Project Title: Enhancing pear psylla biological control through predator recruitment

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Budget 1
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Item	2019	2020 (NCE)	2021 (NCE)
Salaries[1]	23,750	0	0
Benefits[2]	8,723	0	0
Wages	5,760	0	0
Benefits[3]	92	0	0
Equipment	0	0	0
Supplies[4]	8,000	0	0
Travel[5]	5,000	0	0
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	51,325	0	0

Footnotes:
[1] Postdoctoral associate 50% FTE (Y1 -12 months, Y2 – 12 months)
[2] Postdoctoral associate (36.73%)
[3] 1.6%

[4] Includes lab and field supplies.
[5] In state travel.

OBJECTIVE

1. Evaluate the indirect effects of thrips on psylla abundance in the presence and absence of anthocorid predators

SIGNIFICANT FINDINGS

- In the 2021 field experiments *Orius insidiosus* provided weak and insignificant effects on reducing pear psylla abundance.
- In the 2021 field experiments thrips did not directly alter psylla abundance, presumably either through chemical induction, or predation.
- In the 2021 field experiment thrips did significantly not alter predation rates, negatively or positively by serving as alternative prey for *O. insidiosus.*
- In-field variability in the effectiveness chemical defense induction on pear psylla in previous experiments does not appear to be due to variability in thrips feeding, since thrips did not significantly alter psylla abundance.

METHODS

2019

We set out to conduct an inexpensive pilot study in July 2019 to develop methods for the following spring. We conducted the experiment in the pear orchard at the WSU TFREC in Wenatchee, WA. First, we conducted a survey of the plot to identify the most abundant predators, and we designed an experiment focused on these predators to evaluate which combination of predators were most impactful on pear psylla abundance. At this time thrips were not as abundant in the orchard as they were earlier in the season. Therefore, we did not include thrips in the experiment. We observe apparent overlapping psylla generations, such that there was very high variation in psylla reproduction that overwhelmed experimental manipulation. Nonetheless, we describe this experiment below.

We set up a sleeve-cage experiment where sleeves made of fine mesh approximate 2 feet long were placed over the tips of branches including 20 adult psylla and a predator treatment or no-predator control. To set up the cages, on July 24th 2019 we first removed all insects on the branches and added the sleeve. Next (on 7/24/2019), we used beat sheets to collect adult psylla and added 20 adult psylla to each branch. We allowed the psylla 48 hours to establish, after which we counted the psylla by looking through the closed sleeve cages and added predators. We sampled every tree in 2 middle rows of trees for predators, and focused treatments on these predators.

The most common predator species were *Deraeocoris* sp. bugs (D), *Harmonia axyridis* ladybeetles (H), and *Adalia bincutata* lady beetles. Spiders were present too, but there were not enough of the same species to include in an experiment. Thrips were not abundant at this time.

We next designed an experiment to determine which combination of these predators provided the best control of psylla. Each cage included two individuals of either a single predator species, or a pairing of one individual from each of the three species listed above. We also included no-predator controls, and each treatment was replicated 4 times. Predators were introduced on July 26th 2019, and psylla abundances were estimated by peering through mesh sleeve cages, to avoid disruption of psylla treatments by opening cages. We introduced predators immediately after time zero psylla counts. Then, we broke down the experiment on August 12th and counted all psylla and predators.

2020

On March 1, 2020 prior to leaf growth, we set up 40 exclusion sleeve cages on pear trees at the Wenatchee WSU Tree Fruit Research and Extension center in the pear orchard (Fig. 1). To set up the cages, we first removed any overwintering pear psylla from the trees and put the sleeve cages on branches to ensure that all branches were free from psylla and thrips. This would allow us to introduce to the cages four treatments: 1) pear psylla only, 2) thrips and pear psylla, 3) anthocorids and pear psylla, and 4) thrips, anthocorids, and pear psylla. Our plan was to collect anthocorids from surrounding vegetation during bloom and use the most commonly collected anthocorid species for experiment. However, COVID restrictions occurred in March before the trial could be initiated, shutting down the experiment before it could begin. Later, in early summer we were able to develop lab protocols that allowed for methods to conduct research but reduce potential for COVID transmission and began planting pear trees for a similar experiment in growth rooms. However, the employee funded by the project needed to go on family medical leave, and we were not able to hire a new employee. To account for this, we kept 20 trees in a cold room so that we could plant them and grow them in a greenhouse with supplemental light when we were able to restart the experiments. In the fall we established pear psylla colonies, seeded from a colony at USDA Wapato that we kept on potted pear trees, and in November, we planted the pear trees from the cold room to prepare for an experiment (Fig. 2). However, the trees never sprouted, potentially due to either an issue in the cold room, or from the shock of being transplanted to a warmer environment. Therefore, we plan to restart the experiment in Spring 2021.

Figure 1. Sleeve cages on pear trees March 1, 2020 waiting for insect addition.

Figure 2. Pear trees growing in the greenhouse in November 2020 for winter experiments.

2021

On March 10, 2021, prior to leaf growth, we set up 40 exclusion sleeve cages on 10 pear trees (4 cages/tree) at the Wenatchee WSU Tree Fruit Research and Extension center in the pear orchard (Fig. 3). To set up the cages, we first removed any overwintering pear psylla from the trees and put the sleave cages on branches to ensure that all branches were free from psylla and other insects. These cages remained empty on trees from 10 March until 12 April, when the experiment was initiated. Because densities of each, thrips and anthocorids in orchards were low at the start of the experiment, we purchased *Orius insidiosus* from Arbico Organics, and collected western flower thrips (*Frankliniella occidentalis*) from a patch of dandelions growing at the WSU Tree Fruit Research and Extension Center. To each tree we set up four cages, each with a different treatment: 1) pear psylla only, 2) thrips and pear psylla, 3) *O. insidiosus* and pear psylla, and 4) thrips, *O. insidiosus*, and pear psylla. To each cage we introduced 10 female pear psylla on the evening of 12 April 2021 (cages 1-27) or the following morning (cages 28-40). Then, in the afternoon of 13 April 2021 we added 20 adult *Frankliniella occidentalis* thrips per cage to the cages with thrips treatments. Herbivores were allowed at least 72 hours to acclimate, and *O. insidiosus* was introduced to cages on 16 April, which we refer to as day one of the experiment. At day 20, (5 May), we cut all branches with sleeve cages off the trees, leaving the sleeve cage intact, and moved all branches to a refrigerator during sorting. We then visually observed and counted all insects on the branches. This method was effective for adult psylla and thrips, as well as *O. insidiosus*, but was not effective at counting immature psylla or thrips. Therefore, to count nymphs and thrips we also used a leaf brush to remove all insects off 20 randomly selected leaves per sleeve cage. We also counted the leaves within the sleeve cage to use the random leaf sample to estimate the abundance of immature psylla and thrips across the entire cage. To analyze the data, we used a generalized linear mixed model, using a negative binomial error distribution (typical for count data), a log-link function (assumes only positive numbers of insects), and a random effect of tree to account for variability between trees. Because the number of psylla nymphs were estimated, rather than discrete counts we used a gamma distribution to model the error distribution (the negative binomial is only suitable for discrete count data).

Figure 3. Sleeve cages on pear trees March 10, 2021, waiting for insect addition.

RESULTS & DISCUSSION

2019. In our 2019 experiment, we found that in July the most abundant predators were *Deraeocoris* sp. bugs and two species of lady beetles. While adult psyllas were abundant, we observed very few thrips. The experimental approach worked well, except we found very little reproduction. The four no-predator controls had very few psylla in cages, suggesting that reproduction was very low (mean of 3.5 psylla/cage). Numbers of psylla in other cages were highly variable, ranging from 0 to 18 psylla in the predator treatments. Discussion with Louis Nottingham suggested that this was due to a combination of aging adults from the previous

generation that were not reproducing, and newly emerged adults from the next generation. This solidified the benefit of studies early in the season when there is a single generation of psylla, such that psylla reproduction is similar across treatments.

2020. Our spring 2020 experiment was disrupted by the COVID pandemic and needed to be postponed until 2021.

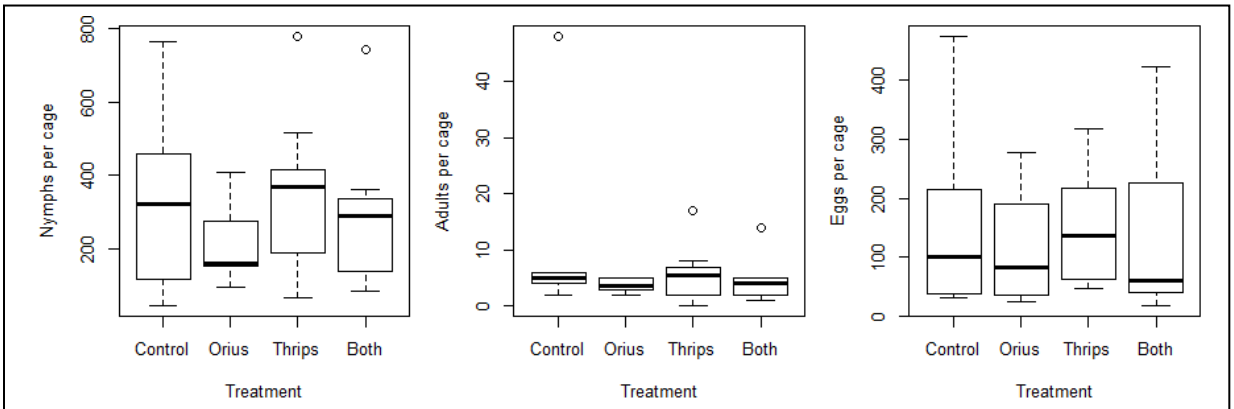

Figure 4. Total number of nymphs (left), adults (middle), or eggs (right) per cage in the presence of Orius insidiosus *(Orius), western flower thrips (Thrips), both* O. insidiosus *and thrips (Both), or none (Control). Boxplots are particularly useful to represent data when the error distribution is not symmetrical, as is often the case in count data. The bold horizontal line inside each box represents the median number of the insects for the treatment. The top and bottom of the box represents the 75th and 25th percentiles of the data, and the whiskers represent the theoretical maximum and minimum of the data. Dots represent outliers.*

2021. In our 2021 cage experiment, we found that *O. insidiosus* slightly reduced numbers of pear psylla nymphs, but the results were not statistically significant (Figure 4, generalized linear mixed model likelihood ratio test: $\chi^2 = 2.63$, $P = 0.105$). Thrips did not significantly alter pear psylla nymph density (Figure 4, generalized linear mixed model likelihood ratio test: $\chi^2 = 0.36$, $P = 0.5055$), or significantly alter the effects of *O. insidiosus* (Figure 4, generalized linear mixed model likelihood ratio test: $\chi^2 = 2.18$, $P = 0.14$). Similarly, none of the treatments affected the number of adult or egg pear psylla in the cages (Figure 4, generalized linear mixed model likelihood ratio tests: all $P > 0.05$).

The results from the experiment are interesting in light of experiments on the use of chemical defense elicitors that serve to promote particular defensive compounds in plants that reduce the ability of pear psylla to grow (Cooper and Horton 2015, 2017). Previous research suggests that chemical elicitors work to reduce pear psylla in the laboratory (Cooper and Horton 2015), but have variable results in the field (Cooper and Horton 2017, Orpet et al. 2021). Here, given the ability for western flower thrips to also influence chemical induction pathways (Steenbergen et al. 2018), we evaluated the potential for thrips to induce defense in the field to see if the variability is driven by inductions by other insects. We found no evidence that the variability in thrips abundances is altering psylla abundance, whether driven by chemical elicitation of defenses, by serving as alternative prey, or through direct predation (Hall 2014), at least within the confinements of cages. Previous research in Europe suggests that pear psylla (*Cacopsylla pyricolla* and *C. pyri*) can induce volatiles that recruit anthocorid predators (Scutareanu et al. 1997). The proposed evaluation of this effect in year 2 was not funded, but the relatively low impact of *O. insidiosus* in this experiment suggest the end result of these impacts

may be minimal as well. Another potential finding that may be worth exploring further is that *C. pyricola* has been shown to induce defenses in nearby pear trees in Europe as well (Scutareanu et al. 1996). If pear trees are communicating, the use of chemical elicitors may not be readily apparent on a tree scale, because control trees are also induced (through communication). Furthermore, if trees are indeed communicating, chemical elicitors may only need to be applied to a subset of trees, with the rest of the trees inducing defense through tree-to-tree communication. Further research may identify whether this mechanism is a way forward to promote defense induction while reducing application costs.

References

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Executive Summary

Project Title: Enhancing pear psylla biological control through predator recruitment

Key words: Pear psylla, biological control, induced defense

Abstract:
Recent research suggests induced defenses can reduce pear psylla growth, potentially improving control in the field. However, while previous lab results were promising, results have been highly variable in the field. This begs the question of whether variability in defense induction is driven by other herbivores feeding on pear trees, altering the hormonal pathways governing chemical defenses. Thrips are often found in pear orchards during bloom, and often induce chemical changes in a range of plant species that make them less palatable to pests. Furthermore, thrips commonly serve as alternative prey for anthocorid bugs that can attack psyllids and have even been observed eating a related herbivore, Asian citrus psyllid. Although thrips are present in pear orchards throughout the year, they generally do not cause economic damage to pears and therefore may provide 3 indirect benefits: *i*) inducing chemical defenses in the plant, *ii*) serving as alternative prey for predators to boost predator reproduction, and *iii*) attracting predators through inducing plant volatiles. Here, we evaluated pathways *i* and *ii* by conducting a field experiment, factorially manipulated thrips abundance and a shared predator (*Orius insidiosus*), known to respond to thrips abundances in other cropping systems. We conducted the experiments within sleeve cages on pear trees and initiated the experiment at bloom. While *O. insidiosus* slightly reduced psylla abundance, the finding was not significant, and thrips provided no impact on either, psylla abundance, or predation by *O. insidiosus*. These findings suggest that variability in chemical defense elicitation in the field is not driven by variation in thrips densities. An avenue of further research would be to evaluate tree to tree communication, to see if chemical defenses induced in one tree promotes defenses in nearby trees.