FINAL PROJECT REPORT

Project Title: Evaluating and improving biological control of WAA

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Cooperators: None

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

Other funding sources

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

WTFRC Collaborative Expenses: None

Budget 1

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

Footnotes:

[1] Project Assistant (Y1 -12 months, Y2 – 3 months); Y2-3 Matt Jones 50% FTE; Tawnee Melton 30% FTE Y1-Y3.

[2] Project Assistant 11.7%, U Chambers (32.1%), T Melton (47.5%)

[3] 2.7%

[4] includes lab and field supplies

[5] w/in state travel

Objectives

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

Significant findings

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

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

Methods

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

Table 1. Information about study sites

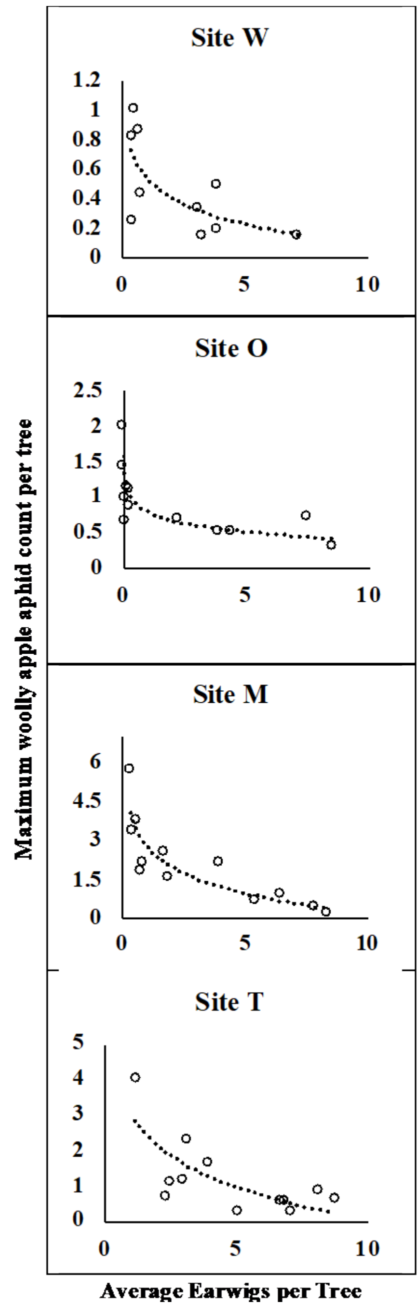
Block name	Nearest town	Variety	Spacing (trees x rows)
M	Quincy	Fuji	3.5' x 12'
T	Quincy	Fuji	7' x 15'
O	Orondo	Fuji	5' x 13'
W	Winchester	Gala	3' x 10'

Insect monitoring and earwig manipulations. WAA and earwigs were sampled at roughly weekly intervals from April to November. We sampled all trees in study sections of sites M, T, and O, and every other tree at site W. WAA colonies were recorded as the number of infested axils on a survey of ten ~1' long twigs plus all colonies on pruning cuts and trunks. Earwigs were monitored by counting the number found in rolled tubes of corrugated cardboard placed in each tree.

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

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

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



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

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

Results and discussion

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

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

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

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

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

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

Methods: During previous experimentation at site W in 2016, samples of 20 earwigs were collected on 6 visits between June 17 and September 21. Collections were made within an hour of sunrise into plastic Ziploc bags stored in a cooler with ice and transported to a -20°C laboratory freezer. Later, each earwig's stomach was dissected the DNA extracted using QIAGEN's DNeasy spin column kit. Each set of extractions included a negative control with no earwig stomach to check for DNA contamination.

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

Results

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

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

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

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

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

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

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

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

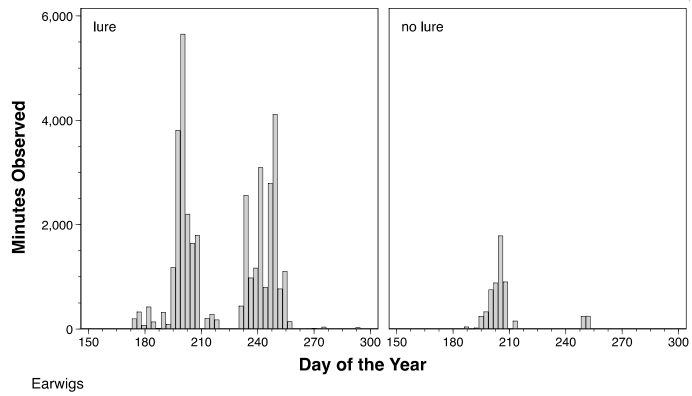
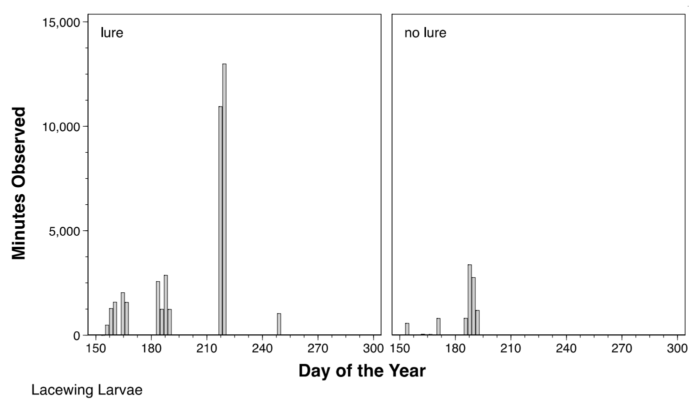


Fig. 3. Number of minutes that lacewing larvae were observed around WAA colonies in the lured and unlured areas throughout the season in 2019.



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

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

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

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

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

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

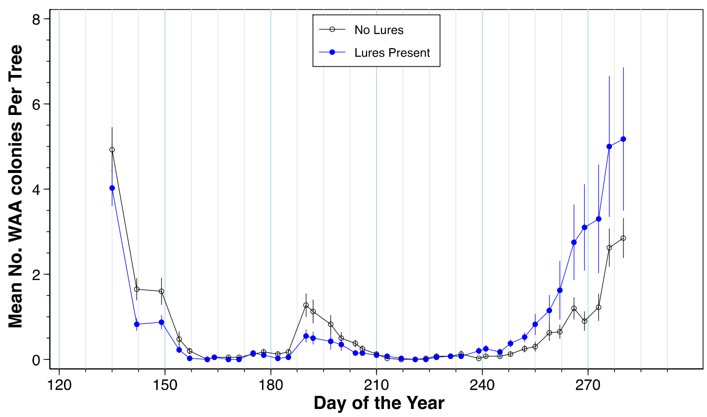
In 2019, we set up a block in Quincy with 20 plots, each consisting of four trees. The plots were randomly assigned to either a no lure or lure treatment. The lures consisted of squalene + an AMP lure as described above. Lures were placed in the orchard on 15 May and then replaced on 25 July. Each lure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

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

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

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

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

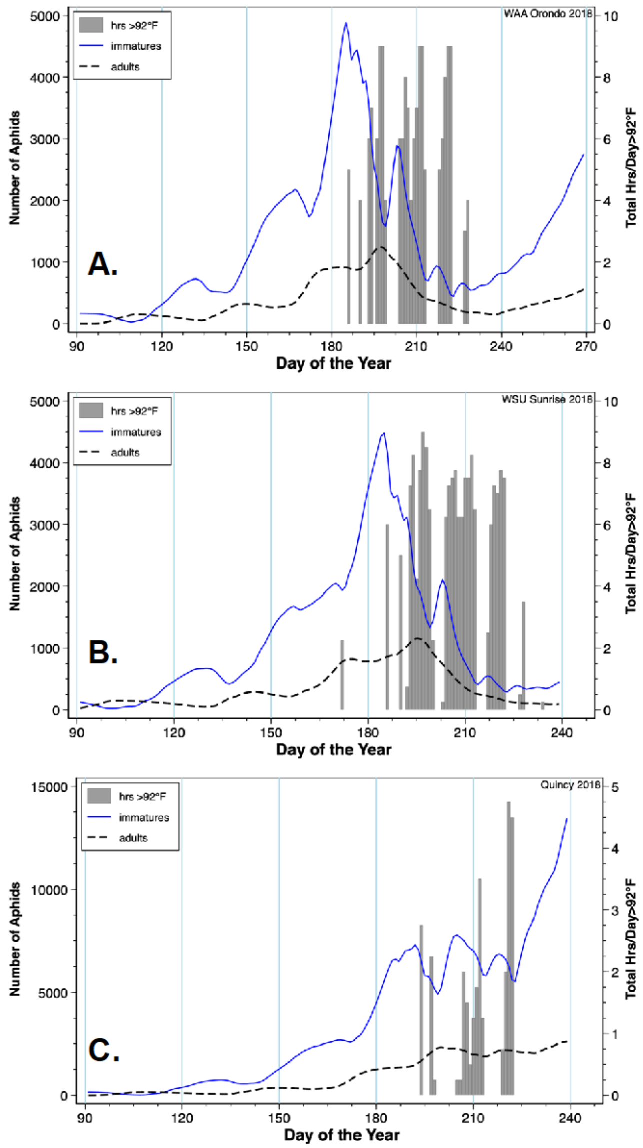


cancelling out the fall performance. If we evaluate the data before the lure ran out, the lure did significantly reduce the number of colonies, but biologically, it was of pretty dubious importance (the mean difference was only 0.36 colonies/tree). Elimination of that one plot showed the control and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

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

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

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



Executive summary

Title: Evaluating and improving biological control of WAA

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

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

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

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

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

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