Final Project Report

Project Title: Bacterial endosymbionts of pear psylla

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Other funding sources: None

Budget 1
Organization Name: USDA-ARS-YARL **Contract Administrator: Chuck Myers**
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Item	**2015**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$12,000		
Travel			
Miscellaneous			
Plot Fees			
Total	$12,000		

Footnotes: Supplies include fluorescence in situ hybridization reagents, PCR and qPCR reagents, TA cloning supplies, gene sequencing costs, and shipping costs.

OBJECTIVES

Summary statement: The overall goal of this one-year study was to document the bacterial endosymbionts of pear psylla.
Objective 1: Compare the prevalence of *Arsenophonus* among pear psylla populations collected pre-budbreak, mid-summer, and autumn from orchards located near Wenatchee, WA, Yakima, WA, Hood River, OR, and Medford, OR.
Objective 2: Document the localization of *Arsenophonus* in specific organs/tissues of pear psylla.
Objective 3: Survey pear psylla collected from various pear growing regions of the Pacific Northwest for undocumented endosymbionts.

SIGNIFICANT FINDINGS

Objective 1: *Arsenophonus* was widespread among pear psylla populations. The prevalence of *Arsenophonus* did not differ among locations.
Objective 2: *Arsenophonus* was predominantly located in the bacteriomes of pear psylla in close proximity to the obligate endosymbiont, *Carsonella ruddii*. *Arsenophonus* was present in the oocytes of each female sampled indicating a high rate of mother to offspring transmission. *Arsenophonus* appeared also capable of colonizing the salivary glands, which may permit plant-mediated horizontal transmission of this endosymbiont.
Objective 3: A survey for additional bacteria associated with pear psylla found that about 20% of pear psylla were carriers of *Phytoplasma pyri*, the pathogen associated with pear decline disease and peach yellow leaf roll disease. The phytoplasma appeared to be more prevalent in Yakima Valley compared with other locations. The endosymbiont *Profftella*, which provides Asian citrus psyllid with protection against parasitoids, may be present in some pear psylla adults.

RESULTS AND DISCUSSION

Objective 1. *Arsenophonus* is widespread among insects and is associated with a wide-range of extended phenotypes expressed in their hosts (Gherna et al. 1991, Novakova et al. 2009, Rana et al. 2012) including providing the red gum lerp psyllid with protection against parasitoids (Hansen et al. 2007). In a preliminary survey of endosymbionts of pear psylla, we identified a strain of *Arsenophonus* that was present in psylla populations sampled from Washington, Oregon, and West Virginia. Sequences of 16S from *Arsenophonus* revealed that this is a strain that is specific to pear psylla.

Based on our preliminary survey, we screened pear psylla populations from various locations for the presence of *Arsenophonus*. This bacterium was prevalent in pear psylla populations at each location (Figure 1). *Arsenophonus* tended to be more prevalent in winterform populations (2014 and spring 2015) than in summerform populations. We are still

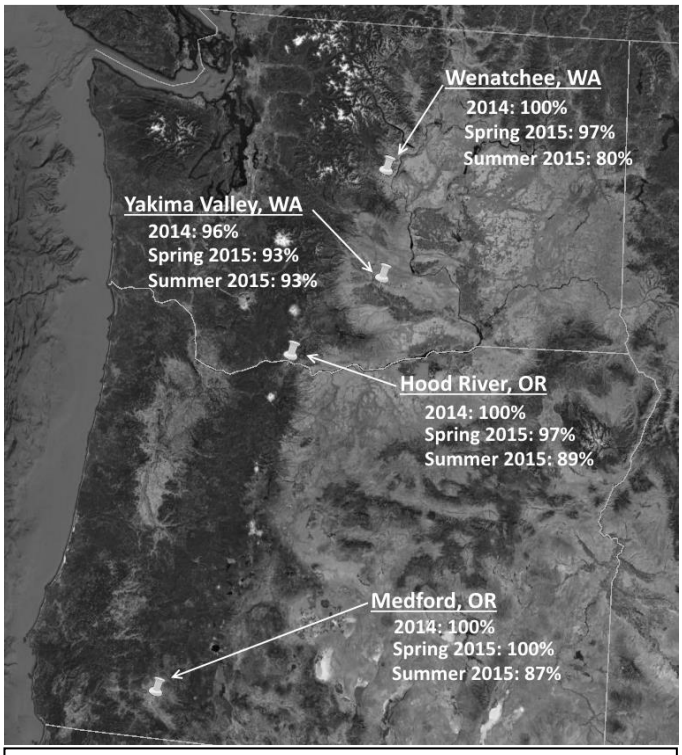

Figure 1. Percent of pear psylla adults harboring *Arsenophonus*

obtaining data for *Arsenophonus* in autumn populations, which will include a mixture of winterform and summerform. We are the first to report the presence of *Arsenophonus* in the pear psylla species that occurs in the United States. The high proportion of psylla carrying *Arsenophonus* suggests that either 1) *Arsenophonus* provides psylla with a selective advantage over psylla lacking this bacterium, or 2) this bacterium readily spreads throughout pear psylla populations without having negative effects on psylla fecundity or survival.

Objective 2. Bacteriomes are specialized organs in insects that house bacterial endosymbionts including the obligate endosymbiont, *Carsonella ruddii*, and many facultative endosymbionts. Using flourescence *in situ* hybridization, we observed *Arsenophonus* in the bacteriomes and oocytes of pear psylla (Figure 2). The occurrence of *Arsenophonus* in oocytes confirms that this endosymbiont is readily transmitted from mother to offspring.

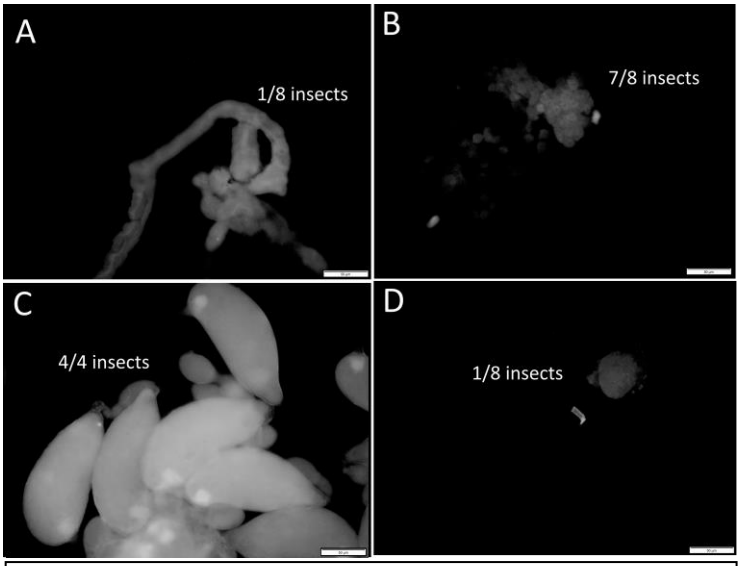

Figure 2. Fluorescent in situ hybridization to identify location of Arsenophonus in pear psylla adults: Alimentary canal (A), bacteriome (B), oocytes (C), and salivary glands (D). Visible tissues appear green through the fluorescent microscope indicating the presence of Arsenophonus. Values represent the number of the total samples positive for the bacterium.

Certain strains of *Arsenophonus* are plant pathogens, and are transmitted to new host plants by colonizing the insects' salivary glands (Novakova et al. 2009). Colonization of insect salivary glands may permit plant-mediated horizontal transmission of endosymbionts even if the bacteria cannot infect the plants (Caspi-Fluger et al. 2012, Gonella et al. 2015, Torres et al. 2015). For plant-mediated transmission to occur, the endosymbiont must be 1) colonize the insect salivary glands, 2) persist in the phloem long enough to be acquired by other insects, and 3) pass through the midgut to colonize the insect's hemolymph (blood). We observed *Arsenophonus* in the salivary glands and the gut of one psylla (Figure 2), but further research is required to confirm this observation and to determine whether colonization of the salivary glands of pear psylla by *Arsenophonus* leads to plant-mediated horizontal transmission.

Objective 3. An in-depth survey of endosymbionts in pear psylla populations did not reveal any new credible associations. In fact, the only bacteria identified using universal PCR primers were *Carsonella, Arsenophonus, Phytoplasma pyri*.

Since sequencing results indicated that *Phytoplasma pyri* was abundant in pear psylla, we used *Phytoplasma*-specific PCR primers to screen populations from different pear growing regions for the presence of this bacterium. This plant pathogen was most abundant in Yakima Valley, and was absent from Hood River. In general, the bacterium was more abundant in winterform populations (Spring 2015) than in summerform populations. We are still obtaining data for *Phytoplasma* in autumn populations, which will include a mixture of winterform and summerform. *Phytoplasma pyri* is the pathogen associated with pear decline disease, and is controlled by grafting pear to *Phtyoplasma* resistant rootstocks. While the use of resistant rootstock prevents the pathogen from overwintering in trees and prevents annual increases in bacterial titers, it is not known whether yearly reinfection with *Phytoplasma* affects tree health and yield. *Phytoplasma pyri* is also associated with peach yellow leafroll disease, which is transmitted to peach by winterform psylla that disperse from pear orchards during the winter (Purcell and Suslow 1984, Blomquist and Kirkpatrick 2002).

Many insect-vectored plant pathogens can alter insect behavior, especially their host preference and propensity to disperse. Further research is required to determine whether *Phytoplasma* alters the behavior of pear psylla.

The use of universal PCR primers will usually only detect the most abundant bacteria. We therefore are screening psylla populations using PCR primers specific for two endosymbionts known to occur in other psyllids, *Wolbachia* and *Profftella*. *Wolbachia* is a common endosymbiont of insects that causes reproductive manipulations. For example, female potato psyllids without *Wolbachia* are not capable of producing offspring with males with *Wolbachia* (Cooper et al. 2015). *Wolbachia* could provide mechanisms of novel psylla control strategies if our ongoing experiments confirm the absence of *Wolbachia* in pear psylla, or reveal a strain different from that of potato psyllid.

PCR amplicons associated with *Profftella* were observed from four pear psylla adults. *Profftella* is abundant in Asian citrus psyllid, and is thought to provide that psyllid with protection against parasitoids (Nakabachi et al. 2013). *Profftella* may also have important interactions with psyllid-vectored plant pathogens (Ramsey et al. 2015). Unfortunately, sequencing data was inconclusive so the product identity could not be confirmed. Further investigation is underway to confirm that PCR bands are associated with the presence of *Profftella*.

Conclusions

This study was first to investigate bacterial endosymbionts of pear psylla in the United States. This initial study is strongly related to our ongoing research on endosymbionts of potato psyllid. Results will be used to justify requests for research funds from other grant funding organizations. Further research on endosymbionts could lead to improved management decisions if results indicate that *Arsenophonus* provides pear psylla with protection from parasitoids or insecticides, as has been found for at least one other psyllid (Hansen et al. 2007). In addition to helping growers make informed pest management decisions, knowledge of psyllid endosymbionts could lead to the development of new control strategies that target endosymbionts to control psylla (Rio et al. 2004, Douglas 2007, Crotti et al. 2012). Researchers are searching for ways to target endosymbionts to control Asian citrus psyllid, aphids, and other insects (Rio et al. 2004, Douglas 2007, Crotti et al. 2012, Bouffard 2014). Our research on endosymbionts of pear psylla could allow these developing technologies to also be applied to pear psylla.

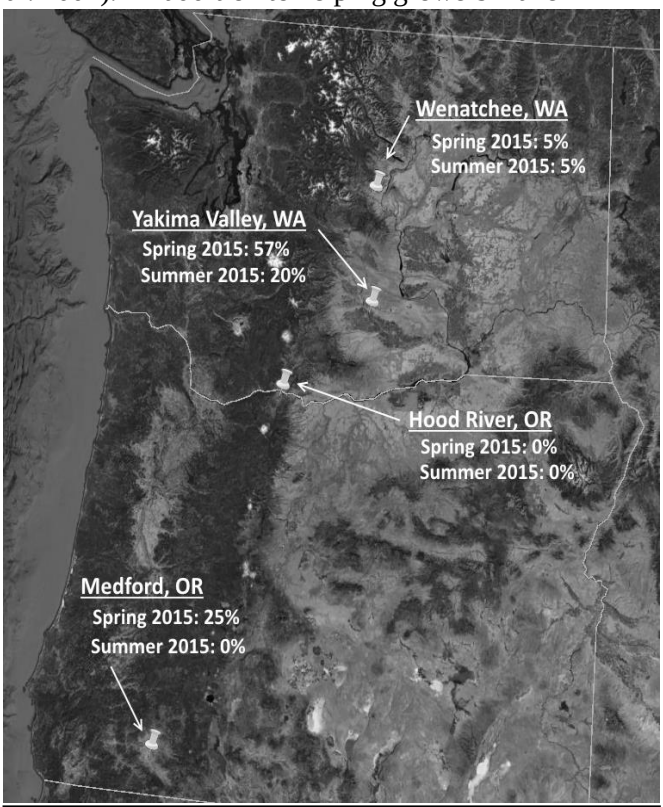

Figure 3. Percent of pear psylla adult harboring *Phytoplasma pyri*

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EXECUTIVE SUMMARY

The objective of this study was to investigate the bacterial endosymbionts of pear psylla, with an emphasis on the endosymbiont, *Arsenophonus*. The information learned from these experiments will help justify requests for research funding from other sources.

Summary of Findings

Results of this study indicate that *Arsenophonus* is prevalent in pear psylla populations throughout the Pacific Northwest. This endosymbiont is predominantly transmitted from mother to offspring, but the observation of *Arsenophonus* in the salivary glands and alimentary canal suggests that plant-mediated transmission is also possible. A survey of bacteria associated with pear psylla did not reveal new associations, but found that *Phytoplasma pyri* is present in some pear psylla populations. *Phytoplasma* appeared to be more prevalent in Yakima Valley than in other regions, and more prevalent in winterform psylla than in summerform psylla.

Future Directions

Bacterial endosymbionts, including plant pathogens, can often alter the behavior and susceptibility to parasitoids and insecticides of their insect hosts. Further research is required to determine whether *Arsenophonus* provides pear psylla with protection against parasitoids as has been demonstrated for another psyllid, or whether *Phtyoplasma* alters flight behavior of pear psylla. Ongoing research efforts seek to control Asian citrus psyllid and potato psyllid by manipulating their bacterial endosymbionts. Further research is needed to determine whether these developing technologies will apply to pear psylla and its endosymbionts.