the capacity provided with the WTFRC to perfom indoor storage research on honey bee colonies. This project investigates queen storage practices using indoor storage

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**	2020
Salaries		15,290	18348
Benefits		2464	2956
Wages		6000	6000
Benefits		1560	1560
Goods and Services	100,000		
Supplies		3000	3000
Total	100,000	**28,314**	31,864

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

Provide a bulleted list of significant findings during the prior year(s) of the project.

- Research on Objective 1 suffered a setback due to equipment failure in one chamber half way through the trial period last winter. The repeated experiment to determine if CO2 can be used to control Varroa will be completed in one week and there were no equipment failures this winter.

- Completed large body of research on Objective 2. We have a Masters student preparing her thesis on the work (graduating this Spring). In addition to the controlled experiments in our container; 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 is now available as an online resource with continual updates on this management practice. The link and document has been accessed more than 2000 times. The PDF version is currently being transferred to a more dynamic web resource with new material added quarterly

- With the combined funds from WTFRC and the Almond Board for the facilities, we were able to secure USDA-NIFA funding to expand the research associated with this project. We also secured funding from Project Apis M to investigate queen banking, and Google X because of the research funding provided by WTFRC that provided the equipment to pursue this line of research.

- 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.

Year 2- indoor wintering - 2019 – completed

October -132 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.
The colonies are distributed so that each group contains, on average, the same Varroa mite load. Both containers were set at 40°F with the manipulated variable being CO_2. One container suffered from catastrophic refrigeration failure. This failure forced us to end the trial just before the end of December. We ran the functional container through the rest of the winter storage period and learned that the containers (when functioning properly) are capable of safely storing honey bee colonies. We were not able to compare high and low CO2 levels on mite levels because of the equipment failure. The refrigeration unit was repaired and functioned properly through the summer trials and is currently operating normally (see below).

Year 3 – Indoor wintering – 2020 (underway)

October – The experimental set up from winter of 2019 was duplicated in year 3, to perform the research that was lost due to equipment failure. (see above)

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

April -June – 160 honey bee colonies were assessed from two different commercial beekeeping colaborators. One set of hives was utilized after almond pollination and a second set of colonies was utilized after Apple pollination. The number of frames of bees and brood was recorded. Samples of bees collected in alcohol to determine the initial Varroa mite load and tracheal and nosema infection in each colony. Each colony was weighed before and after the trial period.

With information about the Varroa mite loads for each colony; colonies will be assigned to one of two controlled atmosphere rooms and a set of colonies remained 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 were placed in the controlled atmosphere chambers for 18 days. One room set at 40°F and in complete darkness. The second room will be held at 50°F in complete darkness. At the end of the 18-day storage period the colonies were 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 were assessed again as they were at the start of the experiment and health, colony size, mite loads were compared. The colonies were assessed again before the end of the season while they were in North Dakota. Data analysis is still ongoing as part of a Masters students thesis. They are graduating in April 2021. The work is being prepared for peer review publication.

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

The initial funding of this project allowed for the ability to acquire additional funding and planning 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 potential 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 where 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 throughout the year and results from this winter will be used to design the experiments planned for summer and winter 2020/2021.

One of the comments/feedbacks 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.

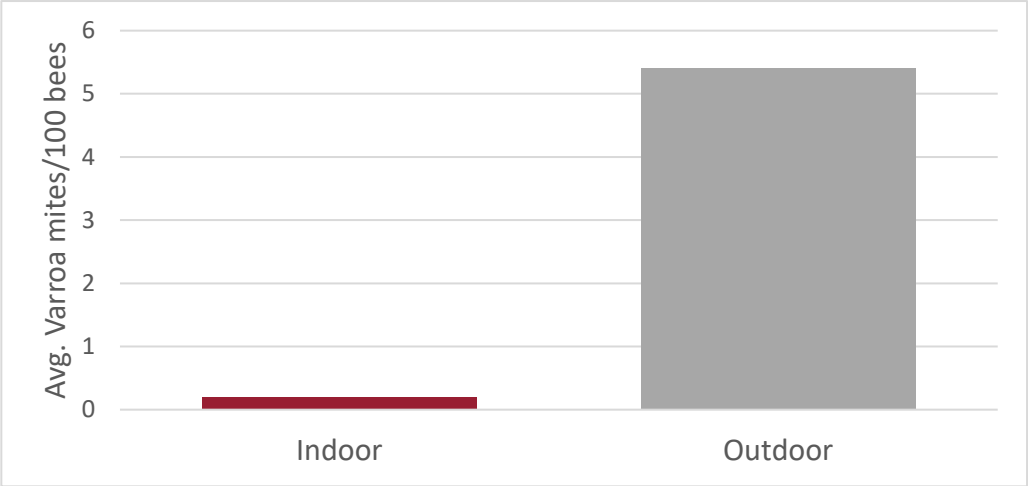

Figure 1: Average Varroa mite populations in colonies that experienced a break in brood production compared to colonies outdoors that continued brood rearing. All colonies experienced the same varroa treatment. The only difference being whether they were moved indoors for 18 days or remained outdoors.

FINAL PROJECT REPORT

Project Title: Implementation of alternative methods to control replant disease

PI:	S. Tianna DuPont	**Co-PI**:	Mark Mazzola
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Cooperators: Mike Robinson, BMR Orchards; Jim Baird, Baird Orchards; Sam Godwin, Box Canyon Orchard

Other funding sources

Agency Name: USDA Crop Protection

Amt. awarded: $195,713

Notes: USDA Crop Protection Grant # 2017-70006-27267 funded two additional sites. Thank you to in kind support from Gold Crown Nursery, Cameron Nursery, Progene Seed, Trident Ag Products, Farm Fuel Inc and generous support of labor, materials and equipment from orchardists Mike Robinson, Jim Baird and Sam Godwin.

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

Budget History:

Item	Year 1: 2017	Year 2: 2018	Year 3: 2019
WTFRC expenses			
Salaries	$19,800	$20,592	$21,416
Benefits	$6,283	$6,534	$6,795
Supplies	$33,457	$6,000	$6,000
Travel	$1,037	$1,037	$1,037
Total	$60,577	$34,163	$35,248

Acknowledgements Thank you to valuable contributions from orchardists hosting project sites Mike Robinson, Jim Baird and Sam Godwin; work and efforts of technicians Abby Kowalski, Ashley Heuchert, Allie Druffel, Chris Strohm; orchard management Cameron Burt.

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

- Brassica seed meal treatments (BSM) successfully altered soil microbial communities and were associated with apple tree growth that was as great or greater than fumigated controls across all three study locations in year one.
- Anaerobic soil disinfestation (ASD) resulted in significant changes in composition of the rhizosphere microbiome and tree growth in year one that was better than the no-treatment control in three of four experiments but not always greater than the fumigated control.

RESULTS AND DISCUSSION

Brassica seed meal treatments (BSM) successfully altered soil microbial communities and were associated with apple tree growth that was as great or greater than fumigated controls across all three study locations in year one. In bioassays, BSM soil amendment lowered *P. penetrans* nematode numbers recovered from apple seedling roots (Table 1) and shifted post treatment microbial composition as assessed by T-RFLP analysis (Fig. 1). In field trials, changes to the microbial community were maintained in concert with lower *P. penetrans* populations in apple roots one-year post-treatment. Significant differences in the apple rhizosphere microbiome and *P. penetrans* root populations were evident between BSM and both the no-treatment and fumigated controls one-year post treatment in Tonasket and Rock Island and two years post-treatment in Othello1 (Fig.2; Table 1). Root pathogens *Illonectria robusta* (all sites) and ***Rhizoctonia*** **spp. (Rock Island)** were significantly diminished in the rhizosphere of BSM treated soil compared to the control and **several fungal genera with potential biocontrol activity including *Talaromyces*, *Chaetomium*, *Gelasinospora* and *Hypocrea/Trichoderma* were present at significantly ($P < 0.05$) greater relative abundance in rhizosphere soil from the BSM than control treatment.** Suppression of plant pathogens and nematodes corresponded with tree growth greater than or equal to the fumigated control at all sites in year 1 (Fig. 3; Table 2).

Anaerobic soil disinfestation (ASD) resulted in significant changes in composition of the rhizosphere microbiome and tree growth in year one that was better than the no-treatment control in three of four experiments but not always greater than the fumigated control. At Rock Island, Tonasket and Othello2, but not Othello1, ASD treated soils attained 50,000 mVhr oxidation reduction potential indicating anaerobic conditions. Post treatment bioassays showed low (comparable to pasteurized control) *P. penetrans* levels g^{-1} root in plants grown in ASD treated soil for Rock Island and Tonasket. At Othello1 and Othello2 sites *P. penetrans* were still present at levels (167 g^{-1} root and 285 g^{-1} root, respectively)

significantly higher than the pasteurized control (Table 1). Bulk soil microbial communities assessed by T-RFLP analysis were transformed significantly in response to ASD at the Rock Island and Tonasket orchards but not Othello1 (Fig. 1). One-year post-treatment, rhizosphere microbial communities from ASD treated plots possessed fewer OTUs that differed in relative abundance from the fumigated and no-treatment control than did BSM treated soil. Bacteria belonging to the Clostridiales and Bacilliales within the Firmicutes, as well as **Actinobacteria and Bacteroidetes shifted to an increased abundance in response to ASD at Rock Island and Othello2.** Amplification of Firmicutes (Mowlick *et al.*, 2013; Liu *et al.*, 2016; Hewavitharana *et al.*, 2019) and Bacteroidetes (van Agtmaal *et al.*, 2015; Mazzola *et al.*, 2018) abundance in soil and the rhizosphere has been documented in response to application of ASD in other crop production systems and was associated with enhanced yields. A progression in composition of the Firmicutes community was correlated with the production of metabolites that possessed antimicrobial, and potentially disease suppressive, activity (Hewavitharana *et al.*, 2019). In this study, sites that exhibited significant rhizosphere bacterial community shifts in response to ASD, such as **at Rock Island, Tonasket, and Othello2, correspondingly exhibited a significant increase in tree growth relative to the control in year one (Figure 3).**

Tree growth and yield measurements of second (and third leaf Othello1) trees showed the early impacts of soil treatments over time. At Othello1 trees grown in BSM treated soil had significantly smaller tree diameter than both the no-treatment and fumigated controls according to repeated measures analysis of variance (Figure 4). Trees at Othello1 in all treatments were large at 33 to 35 mm diameter. At Othello2 trees grown in ASD treated soil were significantly larger than no-treatment controls. At Rock Island cv WA38 trees on G.41 rootstock trees grown in ASD and BSM treated soils were significantly larger than no-treatment and fumigated controls with no difference between ASD and BSM and a significant difference between no-treatment and fumigated controls. At Rock Island cv. WA38 trees on G.41 rootstock trees had significant differences between all treatments but a significant treatment by date interaction. Trees in BSM treated soils were the largest, larger than both no-treatment and fumigated controls. WA38 trees on G.41 trees in ASD treated soils were larger than no-treatment controls but not fumigated controls. At Tonasket tree size was greatest in BSM and fumigated control trees until 14 months after treatment. At month 16 BSM tree size was smaller than those in fumigated control soils. Trees in ASD treated soils were larger than those in no-treatment controls but not fumigated controls. At Othello1 fruit yield in bins per acre in trees grown in BSM treated soil was intermediate for both second and third leaf trees where yield in fumigated control soil trees was greater than that in no-treatment controls. Across sites in second (and third leaf Othello1) trees BSM treated trees were larger than no-treatment control in three of four experiments. Second (and third leaf Othello1) trees in ASD treated soils were larger than those in no-treatment control soils in four of four experiments but smaller than trees in fumigated control soils in two of three experiments.

At Othello1 the combination of a vigorous scion WA38 on a vigorous rootstock G.41 may have contributed to overall large trees and limited differences between treatments at that site. Additionally, no-treatment control plots at Othello1 were small 45 tree plots nested within large approximately one-acre plots. Bioassays of soil across the twelve-acre field site (data not shown) found large within field variability in replant pressure at the site which may not have been captured in small no-treatment control plots.

Table 1 Density (number gram^{-1} root) of Pratylenchus penetrans recovered from tree roots as influenced by soil treatment at the respective orchard field trials. Roots were sampled in October of the first growing season (Tonasket and Rock Island) or the first two growing seasons (Othello1).

	Tonasket†	Rock Island†	Othello1‡ 2018	Othello1‡ 2019
BSM§	5 ± 3 a#	10 ± 7 a	363 ± 129 a	130 ± 38 a
ASD	2 ± 1 a	38 ± 13 b	NA	NA
FUM	9 ± 4 a	44 ± 13 b	1933 ± 449 b	352 ± 45 b
NTC	142 ± 46 b	197 ± 33 c	997 ± 31 b	294 ± 53 b
P value	0.003	<.0001	0.004	0.004

†Analysis performed on log (1+ *P. penetrans*/ gram root)
‡Analysis performed on log (*P. penetrans*/ gram root)
§Treatments: BSM = *Brassica juncea:Sinapis alba* (1:1) seed meal; ASD = anaerobic soil disinfestation; FUM = soil fumigation; NTC = no treatment control
#Means in the same row followed by the same letter are not statistically different ($P > 0.05$).

Table 2. Effect of soil treatments on increase in tree diameter (mm) at 20 cm above the graft union.†

	Tonasket cv. TC2 r. B10	Rock Island cv. Wa38 r. M9	Rock Island cv. Wa38 r. G41	Othello1 cv. Wa38 r. G41	Othello2 cv. Wa38 r. G41
First year growth					
BSM§	5.8 ± 0.4 a	5.3 ± 0.4 a	5.9 ± 0.4 a	7.7 ± 0.2 a	NA
ASD	4.8 ± 0.2 b	4.1 ± 0.2 b	5.4 ± 0.3 a	NA	5.2 ± 0.4 a
FUM	6.1 ± 0.2 a	3.9 ± 0.3 b	5.1 ± 0.4 a	9.1 ± 0.5 a	NA
NTC	4.1 ± 0.2 c	2.9 ± 0.2 c	3.9 ± 0.2 b	8.2 ± 0.6 a	3.2 ± 0.6 b
P value	<0.001	<0.001	0.007	0.2	0.06
Second year growth					
BSM	6.4 ± 0.5 a	7.2 ± 0.4 b	7.1 ± 0.4 a	5.8 ± 1 a	NA
ASD	8 ± 0.3 b	7 ± 0.2 b	7.8 ± 0.2 a	NA	7.5 ± 1 a
FUM	8 ± 0.2 b	8.3 ± 0.3 c	8.1 ± 0.2 a	6.9 ± 0.3 a	NA
NTC	6.4 ± 0.7 a	5.5 ± 0.3 a	7.9 ± 0.3 a	7.2 ± 0.2 a	8 ± 2.1 a
P value	0.049	<0.001	0.11	0.34	0.68
Third year growth					
BSM				4.1 ± 0.4 a	
ASD				NA	
FUM				3.9 ± 0.2 a	
NTC				4.1 ± 0.1 a	
P value				0.83	

†Means in the same column followed by the same letter are not statistically different ($P > 0.05$).
§Treatments: BSM = *Brassica juncea:Sinapis alba* (1:1) seed meal; ASD = anaerobic soil disinfestation; FUM = soil fumigation; NTC = no treatment control

Table 3. Effect of soil treatments on fruit yield (bins/acre).

	Tonasket 2nd leaf				Othello1 2nd leaf				Othello1 3rd leaf			
BSM[§]	1.3	±	0.5	a	12.6	±	1.6	ab	18.8	±	1.3	ab
ASD	0.9		0.3	a	NA				NA			
FUM	2.2	±	0.1	a	16.6	±	0.7	b	20.6	±	0.5	b
NTC	1.4	±	0.5	a	9.1	±	2.3	a	15.6	±	2.2	a

[§]Treatments: BSM = *Brassica juncea:Sinapis alba* (1:1) seed meal; ASD = anaerobic soil disinfestation; FUM = soil fumigation; NTC = no treatment control

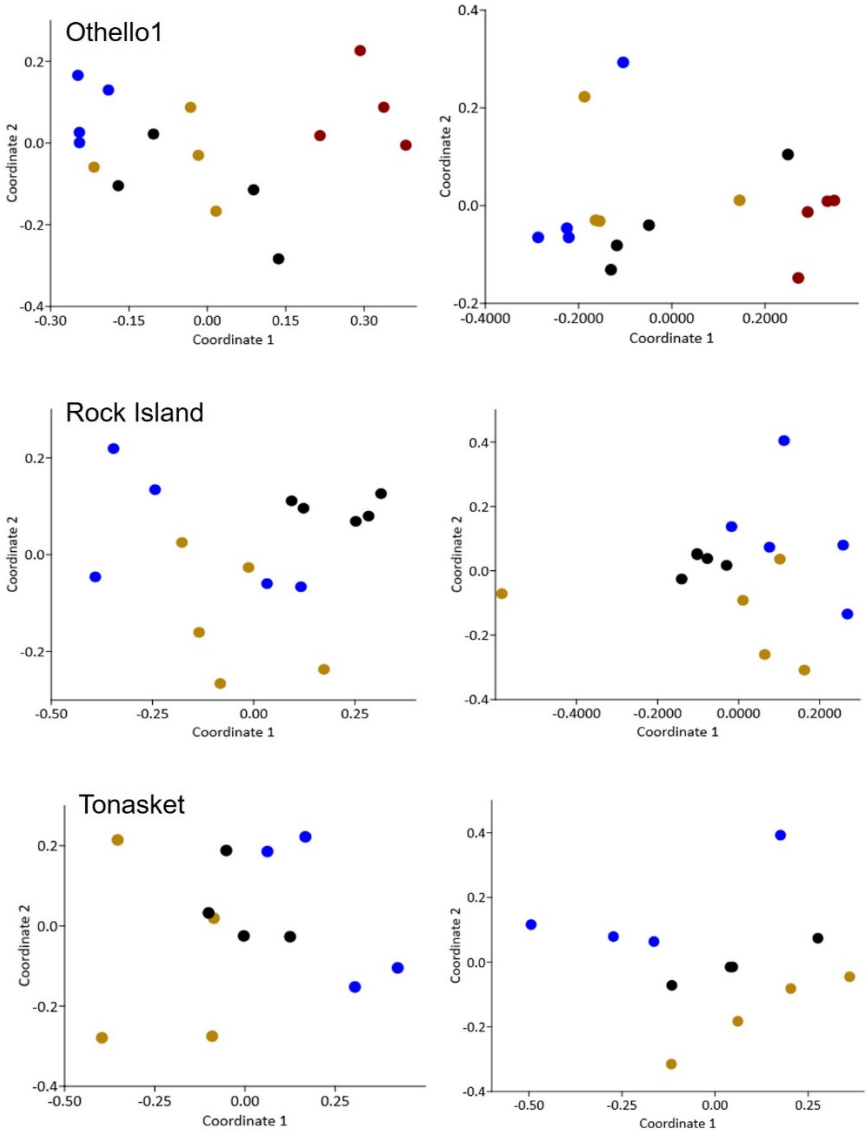

Figure 1 Effect of soil treatment on bulk soil bacterial and fungal community composition at three weeks post-treatment application as assessed by non-metric multidimensional scaling of terminal restriction fragment length polymorphism derived data using the Dice similarity index. Left panels represent bacterial data and right panels represent fungal data. Brassica juncea:Sinapis alba (1:1) seed meal = blue; Anaerobic soil disinfestation = gold; Fumigation = red.

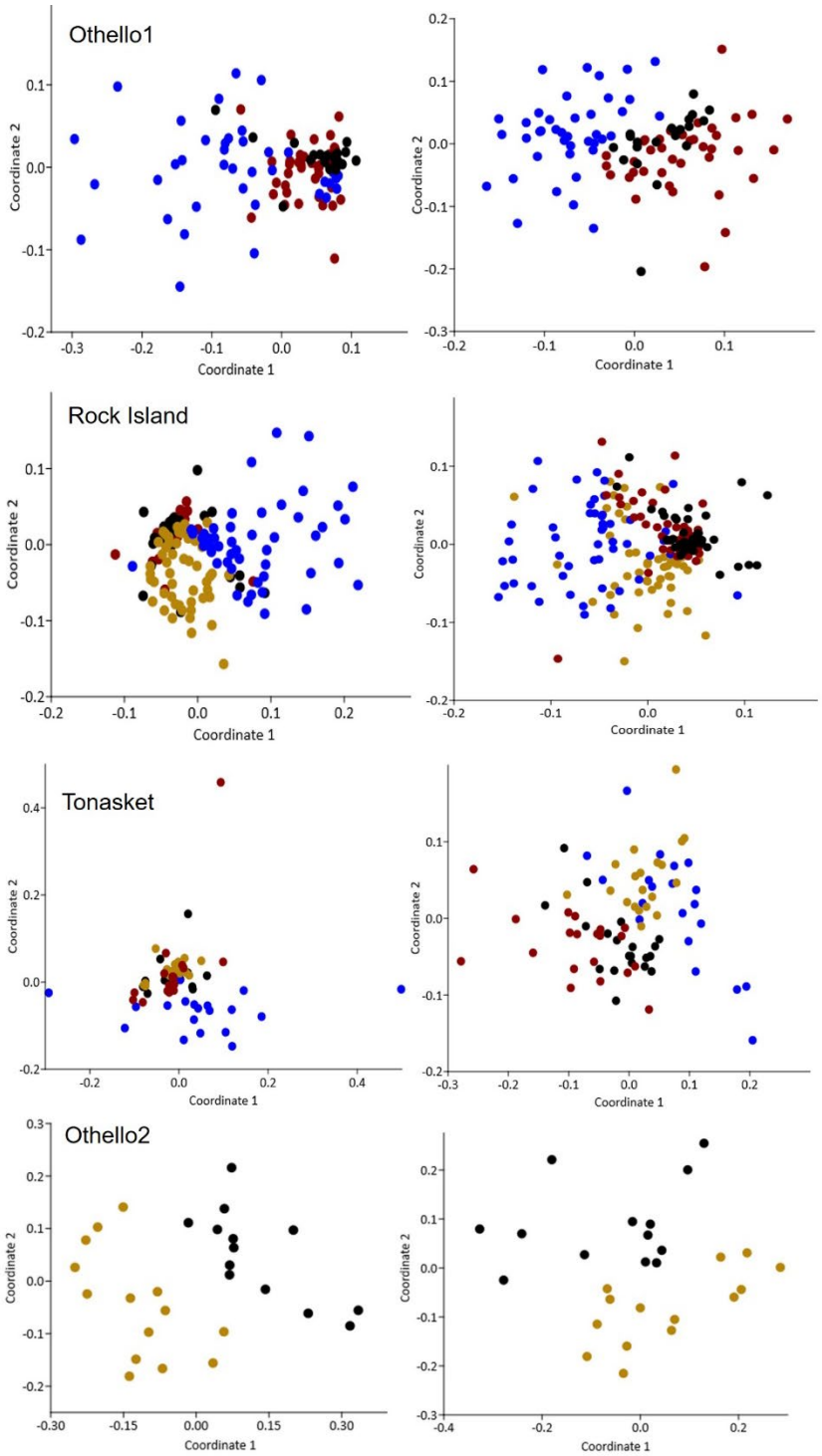

Figure 2. Influence of soil treatment on composition of microbial communities detected in the rhizosphere of apple cultivated in orchard replant soils. Ordination was conducted by non-metric multidimensional scaling (NMDS) of operational taxonomic units (OTUs) using the Bray-Curtis similarity index. Data describe the communities as detected in rhizosphere soil collected one year after treatment application. For all orchards, panels in the left column and right column represent the bacterial and fungal community, respectively. Treatments: Brassica seed meal = blue; anaerobic soil disinfestation = gold; 1,3-dichloropropene/chloropicrin fumigation = red; control = black.

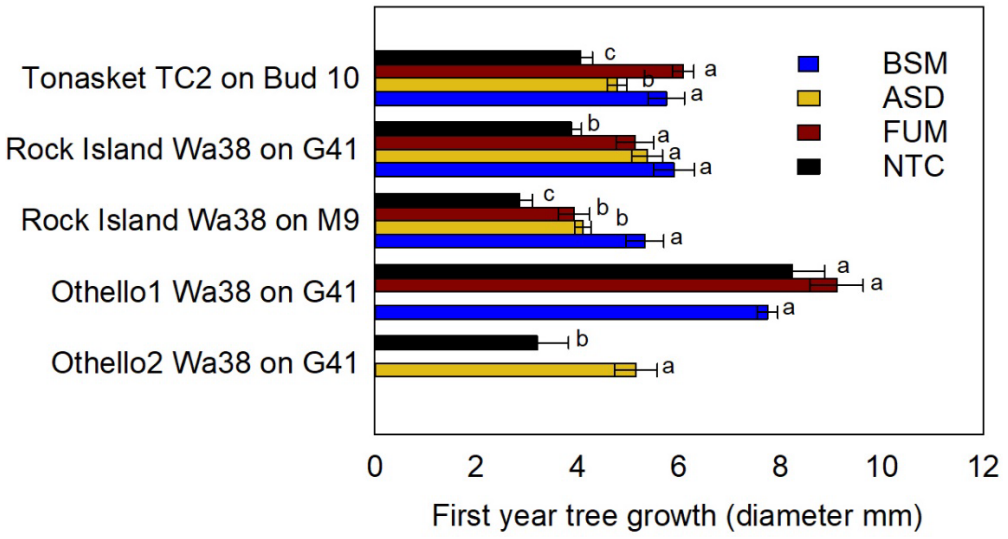

Figure 3. Effect of soil treatments on tree growth in the first year after planting measured as change in trunk diameter (mm) at 20 cm above the graft union. Brassica seedmeal (blue), fumigated control (red), no-treatment control (black).

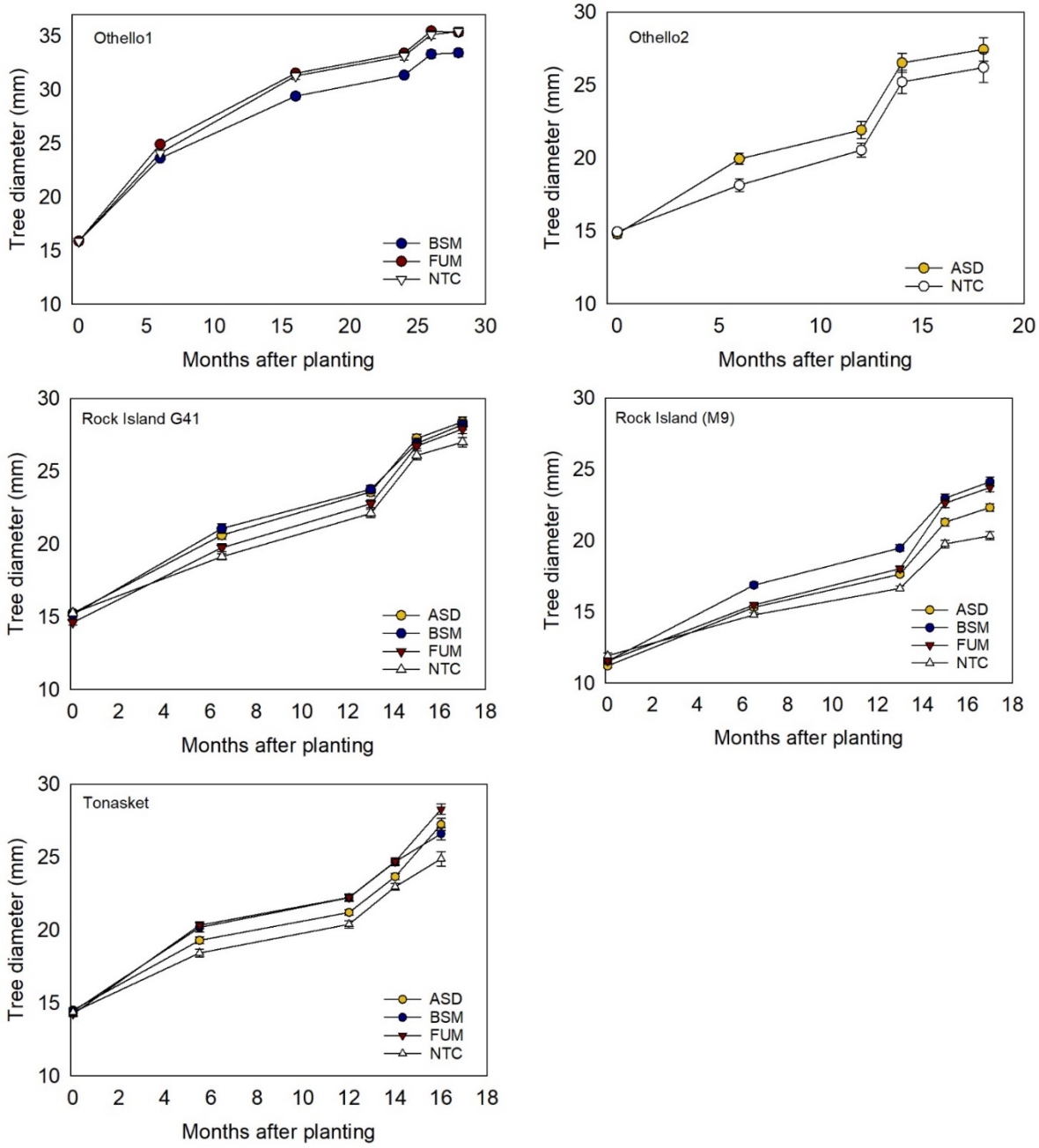

Figure 4. Effect of soil treatments on tree diameter (mm) at 20 cm above the graft union.[§]*Treatments: BSM = Brassica juncea:Sinapis alba (1:1) seed meal; ASD = anaerobic soil disinfestation; FUM = soil fumigation; NTC = no treatment control.*

Application Considerations

Brassica seed meal bio-renovation appears promising as an alternative to fumigating with 1,3-Dichloropropene, Chloropicrin. If considering this option growers should remember that not all brassica seed meals are equal. The seed meal used in this trial was a 1:1 formulation of *B. juncea* and *S. alba*. Seed meals are often processed at different temperatures and with different grinding methods which affect the quantity of active chemistry that is released. Tests should be conducted to determine the type and quantity

of glucosinolate contained in the seed meal that growers intend to use. To date in the US the *Brassica* seed meal we used is labeled only as a fertilizer. Until products have the appropriate labels as a soil fungicide/ nematicide, application for this use is not legal. It is also critical to remember that soil temperature and moisture are important. These are biological processes where moisture and temperature affect the activity of soil biology and the movement through the soil of the compounds they produce. For *Brassica* seed meal, treatment soil should be warm (above 70° C) and moist. Application timing is critical. If used, orchardists will need to consider application timing to avoid phytotoxicity. A previous study demonstrated that applications made in the autumn prior to planting did not yield phytotoxic effects whereas spring applications resulted in tree death in some cases (Mazzola *et al.*, 2015). Recent studies have shown that reduced applications rates (1/3 of current study) can result in the same level of disease control and also resulted in no phytotoxicity when spring applications were made (Wang and Mazzola, 2019). Application rate is important. The rate used in this trial was 0.4 lb per sq ft. Further studies at a large scale are needed to confirm whether reduced rates can be used. Additionally, at rates used in this trial (0.4 lb per sq ft), BSM applications including materials and labor cost approximately $5,900 per acre compared to $900 per acre for fumigation with 1,3-dichloropropene-chloropicrin a cost which may be prohibitively expensive depending on long term benefits (Appendix 4).

Anaerobic soil disinfestation resulted in significant changes in composition of the rhizosphere microbiome and tree growth that was better than the no-treatment control in three of four experiments but not always greater than the fumigated control. **Lack of disease suppression obtained with the ASD treatment at Othello1 can likely be attributed to insufficient moisture, with a resulting failure to achieve anaerobicity and consequent absence of necessary changes to the soil microbiome (Hewavitharana et al. 2019).** It will be essential to keep soil wet (above 30% moisture) and reach anaerobic conditions for success. **Variable success in disease control obtained between sites in these orchard trials may also be influenced by the differences in composition of the replant disease pathogen complex.** In sites with high *Pratylenchus* nematode populations higher carbon inputs and longer incubation times might be necessary for success. Previous studies have shown that grass carbon sources at a target rate of 8 ton per acre were most consistent for the specific apple replant disease pathogen complex present in Washington state (Hewavitharana and Mazzola, 2016) and were used in this study. However, carbon source quality and quantity may need to be adjusted based on the pathogen complex present at any specific orchard site to yield optimal disease control.

Changes in pesticide registration and additional research to refine application rates, methods and timings at a field scale are needed for widespread adoption of anaerobic soil disinfestation or *brassica* seed meal biorenovation. Product registrar Farm Fuels is working with the USDA IR4 program to complete the pesticide registration process. After registration specific use recommendations can be prescribed. Two opportunities for cost effective/practical brassica seedmeal applications include reduced rate spring applications and spot treatment for individual tree replants. Wang and Mazzola, 2019 provide good evidence of the potential of reduced rate applications that can be tested at the field scale. More efficient applications methods for ASD carbon sources such as the use of a bail chopper to apply hay carbon sources should be explored. Additionally, refining target moisture levels so that irrigation can be cycled will be key as continuous drip irrigation for three weeks is not practical when water resources are limited. Yield measurements from current plots on third and fourth year trees will provide more robust return on investment comparisons between treatments.

Extension

Results of research trials were shared with growers and consultants through field days, presentations, newsletter and popular press articles in addition to research reviews. Four field days with 130 total participants were conducted on August 7, 2019 as part of the WSU Sunrise Orchard Field Day and October 27,28,29 at research sites in Tonasket, Rock Island and Othello using social distanced mini-groups. Due to Covid19 events had to be adapted and attendance was limited. Four presentations were made to grower/stakeholder organizations with 260 participants (see below). Of participants surveyed 95% learned a good or great deal and 97% said they were likely to try biorenovation in the future (N=37). Fruit Matters article to be released in February/ March 2020 with publication of peer reviewed article contains application costs, suppliers and application considerations as well as research trial results (contact tianna.dupont@wsu.edu for a pdf prior to publication date). The pesticide label for the *brassica* seed meal product is still pending. Until the product is labeled WSU cannot give recommendations.

Presentations

2020. Alternative Controls for Replant Disease. APAL Australian Growers Association. Webinar. *(invited)*

May 14, 2020. IPM Methods to Control Replant Disease of Tree Fruit. DuPont, S.T., Mazzola, M., Hewavitharana, S. Western Integrated Pest Management Center. Annual Meeting. Webinar.

January 21, 2020. Orchard Biorenovation. DuPont, S.T., Mazzola, M., Hewavitharana, S. GS Long Organic Grower Meeting. Yakima, WA. *(invited)*

February 6, 2020. Replant Disease Project. DuPont, S.T., Mazzola, M., Hewavitharana, S. Northwest Wholesale Grower Meeting. Royal City. WA. *(invited)*

Articles and websites

DuPont, S.T., **S. S. Hewavitharana, M. Mazzola.** Evaluating IPM Methods to Control Apple Replant Disease. Australian Fruit Growers Magazine. V. 14. Issue 3. Spring 2020.

DuPont, S.T., **S. S. Hewavitharana, M. Mazzola.** Evaluating IPM Methods to Control Apple Replant Disease. WSU Fruit Matters. March 2021. http://treefruit.wsu.edu/article/replant_trials/

DuPont, S.T., **S. S. Hewavitharana, M. Mazzola.** Field scale application of *Brassica* seed meal and anaerobic soil disinfestation for the control of apple replant disease. Applied Soil Ecology. Submitted September 26, 2020.

Appendix 1. Field Operations Othello

Anaerobic soil disinfestation application			
Operation	**Implement/Equipment**	**Details**	**Date**
Fertilize			April 2017
Tillage	John Deer 7200/ 15 foot disc		April 2017
seed triticale	John Deer 7200/Great Plains seed drill	95 lbs per acre	April 19, 2017
Irrigation	Hand lines (R33 sprinklers)	6 gal per min, 0.28 in per hr	May-Jun 2017
cut and swath	John Deere R450 swather	4 ft windrow	June 28, 2017
chop	Pak flail	0.7 mi per hr	July 3, 2017
Incorporation	John Deer 7200/ Celli rototiller	8 in depth	July 4, 2017
Tarping	Kubota M8540 / Mulch layer Mechanical Transplanter Co Model 90		July 7, 2017
***Brassica* seed meal application**			
Operation	**Implement/Equipment**	**Details**	
Pre-irrigation	Hand lines (R33 sprinklers)	6 gal per min, 0.28 in per hr	July 15, 2017
Brassica seed meal application	John Deer 5083/ Whatcom mulch spreader	Settings: 4 low, 1700 rpms, belt 5, floor 4, gate 12.5 in	July 19, 2017
Incorporation	John Deer 7200/ Celli rototiller	8 in depth	July 19, 2017
Tarping	Kubota M8540 / Mulch layer Mechanical Transplanter Co Model 90	N/A	July 19, 2017

Appendix 2. Field Operations Rock Island WA

Anaerobic soil disinfestation application			
Operation	**Implement/Equipment**	**Details**	**Date**
Pre-Irrigation	Sprinkler system (R5 sprinklers)/ Big gun system (8 mm nozzle)	1.5 acre-inches applied	July 2-4, 2018
Hay distribution	By hand	8 ton per acre	July 4, 2018
Hay chopping	Flail mower		July 4, 2018
Incorporation	Mascchio Rototiller	8 in depth	July 5, 2018
Tarping	Mechanical Transplanter	N/A	July 5, 2018
Saturation	Drip irrigation to flood soil	0.44 acre-inches per hour	July 6-27, 2018
***Brassica* seed meal application**			
Operation	**Implement/Equipment**	**Details**	**Date**
Brassica seed meal application	Whatcom compost spreader 750	2 for belt and 2 for floor	July 6, 2018
Incorporation	Mascchio Rototiller	8 inch depth	July 6, 2018
Tarping	Mulch layer Mechanical Transplanter Co Model 90	Within 20 min of mustard incorporation.	July 6, 2018

Appendix 3. Field Operations Tonasket WA

Anaerobic soil disinfestation application			
Operation	**Implement/Equipment**	**Details**	**Date**
Pre-Irrigation	Big gun system (8 mm nozzle)	5 acre-in applied in 12 hr sets	Aug 2-6, 2018
Hay distribution	By hand	8 ton per acre	Aug 8, 2018
Hay chopping	Flail mower		Aug 8, 2018
Incorporation	Mascchio Rototiller	8 in depth	Aug 8, 2018
Tarping	Mulch layer Mechanical Transplanter Co Model 90	N/A	Aug 8, 2018
Saturation	Drip irrigation to flood soil	0.44 acre-in per hr	Aug 8-29, 2018
***Brassica* seed meal application**			
Operation	**Implement/Equipment**	**Details**	**Date**
Brassica seed meal application	Mill Creek mulch spreader	1.7 lbs per tree row ft Settings: 4 floor; 4 belt	Aug 9, 2018
Incorporation	Mascchio Rototiller	8 in depth	Aug 9, 2018
Tarping	Mulch layer Mechanical Transplanter Co Model 90	Within 20 min of meal incorporation.	Aug 9, 2018

Appendix 4. Treatment Costs

Anaerobic Soil Disinfestation (ASD) - Carbon grown in place.				
Field activity		**hrs/A**	**$/hr**	**$/A**
tillage		0.25	$40	$10
move irrigation for triticale		4.0	$13	$52
seeding triticale		0.25	$40	$10
cut and swath triticale		custom		$50
flail		1	$40	$40
hay rake		custom		$7
hand rake		2.8	$13	$36
move irrigation for ASD		4.0	$13	$52
lay plastic		2.0	$40	$80
Supplies	**$/unit**	**unit**	**unit/A**	**$/A**
triticale seed	0.32	lb	100	$32
Totally impermeable film	0.06	ft	4200	$252
Equipment	**equip**	**yrs amortized**	**A/year**	**$/A**
hand lines	$650	10	50	$1.30
flail	$4,000	10	50	$8
plastic layer	$2,300	10	50	$5
Total cost				***$635***
Anaerobic Soil Disinfestation (ASD) - Hay carbon source				
Field activity		**hrs/A**	**$/hr**	**$/A**
pre-irrigate		3	$14	$41
apply grass hay (timothy)		16	$14	$213
flail hay to chop		1	$40	$40
place drip lines		5.25	$14	$71
lay plastic		2	$40	$80

Supplies	**$/unit**	**unit**	**unit/A**	**$/A**
grass hay (timothy)	$100	ton	8	$800
hay shipping	$500	ea	1	$500
drip line	$0.07	ft	8400	$546
drip couplings	$3.63	ea	20	$73
Totally impermeable film	$0.06	ft	4200	$267
Equipment	**equip**	**yrs amortized**	**A/year**	**$/A**
flail	$4,000	10	50	$8
plastic layer	$2,300	10	50	$5
Total cost				***$2,642***
Brassica seed meal bio-renovation				
field activity		**hrs/A**	**$/hr**	**$/A**
irrigation		4	$13	$52
Brassica seed meal application		2	$13	$26
incorporation		2	$13	$26
tarping		2	$13	$26
Supplies	**$/unit**	**unit**	**unit/A**	
Brassica seed meal*	$0.85	lb	6720	$5,712
Totally impermeable film	$0.06	ft	4200	$267
Equipment	**equip**	**yrs amortized**	**A/year**	**$/A**
mulch spreader	$22,000	10	100	$22
plastic layer	$2,300	10	50	$5
Total cost				***$6,135***
Fumigation				
Field activity		**hrs/A**	**$/hr**	**$/A**
Total cost		custom		***$900***

*1.6 lbs per tree-row-foot for 4 ft wide tree strips

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EXECUTIVE SUMMARY

Project title: Implementation of Alternative Methods to Control Replant Disease

Key words: replant disease, mustard meal, biorenovation, soil microbiome

Abstract:

Apple replant disease causes stunting and reduced yields when apples are planted in locations previously cropped to tree fruit. Anaerobic soil disinfestation and bio-renovation using mustard seed meals may provide an alternative to fumigation controlling plant pathogens and leading to beneficial long-term changes in soil microbial communities. One-to-twelve acre trials were conducted in Othello, Rock Island and Tonasket to examine alternative techniques at the field scale and to track impacts on tree growth, yield and profits over time. Brassica seed meal treatments (BSM) successfully altered soil microbial communities and were associated with apple tree growth that was as great or greater than fumigated controls across all three study locations in year one. Anaerobic soil disinfestation (ASD) resulted in significant changes in composition of the rhizosphere microbiome and tree growth in year one that was better than the no-treatment control in three of four experiments but not always greater than the fumigated control.

CROP YEAR: 2020

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

Project Title: Pesticide Residues on WA Apples

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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

2020 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 twelve insecticide/acaricides and five fungicides according to either an "aggressive" protocol intended to generate the highest possible residues while observing label guidelines (maximum label rates at minimum retreatment and pre-harvest intervals) or a "standard" protocol following more typical industry use patterns for rates and timings. Each treatment protocol was sprayed at both 100 (concentrate) and 200 (dilute) gallons of water per acre with a Rears Pak-Blast sprayer while holding the rate of pesticide per acre constant. Fruit samples were collected at commercial maturity on August 27 and delivered the next day to Pacific Agricultural Labs (Sherwood, OR) for chemical residue analysis.

TRIAL DETAILS

- 13th 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 0.01" of rain on July 28 and August 6 (30 & 21 days before harvest)

Measured residues vs. maximum residue levels (MRLs) for uniformly applied STANDARD industry apple pesticide programs in 100 or 200 gal water/acre utilizing typical rates, timings, and retreatment intervals. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2020.

Chemical name	Trade name	Application rate	Application timing(s)	100 gal/acre	200 gal/acre	US MRL[1]	India MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
flutianil	Gatten	8	35	<0.01	<0.01	0.15	0.01*	0.01 (many)
isofetamid	Kenja 400SC	12.5	35	0.017	0.018	0.6	0.01*	0.2 (Kor)
abamectin	AgriMek SC	4.25	35	<0.01	<0.01	0.02	0.01*	0.01 (many)
diazinon	Diazinon 50W	16	35	0.019	0.020	0.5	0.01*	0.01 (UAE)
spinetoram	Delegate WG	7	35 & 21	0.02	0.03	0.2	0.01*	0.05 (many)
cyantraniliprole	Exirel	13.5	35 & 21	0.14	0.15	1.5	0.01*	0.8 (many)
spinosad	Entrust	3	35 & 21	0.01	0.01	0.2	0.01*	0.1 (many)
tolfenpyrad	Bexar	27	35 & 21	0.42	0.42	1	0.01*	0.01 (many)
myclobutanil	Rally 40WSP	10	35 & 21	0.20	0.20	0.5	0.01	0.01 (UAE)
fenpropathrin	Danitol	18	35 & 21	0.43	0.37	5	0.01*	0.01 (many)
difenoconazole	Inspire Super	12	28	<0.01	<0.01	5	0.01	0.5 (China)
cyprodinil	Inspire Super	12	28	<0.01	<0.01	1.7	0.01*	0.05 (Indo)
cyflufenamid	Torino	6.8	28	<0.01	<0.01	0.06	0.01*	0.01 (Thai)
buprofezin	Centaur WDG	34.5	28	0.012	<0.01	3	0.01*	1 (Tai)
acequinocyl	Kanemite	31	28	<0.025	<0.025	0.4	0.01*	0.01 (Thai)
afidopyropen	Versys	3.5	28 & 14	<0.05	<0.05	0.02	0.01*	0.01 (many)
bifenazate	Acramite 50WS	16	14	0.045	0.056	0.7	0.01*	0.2 (China)
phosmet	Imidan 70-W**	92	14	1.6	1.2	10	0.01*	2 (Tai)

[1] Top markets for WA apples with established MRLs; 30 Sept 2020. https://nwhort.org/export-manual/, https://bcglobal.bryantchristie.com/
*No tolerance posted; MRL is based on national default value (0.01 ppm in India)
**Imidan 70-W was mixed with a buffering agent to reduce tank pH to 5.5 per standard industry practice

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 against any insect, acarid, or fungal pest, 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.

Measured residues vs. maximum residue levels (MRLs) for uniformly applied AGGRESSIVE industry apple pesticide programs in 100 or 200 gal water/acre utilizing maximum labeled rates, and minimum preharvest and retreatment intervals. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2020.

Chemical name	Trade name	Application rate	Application timing(s)	100 gal/acre	200 gal/acre	US MRL[1]	India MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
flutianil	Gatten	8	21 & 14	0.021	<0.01	0.15	0.01*	0.01 (many)
isofetamid	Kenja 400SC	12.5	35 & 21	0.16	0.062	0.6	0.01*	0.2 (Kor)
abamectin	AgriMek SC	4.25	28	<0.01	<0.01	0.02	0.01*	0.01 (many)
diazinon	Diazinon 50W	16	35 & 21	0.026	0.017	0.5	0.01*	0.01 (UAE)
spinetoram	Delegate WG	7	14 & 7	0.051	0.030	0.2	0.01*	0.05 (many)
cyantraniliprole	Exirel	13.5	14 & 3	0.23	0.16	1.5	0.01*	0.8 (many)
spinosad	Entrust	3	21 & 7	0.022	0.017	0.2	0.01*	0.1 (many)
tolfenpyrad	Bexar	27	28 & 14	0.21	0.10	1	0.01*	0.01 (many)
myclobutanil	Rally 40WSP	10	21 & 14	0.33	0.15	0.5	0.01	0.01 (UAE)
fenpropathrin	Danitol	18	28 & 14	0.26	0.12	5	0.01*	0.01 (many)
difenoconazole	Inspire Super	12	21 & 14	0.11	0.058	5	0.01	0.5 (China)
cyprodinil	Inspire Super	12	21 & 14	0.19	0.11	1.7	0.01*	0.05 (Indo)
cyflufenamid	Torino	6.8	14	0.036	0.021	0.06	0.01*	0.01 (Thai)
buprofezin	Centaur WDG	34.5	14	1.3	0.84	3	0.01*	1 (Tai)
acequinocyl	Kanemite	31	35 & 14	<0.025	<0.025	0.4	0.01*	0.01 (Thai)
afidopyropen	Versys	3.5	14 & 7	<0.05	<0.05	0.02	0.01*	0.01 (many)
bifenazate	Acramite 50WS	16	7	0.076	0.072	0.7	0.01*	0.2 (China)
phosmet	Imidan 70-W**	92	21 & 7	5.3	2.6	10	0.01*	2 (Tai)

[1] Top markets for WA apples with established MRLs; 30 Sept 2020. https://nwhort.org/export-manual/, https://bcglobal.bryantchristie.com/
*No tolerance posted; MRL is based on national default value (0.01 ppm in India)
**Imidan 70-W was mixed with a buffering agent to reduce tank pH to 5.5 per standard industry practice

CONCLUSIONS

As we have observed in every study since 2011, no spray program produced a residue that exceeded the tolerance level set by the US Environmental Protection Agency; these findings are further evidence that apple growers following directions on product labels should expect their fruit to be in full compliance for domestic sales regarding pesticide residues. Several products we tested, however, did produce **residues which exceed Maximum Residue Levels** (MRLs) set in important export markets for Washington apples including: **Gatten, Diazinon 50W, Delegate WG, Bexar, Rally 40WSP, Danitol, Inspire Super, Torino, Centaur WDG, and Imidan 70-W**. India has yet to post tolerances for most pesticides used by WA apple growers; in the absence of a posted MRL, the default tolerance in India is 0.01 ppm, essentially meaning that any product which produced a detectable residue in our study would potentially violate India's standards. Trade representatives from the USDA and Northwest Horticultural Council continue to work with Indian authorities to encourage them to post more MRLs, which should make compliance more feasible.

Results from this year's study revealed a trend for higher detectable residues being recorded from concentrate (100 gal water/acre) than dilute (200 gal water/acre) applications under the aggressive protocol; under the standard protocol, however, there was virtually no difference in residue levels produced by spraying dilute vs. concentrate. Given that most materials in the standard protocol were applied earlier in the season and had more time to degrade than in the aggressive protocol, any treatment differences between dilute and concentrate applications may have diminished over time. The results of several years of testing the effect of carrier volume on pesticide residues have been largely inconsistent and therefore inconclusive.

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.org. We encourage growers and consultants to stay abreast of current information on international MRLs, which often change in response to trade negotiations and/or political developments. For more information, visit the Northwest Horticultural Council website, www.nwhort.org.

WASHINGTON TREE FRUIT RESEARCH COMMISSION

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

CONTINUING PROJECT REPORT **YEAR:** No-Cost Extension

Project Title: Development of New Biocontrol Strains from Washington Native Trees

PI: Professor Sharon Doty
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Dr. Tianna DuPont (WSU-Wenatchee), tianna.dupont@wsu.edu
Dr. Patricia Okubara (WSU-Pullman), patricia.okubara@usda.gov

Total Project Request: **Year 1: $46,229**

WTFRC Budget: None

Budget 1
Organization Name: University of Washington
Contract Administrator: Carol Rhodes, Director, Office of Sponsored Programs
Telephone: (206) 543-4043
Email address: osp@uw.edu

Item	2020	2021
Salaries	$28,632	
Benefits	$8,775	
Supplies	$5,000	
Travel	$822	
Miscellaneous	$3,000	
Total	$46,229 (Total Year 1)	0

OBJECTIVES

1) Testing for biocontrol of *Erwinia amylovora* (causal agent of fire blight) using native plant microbiota from Washington State
    a. Assay development
    b. Testing of our fully characterized biocontrol strains in the assays optimized in Objective 1a
    c. Screening for new microbial strains with activity against *E. amylovora*.
    d. Genomic sequencing of selected strains
2) Testing for biocontrol of pre- and post-harvest apple fruit pathogens using native plant microbiota from Washington State
    a. Testing our fully characterized biocontrol strains
    b. Screening for new microbial strains with activity against the pre- and post-harvest apple pathogens
    c. Genomic characterization of selected strains.

SIGNIFICANT FINDINGS

- Through this project, we isolated over a hundred new endophyte strains from the Wenatchee, Entiat, Yakima, and Methow areas
- 14 strains showed inhibition of *Penicillium expansum*. Since the strains grew in the presence of this fungus known to produce the antimicrobial compound, patulin, they may have the capacity to degrade it
- 27 strains inhibited *Botrytis cinerea*
- 21 strains inhibited *Neofabraea perennans*
- 38 strains inhibited *Phacidiopycnis washingtonensis*
- 40 strains inhibited *Erwinia amylovora*
- Several of the strains appeared to inhibit the pathogenic fungi through production of volatile compounds
- **The current status of this project (continuing through a No Cost Extension) is the preparation of genomic DNA and full genomic sequencing of the top-performing candidate strains**

METHODS

Isolation of new endophyte strains from natural areas near to the fruit tree growing areas. Doty obtained the required permits and sampled a variety of native plants in natural sites in the Wenatchee, Entiat, Yakima, and Methow areas. Microbial endophyte strains (bacteria and yeast from within plant tissues) were isolated through maceration in bacterial media and streak purification.

In vitro assay for inhibition of the post-harvest decay pathogens, *Penicillium expansum*, *Botrytis cinerea*, *Neofabraea perennans*, and *Phacidiopycnis washingtonensis*. The fungal samples were obtained from the Amiri Lab at the WSU Tree Fruit Research and Extension Center in Wenatchee. Using a modification of the dual plate assay we had used previously (Kandel et al. 2017), we pipetted 10 µl fungal spore/suspended hyphae preparations to the center of agar plates containing medium appropriate for fungal growth, PDA (potato dextrose agar). The fungi were allowed to grow at room temperature until robust fungal growth was evident in the center of the plate. Endophyte isolates, which had been grown on rich media (MGL), were then spotted around the edge of the plate with up to eight isolates per plate (**Figure 1**). Due to the slower growth of *Neofabraea perennans* only up to four isolates were spotted per plate and half the distance from the hyphae edge (**Figure 2A**). Growth of the fungus was monitored and

inhibition was scored when the fungal growth reached the perimeters of the plates, except *Neofabraea perennans* which was scored based on hyphae growth disruption (**Figure 2B**).

***Erwinia amylovora in vitro* inhibition assay**. Three *Erwinia amylovora.* isolates were provided by Dr. Tianna DuPont, however after initial testing indicated the three strains displayed identical inhibition patterns, assays were carried out on a mixture of the three strains provided. 100 µl of *Erwinia amylovora* with an optical density of 0.01 at 600nm were spread onto rich medium appropriate for *Erwinia* (NYDA). Endophyte isolates were grown on MGL and then spotted onto these plates of *Erwinia amylovora*. Clear zones on the *Erwinia* lawns were scored as inhibitory activity (**Figure 3**).

RESULTS AND DISCUSSION

A total of 38 strains inhibited the growth of *Phacidiopycnis washingtonensis*, 21 strains inhibited *Neofabraea perennans*, 27 strains inhibited *Botrytis cinerea,* and 14 strains inhibited *Penicillium expansum*. Many of the strains strongly inhibited the growth of *Erwinia amylovora*, with a total of 40 inhibitory strains. (**Table 1**).

Figure 1. Inhibition of *Botrytis cinerea* by some of the endophyte strains. A) Example plate showing no inhibition of the fungus. B) Strong inhibition of the fungus by sample # 88. C) Apparent inhibition by volatiles produced by some of the strains, as indicated by the overall reduced fungal growth and the bubbling appearance of some of the samples.

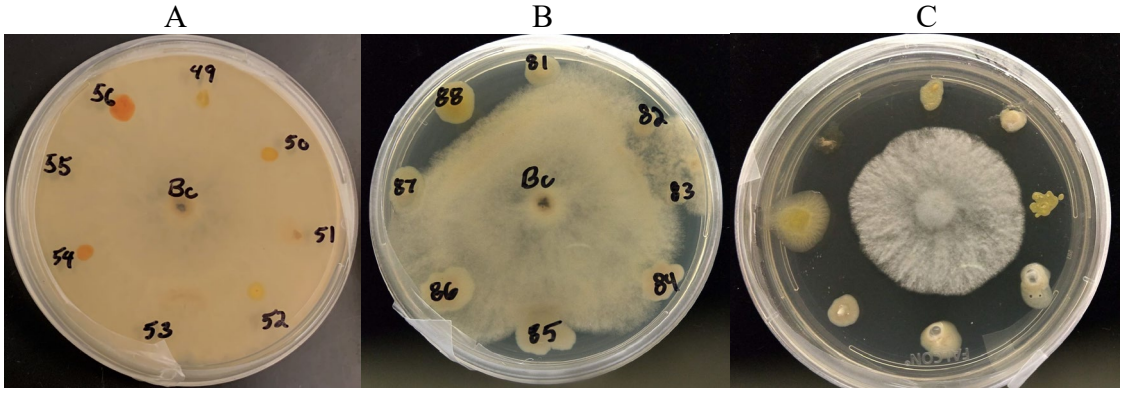

Figure 2. Inhibition of *Neofabraea perennans*. A) Example of four spotted endophyte isolates. B) Close up of disrupted hyphae growth near sample #4, with the leading edge of fungal growth becoming filiform as opposed to the smooth edge seen near sample #1.

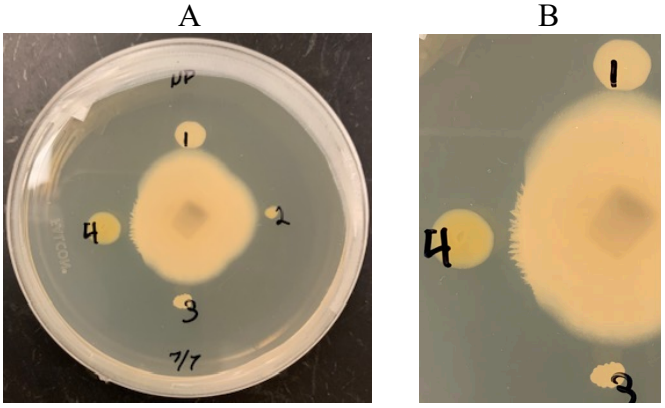

Figure 3. Inhibition of *Erwinia amylovora*. The pathogen was inhibited by several of the endophyte strains as indicated by clearing zones on the lawn of *Erwinia* growth. A. Overall screening results. B. Close up of one of the assay plates showing the strong inhibition of *Erwinia* growth.

A B

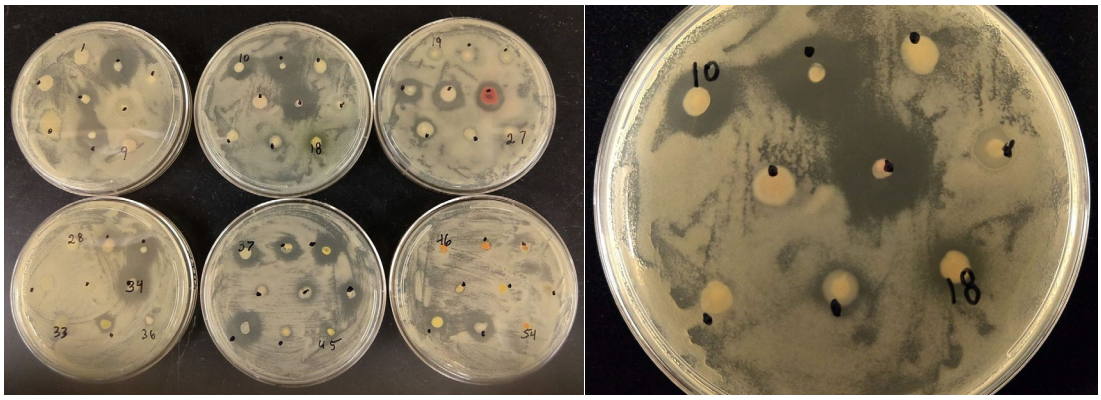

Table 1. Apple biocontrol project screening results. Endophyte strains with any activity against each of the pathogens. Pw *Phacidiopycnis washingtonensis*, Np *Neofabraea perennans*, Bc *Botrytis cinerea*, Pe *Penicillium expansum*, and Ea *Erwinia amylovora*. Strain names preceded by a number indicate the site from which they were isolated: 1 Wenatchee area, 2 Entiat/Okanagan area, 3 Yakima River area near Ellensburg, and 4 Methow area.

Pw	Np	Bc	Pe	Ea
1SS-L-D	1SS-L-D	1SS-L-D	1SS-L-H	1 SS-L-C
ISS-L-E	1SS-L-F	4 2 2	1Cv-L-C	4RDLD
1SS-L-F	1SS-L-J	WP 40	WP 40	2RDLC
1SS-L-H	4 5 3	WP 41	WP 41	2RDLD
1SS-L-I	4 4 2	WP 42	WP 42	3Pop12L1
1SS-L-J	WP 40	AFE 4A	AFE 4A	3YPLB
1Cv-L-C	WP 41	AFE 21B	WPB	2ALE2
1 SS-S-A	WP 42	AFE 5	AFE 3	2PtLE
4 2 2	AFE 4A	1 SS-A	2PtLD	2OPSB
4 5 3	AFE 21B	1 SS-B	3YPLB	3RS1
4 3 2	1 SS-S-B	1 Cv-S-A	4ASD	3RS3
4 4 2	WW7B	AFE 8	4RDLI	3Pop12L4
WP 40	AFE5	WPB	4RDLJ	3YPS2
WP 41	AFE9	4RLD	4RDLG	3YPS3
WP 42	AFE14	4RDLD		2 OPSB
PTD1	2PtLD	3ThS2		2PtLC2
AFE 4A	2SASA	2OPSA		2PTLF1
AFE 21B	2ALE2	2PtLD		2SASD
4RDLD	2RDSA	2SASA		2RDSB

3ThS2	2PtLE	2RDLC		2RDLA
2PtLD	2OPSB	2RDLD		2ALA1A2
2SASA		4SBLB-		2ALB
2RDLC		3WL2		4RLA
2RDLD		3WL3		4RLE
3WL2		3Pop12L1		4RFA
3WL3		3YPLB		4RFB
3Pop12L1		3YPLD		4RSC
3YPLB				4ASA
3YPLD				4ALB
3RS1				4ALC
4ASD				4RDLA
3RS3				4RDLE
3ThS1				4RDLF
3Pop12S3				4HNLA
3Pop12L3				4HNLB
3Pop12L4				4SBLA
3YPS2				3RF1
3YPS3				3RL2
				3ThS3
				3ThL1

Table 2. Endophyte isolates chosen for sequencing after rRNA preliminary identification.

3YPLB	*Pseudomonas*
1SS-L-D	*Erwinia*
3Pop12L1	*Pseudomonas*
1SS-L-F	*Schwanniomyces*
3ThS2	*Pseudomonas*
3WL2V	*Acinetobacter*
3WL3V	*Acinetobacter*
3YPLD	*Pseudomonas*
3RS1	*Enterobacter/Pantoea*
4ASD	*Sphingomonas*
3YPS3	*Pseudomonas*
4RDLJ	*Sphingomonas*
4RDLG	*Rhizobium*
1SS-L-J	*Rhodotorula*
2PtLD	*Serratia*

Genomic DNA Sequencing. Fifteen strains were chosen for full genomic sequencing based on the number of pathogens towards which the strain was inhibitory, the strength of the inhibitory activity, and uniqueness of the strain compared to the other top-performing strains (**Table 2**). We now have an agreement with GeneWiz for the sequencing of these strains and can begin preparing the genomic DNA. Sequence data is expected by late March, prior to the start of our proposed Phase 2 project.

KEYWORDS, ABSTRACT AND EXECUTIVE SUMMARY

Keywords: Fire blight; *Erwinia amylovora*, post-harvest decay; *Penicillium expansum*, *Botrytis cinerea*, *Neofabraea perennans*, *Phacidiopycnis washingtonensis*

New microbial endophyte strains were isolated from native plants in natural areas near apple tree growing areas of Wenatchee, Entiat/Okanagan, Yakima, and Methow. A total of 119 strains (15 previously characterized and 104 new isolates) were screened using *in vitro* assays for inhibition of the post-harvest decay pathogens, *Penicillium expansum*, *Botrytis cinerea*, *Neofabraea perennans*, and *Phacidiopycnis washingtonensis*, as well as the causal agent of fire blight, *Erwinia amylovora*. Two to three dozen inhibitory strains for each pathogen were identified. Preliminary rRNA sequence characterization was used to screen for isolates related to potential human pathogens and a subset of the isolates with the strongest or broadest activities was selected for full genomic sequencing.

FINAL PROJECT REPORT
CP-18-104

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

PI:	Elizabeth Beers	**Co-PI (2):**	David Crowder
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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: **Contract Administrator:** Shelli Tompkins
Telephone: 509-293-8803 **Email address:** 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 sterile insect release (SIR).*
   We have completed all three years of releases in the replicated plot study in the Tonasket/Malott area. We collected moth recapture and fruit damage data for all three years.
2. *Compare the non-target effects of broad-spectrum pesticide use versus SIR as a supplement to mating disruption in organic orchards.*
   A broad range of secondary pest and natural enemy samples were taken during the 2018 season. 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 was unsuccessful.
4. *Investigating sterile moth recapture and behavior.*
   This objective was added in 2019. We investigated recapture of sterile insects near net installations, the effect of high densities of sterile females, and the apparent longevity/viability of sterile moths in the field.

Significant Findings:

- Recapture of sterile moths was low in spring and fall and peaked in midsummer (July and August). The higher (3x) rate was reflected in recaptures in 2018 and 2020, but not in 2019.
- Overflooding ratios ranged from 2 to 20 (2018), 1 to 15 (2019) and 1 to 13 (2020) depending on treatment and time of season.
- Codling moth fruit damage (stings+entries) were significantly lower in the two SIR treatments in 2018, but no significant differences occurred thereafter. Overall, the number of fruit damaged declined each year of the study but did so in all treatments.
- Moths released outside of caged orchards were captured at much lower rates than uncaged orchards, indicating good exclusion by the nets. Moths released over a cage by drone were recaptured at the same rate as moths released by hand inside the cage; in addition, moths sprinkled in the canopy were recaptured at much higher rates than moths sprinkled on the ground (hand release).
- When higher densities of female moths were released, the percent recapture of females was significantly lower than the lower release density treatments.
- Sterile moth longevity/viability in field: In a preliminary study, moth recapture peaked at 3 days post-release with the last detection at 9 days; a larger study found peak recapture 6 days post-release with a final detection at 18 days. The majority of recapture occurred during the first week post-release.

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

The plots identified in 2018 were used throughout the project with a few exceptions. In 2019, the trees in one block were removed, and a different block on the same ranch was substituted. In 2020, two check plots were removed (blocks on the same ranch substituted), and two blocks were reduced in size, but remained in the program. 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 to 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.

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

Moths were transported from the Osoyoos facility and released on Tuesdays using a hexacopter (Hermes V2 UAS) by M3 Consulting of Phoenix, AZ to the eight SIR-treated plots (Trts. 1 and 2). Moths were released weekly for 22 weeks (late April – mid-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 received only 2.4-fold the number of moths as the 1x treatment.

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 a grid pattern inside the plot boundaries, with four additional perimeter traps ca. 50 ft from the center of each edge. Liners were changed weekly, and the number of moths recorded by sex (Fig. 1B, C), mating status (females only), and origin (sterile, wild). Sterile moths were identified by crushing the abdomen to see the internal red dye used in the larval diet in the Osoyoos rearing facility. Lures were changed every 6 weeks as per manufacturer's recommendations. Trap captures were counted the entire release season plus one week before the initial and one week after the 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. 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.

Results. In all years of the project, sterile moth recapture peaked in mid-summer (mid-July-August) and was lower in the spring and fall (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; however, wild moth flight is also affected by environmental conditions. Overall, the interior traps in the 3x treatment recaptured almost twice as many moths as the 1x treatment in 2020 and 2018. In 2019, the recapture in the 1x and 3x treatments was almost identical. The percentage recapture of sterile moths in 2020 was a little higher for the 1x (0.08%) compared to the 3x (0.05%). 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 highest during mid-summer and early fall, and somewhat higher in the 3x rate (0.9:9.9, mean 5.32) than in the 1x rate (0.25:12.6, mean 3.74). The proportion of moths captured by the interior vs perimeter traps was consistently higher over time, irrespective of treatment (data not shown), although 30-40% of the total moths recaptured were in the perimeter traps, indicating a moderate amount of off-target drift.

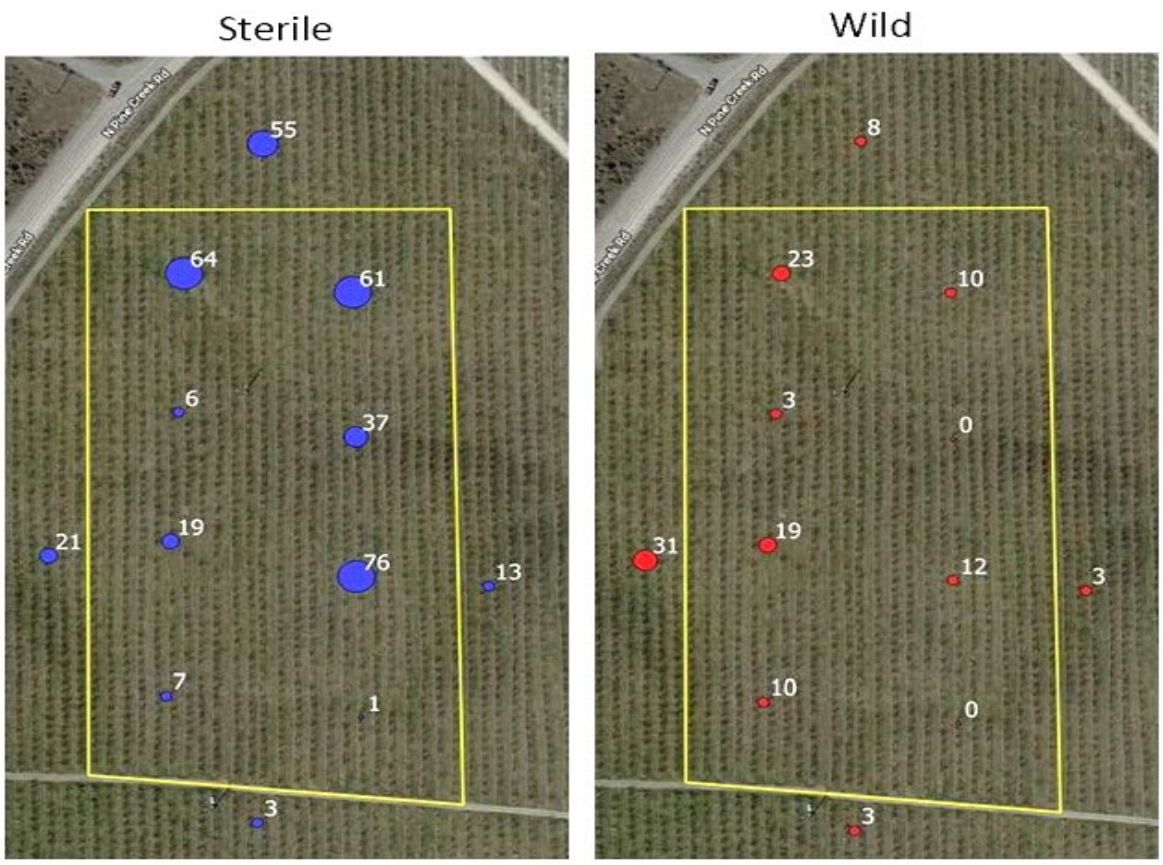

Fig. 2. Bubble maps of weekly trap catches (wild and sterile), 29 May 2018.

Wild moth densities showed a first-generation peak, with the strongest peak in late May (Fig. 3). Wild moth captures declined over the course of the study in all treatments. In 2018, there was a peak of ca 10 moths/trap, but in 2019 and 2020, there were <4 moths/trap/week throughout the season. The second flight in July was fairly distinct in 2018 but was difficult to discern in 2019 and 2020. There were no significant differences between wild moth capture between the treatments.

Plate 1. Visually recording stings and entries, 2018.

The success of the SIR treatments was assessed by intensive preharvest fruit damage samples. Codling moth stings and entries were recorded ca. 7 to 10 days before each block was harvested by visually observing both sides of each row of the blocks and recording the latitude and longitude of each damaged apple observed (Plate 1). This allowed to us to determine and map the spatial pattern of damage in the blocks (Fig. 4). The total number of apples observed was recorded independently to provide information on percent damaged fruit.

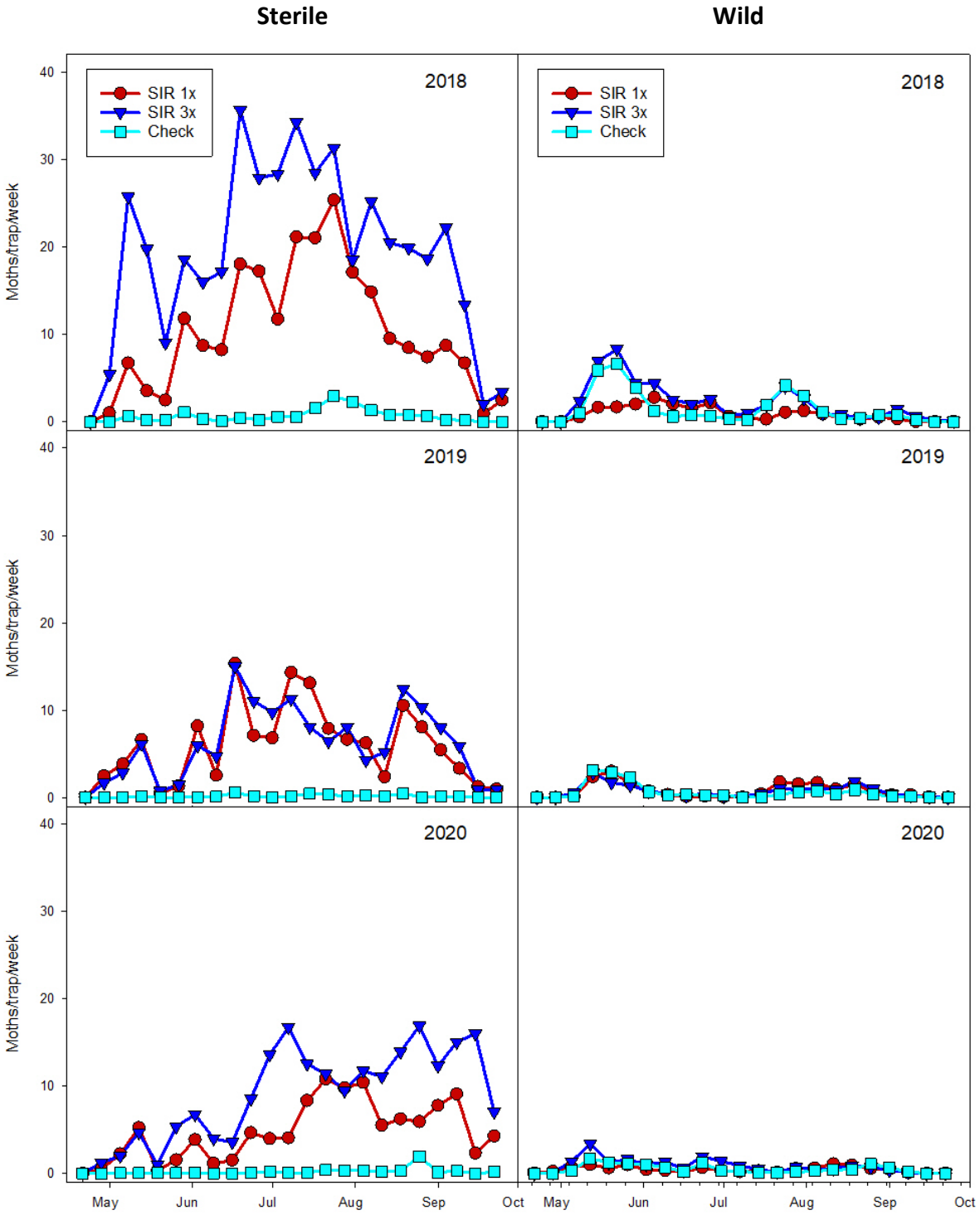

Fig. 3. Sterile and wild moth trap captures in three treatments, 2018-2020.

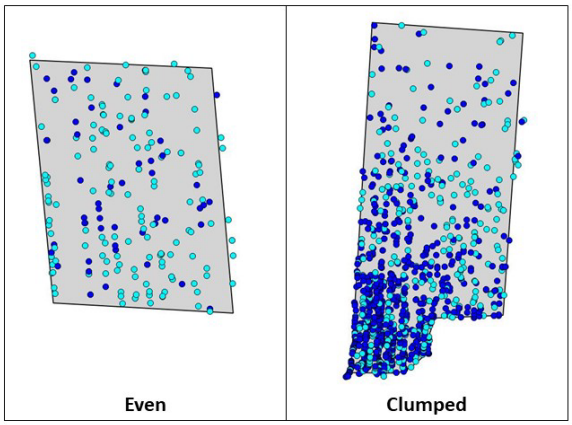

Fig. 4. Even (left) vs clumped (aggregated) (right) distribution of codling moth fruit damage.

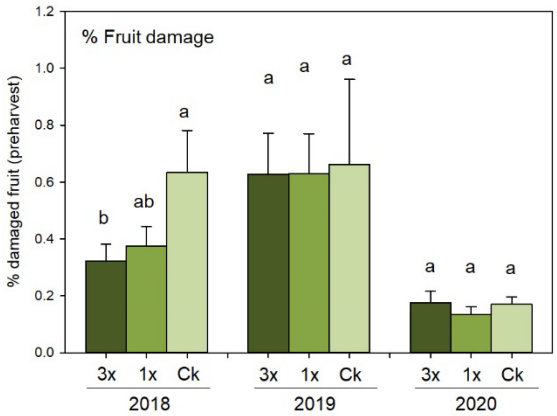

Fig. 5. Percentage codling moth fruit damage, 2018-2020. Replicate 1B was dropped from the analysis in 2020 due to atypical orchard management.

The average fruit damage was <0.66% in all three years of the study for all treatments. Individual blocks were more variable, but never exceeded 1.6% damage. In 2018, codling moth damage was significantly lower in the 3x SIR treatment in comparison to the check, with the 1x treatment intermediate (Fig. 5). There were no significant differences in mean fruit damage thereafter. Fruit damage was about 0.65% in all treatments in 2019, and about 0.16% in 2020.

The georeferenced fruit damage assessments indicated that some blocks had evenly distributed damage, while some were concentrated in one section of the block (Fig. 4). Information of this type can be very useful to help growers address 'hotspots' in their blocks. Remedial measures could include bands, sprays, sanitation, intensive trapping, or higher rates of SIR moths in the high-damage area.

The spatial fruit damage pattern also allowed us to see a noticeable diminution in damage in a hotspot area in one block over the course of the study (Fig. 6). This occurred even though the moths were distributed evenly in all years of the study; altering moth distribution to address this area may be a future option. There was some correspondence between the trap capture of wild moths (Fig. 7) and the resulting fruit damage (Fig. 4, clumped) in high pressure situations, but in general, the capture of wild moths did not provide an accurate predictor of fruit damage.

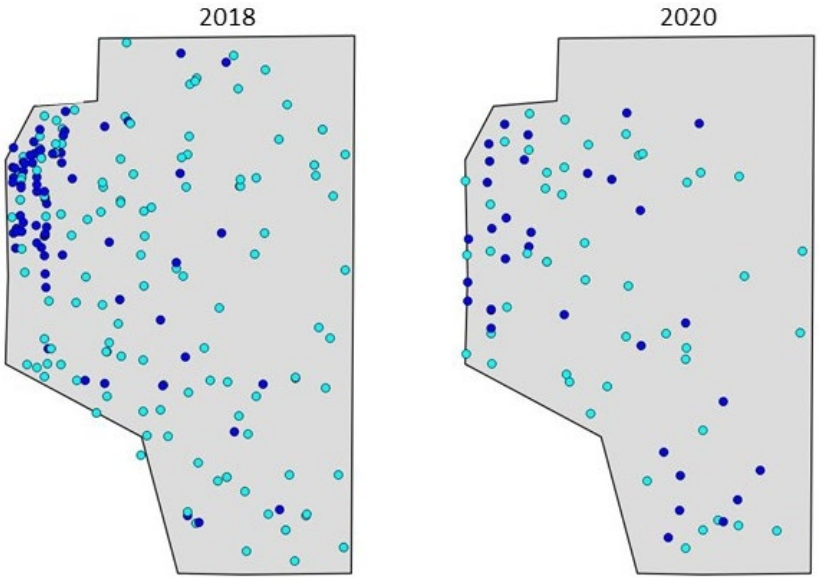

Fig. 6. Amelioration of a codling moth hotspot from 2018 to 2020.

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.

Fig. 7. Aggregated wild moth distribution in pheromone traps (see Fig. 4, right).

Results. 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. We continued this effort in 2020, but were still unable to find a viable molecular marker to distinguish between the colony and wild moths.

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.

CM exclusion. We conducted CM exclusion experiments in 2019 and 2020 in an orchard in Douglas County, WA which had an existing installation of both a full net cage and an overhead net, as well as orchards without nets (check). In 2019, the caged plot was compared to the overhead net and check, but in 2020, only the cage and check were compared. Plots ranged from 10 to 26 acres/plot in 2019, and 10 to 15 acres/plot in 2020. We deployed traps (CM-DA+AA lures in an orange plastic Delta trap) 50 ft inside the perimeter of the blocks. Sterile moths from the OK-SIR facility in Osoyoos, BC, were marked with fluorescent powder and released 50 ft outside of the perimeter of the blocks, with 1 to 6 release points per

edge depending on the block size and surroundings (Fig. 8). There were about two-thirds as 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) from late July to early September of both years. In 2019, the number of moths available each week was variable; the moths were divided evenly among release cups. In 2020, each release point received a full dish of 800 moths, except for one week, which received 400 moths/release point. The total number of moths released per block per week was the product of moths/release point × release points/block. In 2019, the marked moths were placed in plastic cups which were suspended from a trellis wire about 6 ft from the ground; in 2020, the moths were sprinkled on the tree canopy over a few feet around a flagged release point. The recapture was expressed as the number of released moths recaptured per 1,000 moths released on a whole-block basis.

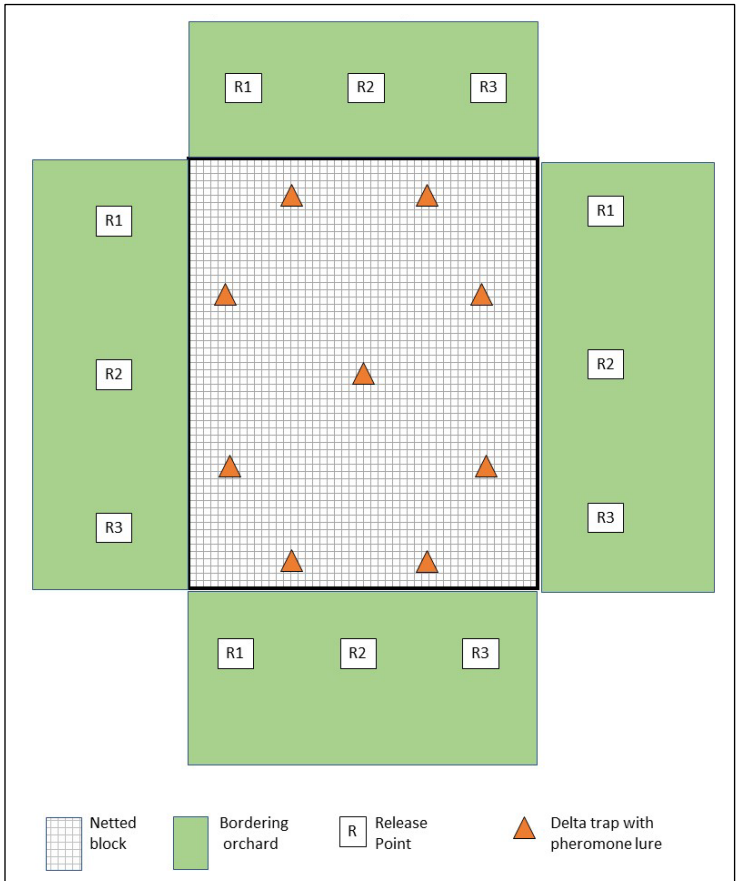

Fig. 8. Treatment schematic for testing codling moth exclusion

Results: Overall, the recapture rate of moths was much lower in 2019 than in 2020 (note different y-axes in Fig. 9); this corresponds to lower recapture rates in the SIR pilot project plots in this year. In 2019, the recapture in the overhead net block was slightly lower than the check plot, but not significantly so. In both years, the recapture of moths in the caged block was substantially lower than the check plots (Fig. 10). Interestingly, the same trend occurred each year for wild moths in the cage vs check plots, although different check plots were used, and the underlying wild moth population was unknown.

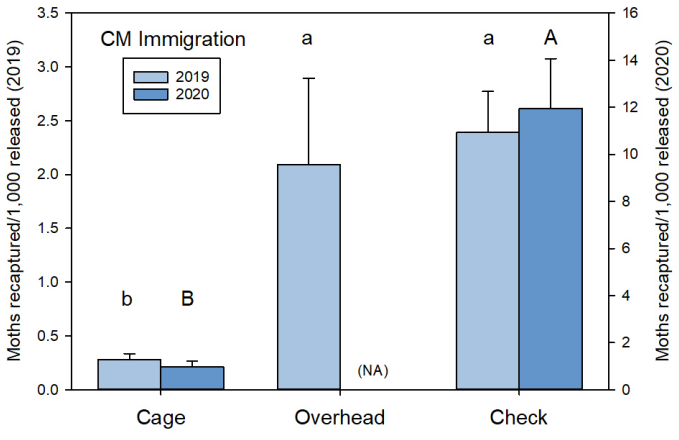

Fig. 9. Exclusion of codling moth from caged and overhead net blocks, 2019-2020.

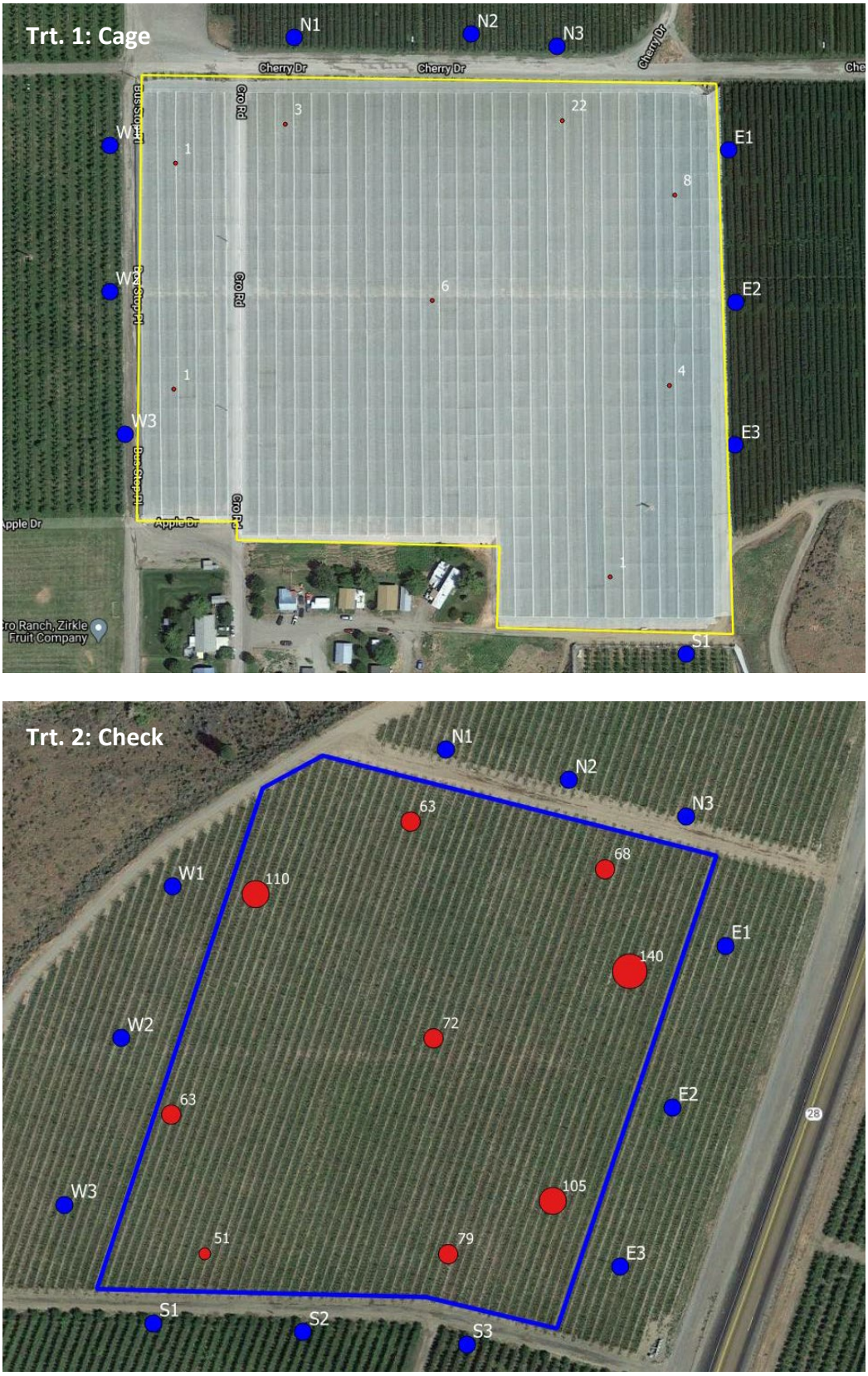

Fig. 10. Recapture of marked sterile moths released outside of a caged (top) or uncaged (bottom) orchard.

CM drone releases over nets. In 2020, we studied the potential for moth release over a caged orchard by drone. The alternative for SIR treatment in caged orchards (or those with overhead nets) is to revert to ground release, either by hand or all-terrain vehicle (ATV). Either method would significantly slow the workflow of releases, and possibly result in poorer moth quality. Previous immigration/emigration experiments indicated that behavior, rather than physical exclusion, plays the greater role in the propensity of moths to traverse a net barrier. Rapid escape from small cages clearly indicated that moths are physically able to cross a 5 x 2 mm mesh of the type commonly used for sunburn protection. However, our 2019 cage experiments (above) also indicated they have little tendency to enter a cage when released outside it at ca. 6 ft. Moths released above a net may exhibit different behavior in order to reach a host tree. If moths can be released by drone above nets, a substantial savings of time would result. The study site was a recently built cage over an 8.3-acre apple orchard. Three of four sides were completely enclosed to ground level, but one side (west) had a partial wall to allow entry/exit of farm equipment. In addition, the top net had apertures at intervals that, while still providing sunburn protection, allowed possible entry points for moths (Fig. 11). We compared drone release above this caged orchard with an uncaged orchard immediately adjacent to the west. In addition, we made releases by hand beneath in both the caged and uncaged orchard, using different colors of fluorescent powder to determine the origin of the moths. Two types of hand releases were compared: spreading the moths on the ground (simulating the Canadian ATV release method) and sprinkling the moths in the tree canopy about 6 to 7 ft above the ground. Moth recapture was determined by a grid of delta traps (1/acre) baited with a CM-DA-AA lure. For all methods, the same rate of moth release was used (800 moths/acre). The drone releases were made by M3 Consulting using their normal flight path for even distribution, and the hand releases were made using 1 release point per acre, thus 1 dish of 800 moths per release point. The treatments were replicated over time for 6 to 10 weeks from mid-July to late August.

Fig. 11. Upper net of cage testing drone versus hand release, 2020 (note gaps).

Results. In the caged plot, releases made by drone were not significantly different than releases made by hand (sprinkling moths in the canopy) (Fig. 12); this is a positive indicator that under certain conditions, SIR moths can be distributed by drone with no penalty in moth performance. The two hand-release methods gave strikingly different results; placing the moths in the canopy (Plate 2) resulted in higher recapture than placing them on the ground (Fig. 13). This difference was apparent in both the caged and uncaged plots. Interestingly, the drone release resulted in 4× higher recapture of the caged plot than over the uncaged plot (data not shown); the reasons for this are not immediately apparent, but are still a hopeful indicator that drone releases over nets will be as effective as those made by hand

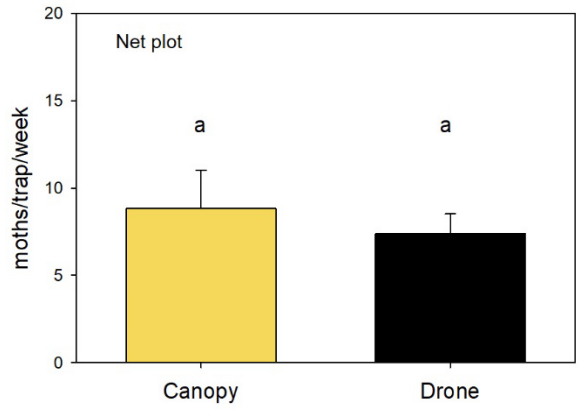

Fig. 12. Recapture of marked sterile moths released by drone or by hand (sprinkled in canopy).

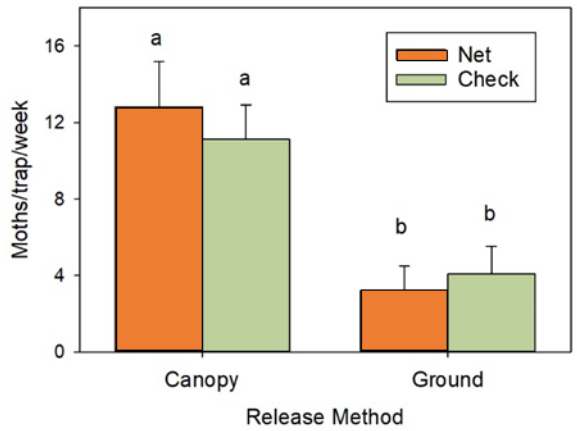

Fig. 13. Recapture of marked sterile moths released by hand in the canopy or on the ground.

Sterile moth accumulation in traps over time

Two experiments were conducted to see how long sterile codling moths remain viable in the field, as evidenced by trap recapture. The first preliminary experiment was in a small 2-acre research pear orchard not under mating disruption at the WSU Tree Fruit Research and Extension Center in Wenatchee, WA. There was a single release of 3,200 moths (4 points x 800 moths/point) on 8 July. Recapture was determined by a grid of 8 traps all within ca. 200 ft of the release point. Traps were check daily until no more moths were recorded.

Plate 2. Marked codling moths in orchard canopy.

The second experiment was conducted in a 21.6-acre organic apple block under mating disruption in a commercial apple orchard near Rock Island, WA. We had 12 traps baited with CM-DA-AA lures. Traps in the release block were placed 200 ft apart in a north-south line (10 traps), with an additional 2 traps 200 ft to the east and west of the release point. These were eight

additional traps (400 ft apart) in surrounding blocks to the west (the east side abutted a cliff); four in Fuji organic transition and four in conventional Granny Smith (Fig. 14). There was a single release of moths on 15 July 2020 of 10 dishes (8,000 moths) at the central release point. Traps were equipped with cameras, and a photo of the trap liner and moths was taken ca. 10 am daily. Liners were changed weekly and brought into the lab to match the moths captured to the photos to determine the day of capture. The traps were left in place for three weeks post-release. This experimental design allowed us to look at movement of moths over a greater distance, and well as the recapture curve.

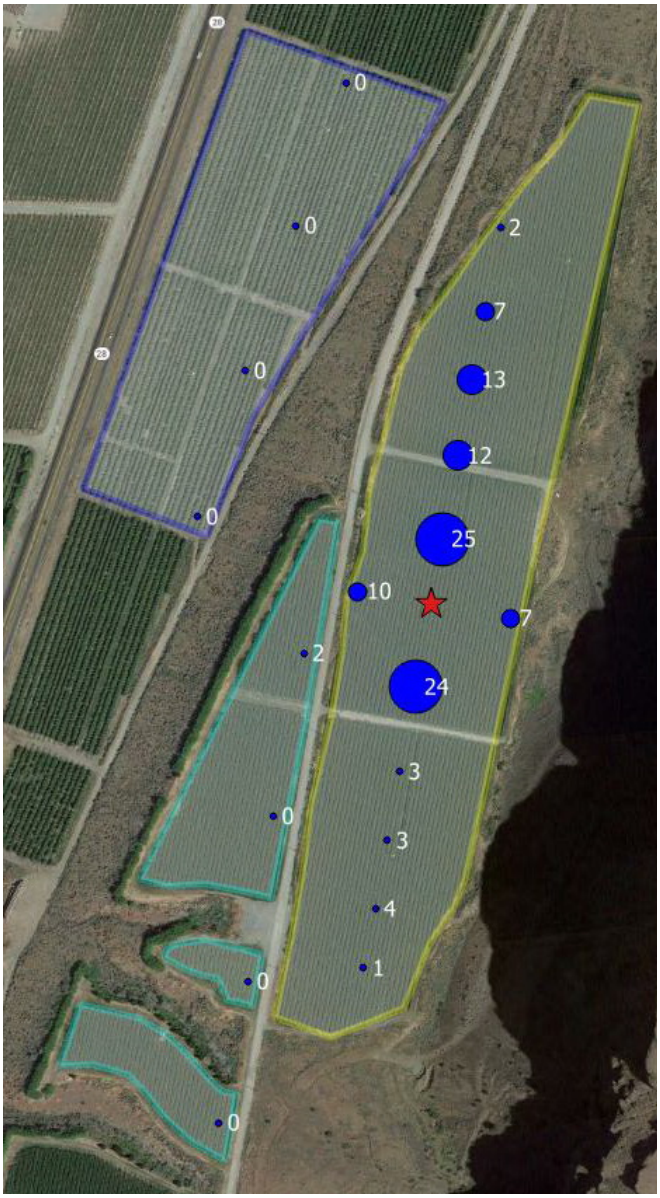

Fig. 14. Recapture pattern of moths in a 21-acre organic apple block, 2020.

Results. In the preliminary experiment, 93 of the 3,200 moths released in the pear block were recaptured, or 2.9%. Peak recapture occurred on 3 days after release, and 98% of all moths recaptured occurred in 7 days. The last moth was detected 9 days after release (Fig. 15A).

In the second experiment, a total of 113 moths released in the apple block were recaptured, or 1.4% of the total released (avg. 5.65 ± 1.71 moths/trap). Moth recapture was low on day 1 and peaked on day 6. Recapture steadily decreased quickly after day 6 (Fig. 15B). There was a small peak of recapture on day 12. This suggests that sterile moths may have greater longevity than we may have previously thought. There was one moth recaptured on day 18, almost three weeks after release. Overall, the majority of recaptured moths were caught in 8 days, and in the release block; only 2 were found in a neighboring block. The greatest numbers (43%) were found in the two adjacent traps along the north-south transect, and 58% in the four traps in four cardinal directions (all 200 ft from the release point) (Fig. 14). Although treated as a single management unit, the block had two bisecting east-west road that may have impeded movement. Recovery ≥800 ft from the release point was extremely low.

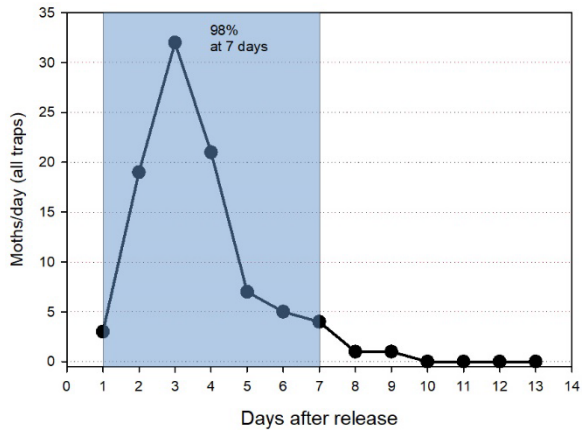

Fig. 15A. Recapture of released sterile moths over time, pear block, 2019.

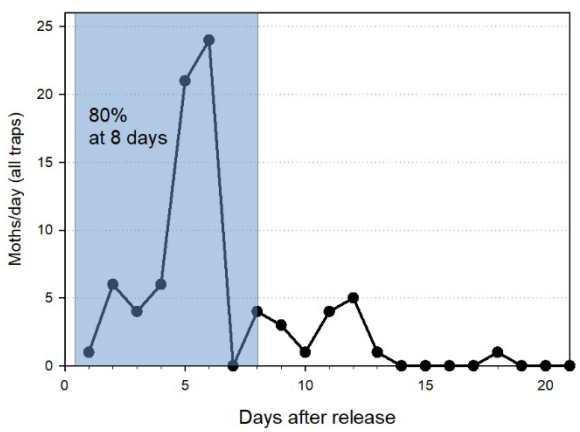

Fig. 15B. Recapture of released sterile moths over time, apple block, 2020.

EXECUTIVE SUMMARY

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

Keywords: sterile insect release, SIR, codling moth

Abstract: The Canadian SIR program in British Columbia has been in place since the early 1990s, and had successfully suppressed this key pest of apples and limited the need for pesticides. Interest in this technique intensified in Washington in recent years due to the availability of excess moths from the Canadian facility in Osoyoos, a few miles from the Washington border. A pilot project was conducted in Washington from 2018 to 2020 in Okanogan County, WA to determine if sterile moth release could be used as an IPM tactic outside of an areawide program, the latter being the norm for most SIR projects. Two rates of sterile moth releases were compared to a check without SIR. Fruit damage in the first year was significantly reduced in the SIR treatments, but no differences occurred in subsequent years. However, fruit damage declined steadily in most of the plots throughout the study. This preliminary study provides a positive indicator that SIR can be helpful in suppressing codling moth when used on limited acreage, although more experience is needed to confirm this.

Summary: Codling moth (CM) has been the key pest of Washington apples since the early 1900s and remains so today. The development of pheromone mating disruption (MD) in the late 1980s helped stabilize pest management programs under constant threat of pesticide resistance and became the foundation of codling moth management for ca. 85 to 90% of the state's apple acreage.

Despite the efficacy and widespread use of MD, codling moth pressure has been building in recent years in Washington. The problem is more acute in organic orchards, where insecticidal inputs lack the high mortality levels and long residual control of conventional materials. Sterile insect release (SIR) provides a potential new tool that can be used in both organic and conventional production, with advantages to resistance management in both regimes. However, SIR is normally used on an areawide basis, with compulsory releases on all affected acreage in a region under a sponsored government program. The paradigm of SIR on a voluntary, open market, and block-by-block basis is generally considered unfeasible due to the high initial input costs of rearing, sterilization, and distribution. The availability of sterile moths from the (existing) Canadian program makes this new approach possible. Unsurprisingly, very little of the foundational research addressed this type of use; thus exploration of the integrated pest management (IPM) approach was needed.

To explore this concept, we released Canadian SIR moths from 2018 to 2020 in Okanogan County, WA. We used three treatments, the standard release rate of sterile moths (1x, or 800 moths/acre/week), a high rate of moths (3x), and a control where no moths were released. The recapture rate of sterile moths throughout all three growing seasons did not always mirror the release rates. There was also much better recapture of SIR moths in the middle (warmer) part of the season, with poor rates in spring and fall. The overflooding ratios (sterile:wild moths), a key concept to SIR, varied correspondingly.

The true test of efficacy of any IPM technique is fruit damage. In the first year, the use of SIR moths significantly reduced damaged when compared to the check. No differences among treatments were found in succeeding years, although damage had declined overall by the third year, irrespective of treatment. Our experience in this pilot project indicates that the underlying insect pressure and management intensity greatly affects the outcome for a given block, suggesting that each block is in effect a case study. The transition from a single-tactic approach to a multi-tactic approach thus is dependent on the efficacy of deployment of available tactics, and their integration into a comprehensive program.

FINAL PROJECT REPORT

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:** **$10,000**

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

Total Project Funding: $70,000
Budget History:

Item	Year 1: 2019	Year 2: 2020
Salaries	-	19,500
Wages	-	6,500
Equipment	-	-
Supplies	-	15,500
Travel	$-18,000	10,500
Total	$18,000	$52,000

Funds ($35,000) approved for Dr. Knight in 2019 were withdrawn by the Commission and only funds for travel of the three co-PIs were paid. Funds in 2020 were paid in full to Drs. Knight, Basoalto and Preti ($6,500 each for supplies), and $4,000 was returned by Dr. Mujica.

Objectives:

1. Evaluate the effectiveness of a new commercial non-pheromone lure (MegaLure 4K) comprised of pear ester, nonatriene, linalool oxide and acetic acid in apple and pear orchards.
2. Evaluate 19 other plant volatile blends compared with the 4K blend.
3. Evaluate the use of the 4K lure to establish codling moth Biofix.
4. Evaluate the addition of attractive plant volatiles to improve mating disruption.
5. Evaluate the use of female removal with the 4K lure to manage codling moth in apple and pear.

Significant Findings:

- A new more attractive blend for male and female codling moth consisting of plant and microbial volatiles was discovered and commercialized as the MegaLure 4K.
- Field trials showed that the 4K lure catches ca. 3-fold more females than any previous lure.
- The new PVC Combo lure used with acetic acid was much more effective than the previous septum lure used by the Industry for the past 15 years.
- The addition of sex pheromone to the 4K lure (5K) increased males but decreased female catches.
- All of the new PVC lures were effective for at least 8 weeks.
- The 4K lure outperformed the standard Combo septum lure when traps were placed at head height, and catches were greater if the trap was placed higher in the canopy.
- Establishing a Biofix to predict egg hatch with either Combo-P with acetic acid or 4K lures and with either total moth or female counts were equally effective.
- Adding acetic acid co-dispensers next to MD dispensers loaded with sex pheromone, pear ester, and one of two plant volatiles increased moth catches within replicated plots. No difference in levels of fruit injury occurred at mid-season.
- Several plant volatiles were found to decrease moth catches when added to the pear ester, nonatriene, and acetic acid lure set. These were associated with rosy apple aphid feeding, i.e., one orchard in 2019 with extremely high levels of RAA damage had an unexplained lack of codling moth injury and a record high level of virgin codling moth females trapped.
- Four volatiles were found to be effective as substitutes for linalool oxide in the four-component blend. Further studies are ongoing in Chile and planned for 2021 to develop alternative blends.
- The most effective trap for female codling moth with the new PVC Combo-P + AA lure set was a bucket trap with a green top and clear bottom. Orange delta traps outperformed the clear and green bucket traps. Standardization of how to use milk jug traps needs more work.
- Small, inexpensive solar-powered UV lights added to traps significantly increased catches of codling moth, oriental fruit moth, eye-spotted budmoth, and oblique banded leafroller including increase in females from 2-10-fold.
- Adding the UV light to delta traps baited with the OFM Dual lure significantly increased both OFM and CM catch and may allow the trap to be used for both species.
- 25 and 16 paired studies were conducted in apple and pear, respectively; to evaluate the effectiveness of a female removal strategy during 2019-20.
- Levels of codling moth injury were reduced on average by 56% with the use of 24 traps per acre across these 41 studies. Levels of injury reductions were similar in apple and pear.
- Female removal works best in combination with mating disruption because a higher proportion of unmated females are trapped.
- Trap density can be increased to at least 40 per acre without trap competition. Recommendations for 2021 using the Combo-P+AA lure are 50 traps per acre.
- Traps (50 per acre) baited with the 4K lure were placed in the 1.6-acre corner of a conventional orchard next to a bin pile stacked in August removed 1,770 females, and only a few injured fruits occurred on the row bordering the bins.

Results: Traps captured large numbers of codling moth during the lure evaluation trial in 2019 (Table 1). All three PVC lures outperformed the standard Combo-septum lure. The 4K and 5K lures captured significantly more females than either combo lures. The addition of PH in the multi-component PVC lure significantly reduced the capture of females

Table 1. Mean (± SEM**)** cumulative captures of codling moth from 4 May to 22 July 2019 in orange delta traps baited with combinations of sex pheromone and pear ester (Combo lures in either the standard grey septa or a new PVC matrix) and two new PVC lures loaded with pear ester, nonatriene, and linalool oxide (4K) or with this plus sex pheromone (5K), N = 8.

	Mean (± SEM) moth capture per trap	
Lure	Total	Females
Combo – septum + AA	94.6 ± 9.5 a	39.1 ± 4.4 a
Combo – PVC + AA	281.0 ± 19.9 b	52.0 ± 5.2 a
4K – PVC + AA	244.3 ± 17.8 b	136.6 ± 9.2 c
5K – PVC + AA	233.6 ± 16.3 b	80.1 ± 4.8 b
ANOVA: df = 3, 28	F = 42.40, P < 0.001	F = 39.15, P < 0.001

The new 4K lure was significantly more attractive than the standard Combo septa lure over the course of the season in 10 pear orchards monitored in California during 2020 (Table 2). However, it was not more effective than the use of the Combo-P lure with the AA co-lure added in a 6-week pear trial in the Delta region. Both data sets showed that placing traps higher in the canopy is advantageous, but overall, the non-pheromone 4K lure can be used at head height instead of the standard use of the Combo lure placed high to monitor codling moth. Similar supporting datasets were generated in 2019 in Oregon and Washington but are not shown due to space limitations (see previous report).

Table 2. The influence of trap height on the capture of codling moth with either MegaLure 4K or the Combo-S or Combo-P +AA lure set in Bartlett pear, California, 2020.

Lake County and other Areas 6 April – 21 Sept Mean (SE) catch		Sacramento-Delta Region 9 April – 16 May Mean (SE) catch		
Lure/height	Total	Lure/height	Females	Total
Combo – low, 6’	2.3 (1.5)b	Combo-P+AA – low 6’	11.0 (4.4)	30.0 (6.5)
Combo – high 13.5’	6.7 (4.1)b	Combo-P+AA – high 10’	15.5 (4.3)	44.8 96.6)
4K – low 6’	9.5 (4.0)ab	4K – low 6’	17.0 (3.1)	27.0 (2.0)
4K – high, 13.5’	37.7 (17.0)a	4K high, 10’	22.5 (6.0)	38.3 (8.3)
ANOVA	$F_{3, 36}$ = 7.37	ANOVA	$F_{3, 36}$ = 1.07	$F_{3, 36}$ = 1.63
	P = 0.0006		P = 0.40	P = 0.23

Canopies averaged 14-16’ in the two studies.

The green/clear bucket trap caught significantly more total moths than the other two bucket traps and similar numbers to the orange delta (Table 3). However, the green/clear bucket caught significantly more females than any other trap. Interestingly, the proportion of females was similar among traps baited with the 4K lure, but both the clear and the green/clear bucket outperformed the orange delta and all-green bucket when baited with the Combo-P+AA lure set. During 2019 the milk jug trap outperformed all of these traps over a short trial period with extremely high moth catch, i.e., 500 moths per trap. However, in 2020 the milk jug traps in the first half of the season did not work well as the liquid often spilled and lure placement and potential degradation of the lures became an issue. Thus, milk jugs were not included in the 2020 trap study.

A core goal of our research developing new, more attractive blends for codling moth over the past 20 years has been to increase catches of females. Female-based monitoring has been shown to provide a more direct prediction of key life history events, *i.e.*, egg hatch and to establish action thresholds used to trigger supplemental insecticide sprays. The higher female moth catches with the 4K increases this opportunity. Studies found that the use of the 4K versus the Combo septum increased the ability to set a Biofix. However, the use of the new Combo-P with an acetic acid co-lure also allowed a Biofix to be set. No improvement was found in predicting egg hatch with these two lures using either sustained male or female catches during 2020.

Table 3. Comparison of codling moth catches in four trap types baited with either Combo-P or the 4K lure in apple, Washington 2020.

Trap	# traps	Lure	Mean (SE) catch per trap [a] Males	Females	Total	Proportion females [b]
Orange delta	64	4K	3.5 (0.4)Ab	3.9 (0.3)Ba	7.3 (0.6)Ab	0.53a
	47	Combo-P	14.0 (1.3)Aa	1.6 (0.2)Bb	15.6 (1.4)Aa	0.10c
Clear bucket	51	4K	1.6 (0.2)Bc	3.2 (0.4)Ba	4.9 (0.6)Bb	0.65a
	35	Combo-P	6.5 (1.5)Bb	3.7 (0.8)Bb	10.2 (2.4)Ba	0.36b
Green bucket	7	4K	0.9 (0.3)Bbc	1.7 (0.5)Ca	2.6 (0.6)Bb	0.65a
	15	Combo-P	3.7 (1.2)Bbc	0.6 (0.3)Cb	4.3 (1.5)Ba	0.14c
Green top/ clear bucket	25	4K	3.2 (0.6)Abc	6.9 (1.4)Aa	10.1 (1.9)Ab	0.68a
	14	Combo-P	10.0 (1.4)Aa	5.2 (1.0)Ab	15.2 (2.3)Aa	0.34b
ANOVA:	Trap: df = 3, 250		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	
	Lure: df = 1, 250		$P < 0.0001$	$P < 0.001$	$P < 0.001$	
	Trap*Lure: df = 3, 250		$P < 0.05$	$P = 0.06$	$P = 0.26$	

The greatly improved attraction of the 4K lure for female codling moth now provides an opportunity to develop more effective 'lure and kill' strategies to manage populations. However, concerns about lure performance over extended periods, chemical stability, cost of active materials, and potential registration difficulties suggested that additional volatiles should be evaluated in combination with pear ester, acetic acid, and nonatriene.

Studies examined whether one or more host plant volatiles could be substituted for linalool oxide in the 4K blend to increase moth catches. Nineteen pome fruit and walnut volatiles were evaluated in a series of field studies in apple. The volatiles are not identified prior to the acceptance of the manuscript for publication due to the wishes of several coauthors. Several compounds were found to significantly lower total or female catches when added to the 3K blend (Table 4). These data suggested that the anomalous results obtained in an apple block heavily impacted by RAA (several of these compounds known to be released by aphid feeding) could account for the observed lack of fruit injury and record high levels of virgin females despite high codling moth pressure which unexpectedly occurred. Other substituted compounds did not increase moth catches (Table 5), and a few added compounds were found to provide a good substitute for linalool oxide in the 4K blend (Table 6). However, no volatile substitution improved the performance of the 4K blend. Our interesting results suggest that further evaluations of more complex blends and variable component ratios and emission rates should be conducted.

Table 4 Summary of mean (+ SEM) of adult *Cydia pomonella* caught in orange delta traps baited with a ternary combination of pear ester, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and acetic acid and quaternary bends with various volatiles, N = 8 lure replicates.

Trial		Mean ± SEM moth catch per trap		
#	Volatile added	Females	Males	Total
1	-	9.6 ± 2.5 ab	2.0 ± 0.4 ab	11.6 ± 2.7 ab
	1	1.8 ± 0.5 c	1.0 ± 0.4 b	2.8 ± 0.8 c
	2	1.3 ± 0.6 c	1.1 ± 0.5 b	2.4 ± 0.8 c
	3	2.8 ± 0.8 c	1.9 ± 0.4 ab	4.6 ± 0.9 bc
	4	4.0 ± 0.9 bc	2.3 ± 1.0 ab	6.3 ± 1.7 bc
	5	1.3 ± 0.5 c	0.9 ± 0.4 b	2.1 ± 0.8 c
	6	2.5 ± 1.1 c	2.6 ± 1.0 ab	5.1 ± 1.8 bc
	Linalool oxide (4K)	14.1 ± 2.6 a	5.0 ± 1.4 a	19.1 ± 3.8 a
	ANOVA, df = 7, 56	$F = 11.10$, $P < 0.0001$	$F = 2.87$, $P = 0.012$	$F = 8.21$, $P < 0.001$

Table 5 Summary of mean (+ SEM) of adult *Cydia pomonella* caught in orange delta traps baited with a ternary combination of pear ester, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and acetic acid and quaternary bends with the addition of a fourth volatile, N = 8-10 lure replicates.

Trial		Mean ± SEM moth catch per trap		
#	Volatile added	Females	Males	Total
2	-	4.6 ± 0.6 a	2.0 ± 0.3 a	6.6 ± 0.8 a
	7	4.9 ± 0.8 a	2.8 ± 0.4 a	7.7 ± 1.1 a
	Linalool oxide (4K)	5.9 ± 0.9 a	3.2 ± 0.5 a	9.1 ± 1.2 a
	RCB ANOVA, df = 2, 85	$F = 0.92$, $P = 0.401$	$F = 2.18$, $P = 0.120$	$F = 2.03$, $P = 0.138$
3	-	3.7 ± 0.7 a	1.8 ± 0.3 a	5.5 ± 0.9 a
	8	5.3 ± 1.2 a	2.7 ± 0.5 a	8.0 ± 1.5 a
	Linalool oxide (4K)	4.9 ± 0.9 a	3.1 ± 0.6 a	8.0 ± 1.4 a
	RCB ANOVA, df = 2, 57	$F = 0.50$, $P = 0.601$	$F = 1.45$, $P = 0.244$	$F = 2.01$, $P = 0.143$
4	-	5.5 ± 0.8 b	1.9 ± 0.4 a	7.4 ± 1.0 b
	9	5.2 ± 0.9 b	3.6 ± 0.8 a	8.8 ± 1.5 b
	Linalool oxide (4K)	9.4 ± 1.1 a	4.1 ± 0.8 a	13.6 ± 1.5 a
	RCB ANOVA, df = 2, 50	$F = 6.77$, $P = 0.003$	$F = 2.04$, $P = 0.141$	$F = 5.62$, $P = 0.006$
5	-	4.1 ± 1.2 b	1.4 ± 0.3 a	5.5 ± 1.2 b
	10	6.3 ± 1.5 ab	4.3 ± 1.1 a	10.5 ± 2.0 ab
	11	6.4 ± 1.6 ab	3.8 ± 1.1 a	10.1 ± 2.5 ab
	Linalool oxide (4K)	10.9 ± 1.9 a	3.5 ± 1.2 a	14.4 ± 2.4 a
	ANOVA, df = 3, 28	$F = 3.05$, $P = 0.045$	$F = 1.53$, $P = 0.230$	$F = 3.41$, $P = 0.031$
6	-	6.6 ± 0.9 a	2.5 ± 0.8 ab	9.1 ± 1.4 a
	12	5.4 ± 1.2 a	1.8 ± 0.5 ab	7.1 ± 1.1 a
	13	5.9 ± 2.4 a	1.1 ± 0.4 b	7.0 ± 2.4 a
	14	8.4 ± 1.3 a	2.6 ± 0.5 ab	11.0 ± 1.5 a
	Linalool oxide	8.9 ± 1.8 a	3.5 ± 0.8 a	12.4 ± 2.3 a
	ANOVA, df = 4, 35	$F = 1.43$, $P = 0.245$	$F = 2.71$, $P = 0.046$	$F = 2.07$, $P = 0.106$

Table 6 Summary of mean (+ SEM) of adult *Cydia pomonella* caught in orange delta traps baited with a ternary combination of pear ester, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and acetic acid and quaternary bends with a fourth volatile added, N = 8-10 lure replicates, trials conducted on 2-4 dates.

Trial		Mean ± SEM moth catch per trap		
#	Volatile added	Females	Males	Total
7	-	4.8 ± 0.6 b	1.8 ± 0.3 b	6.6 ± 0.8 b
	15	7.0 ± 0.8 ab	4.7 ± 0.9 a	11.7 ± 1.6 a
	Linalool oxide (4K)	7.8 ± 0.9 a	3.5 ± 0.6 ab	11.2 ± 1.3 a
	RCB ANOVA, df = 2, 73	$F = 4.42$, $P = 0.015$	$F = 5.11$, $P = 0.008$	$F = 6.58$, $P = 0.002$
8	-	5.5 ± 0.6 b	2.2 ± 0.4 a	7.7 ± 0.8 b
	16	8.9 ± 1.0 a	3.7 ± 0.6 a	12.6 ± 1.5 a
	Linalool oxide (4K)	7.2 ± 0.8 ab	3.5 ± 0.5 a	10.6 ± 1.2 ab
	RCB ANOVA, df = 2, 73	$F = 4.83$, $P = 0.011$	$F = 2.99$, $P = 0.057$	$F = 4.83$, $P = 0.011$
9	-	3.7 ± 0.5 b	1.6 ± 0.2 b	5.3 ± 0.6 b
	17	5.5 ± 0.8 a	3.4 ± 0.6 a	8.9 ± 1.2 a
	Linalool oxide (4K)	5.9 ± 0.8 a	2.9 ± 0.5 ab	8.9 ± 1.1 a
	RCB ANOVA, df = 2, 111	$F = 3.25$, $P = 0.042$	$F = 3.25$, $P = 0.042$	$F = 5.24$, $P = 0.007$
10	-	3.8 ± 0.6 b	1.7 ± 0.3 a	5.5 ± 0.7 b
	18	6.8 ± 1.1 a	2.8 ± 0.5 a	9.6 ± 1.4 a
	Linalool oxide (4K)	6.5 ± 1.0 a	3.2 ± 0.6 a	9.7 ± 1.3 a
	RCB ANOVA, df = 2, 83	$F = 4.38$, $P = 0.016$	$F = 2.23$, $P = 0.114$	$F = 5.73$, $P = 0.005$
11	-	6.6 ± 0.7 a	2.4 ± 0.5 b	9.1 ± 0.9 a
	19	8.8 ± 1.3 a	5.1 ± 0.8 a	13.9 ± 1.8 a
	Linalool oxide (4K)	8.6 ± 1.0 a	4.1 ± 0.7 ab	12.7 ± 1.5 a
	RCB ANOVA, df = 2, 50	$F = 1.09$, $P = 0.343$	$F = 4.34$, $P = 0.018$	$F = 2.47$, $P = 0.095$

Another approach to increase the catch of female codling moths is to add an inexpensive solar-powered UV light to the trap. Lights were obtained directly from China as "Mosquito Zappers" for $4/unit. We tested three types of solar lights in 2020 with the bisexual lures I have developed for codling moth, oriental fruit moth, eye-spotted budmoth, and several leafroller species. The smaller light was discontinued due to poor reliability of the product. The addition of either of the two larger UV lights increased codling moth catch ca. 2-fold (Table 7). However, previous studies in Chile with UV lights on milk jugs increased catch 5-fold. New ongoing studies in Chile are comparing UV lights placed on several trap designs. Also, data is being developed on the reliability and longevity of these lights when used within orchard canopies.

Table 7. Comparison of codling moth catches in delta traps baited with 4K lure in traps with two different sizes of solar-powered UV lights added, Washington 2020.

Light/ date	Lure	UV added	Males	Females	Total
Medium UV light	4K	Yes, N = 25	10.6 (1.4)a	10.0 (0.8)a	20.6 (1.9)a
		No, N = 47	7.4 (0.8)b	6.8 (0.5)b	14.2 (1.0)b
7 July – 20 July	ANOVA df = 1, 70		$F = 6.06$	$F = 11.13$	$F = 12.68$
			$P < 0.05$	$P < 0.01$	$P < 0.001$
Large UV light	4K	Yes, N = 27	23.1 (1.8)	14.1 (1.2)a	37.1 (2.5)a
		No, N = 13	21.7 (3.3)	8.7 (1.1)b	29.4 (4.0)b
7 July – 20 Aug.	ANOVA df = 1, 38		$F = 1.11$	$F = 7.57$	$F = 5.11$
			$P = 0.29$	$P < 0.01$	$P < 0.05$

Interestingly, results were obtained in our 2020 studies with OFM in two apple orchards using the UV lights added to delta traps (Table 8). First, adding the UV light significantly increased male and female OFM catch, 2- and 3-fold, respectively. Second, we saw that delta traps with the UV light became effective (nearly 6-fold increase) in catching both sexes of CM (Table 8). These total counts were about half of what was caught in a single delta trap baited with the 4K lure for CM in each orchard, but the numbers of females caught were similar. It appears that the attraction of both CM sexes to the light partially overcomes any short-range repellency of the OFM sex pheromone blend normally has for codling moth. This result suggests that the OFM Dual lure could be used effectively to monitor both pests and supports the development of 'Smart traps' to remotely monitor both key pests.

Table 8. Comparison of moth catches in delta traps with or without a solar UV light attached in two apple orchards situated near Sunnyside, WA, baited with the OFM Combo lure from 15 July to 24 August 2020. N = 10

Pest	Trap	Males	Females	Total
OFM	Solar UV	114.1 (12.2)a	33.3 (4.5)a	147.4 (13.0)a
	No solar	50.1 (4.7)b	10.2 (0.7)b	60.3 (5.1)b
ANOVA df = 1, 17		$F = 28.73$	$F = 45.62$	$F = 45.00$
		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
CM	Solar UV	13.2 (2.1)a	15.3 (0.8)a	28.5 (1.9)a
	No solar	2.6 (0.5)b	2.7 (0.8)b	5.3 (1.2)b
ANOVA df = 1, 17		$F = 31.44$	$F = 98.61$	$F = 125.79$
		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

The major effort of this project was to evaluate the use of traps baited with these new lures to remove female codling moth and thus reduce levels of fruit injury. Studies were successful in both apple and pear in 2019 and 2020 in the USA and in 2020 in Italy (Tables 9, 10). Over 1,000 traps were deployed during both years. Levels of fruit injury reductions with the trap deployments averaged 56% over all studies and ranged up to 75%. Other factors, such as lure, trap, and duration of trials varied over the course of these studies. Dissections of codling moth during 2019 provided an interesting view of the importance of mating disruption (Table 11). Proportions of female codling moth that were mated was reduced with mating disruption by < 15%. Multiple matings by females was more strongly impacted by deploying mating disruption, in agreement with our previous studies. Data from 2020 are still being gathered from Drs. Mujica and Basoalto.

Table 11. Summary of the mating success of female codling moth collected from orchards either untreated or treated with sex pheromone dispensers or sex pheromone/pear ester dispensers, 2019.

Treatment	Number of blocks	1st generation Proportion Mated F	1st generation Proportion Multiple-mated females	2nd generation Proportion Mated F	2nd generation Proportion Multiple-mated females
Untreated	9	0.77	0.17	0.88	0.41
Sex pheromone dispensers/aerosols	7	0.58	0.03	0.73	0.12
Sex pheromone / pear ester dispensers	6	0.70	0.04	0.72	0.05

Discussion: Interesting developments occurred during 2020 beyond our variable levels of hibernation due to the virus. First, the MegaLure 4K was not allowed in organic orchards in 2020, but this appears to be unclear based on my own correspondence, and it should be available for at least monitoring in 2021. Meanwhile, studies are underway to develop more organic-friendly formulations with the same plant compounds but from natural sources. However, FMD-FR studies planned for 2021 are based on the use of the Combo-P+AA lure set in green/clear buckets. Second, during 2020 I learned that milk jugs can be used inappropriately and are not as effective as other traps when they are misused. One issue is the deterioration of the liquid due to excessive captures of nontargets (flies) that could repel moths. The problem may also be associated with the positioning of the lures near the 2" holes cut into the jug allowing direct exposure to UV light and higher wind velocity which might deplete the lures faster. A third idea for their poor performance in my studies is that having the AA lure placed near the sex pheromone containing lure (Combo-P) in these traps may generate codlemone acetate which is a known repellant. Previous studies suggesting that milk jugs were a cost-effective trap design were only conducted over 10-14 d and apparently, I was mistaken about their utility over longer periods of use. In WA, I switched all my jug traps to orange deltas in June. However, at least one pear grower used milk jugs with Combo-P+AA lures in 2020 to clean up a severe codling moth problem overwintering from 2019. She placed the lures directly under the cap of the jug, unlike what I typically did with lures hanging down near the holes in the trap.

Studies conducted in August in WA apple identified a bucket trap with a green top and a clear bottom as a much more effective trap than the all-clear or an all-green bucket trap in terms of both catch size and a much higher proportion of females trapped. This new result suggests that the Combo-P+AA lure could be used in organic orchards in 2021, but likely at a higher density (ca. 50/acre) using green/clear traps.

The use of female removal was shown to be an effective component of an integrated program to manage codling moth. Significant reductions in fruit injury were demonstrated with female removal in Italy, California, Oregon, and Washington. FR should be most effective if females can be removed before they mate or lay eggs. We found that FR removed a somewhat higher proportion of unmated female codling moth in orchards treated with mating disruption. During 2020 the addition of small solar-powered UV lights added to delta traps significantly increased female moth catches of codling moth and oriental fruit moth. Studies with these units are continuing in South America to develop more expertise with their reliability and effectiveness. Thus, it is likely that the Combo-P+AA lure can be used with the solar-powered UV lights to improve the effectiveness of MD-FR for codling moth. The lights also made the OFM Dual lure much more attractive for both oriental fruit moth and codling moth creating an opportunity to monitor both pests together with standard traps or with remote 'Smart traps'.

Growers are fully aware that MD does nothing to prevent mated females from entering an orchard and laying eggs. Thus, our current recommendation is to continue to use green/clear bucket traps along borders of orchards adjacent to unmanaged sources of codling moth and to reduce populations of female codling moth with clusters of traps placed within hot spots, such as borders, near bin piles, and any uphill edges of blocks with a history of pest injury. One of the most remarkable experiences of my 30-year career with CM management occurred in 2020 in a conventional apple block. I placed 80 orange delta traps at a high density (50/acre) baited with the 4K lure in the corner of this orchard next to where a bin pile was established in early August. Over the next 6 weeks we removed 1,800 females from this corner of the orchard, and only a few apples were damaged and only on the outside row at harvest. I have no explanation for where these moths came from except the bin pile, and I am amazed that the crop could be saved using MD/FR!

Table 9 Summary of codling moth "female removal" field trials conducted during 2019.

Year – trials Crop / country	Treatments 1st / 2nd flight	# moths caught per trap	Prop. females	Prop. unmated females	Prop. fruit injury	# moths caught per trap	Prop. females	Prop. Unmated females	Prop fruit injury
2019 N = 8 Apple orchard pairs USA	PH/PE+AA / 4K	20.1 (3.1)	0.25 (0.04)	0.36 (0.05)	0.019b (0.008)	14.9 (4.9)	0.50 (0.05)	0.36 (0.06)	0.024b (0.010)
	No traps	-	-	-	0.031a (0.012)	-	-	-	0.048a (0.013)
Summary	**502 traps**	**10,090 moths**	**3,362 females**	**Paired t-test $t_7 = 6.85$, $P = 0.0002$**		**7,480 moths**	**3,734 females**	**Paired t-test $t_7 = 3.78$, $P = 0.007$**	
2019 N = 8 Apple orchard pairs USA	PH/PE+AA / 5K	19.4 (3.7)	0.46 (0.08)	0.31 (0.03)	0.015b (0.006)	13.3 (2.1)	0.69 (0.08)	0.18 (0.04)	0.045b (0.023)
	No traps	-	-	-	0.031a (0.010)	-	-	-	0.086a (0.036)
Summary	**423 traps**	**8.206 moths**	**3,775 females**	**Paired t-test $t_7 = 4.88$, $P = 0.002$**		**5,626 moths**	**3,882 females**	**Paired t-test $t_7 = 3.53$, $P = 0.010$**	
2019 N = 5 Pear orchard pairs USA	PH/PE+AA / 5K	7.2 (3.4)	0.41 (0.08)	0.32 (0.05)	0.030b (0.019)	4.5 (1.6)	0.58 (0.08)	0.09 (0.05)	0.039b (0.020)
	No traps	-	-	-	0.062a (0.040)	-	-	-	0.111a (0.047)
Summary	**156 traps**	**1,123 moths**	**461 females**	**Paired t-test $t_4 = -3.37$, $P = 0.028$**		**702 moths**	**407 females**	**Paired t-test $t_4 = 2.80$, $P = 0.049$**	

Table 10. Summary of codling moth 'female removal' field trials conducted during 2020

Year – trials Crop / country	Treatments 1st / 2nd flight	# moths caught per trap	Prop. females	Prop. unmated females	Prop. fruit injury	# moths caught per trap	Prop. females	Prop. Unmated females	Prop fruit injury
2020 N = 9 Apple orchard pairs USA	-/ 4K	-	-	-	-	19.9 (6.5)	0.45 (0.02)	0.38 (0.02)	0.015 (0.006)b
	No traps	-	-	-	-	-	-	-	0.071 (0.036)a
Summary	**641 traps**	**-**	**-**	**-**	**-**	**12,756 moths**	**5,740 females**	**Paired t-test t_8 = 3.69, P = 0.006**	
2020 N = 6 Pear orchard pairs USA	PH/PE+AA / 4K	20.3 (7.2)	0.52 (0.02)	0.23 (0.03)	0.004b (0.002)	10.6 (2.7)	0.55 (0.05)	0.35 (0.03)	0.052b (0.034)
	No traps	-	-	-	0.010a (0.005)	-	-	-	0.113a (0.039)
Summary	**132 traps – 1st 306 traps – 2nd**	**8.206 moths**	**3,775 females**	**Paired t-test t_2 = 2.89, P = 0.034**		**3,244 moths**	**1,784 females**	**Paired t-test t_7 = 3.53, P = 0.007**	
2020 N = 5 Pear orchard pairs Italy	4K	5.3 (0.8)	0.43 (0.03)	0.17 (0.03)	0.015b (0.007)	7.1 (0.8)	0.61 (0.04)		0.063b (0.020)
	No traps				0.019a (0.009)				0.094a (0.02)
Summary	**258 traps**	**4,512 moths**	**1,940 females**	**Paired t-test t_4 = 3.30 , P = 0.046**		**1,832 moths**	**1,117 females**	**Paired t-test t_4 = 4.65 P = 0.019**	

Executive Summary

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

Key Words: plant volatiles, fruit injury, *Cydia pomonella*, oriental fruit moth, mating disruption

Abstract: The serendipitous development of the 4K lure for codling moth occurred through a collaborative synthesis of world-wide expertise. Its discovery gives us the ability to monitor codling moth without the use of sex pheromones and thus avoid any disruption of traps in orchards treated with variable levels of mating disruption technologies. The 4K lure provides us the ability to seasonally track female codling moths and improve predictive timing models for key phenological events. The attractiveness of the 4K lure allows traps to be placed at a more user-friendly height in the canopy and the lure is effective for at least 8 weeks. Ongoing studies have identified other host plant volatiles that can be used to create additional effective blends. The addition of UV light to traps baited with the 4K lure creates new opportunities to catch more moths, thus improving our ability to monitor and remove female moths from our orchards. The power of this dual modality can also be used for monitoring and female removal of other tortricid pests, such as oriental fruit moth, eye-spotted bud moth, and leafrollers. Development of optimized non-saturating trap-lure combinations allows us to use the power of the 4K lure to remove substantial numbers of both virgin and mated resident and immigrant female codling moths from our orchards before they lay eggs. Studies conducted over the past two years in apple and pear have consistently shown that female removal strategies can be effective in reducing levels of fruit injury by > 50%. Female removal should be used with mating disruption to increase the removal of unmated females. This MD-FR approach when used for orchards' borders and surrounding bin piles can serve as a key bulwark to protect the orchard from immigrant moths. Future studies building upon these initial findings will strive to increase this impact. Much has been accomplished and more fine tuning is needed by growers and farm managers to deploy and integrate this useful tactic into their programs.