

## FINAL PROJECT REPORT

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

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### Objectives

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

### Significant Findings

- Transcriptome libraries of summerform, diapausing winterform, and post-diapause winterform pear psylla were created and analyzed, substantially improving knowledge of seasonal biology of pear psylla.
- Differentially expressed genes that are likely related to changes in seasonal biology, including those involved in reproduction, immunity/defense, olfaction, sight, and muscle development, were identified and analyzed. This dataset will aid future studies on the overwinter biology and control of pear psylla.
- Funds for this project were used to leverage additional funds to sequence the pear psylla genome and to obtain long-read transcriptome sequences.
- Adding the transcriptomic and genomic data to the open access AgriVectors.org database will allow streamlined comparisons with transcriptomes and genomes of citrus psyllid and potato psyllid, enabling researchers to adapt gene-based therapies developed for other psyllids and related insect pests.

### Results and Discussion

#### *Background*

Pear psylla occurs as two distinct seasonal morphotypes - summerform and winterform - that differ with respect to diapause, feeding behavior, plant attraction, and association with bacterial endosymbionts (Figure 1) (Ullman and McLean 1988, Krysan and Higbee 1990, Krysan and Horton 1991, Horton et al. 1998, Civolani et al. 2011, Cooper et al. 2017). Summerforms undergo several overlapping generations each year. The nymphs develop exclusively on pear, and summerform adults

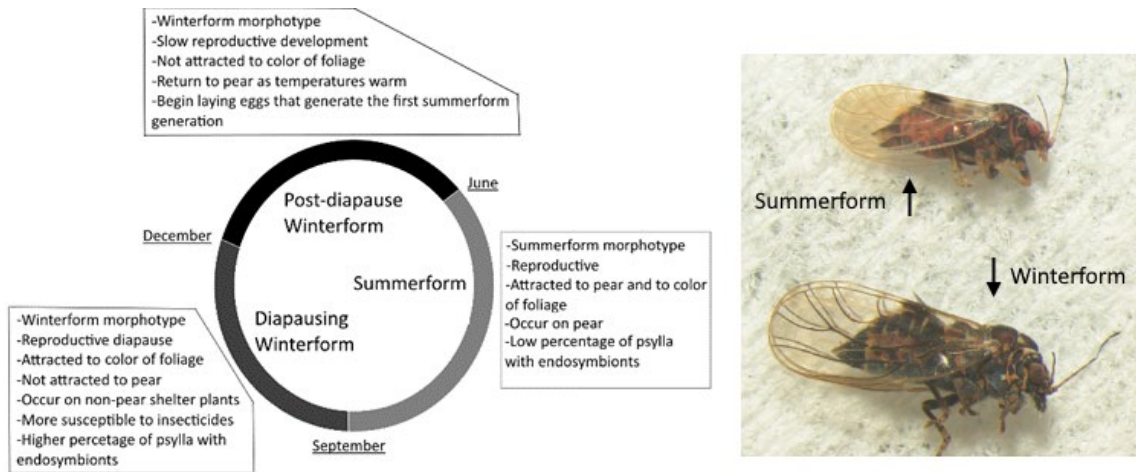


Figure 1. Annual occurrence and phenological traits associated with summerform (light grey), diapausing winterform (dark grey), and post-diapause winterform (black) pear psylla.

are rarely found on other plants (McMullen and Jong 1967). Nymphs develop into winterform in response to shortening photoperiods of early autumn. Winterform are larger and darker compared with summerform and occur as a single overwintering generation (Horton et al. 1998, Mustafa and Hodgson 2984). The winterforms begin autumn and winter in reproductive diapause characterized by a lack of mating and ovarian development. Reproductive diapause seems to be associated with reduced tolerance to certain insecticides (Unruh and Krysan 1994), and winterforms are more likely than summerforms to harbor certain bacterial endosymbionts (Cooper et al. 2017). Autumn leaf drop displaces winterforms from pear trees prompting many psylla to disperse from orchards (Horton et al. 1994). Diapausing winterforms remain attracted to the color of foliage, and often visit or settle upon evergreen trees and shrubs, or deciduous trees with leaf drop occurring later than in pear (Kaloostian 1970). Winterforms break diapause in late December, but reproductive development remains slow due to cold temperatures (McMullen and Jong 1967). As temperatures warm in February and March, post-diapause winterforms return to pear and begin laying eggs destined to become the first summerform generation.

Although changes in behaviors and phenotypes associated with summerform, diapausing winterform, and post-diapause winterform psylla are well-documented, the timing for these behavioral changes and mechanisms controlling behaviors are not currently understood. Comparative transcriptomics has proven highly useful to examine the seasonal or other life cycle shifts in behavior or physiology by other insect pests. The goal of our study was to use complete transcriptomes to compare gene expression among summerform, diapausing winterform, and post diapause winterform, which will allow us to pinpoint the exact timing for these changes and to develop gene-based insecticides to control pear psylla.

## Methods

Pear psylla were collected in July (Summerform), December (diapausing winterform), and February (post-diapausing winterform) from a Bartlett pear orchard located at the USDA-ARS research farm near Moxee, WA. RNA was extracted from the insects using a commercial kit and was sequenced by Novogene. The transcriptomes were assembled and analyzed using BLAST2GO by co-PIs Krey and Saha. BLAST2GO software identifies the putative function of gene transcripts and categorizes the genes based on gene ontology. We observed substantial differences in gene expression that were mostly consistent with the differences in biology and behavior among the three

lifecycles of pear psylla. In 2020, quantitative PCR was used to confirm differential expression of a subset of genes involved in reproduction, immunity, defense, muscle function, and sensory.

## Results

We first looked at overall numbers of differentially expressed genes among summerform, diapausing winterform, and post-diapausing winterform pear psylla. A higher number of differentially expressed genes indicates larger differences in physiology. Generally, there was a high degree of similarity in gene expression profiles among replications of each stage (Figure 2; like shading/fill = similar gene expression profiles). The exception was rep 3 of post-diapausing winterform, which exhibited a more similar gene expression profile to summerforms collected several months later (Figure 2B and C). However, the major differences in gene expression were observed between summerform and diapausing winterform (Figure 2A). Post-diapausing winterform exhibited gene expression profiles that were intermediate to those of diapausing winterform and summerforms (Figure 2). Overall, these results suggest that pear psylla undergo substantial physiological changes in autumn, but winterforms that are present in spring are more similar to summerforms than to autumn winterforms.

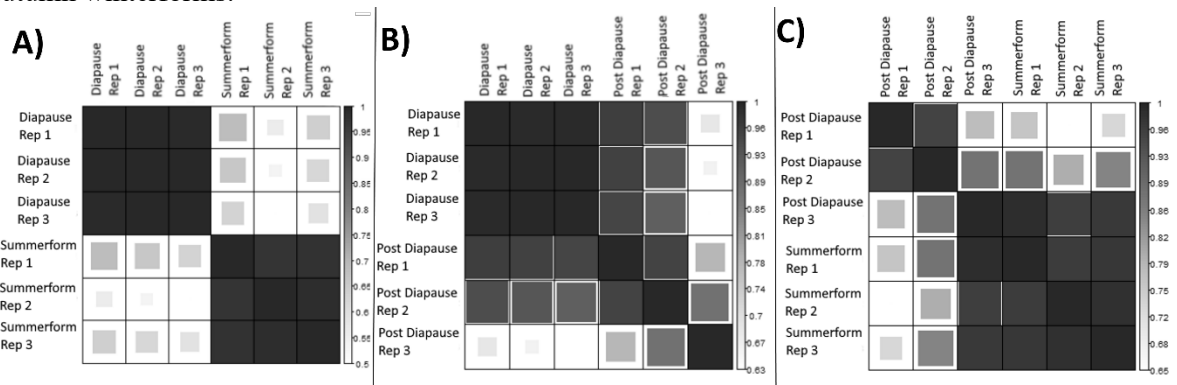


Figure 2. Relative differences in overall gene expression between summerform and diapausing winterform (A), diapausing and post diapausing winterform (B), and post-diapause and summerform (C). Similar shading and fill represent greater similarity in gene expression among specimens.

BLAST2GO analysis was used to assign gene ontologies to differentially expressed genes. Results of that analysis are too broad for the scope of this report but will be included in a forthcoming manuscript submitted to a peer-reviewed journal. From the ~15,000 differentially expressed genes, we identified a subset of genes that are involved in reproduction, defense, immunity, photoreception, olfactory, and muscle structure and function (Figure 3). Consistent with the results presented in Figure 2, the largest variations in selected differentially expressed genes was observed in diapausing winterforms (Figure 3). A large number of genes were up- and down-regulated in diapausing winterforms relative to summerforms. The largest number of differentially expressed genes were related to muscle structure and function (Figure 3A). Winterforms are larger than summerforms (Figure 1) and likely have more muscle mass for long-distance dispersal. The differences in expression of genes between summerforms and winterforms may be related to this behavior.

We previously found that pear psylla collected in spring are more likely than those collected in summer to harbor the plant pathogen that causes pear decline, *Phytoplasma pyri* (Cooper et al. 2017), and winterforms are more susceptible to certain classes of insecticides compared with summerform (Unruh and Krysan 1994). The largest variation in differentially expressed genes associated with immunity and defense was observed between summerform and diapausing winterform (Figure 3), which may alter pear psylla's susceptibility to infection or pesticides. Further

research is needed to examine whether pear psylla are more susceptible to entomopathogens during the orchard re-entry phase in early spring.

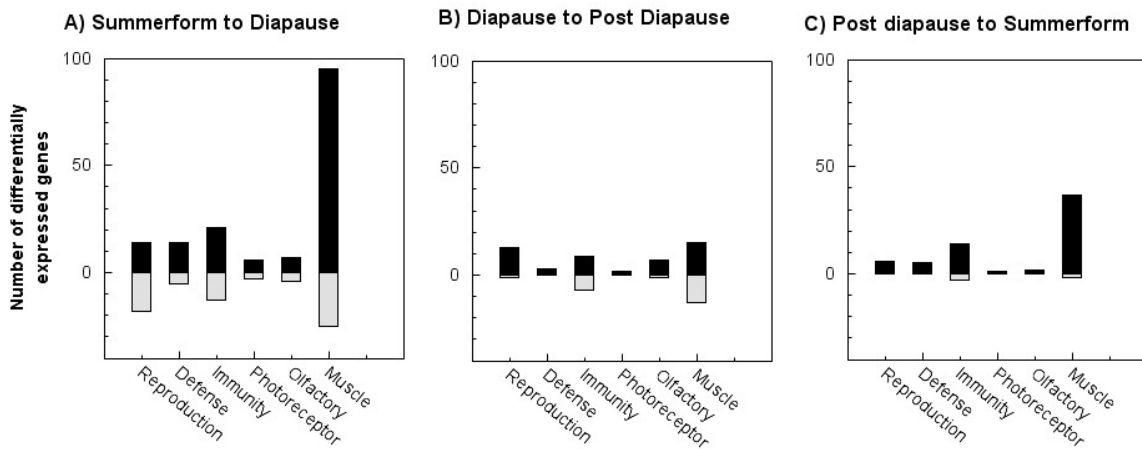


Figure 3. Number of genes that were up (black bars) or down (grey bars) regulated in diapausing versus summerform (A), post-diapausing versus diapausing winterform (B), and summerform versus post-diapausing winterform (C) pear psylla.

We identified 15 sensory receptor proteins putatively involved in sight, olfaction, or hearing that were upregulated in diapausing winterform psylla. Winterform psylla are attracted to color of foliage in autumn but are not attracted to pear specifically (Figure 1). Because they are attracted to the color of foliage, they often disperse from pear orchards after leaf drop and overwinter on evergreen conifers. Post-diapause winterforms are not attracted to the color of foliage and disperse from conifers to pear trees. We currently do not know what cues pear psylla use to locate pear trees, but preliminary evidence suggests that pear psylla are attracted to pear volatiles in early spring. It is currently not known whether winterform pear psylla are also attracted to volatiles released by conifers. The upregulation of sensory receptors in diapausing winterform psylla (Figure 3A) collected in December may be due to a change in which senses (olfactory versus visual) regulate pear psylla dispersal and behavior.

#### ***Anticipated benefit to the industry.***

It is not surprising that gene expression differed between winterform and summerform pear psylla. But the transcriptomes produced by this project provide valuable insight into the basic biology of seasonal morphotypes that will improve design and interpretation of future studies on the biology and management of overwintering psylla. With matching funding related to this project, we are working with AgPest100 Initiative (<http://i5k.github.io/ag100pest>) to sequence the genome of pear psylla, which will be the first genome of any psyllid pest of temperate tree fruits. In addition, we are working with collaborators to obtain long-read transcriptome sequences that will improve annotation of the transcriptomes developed from this current project. Once we obtain and analyze the genome of long-read transcriptomes, we will prepare a peer-reviewed manuscript changes in gene expression that correspond with changes in seasonable biology and management of pear psylla.

We are making these transcriptome libraries available on AgriVectors.org, an online bioinformatics tool developed by the PIs (Saha et al. 2021). This portal provides an open access platform that allows researchers to easily compare datasets across multiple pathosystems, including citrus psyllid and potato psyllid. Contemporary research is progressing toward the ability to use highly-specific gene-based therapies to target insects and pathogens in crops (Hunter 2017, Ghosh et

al. 2018, Das and Sherif 2020, Hunter et al. 2021, Hunter and Wintermantel 2021). In fact, the precursor to AgriVectors.org (citrusgreening.org) has already helped researchers develop several RNAi biopesticides which have been patented for control of citrus psyllid (US patent 10,344,291\_B2), and several patented antisense oligos (US patent 11,001,842 B2) that target and reduce pathogens. By comparing transcriptomes between citrus and potato pathosystems, we adapted an RNA-targeting therapy developed for citrus greening disease to target RNA of the zebra chip pathogen in potato (Hunter et al. 2021), and are currently adapting RNA-based insecticides developed for citrus psyllid to target potato psyllid. ***The pear psylla transcriptomes will allow us to also adapt these bioinsecticides to target pear psylla.***

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## **EXECUTIVE SUMMARY**

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### **Abstract:**

Pear psylla occurs as two distinct seasonal morphotypes - summerform and winterform - that differ with respect to diapause, feeding behavior, plant attraction, and association with bacterial endosymbionts. Winterforms are further divided into diapausing winterforms that occur in autumn and early winter, and post-diapausing winterforms that occur in late winter and spring. Changes in behaviors associated with summerform, diapausing winterform, and post-diapause winterform psylla are well-documented, but the timing and regulatory mechanisms controlling these behavioral changes remain unknown. Comparative transcriptomics has proven to be highly effective for examining the seasonal and other life cycle shifts in behavior or physiology in other insect pests. We used complete transcriptomes to compare gene expression among summerform, diapausing winterform, and post diapause winterform pear psylla, thus providing a better understanding of the expression-level changes underlying the seasonal biology of pear psylla. We also used funds to leverage opportunities to sequence the pear psylla genome using the latest sequencing technologies. These transcriptomic and genomic libraries will enable researchers to adapt gene-based therapies that have been developed and that are currently being tested for control of citrus and potato psyllids to control winterform and summerform pear psylla.