"*Candidatus* Phytoplasma pyri" Affects Behavior of *Cacopsylla pyricola* (Hemiptera: Psyllidae)[1]

Mireya Cruz[2], W. Rodney Cooper[3], David R. Horton, and Nina M. Barcenas[2]

USDA-ARS-Yakima Agricultural Research Laboratory, Wapato, Washington 98951 USA

J. Entomol. Sci. 53(3): 361–371 (July 2018)

Abstract Pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), is a key pest of pear and is a vector of "*Candidatus* Phytoplasma pyri", the pathogen associated with pear decline disease. Although commercial pear trees are grafted to Phytoplasma-resistant rootstock, a recent report indicated that many *C. pyricola* in Washington and Oregon harbor this bacterium. Using fluorescence in situ hybridization, we confirmed that Phytoplasma invades internal tissues of *C. pyricola*, suggesting the pathogen is persistently transmitted. Because many persistently transmitted plant pathogens alter the flight behavior of their insect vectors, we examined the effects of Phytoplasma infection on dispersal of *C. pyricola*. Flight behavior was investigated using greenhouse bioassays, which demonstrated that Phytoplasma-infected psylla were less likely than uninfected psylla to emigrate from trees and become captured on yellow traps. Pear psylla occurs as two seasonal morphotypes—summerform and winterform—and the effects of Phytoplasma were observed for both morphotypes. Results provide direction for future study of *C. pyricola* ecology and interactions between Phytoplasma and psyllid vectors.

Key Words pear psylla, winterform, summerform, mycoplasma-like organisms

Pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), is an important pest of pear in the United States and Europe. The nymphs produce copious amounts of honeydew, which promotes growth of sooty mold, marks the pear fruit, and complicates harvest (Westigard and Zwick 1972). A recent survey for bacteria associated with *C. pyricola* revealed that many psylla collected from pear-growing regions of the Pacific Northwest harbored "*Candidatus* Phytoplasma pyri" (Cooper et al. 2017). This bacterium causes pear decline disease in susceptible pear, which was responsible for substantial economic losses to U.S. pear production in the mid–20th century (Westigard and Zwick 1972). Growers at the time observed that trees grafted to certain rootstocks were immune to pear decline, and diseased orchards were replanted with trees having Phytoplasma-resistant rootstocks. Most Phytoplasmas are persistently transmitted by their insect vectors (Weintraub and Beanland 2006). Many persistently transmitted plant pathogens, including Phytoplasmas, share biological traits with other insect endosymbionts including ability to alter insect transcription profiles, fitness, and behavior (Davis et al. 2012;

[1]Received 05 October 2017; accepted for publication 14 December 2017.
[2]Heritage University, Department of Natural Sciences, Toppenish, WA 98948 USA.
[3]Corresponding author (email: Rodney.Cooper@ars.usda.gov).

Hogenhout et al. 2008; Mann et al. 2012; Nachappa et al. 2012a, 2012b; Mas et al. 2014; Ramsey et al. 2015; Rid et al. 2016; Vyas et al. 2015; Weintraub and Beanland 2006). While the use of resistant rootstock effectively manages plant pathogenic life stages of "*Ca*. Phytoplasma pyri", the biological and applied consequences of the insect endosymbiotic life stages of this bacterium are entirely unknown.

Cacopsylla pyricola occurs as two distinct seasonal morphotypes—summerform and winterform—that differ with respect to diapause, feeding behavior, and plant attraction (Civolani et al. 2011; Horton et al. 1998, 2007; Krysan and Higbee 1990; Ullman and McLean 1988). Although summerforms are rarely found on plants other than pear, winterforms often emigrate from pear and overwinter on a variety of shelter plant species including on other deciduous fruit trees, in windbreaks, and on various conifers. Phytoplasma infection rates appear to be greater in winterform than summerform populations (Cooper et al. 2017, Davies and Eyre 1996). These previous reports require cautious interpretation because winterform psylla were collected from pear orchards, and Phytoplasma infection rates in psylla collected from nonpear shelter plants have not been estimated. Two possible conditions could, therefore, explain the apparent differences in Phytoplasma infection between surveyed morphotypes: (a) winterform adults are more likely to harbor the bacterium than summerforms; or (b) Phytoplasma lowers the propensity for winterform psylla to emigrate from pear, thus samples of winterforms collected from orchards would have estimated rate of infection higher than actually occurring in the population as a whole.

Our study had two primary objectives. We first used fluorescence in situ hybridization to confirm that "*Ca*. Phytoplasma pyri" invades the internal organs of *C. pyricola*, which is likely needed for the bacterium to cause changes in insect behavior. Our second objective was to investigate the potential effects of Phytoplasma on *C. pyricola* flight behavior and dispersal. Greenhouse assays compared flight behavior of Phytoplasma-infected and -uninfected psyllids under controlled conditions. Field surveys were conducted to examine the two possible conditions to explain the apparent differences in Phytoplasma infection between morphotypes (Cooper et al. 2017, Davies and Eyre 1996). If Phytoplasma lowers the propensity for winterform psylla to emigrate from pear, then we would expect infection rates to be higher among psylla that remained on pear versus those the migrated, but overall infection rates in winterforms to not be substantially different from those in summerforms.

Material and Methods

Location of "*Ca*. Phytoplasma pyri" in *C. pyricola*. Fluorescence in situ hybridization was performed as described earlier for observing "*Ca*. Liberibacter solanacearum" in *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) and *Carsonella* in *C. pyricola* and *B. cockerelli* (Cooper et al. 2014, 2015). Adult psylla collected from a laboratory colony were dissected in phosphate-buffered saline, and the salivary glands, alimentary canal, and ovaries were transferred to positively charged microscope slides (Fisher Scientific, Pittsburgh, PA). Spermatophores were removed from the reproductive organs before transferring them to the slides.

The dissected tissues were fixed in Carnoy's solution (Electron Microscopy Sciences, Hatfield, PA), then briefly rinsed with 100% ethanol and washed three times in hybridization buffer (20 nM Tris-HCl, 0.9 M NaCl, 0.01% SDS, and 30% formamide) (Cooper et al. 2014, 2015). Samples were hybridized overnight with 100 μl of 250 pmol/ml of the HPLC-purified oligonucleotide probe GAC CAT AGA CTT ATT AAA CCG (Raddadi et al. 2011) labeled with Alexa Fluor 488 on the 5′ end (Invitrogen, Carlsbad, CA). During probe hybridization, samples were kept under dark and humid conditions at 25°C. After incubation, samples were rinsed in hybridization buffer and in tris-buffered saline. The presence of Phytoplasma was detected at 20× magnification using a fluorescence microscope (Exposure = 265.196 ms, Sensitivity = ISO 200, Resolution = 1,280 × 960). Tissues and organs were photographed using a DP25 camera mounted to the microscope and operated using the CellSens software (Olympus America Inc., Central Valley, PA).

Laboratory studies on effects of Phytoplasma infection on psyllid dispersal. Adult *C. pyricola* were collected from a pear orchard located at the USDA research farm near Moxee, WA. Insects were dislodged from trees onto a 0.5-m^2 beat sheet by tapping branches with a 0.2-m-long rubber hose. Summerform psylla were collected in June and July of 2016, whereas diapausing winterform psylla were collected on 4 October 2016. Psyllids were collected immediately before they were to be used in the laboratory experiments, and were kept cool during transport from the orchard to the laboratory by keeping them in an ice chest.

Dispersal tendencies of *C. pyricola* were assessed as described by Horton and Lewis (1995). Studies were performed in a greenhouse maintained at 20–25°C with supplemental lighting to provide a 16:8 h (L:D) photoperiod. A 30- to 50-cm-tall potted Bartlett pear seedling was placed on the south side of each of five mesh dome cages (61 × 61 × 61 cm; Bioquip BugDorms) with a few leaves touching the wall. The doors of each cage faced north. Cages were then covered with cardboard, and a rectangular window (20 × 27 cm) was cut in the cardboard on the south side of each cage to allow light to enter. The purpose of the window was to orient psylla toward the light and prompt them to settle on the tree seedling. One hundred psyllids comprising an equal number of female and male insects were released at the base of each seedling. After 24 h of settling time, the cardboard was removed from the north side of the cage and a yellow card trap was placed near the cage door. Psyllids were collected from each tree and trap 24 h after having placed the trap in the cage. The experiment was conducted twice (= trial) with summerform psylla, and once with diapausing winterform psylla. All psylla collected from traps were tested for the presence of Phytoplasma, using polymerase chain reaction (PCR) as described in the "Diagnostic PCR for Phytoplasma" subsection. A sample of psyllids remaining on the pear seedling was also examined for Phytoplasma. In this case, because far more psylla remained on the tree than were captured in traps, only a subset of insects collected from each tree were tested for Phytoplasma. Each subsample examined for Phytoplasma was intentionally equivalent in numbers of psyllid and in sex ratio to the numbers and sex ratios of psyllid on the corresponding trap. Foliage from the terminal of each tree was also tested for the presence of Phytoplasma.

The probability for psylla to disperse from the tree and become captured in the yellow trap was compared between Phytoplasma-infected and -uninfected insects by logistic regression using the GLIMMIX procedure of SAS 9.4 (SAS Institute

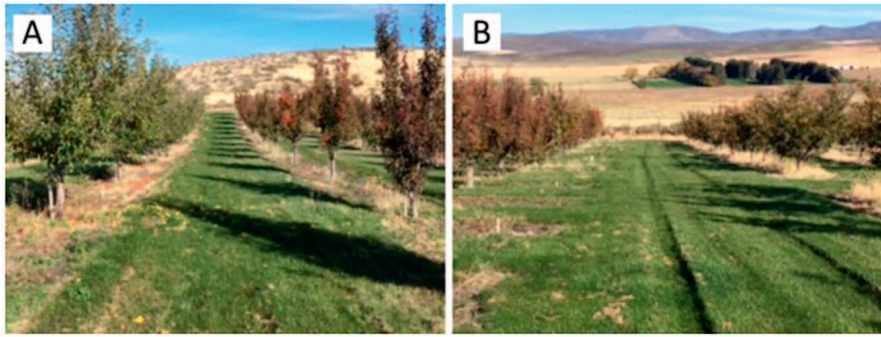

Fig. 1. Diapausing winterform *C. pyricola* were collected from adjacent rows of apple and pear in two separate plots. Both photographs were captured facing north. In the first plot (A), the apple row was located to the west of the pear row. In the second block (B), the apple row was located to the east of the pear row.

2013). The number of Phytoplasma-infected psylla divided by the total number of psylla sampled in each cage was the dependent variable. Treatment (dispersed versus remained on the tree), insect morphotype, and the main effect interactions were included as the fixed effects. Sex and cage nested within trial were included as random variables.

Field study on the effects of Phytoplasma on dispersal of winterform psylla. Adult diapausing winterform psylla were collected from adjacent rows of apple (*Mallus domestica* Borkh) and pear trees located at the USDA research farm near Moxee, WA. Insects were sampled from two separate plots. The apple row was west of the pear row in one plot, and east of the pear row in the second plot (Fig. 1). Insects were collected on 25 October 2016 when foliage was dropping from pear, but not from apple (Fig. 1). Psylla were then sampled for the presence of Phytoplasma as described in the "Diagnostic PCR for Phytoplasma" subsection. The proportion of Phytoplasma-infected psylla collected from apple and pear was analyzed using the GLIMMIX procedure of SAS 9.4 with the incidence of Phytoplasma infection as the dependent variable, tree species (pear versus apple) as the fixed effect, and block as the random variable. Data were modeled with a binomial distribution using the DIST=BINOMIAL option of the MODEL statement.

Diagnostic PCR for Phytoplasma. DNA was extracted from psylla using a cetyltrimethylammonium bromide (CTAB) precipitation method and was suspended in 50 µl of sterile water (Crosslin et al. 2011). Similarly, DNA was extracted from plant samples using a modified CTAB precipitation method (Munyaneza et al. 2010) and was suspended in 100 µl of sterile water. PCR diagnosis of Phytoplasma was performed using a previously published nested PCR procedure (Deng and Hiruki 1991, Lee et al. 1995, Smart et al. 1996). The first PCR step included primers P1 (AAG AGT TTG ATC CTG GCT CAG GAT T) and P7 (CGT CCT TCA TCG GCT CTT). PCR conditions for the first step included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 10 min. The second step was performed with a 1:20

dilution of PCR product from the initial PCR step using primers, R16 (x)-F1 (GAC CCG CAA GTA TGC TGA GAG ATG), and R16(x)-R1 (CAA TCC GAA CTG AGA CTG). PCR conditions for the second step included 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 10 min. For both PCR steps, each 10-μl reaction included Invitrogen Amplitaq Gold 360 Master Mix (Invitrogen), 200 nM of each primer, and 1 μl of template DNA. PCR products were observed on 1.5% agarose gels stained with ethidium bromide.

Results

Location of "*Ca*. Phytoplasma pyri" in *C. pyricola*. Green fluorescence was not observed in the no-probe controls (Fig. 2A–C), confirming that *C. pyricola* tissues do not auto-fluoresce. Phytoplasma was observed in the alimentary canal of 54% of adults (Fig. 2D), and in the salivary glands of 26% of adults (Fig. 2E). These observations confirm that Phytoplasma is transmitted by *C. pyricola* via a circulative and persistent mode of action. Phytoplasma was also observed in the developing oocytes of 43% of females (Fig. 2F).

Laboratory studies on the effects of Phytoplasma infection on psyllid dispersal. We did not detect Phytoplasma in leaf samples collected from trees used in our bioassays. However, 32% of the 264 summerform psylla and 48% of the 71 winterform psylla collected from our field population as sources of insects used in the assay harbored the bacterium. Results of greenhouse assays indicated that noninfected psylla exhibited a greater propensity to move from trees to yellow card traps than did infected psylla ($F = 11.7$; df = 1, 35; $P = 0.002$; Fig. 3). There were no differences in dispersal between summerform and winterform morphotypes ($F = 1.6$; df = 1, 35; $P = 0.209$). The lack of a significant main effect interaction indicated that the effects of Phytoplasma on psyllid dispersal were similar between morphotypes ($F = 0.1$; df = 1, 35; $P = 0.778$). Although about equal numbers of males and females were released in cages, samples collected for diagnostic PCR of Phytoplasma were female-biased (212 females versus 123 males).

Field study on the effects of Phytoplasma on dispersal of winterform psylla. At the time of collections, pear trees were defoliating whereas apple trees were still green and retaining their leaves (Fig. 1A, B) and psylla were present on both tree species. However, the proportion of psyllids found to be infected did not differ between apple-collected specimens and pear-collected specimens (Fig. 4; $F = 0.57$; df = 1, 91; $P = 0.453$). Approximately 46% (44 of 95 psyllids sampled) of psyllids were found to harbor the Phytoplasma (Fig. 4), irrespective of orchard source.

Discussion

Using fluorescence in situ hybridization, we observed "*Ca*. Phytoplasma pyri" in the alimentary canal and salivary glands of *C. pyricola* adults, confirming that this pathogen invades the internal tissues of its vector and is likely transmitted persistently. We also observed Phytoplasma in the developing oocytes of adults, suggesting that the pathogen is transovarially transmitted. Transovarial transmis-

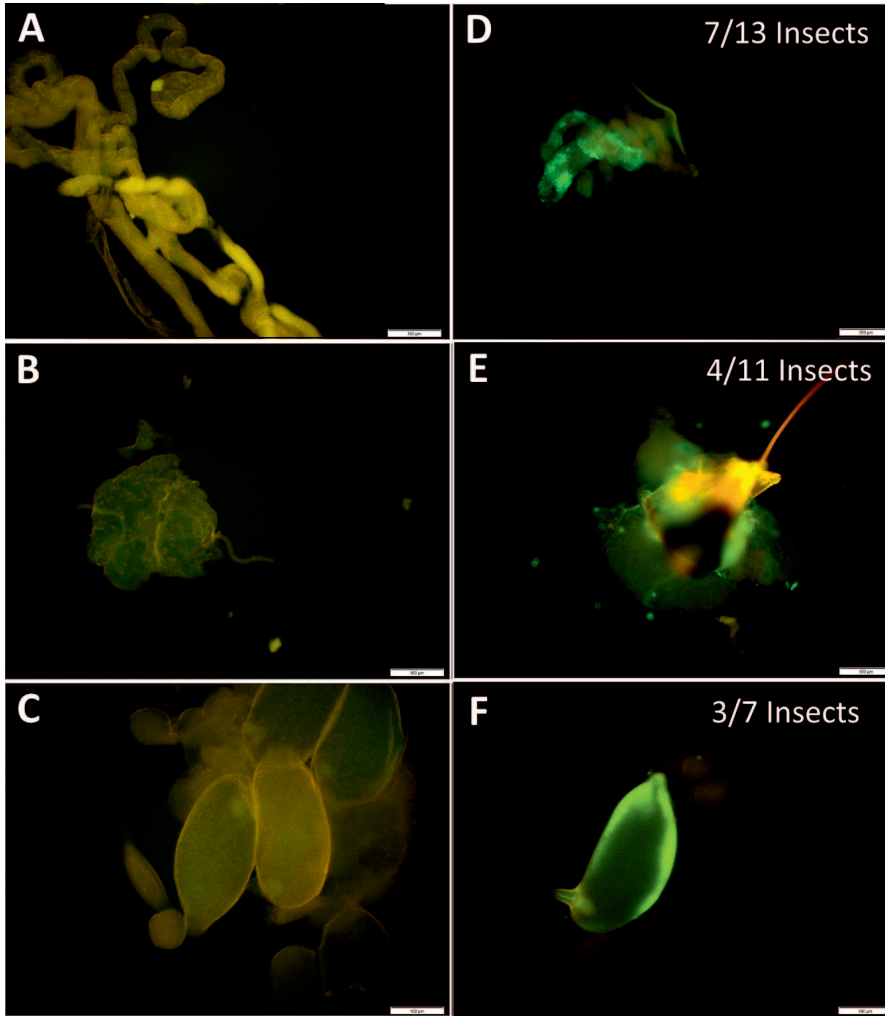

Fig. 2. Yellow coloration of *C. pyricola* alimentary canal (A), salivary glands (B), and oocytes (C) hybridized without the fluorescent probe designed to bind to "*Ca.* Phytoplasma pyri". The green florescence in alimentary canal (D), salivary gland (E), and oocyte (F) indicates the presence of the bacterium. The number of psyllids with each respective tissue colonized by Phytoplasma per the total number of observed psyllids is indicated in panes E–F. Tissues from 14 insects were prepared for hybridization, but some tissues were lost during processing.

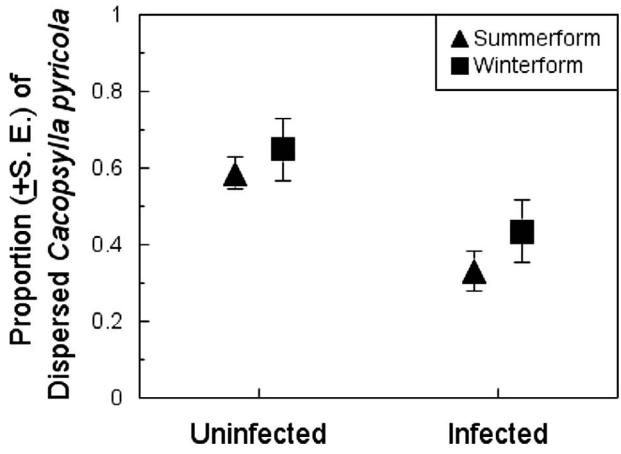

Fig. 3. Proportion of uninfected and infected *C. pyricola* dispersing from pear trees and captured in yellow traps in greenhouse assays.

sion, if it indeed occurs, would help explain the relatively high rates of infection among psyllids in the Pacific Northwest despite the widespread plantings of pear trees grafted to Phytoplasma-resistant rootstock (Cooper et al. 2017). Since Phytoplasma and other tissue-invading bacteria may alter flight and dispersal behavior or overwintering ability of their insect vectors (Davis et al. 2012, Ebbert and Nault 1994, Mann et al. 2012, Mas et al. 2014, Nachappa et al. 2012b, Weintraub and Beanland 2006), we conducted greenhouse and field assays to assess potential associations between Phytoplasma and dispersal behaviors.

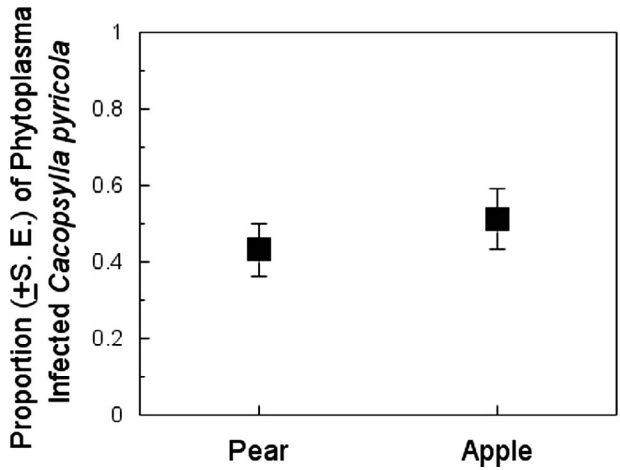

Fig. 4. Proportion of Phytoplasma-infected winterform psylla remaining in the pear orchard versus dispersing to adjacent apple trees.

Results of our greenhouse assays indicated that Phytoplasma infection was associated with a reduced propensity for psylla to disperse from the pear tree and become captured in a yellow trap. Several factors, which are not mutually exclusive, could explain this observed association between Phytoplasma and psyllid dispersal including direct effects of Phytoplasma on the psyllid, and confounding factors such as insect age and sex. Phytoplasmas manipulate their plant and insect hosts to improve their own dispersal and survival (Hogenhout et al. 2008). It is therefore possible that Phytoplasma directly altered dispersal behavior of *C. pyricola* or reduced the insect's attraction to yellow traps. Yellow may visually mimic diseased plants more than uninfected plants. Phytoplasmas can also influence survival and fecundity of insect vectors, and can have either negative or positive impacts on vector performance (Weintraub and Beanland 2006). Age, sex, and egg load are known to influence dispersal of *C. pyricola*, and age structures and sex ratios of psylla captured in flight traps do not always correspond with age and sex ratios of psylla present on trees (Horton 1994, Horton and Lewis 1997). Phytoplasma, therefore, may have indirectly influenced psyllid dispersal by altering the age structure or reproductive maturity of field-collected infected and uninfected psylla used in our assays. Regardless of the mechanism(s), results of our study indicate that Phytoplasma infection is associated with altered dispersal behavior.

Plants infected with a different phloem-limited bacterial plant pathogen, Liberibacter, are more attractive to uninfected psyllid vectors compared with uninfected plants, but uninfected plants are more attractive to infected psyllids (Mas et al. 2014). These manipulations likely increase the probability that uninfected psyllid vectors acquire the pathogen, and that infected psyllids transmit the pathogen to uninfected hosts. There are no comparable studies to determine whether similar manipulations occur in Phytoplasma-infected insects. If Phytoplasma alters vector responses to diseased plants, then different results would be expected if trees used in our bioassays were infected with Phytoplasma. Efforts to detect Phytoplasma in trees at our research farm near Moxee did not identify infected trees to use in assays comparing responses of uninfected and infected psylla to trees with or without Phytoplasma (data not shown). It is possible that the bacterium was present but not detected using our methods due to low titers or spatial variability within the plant canopy.

Consistent with previous surveys (Cooper et al. 2017, Davies and Eyre 1996), a greater percentage of winterform (48%) than summerform psylla (32%) were infected with Phytoplasma. We did not observe a difference in Phytoplasma infection rates between winterform psylla collected from pear trees versus those that had moved to apple. Emigration of winterform psyllids is triggered by leaf fall in autumn. These psyllids are known to be attracted to the color of foliage, and thus disperse to evergreen trees or to deciduous tree species having leaf drop later in autumn than shown by pear trees (Horton et al. 1994). It is possible that factors triggering winterform emigration (i.e., leaf-fall displacement of psyllids) have greater influence on psyllid dispersal than Phytoplasma infection. Our findings therefore suggest that observed differences in infection rates between morphotypes observed here and elsewhere (Cooper et al. 2017, Davies and Eyre 1996) cannot be explained by migration tendencies of infected versus uninfected winterform psyllids from pear. But it is also possible that our experimental design using adjacent rows of pear and apple trees did not accurately compare migration of

infected and uninfected psylla away from pear. More sophisticated techniques using gut content analysis (Cooper et al. 2016) of psylla returning to orchards in spring may provide more precise comparisons of migration tendencies between Phytoplasma-infected and -uninfected psylla.

Although our results do not help explain reports that a larger percentage of winterform *C. pyricola* harbor Phytoplasma than summerforms (Cooper et al. 2017, Davies and Eyre 1996), results do provide direction for further research and will aid the interpretation of results from more sophisticated studies. Studies are required to confirm and determine rates of transovarial transmission of "*Ca*. Phytoplasma pyri" by *C. pyricola*, and assess potential effects of Phytoplasma on psylla fitness. This information may help explain why such a large percentage of *C. pyricola* (up to 60%) harbor "*Ca*. Phytoplasma pyri" in some pear-growing regions of the Pacific Northwest (Cooper et al. 2017). Results of our greenhouse study suggest that yellow traps may be biased toward capture of uninfected psyllids. It is important to elucidate the mechanisms for this bias to determine potential effects of Phytoplasma infection on sampling efficiency for *C. pyricola*. Yellow traps are commonly used to monitor incidence of "*Ca*. Liberibacter" infection of *B. cockerelli* and *Diaphorina citri* Kuwayama, so it is also important to determine if Liberibacter also affects capture of psyllids on yellow traps. Lastly, further research is needed to assess factors that affect Phytoplasma infection rates in winterform and summerform *C. pyricola*. Cooper et al. (2017) reported that in addition to higher rates of Phytoplasma, winterform psylla had slightly higher rates of the insect endosymbiont, *Arsenophonus*. This bacterium was present in nearly every *C. pyricola* sampled, so the differences between winterform and summerform psylla were not as obvious as the differences in Phytoplasma infection. It is possible that winterform physiology is more conducive than that of summerforms for growth or horizontal transmission of bacterial endosymbionts. Abiotic factors such as seasonal or diurnal temperature changes may also affect seasonal changes in incidence of bacterial endosymbionts. Documenting physiological or abiotic factors that influence incidence of bacterial endosymbionts, including Phytoplasma, is needed to elucidate potential effects of these bacteria on ecology, behavior, and management of psyllids.

Acknowledgments

Mireya Cruz was supported by National Science Foundation Research Experience for Undergraduates Award Number 1460733 to Nina Barcenas of Heritage University. We thank Pauline Anderson, Heather Headrick, and Jennifer Stout for technical assistance. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture. USDA is an equal opportunity provider and employer.

References Cited

Civolani, S., M. Leis, G. Grandi, E. Garzo, E. Pasqualini, S. Musacchi, M. Chicca, G. Castaldelli, R. Rossi and W.F. Tjallingii. 2011. Stylet penetration of *Cacopsylla pyri*: An electrical penetration graph (EPG) study. J. Insect Physiol. 57: 1407–1419.

Cooper, W.R., S.F. Garczynski and D.R. Horton. 2015. Relative abundance of *Carsonella ruddii* (Gamma Proteobacterium) in females and males of *Cacopsylla pyricola* (Hemiptera: Psyllidae) and *Bactericera cockerelli* (Hemiptera: Triozidae). J. Insect Sci. 15: 65.

Cooper, W.R., S.F. Garczynski, D.R. Horton, T.R. Unruh, E.H. Beers, P.W. Shearer and R.J. Hilton. 2017. Bacterial endosymbionts of the psyllid *Cacopsylla pyricola* (Hemiptera: Psyllidae) in the Pacific Northwestern United States. Environ. Entomol. 46: 393–402.

Cooper, W.R., D.R. Horton, T.R. Unruh and S.F. Garczynski. 2016. Gut content analysis of a phloem-feeding insect, *Bactericera cockerelli* (Hemiptera: Triozidae). Environ. Entomol. 45: 938–944.

Cooper, W.R., V.G. Sengoda and J.E. Munyaneza. 2014. Localization of '*Candidatus* Liberibacter solanacearum' (Rhizobiales: Rhizobiaceae) in *Bactericera cockerelli* (Hemiptera: Triozidae). Ann. Entomol. Soc. Am. 107: 204–210.

Crosslin, J.M., H. Lin and J.E. Munyaneza. 2011. Detection of '*Candidatus* Liberibacter solanacearum' in the potato psyllid, *Bactericera cockerelli* (Sulc) by conventional and real-time PCR. Southwest. Entomol. 36: 125–135.

Davies, D.L. and S. Eyre. 1996. Detection of Phytoplasma associated with pear decline in pear psylla by polymerase chain reaction. Proc. Br. Crop Prot. Symp. 65: 67–75.

Davis, T.S., D.R. Horton, J.E. Munyaneza and P.J. Landolt. 2012. Experimental infection of plants with an herbivore-associated bacterial endosymbiont influences herbivore host selection behavior. PLoS One 7: e49330.

Deng, S. and C. Hiruki. 1991. Amplification of 16S RNA genes from culturable and nonculturable mollicutes. J. Microbiol. Meth. 14: 53–61.

Ebbert, M.A. and L.R. Nault. 1994. Improved overwintering ability in *Dalbulus maidis* (Homoptera: Cicadellide) vectors infected with *Spiroplasma kurnkelli* (Mycoplasmatales: Sprioplasmataceae). Environ. Entomol. 23: 634–644.

Hogenhout, S.A., K. Oshima, E.D. Ammar, S. Kakizawa, H.N. Kingdom and S. Namba. 2008. Phytoplasmas: Bacteria that manipulate plants and insects. Mol. Plant Pathol. 9: 403–423.

Horton, D.R. 1994. Relationship among sampling methods in density estimates of pear psylla (Homoptera: Psyllidae): Implications of sex, reproductive maturity, and sampling location. Ann. Entomol. Soc. Am. 87: 583–591.

Horton, D.R., D.A. Broers, T. Hinojosa and T.M. Lewis. 1998. Ovarian development in overwintering pear psylla, *Cacopsylla pyricola* (Homoptera: Psyllidae): Seasonality and effects of photoperiod. Can. Entomol. 130: 859–867.

Horton, D.R., E.C. Burts, T.R. Unruh, J.L. Krysan, L.B. Coop and B.A. Croft. 1994. Phenology of fall dispersal by winterform pear psylla (Homoptera: Psyllidae) in relation to leaf fall and weather. Can. Entomol. 126: 111–120.

Horton, D.R., C. Guedot and P.J. Landolt. 2007. Diapause status of females affects attraction of male pear psylla, *Cacopsylla pyricola*, to volatiles from female-infested pear shoots. Entomol. Exp. Appl. 123: 185–192.

Horton, D.R. and T.M. Lewis. 1995. Interplant movement by pear psylla (Homoptera: Psyllidae): Effects of sex ratio and reproduction status. J. Insect Behav. 8: 687–700.

Horton, D.R. and T.M. Lewis. 1997. Quantitative relationship between sticky trap catch and beat tray counts of pear psylla (Homoptera: Psyllidae): Seasonal, sex, and morphotypic effects. J. Econ. Entomol. 90: 170–177.

Krysan, J.L. and B.S. Higbee. 1990. Seasonality of mating and ovarian development in overwintering *Cacopsylla pyricola* (Homoptera: Psyllidae). Environ. Entomol. 19: 544–550.

Lee, I.M., A. Bertaccini, M. Vibio and D.E. Gundersen. 1995. Detection of multiple Phytoplasma in perennial fruit trees with decline symptoms in Italy. Phytopathology 85: 728–735.

Mann, R.S., R.G. Ali, S.L. Hermann, S. Tiwari, K.S. Pelz-Stelinski, H.T. Alborn and L.L. Stelinski. 2012. Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLoS One 8: e1002610.

Mas, F., J. Vereijssen and D.M. Suckling. 2014. Influence of the pathogen *Candidatus* Liberibacter solanacearum on tomato host plant volatiles and psyllid vector settlement. J. Chem. Ecol. 40: 1197–1202.

Munyaneza, J.E., T.W. Fisher, V.G. Sengoda, S.F. Garczynski, A. Nissinen and A. Lemmetty. 2010. Association of '*Candidatus* Liberibacter solanacearum' with the psyllid *Trioza apicalis* (Hemiptera: Triozidae) in Europe. J. Econ. Entomol. 103: 1060–1070.

Nachappa, P., J. Levy and C. Tamborindeguy. 2012a. Transcriptome analyses of *Bactericera cockerelli* adults in response to "*Candidatus* Liberibacter solanacearum" infection. Mol. Genet. Genomics 287: 803–817.

Nachappa, P., A.A. Shapiro and C. Tamborindeguy. 2012b. Effect of '*Candidatus* Liberibacter solanacearum' on fitness of its insect vector, *Bactericera cockerelli* (Hemiptera: Triozidae), on tomato. Phytopathology 102: 41–46.

Raddadi, N., E. Gonella, C. Camerota, A. Pizzinat, R. Tedeschi, E. Crotti, M. Mandrioli, P.A. Bianco, D. Daffonchia and A. Alma. 2011. '*Candidatus* Liberibacter europaeus' sp. nov. that is associated with and transmitted by the psyllid *Cacopsylla pyri* apparently behaves as an endophyte rather than a pathogen. Environ. Microbiol. 13: 414–426.

Ramsey, J.S., R.S. Johnson, J.S. Hoki, A. Kruse, J. Mahoney, M.E. Hilf, W.B. Hunter, D.G. Hall, F.C. Schroeder, M.J. MacCross and M. Cilia. 2015. Metabolic interplay between the Asian citrus psyllid and its *Profftella* symbiont: An Achilles' heel of the citrus greening insect vector. PLoS One 10: e0140826.

Rid, M., C. Mesca, M. Ayasse and J. Gross. 2016. Apple proliferation phytoplasma influences the pattern and plant volatiles emitted depending on pathogen virulence. Front. Ecol. Evol. 3: 152.

SAS Institute. **2013**. SAS release 9.4 ed. SAS Institute Inc., Cary, NC.

Smart, C.D., B. Schneider, D.I. Blomquist, I.J. Guerra, N.A. Harison, U. Ahrens, K.H. Lorenz, E. Seemuller and B. Kirkpatrick. 1996. Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. Appl. Environ. Microbiol. 62: 2988–2993.

Ullman, D.R. and D.L. McLean. 1988. Feeding behavior of the winter-form pear psylla, *Psylla pyricola* (Homoptera: Psyllidae), on reproductive and transitory host plants. Environ. Entomol. 17: 675–678.

Vyas, M., T.W. Fisher, R. He, W. Nelson, G. Yin, J.M. Cicero, M. Willer, R. Kim, R. Kramer, G.A. May, J.A. Crow, C.A. Soderland, D.R. Gang and J.K. Brown. 2015. Asian citrus psyllid expression profiles suggest *Candidatus* Liberibacter asiaticus-mediated alteration of adult nutrition and metabolism, and of nymphal development and immunity. PLoS One 10: e0130328.

Weintraub, P.G. and L. Beanland. 2006. Insect vectors of Phytoplasma. Annu. Rev. Entomol. 51: 91–111.

Westigard, P.H. and R.W. Zwick. 1972. The pear psylla in Oregon. Agric. Exp. Sta., Oregon State Univ., Tech. Bull. 122.