

Potato Psyllids and Their Bacterial Allies: Two Fronts in the War Against Zebra Chip Disease

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What are endosymbionts and why do we care?

Many people think that an insect is only an insect, but this isn't completely true. Insects' biology, ecology, and behavior are dependent in-part on the bacteria found living within them. These bacteria, called endosymbionts, generally fall into one of three categories: obligate endosymbionts, facultative endosymbionts, and insect-vectoring plant pathogens. The obligate endosymbiont in psyllids, *Carsonella*, produces essential amino acids that are lacking in the insects' diet (Thao et al. 2007). *Carsonella* is found in all psyllids, and elimination of this bacterium using antibiotics leads to death of the insect. Unlike the obligate endosymbiont, the presence of facultative endosymbionts may vary among insect populations. Although they are not required for insect survival, many of these bacteria provide some benefit to the insect. Facultative endosymbionts can cause reproductive incompatibilities in insects, can increase insects' resistance to insecticides, plant defenses, and natural enemies, or may alter the geographical range of insects by changing their tolerance to extreme temperatures (Oliver et al. 2003, Scarborough et al. 2005, Russell and Moran 2006, Hansen et al. 2007, Ghanim and Kontsedalov 2009, Kikuchi et al. 2012, Su et al. 2015). Insect-vectoring plant pathogens are not generally thought of as endosymbionts, but many pathogens, including the zebra chip pathogen, colonize the internal organs of insects and may alter insect behavior.

Research on psyllid endosymbionts:

- 1) Help growers make informed pest management decisions
- 2) Lead to novel ways to control psyllids by targeting endosymbionts

Little is known of what endosymbionts are present in potato psyllid, whether they alter their susceptibility to pesticides, plant defenses, or natural enemies, or whether the presence of endosymbionts vary among the four identified potato psyllid haplotypes (Western, Central, Northwestern, and Southwestern). This information could be one key to making informed pest management decisions, and is especially important for growers in the Pacific Northwest where at least three of the four psyllid haplotypes have been found. In addition to allowing growers to make informed pest management decisions, research on psyllid endosymbionts may provide the framework necessary for the development of novel insect control strategies that target the endosymbionts. Research is underway to target endosymbionts for the control of Asian citrus psyllid, aphids, and other insects (Rio et al. 2004, Douglas 2007, Crotti et al. 2012, Bouffard 2014). A better understanding of potato psyllid endosymbionts may allow us to use these technologies developed for other insects to control potato psyllid.

Endosymbiont Research at the USDA-ARS Yakima Agricultural Research Laboratory

Ongoing research at the USDA-ARS Yakima Agricultural Research Laboratory in Wapato, WA seeks to identify bacterial endosymbionts of potato psyllid, determine the

biological role of potato psyllid endosymbionts, and develop novel ways to control potato psyllid by targeting endosymbionts. This research involves each of three categories of endosymbionts - the obligate endosymbiont *Carsonella*, facultative endosymbionts, and the zebra chip pathogen transmitted by the potato psyllid. Our research on potato psyllid endosymbionts is closely aligned with research on pear psylla endosymbionts also being performed at the ARS in Wapato, and complements contemporary research on endosymbionts of other psyllids including Asian citrus psyllid.

Obligate Endosymbiont. Our work on the obligate endosymbiont, *Carsonella*, has thus far focused on developing methods to accurately estimate the size of *Carsonella* populations in psyllids. The ability to accurately estimate *Carsonella* densities is critical to developing ways of controlling psyllids by targeting *Carsonella*. We developed two methods to estimate *Carsonella* populations, namely fluorescence *in situ* hybridization (FISH) and qPCR. FISH uses a probe that binds specifically to *Carsonella* DNA allowing us to view the bacteria under a fluorescence microscope (Figs. 1 and 2). *Carsonella* occurs inside specialized insect cells called bacteriocytes (Fig. 1) and in developing eggs of females (Fig. 2). The intensity of fluorescence is correlated with the density of *Carsonella* within these structures. Our other method of quantifying *Carsonella*, qPCR, is used to estimate the number of *Carsonella* in whole insects. Our future studies will use both methods to determine whether certain types of plant defenses and antibiotic insecticides reduce survival of psyllids by reducing *Carsonella*.

Facultative Endosymbionts. Our work on facultative endosymbionts has primarily focused on the presence and role of *Wolbachia*, which causes reproductive incompatibilities in numerous insects (Werren 1997). The most common reproductive incompatibility caused by this bacterium is cytoplasmic incompatibility, which is characterized by the death of eggs produced by a female without *Wolbachia* that has been mated by a male with *Wolbachia* (Fig. 3). In a previous study, we found that females of the Northwestern haplotype that had been mated by males of either the Western or Central haplotype failed to hatch (Fig. 4; Mustafa et al. 2015). Because this pattern is similar to that caused by *Wolbachia* (Figs. 3 and 4), we tested the psyllids for the presence of *Wolbachia*. Results indicated that psyllids of the Western and Central haplotypes both harbored *Wolbachia* whereas psyllids of the Northwestern haplotype did not (Fig. 5; Cooper et al. 2015). We then tested field-collected psyllids of each haplotype (including Southwestern) for *Wolbachia*. Results using wild insects confirmed our finding that psyllids of the Western and Central haplotypes have *Wolbachia* while psyllids of the Northwestern haplotype are *Wolbachia*-free, and indicated that psyllids of the southwestern haplotype were also *Wolbachia*-free. These findings suggest that the four haplotypes are at least partially reproductively isolated and that 'potato psyllid' is not a homogenous classification in the Pacific Northwest. The reproductive incompatibilities caused by *Wolbachia* may reduce population growth of potato psyllid under certain conditions, which is being investigated at ARS in Wapato.

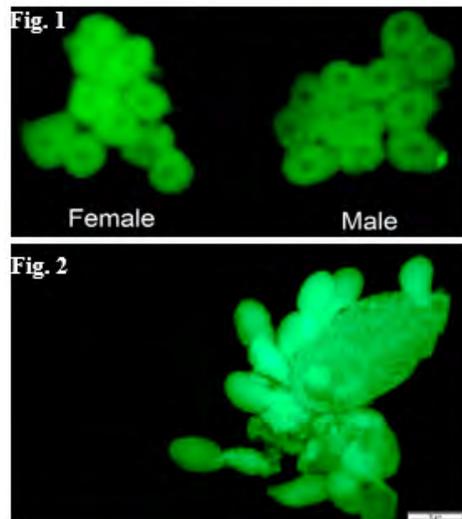


Fig. 1. *Carsonella* within specialized psyllid cells, bacteriocytes, dissected from female and male psyllids. **Fig. 2.** *Carsonella* within developing eggs dissected from a female potato psyllid. In both figures, *Carsonella* appears bright green because it has been labeled with a fluorescent probe.

Fig. 3	♂ +	♂ -
♀ +	Yes	Yes
♀ -	No	Yes

Fig. 4	♂ C or W	♂ NW
♀ C or W	Yes	Yes
♀ NW	No	Yes

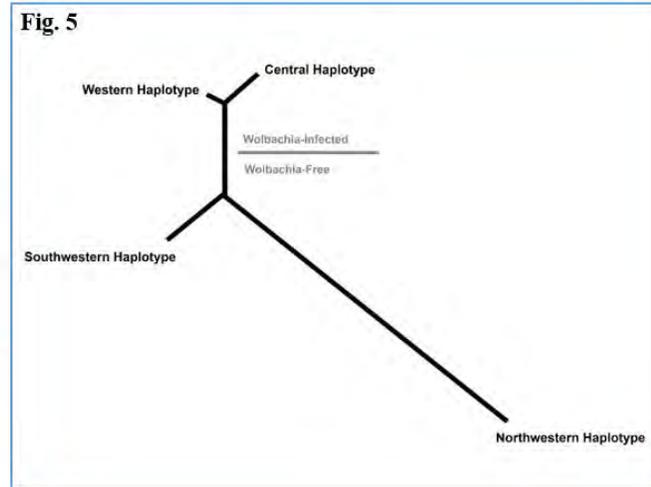


Fig. 3. Reproductive incompatibilities caused by *Wolbachia*; + indicates *Wolbachia* is present, - indicates *Wolbachia* is absent; yes and no refers to the ability for each cross to produce viable eggs. **Fig. 4.** Reproductive incompatibilities observed among potato psyllid haplotypes; C=Central, W=Western, NW=Northwestern. **Fig. 5.** Relationship between psyllid haplotype and the presence of *Wolbachia*; the length of each branch is proportional to the genetic distance (the longer the branch between haplotypes, the less similar the haplotypes).

In addition to causing reproductive manipulations, *Wolbachia* can reportedly alter insects' host-plant preference and tolerance to cold temperatures. Psyllids of the Northwestern haplotype (*Wolbachia*-free) appear to prefer bittersweet nightshade over potato as a host whereas psyllids of the Western and Central haplotypes (*Wolbachia*-present) appear to prefer potato. In addition psyllids of the Northwestern haplotype overwinter in the Pacific Northwest whereas psyllids of Western and Central haplotypes do not appear to readily overwinter in this region. It is not yet known whether *Wolbachia* is a cause for the differences in host plant preference and overwintering ecology, but this will be a subject of our future studies.

We recently discovered an undocumented facultative endosymbiont in potato psyllid called *Arsenophonus*. This bacterium is widespread in insects, and appears to provide another psyllid with protection against parasitoids (Hansen et al. 2007). It is not known whether this endosymbiont varies among psyllid haplotypes, or if it provides potato psyllid with protection against parasitism. Ongoing studies seek to compare the prevalence of *Arsenophonus* among different potato psyllid haplotypes.

Our list of facultative endosymbionts of potato psyllid is incomplete and facultative endosymbionts other than *Wolbachia* and *Arsenophonus* may be present in some populations. Recognition of these undocumented endosymbionts is important because they may alter the insect's biology and pest-status. Our ongoing studies seek to identify potential undocumented facultative endosymbionts of potato psyllid.

Insect-Vectored Plant Pathogens. Our work on the zebra chip pathogen, *Liberibacter*, has focused on the location of this bacterium in potato psyllid with regard to whether or not the psyllid can transmit the pathogen to non-infected host plants. Using fluorescence *in situ* hybridization, we observed *Liberibacter* in every psyllid tissue/organ studied: the gut, hemolymph (blood), bacteriocytes (location where the obligate symbiont resides), reproductive organs, and salivary glands (Figs. 6 and 7; Cooper et al. 2014). Although it was found in all tissues of some psyllids, in most specimens, the pathogen was observed only in the insect's gut. Our work then focused on infection of the gut and salivary glands. We postulated that if psyllids

can transmit *Liberibacter* to host plants when the pathogen was located in the gut only, then transmission could occur by gut regurgitation or by transfer on the insect's mouthparts. This was not the case; in fact, we observed a strong relationship between infection of the potato psyllid salivary glands and transmission of *Liberibacter* (Sengoda et al. 2014). Our study provided evidence for what many have assumed without data - that *Liberibacter* exhibits a circulative and persistent mode of transmission.

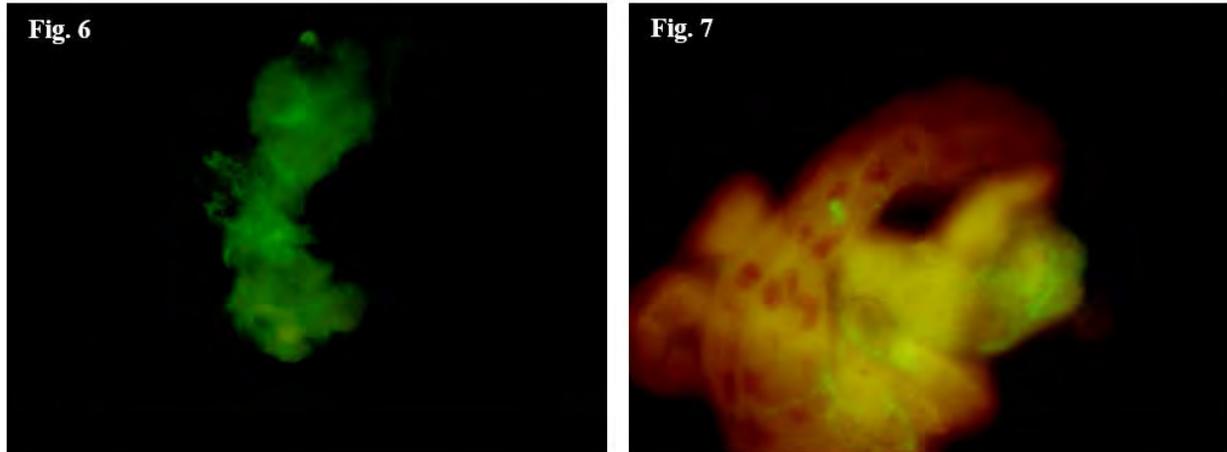


Fig 6. *Liberibacter* infection of the salivary glands. **Fig. 7** *Liberibacter* infection of the gut. In both figures, *Liberibacter* appears green.

Conclusions and Future Directions

ARS research on endosymbionts of potato psyllid has 1) led to the development of molecular tools needed to discover ways to control potato psyllids by targeting an obligate endosymbiont of the insect, 2) identified ecologically important variations in facultative endosymbionts among potato psyllid haplotypes, 3) identified a previously undocumented facultative endosymbiont of potato psyllid, and 4) improved our knowledge of biological interactions between potato psyllid and the zebra chip pathogen. Although progress has been made toward a better understanding of psyllid endosymbionts, many questions remain unanswered. Can *Wolbachia* reduce population growth of potato psyllid? Does *Wolbachia* contribute to biological differences among potato psyllid haplotypes? What role, if any, does *Arsenophonus* have in altering potato psyllid biology? Does *Liberibacter* colonization of internal organs such as the salivary glands lead to altered psyllid behavior or biology? And most importantly, can we develop new chemistries that target endosymbionts and provide psyllid control? In the short-term, our research will help growers make informed IPM decisions by improving knowledge of pest biology. In the long-term, our research may lead to the development of new psyllid control strategies.

References

- Bouffard, K. 2014. Entomology Research could yield solution to citrus' woes. The Ledger. Published Sunday, December 14, 2014.
- Cooper, WR, VG Sengoda, and JE Munyaneza. 2014. Localization of '*Candidatus Liberibacter solanacearum*' (Rhizobiales: Rhizobiaceae) in *Bactericera cockerelli* (Hemiptera: Triozidae). Ann. Entomol. Soc. Am. 107: 204-210.

- Cooper, WR, KD Swisher, SF Garczynski, T Mustafa, JE Munyaneza, and DR Horton. 2015. *Wolbachia* infection differs among divergent mitochondrial haplotypes of *Bactericera cockerelli* (Hemiptera: Triozidae). Ann. Entomol. Soc Am. in press.
- Crotti, E, A Balloi, C Hamdi, L Sansonno, M Marzorati, E Gonella, G Favia, A Alma, and D Daffonchio. 2012. Microbial symbionts: a resource for the management of insect-related problems. Microb. Biotechnol. 5: 307-317.
- Douglas, AE. 2007. Symbiont microorganisms: untapped resources for insect pest control. Trends Biotechnol. 25: 8.
- Ghanim, M, and S Kontsedalov. 2009. Susceptibility to insecticides in the Q biotype of *Bemisia tabaci* is correlated with bacterial symbiont densities. Pest Manage. Sci. 939-942.
- Hansen, AK, G Jeong, TD Pain, and R Stouthamer. 2007. Frequency of secondary symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic scale in California. Applied and Environ. Microbiol. 73: 7531-7535.
- Kikuchi, Y, M Hayatsu, T Hosokawa, A Nagayama, K Tago, and T Fukatsu. 2012. Symbiont-mediated insecticide resistance. Proc. Nat. Acad. Sci. USA. 109: 8618-8622.
- Mustafa, T., DR Horton, WR Cooper, KD Swisher, RS Zack, and JE Munyaneza. 2015. Interhaplotype fertility and effects of host plant on reproductive traits of three haplotypes of *Bactericera cockerelli* (Hemiptera: Triozidae). Environ. Entomol. in press.
- Oliver, KM, JA Russell, NA Moran, and MS Hunter. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasp. Proc. Nat. Acad. Sci. USA. 100: 1803-1807.
- Rio, RVM, Y Hu, and S Aksoy. 2004. Strategies of the home-team: symbioses exploited for vector-borne disease control. Trends Microbiol. 12:7.
- Russell, JA, NA Moran. 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. Proc. R. Soc. Biol. Sci. 273: 603-610.
- Scarborough, CL, J Ferrari, and HCJ Godfray. 2005. Aphid protected from pathogen by endosymbiont. Science. 310: 1781.
- Sengoda, VG, WR Cooper, KD Swisher, DC Henne, and JE Munyaneza. 2014. Latent period and transmission of "*Candidatus Liberibacter solanacearum*" by the potato psyllid *Bactericera cockerelli* (Hemiptera: Triozidae). PLoS One. 9: e93475.
- Su, Q, KM Oliver, W Xie, Q Wu, S Wang, and Y Zhang. 2015. The whitefly-associated facultative symbiont *Hamiltonella defensa* suppresses induced plant defenses in tomato. Functional Ecol. in press.
- Thao, ML, NA Moran, P Abbot, EB Brennan, DH Burckhardt, and P Baumann. 2000. Cospeciation of psyllids and their primary prokaryotic endosymbionts. Appl. Environ. Microbiol. 66: 2898-2905.
- Werren, JH. 1997. Biology of *Wolbachia*. Ann. Rev. of Entomol. 42: 587-609.