FINAL PROJECT REPORT

Project Title: Suppression of pear psylla using elicitors of host-defenses

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Total Project Request: **Year 1:** $25,000 **Year 2:** $25,000 **Year 3:** $5,700

Other funding sources: None

Budget 1
Organization Name: USDA-ARS-YARL **Contract Administrator: Chuck Myers**
Telephone: 510/559-5769 **Email address: Chuck.Myers@ars.usda.gov**

Item	**2014**	**2015**	**2016**
Salaries	$16,000	$16,000	$5,000
Benefits	$1000	$1000	$200
Wages			
Benefits			
Equipment			
Supplies	$5000	$5000	
Travel			
Plot Fees	$3000	$3000	$500
Miscellaneous			
Total	$25,000	$25,000	$5,700

Footnotes:
[1] Partial funding for a temporary employee to help with field studies

OBJECTIVES

1) Test the effects of commercially available elicitors of host defenses on pear psylla population growth in pear orchards.
2) Test the effects of defense elicitors on recruitment of natural enemies.
3) Test the combined effects of defense elicitors and potassium or magnesium fertilization on pear psylla numbers.
4) Test the effects of defense elicitors on obligate bacterial symbionts of pear psylla.

SIGNFICANT FINDINGS

1) Both Actigard and ODC reduced pear psylla nymph populations by about 20% during peak populations.
2) Magnesium sulfate treatment reduced pear psylla numbers, but did not enhance Actigard-activated defenses against psylla beyond the effects of Actigard alone.
3) Adults collected from pear trees treated with Actigard had significantly reduced titers of the obligate symbiont, *Carsonella ruddii*, than did adults collected from untreated trees.

RESULTS AND DISCUSSION

Analysis of variance did not reveal significant week by treatment interactions for either nymphs or adults regardless of sampling year (Table 1), but counts of nymphs and adults varied by sampling week within each year (Table 1). Nymphal populations exhibited two generation peaks, which occurred in late April to early May and in June of each year (Figs. 1A-3A). The second generation of nymphs was nearly 3 to 4 times larger than the first generation in all three years (Figs. 1A-3A). Adult populations also exhibited two generation peaks, which occurred about two weeks after observed peaks in nymphal populations (Figs. 1B-3B). The relative size of the two peaks varied among years. In 2014, the second generation of adults was numerically larger than the first generation, but the second generation was small compared to the first generation in both 2015 and 2016 (Figs. 1B-3B).

Table 1. Statistical analyses examining the effects of foliar applications of defense elicitors on pear psylla populations.

Variable	2014	2015	2016
Nymphs			
Week	$F_{18, 72}$=40.6; P<0.001	$F_{16, 64}$=24.9; P<0.001	$F_{18, 72}$=32.6; P<0.001
Treatment	$F_{3, 12}$=5.7; P=0.013	$F_{3, 12}$=1.6; P=0.253	$F_{3, 12}$=4.8; P=0.020
Week × Treatment	$F_{54, 216}$=1.4; P=0.056	$F_{48, 192}$=0.8; P=0.818	$F_{54, 216}$=1.1; P=0.295
Adults			
Week	$F_{18, 72}$=58.1; P<0.001	$F_{16, 64}$=43.8; P<0.001	$F_{18, 72}$=7.3; P<0.001
Treatment	$F_{3, 12}$=6.3; P=0.008	$F_{3, 12}$=0.4; P=0.763	$F_{3, 12}$=2.6; P=0.097
Week × Treatment	$F_{54, 216}$=0.8; P=0.806	$F_{48, 192}$=0.7; P=0.900	$F_{54, 216}$=0.9; P=0.643

Analyses revealed significant differences in numbers of nymphs among foliar treatments in 2014 and 2016, but not in 2015 (Table 1). In 2014, significantly fewer nymphs were observed on trees treated with Actigard, Employ, or ODC than on untreated controls pooled over sampling dates (Fig. 1A: right panel). Although not statistically significant in 2015, nearly 20 to 30% fewer nymphs were observed on trees treated with Actigard, Employ, or ODC than on untreated controls (Fig. 2A: right panel). Paired contrasts suggested marginally significant reductions (α<0.1) of nymphs on trees treated with Actigard in 2015 compared with untreated controls (t=2.1; P=0.058; Fig. 2A: right panel). As observed in 2014, significantly fewer nymphs (Fig. 3A: right panel) were recorded from trees treated with Actigard, Employ, or ODC than on untreated controls in 2016. Overall, results from the three sampling years were consistent and suggested that treating trees with defense elicitors leads to a modest (20-30%) reduction in populations of pear psylla nymphs. These reductions were most obvious during the second generation population peak. Results of these field trials were also consistent with our previous laboratory study, which indicated that treating pear with foliar applications of Actigard, Employ, or ODC induced systemic defenses that increased mortality of psyllid nymphs (Cooper and Horton 2015).

Significantly more adults were collected from untreated trees than from trees treated with Actigard, Employ, or ODC pooled over all sampling weeks in 2014 (Fig. 1B: right panel). As observed for nymphs, the differences in treatments were most obvious during the second generation peak, which occurred in July of 2014 (Fig. 1B). This pattern was not observed in 2015 (Fig. 2B) or 2016 (Fig. 3B), probably because the second generation both years was extremely small in all treatments. Although the overall treatment effect was not significant at the $\alpha=0.05$ confidence interval in 2016 (Table 2; $P=0.097$), paired contrasts indicated that significantly fewer adults were collected from trees treated with Employ than from untreated trees ($t=2.67$; $P=0.020$), and marginally fewer adults were collected from trees treated with Actigard than from untreated trees ($t=1.96$; $P=0.073$) (Fig. 3B). Our previous laboratory study did not indicate that defense elicitors led to decreased adult survival, but adults did tend to settle and oviposit on untreated trees more often than on trees treated with Actigard, Employ, or ODC in choice assays (Cooper and Horton 2015). It is possible that differences among treatments observed in 2014 were due to reduced numbers of nymphs developing to adults on treated trees, and due to movement of adults to adjacent untreated trees. It is unclear whether treatment differences attributed to adult preference would be replicated if an entire orchard were treated with an elicitor product.

Results of our study demonstrate that foliar applications of Actigard, Employ, or ODC reduced densities of pear psylla nymphs under field conditions. These results are consistent with those of our previous laboratory bioassays (Cooper and Horton 2015), and with other reports that elicitors of salicylic acid-dependent defenses reduce performance of other phloem-feeding insects (Dong et al. 2004, Cooper et al. 2004, Cooper and Goggin 2005, Li et al. 2006, Boughton et al. 2006, Gao et al. 2007, Zhang et al. 2012). The modest reduction in pear psylla nymphs observed here does not warrant the use of elicitors alone for the control of pear psylla. However, elicitors are often used in pear orchards to manage fire blight, and knowledge that these products may also partially suppress pear psylla populations could be useful for system-wide integrated pest management approaches. More trials are required to evaluate the efficacy of these products applied to entire orchards and used in a spray schedule typical for fire blight management.

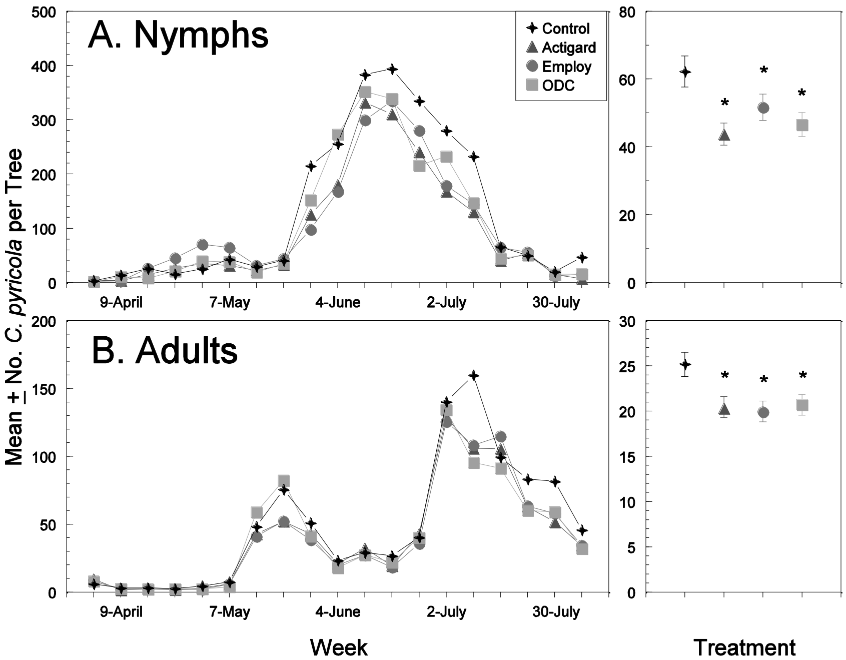

Figure 1. Mean number of pear psylla nymphs per shoot (A) and adults per beat sheet sample (B) in 2014. Dates provided on the x-axis indicate days on which foliar applications were applied. Figures on the right show the overall effects of treatment regardless of sampling week. Error bars denote standard errors and asterisks indicate that values are significantly different from the untreated control treatment.

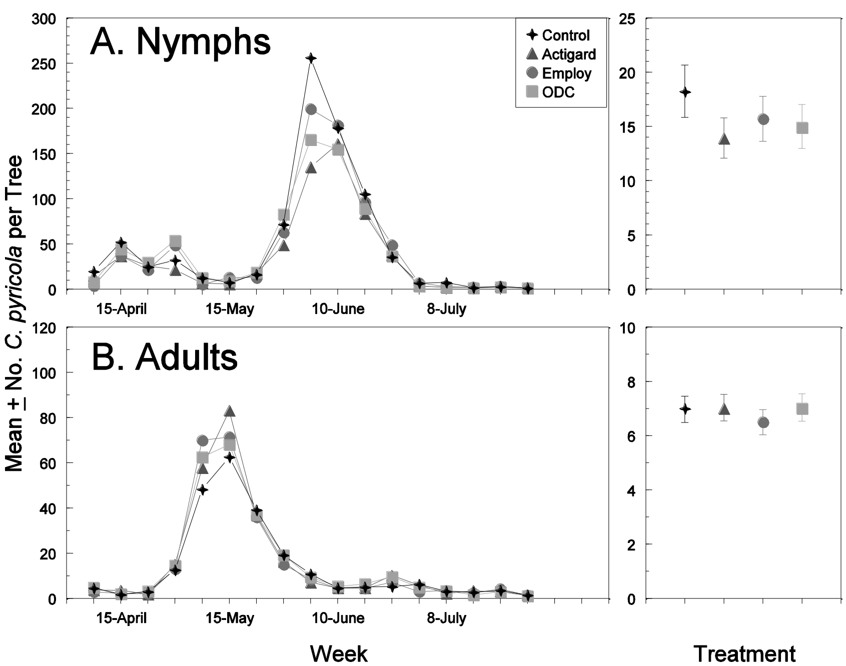

Figure 2. Mean number of pear psylla nymphs per shoot (A) and adults per beat sheet sample (B) in 2015. Dates provided on the x-axis indicate days on which foliar applications were applied. Figures on the right show the overall effects of treatment regardless of sampling week. Error bars denote standard errors.

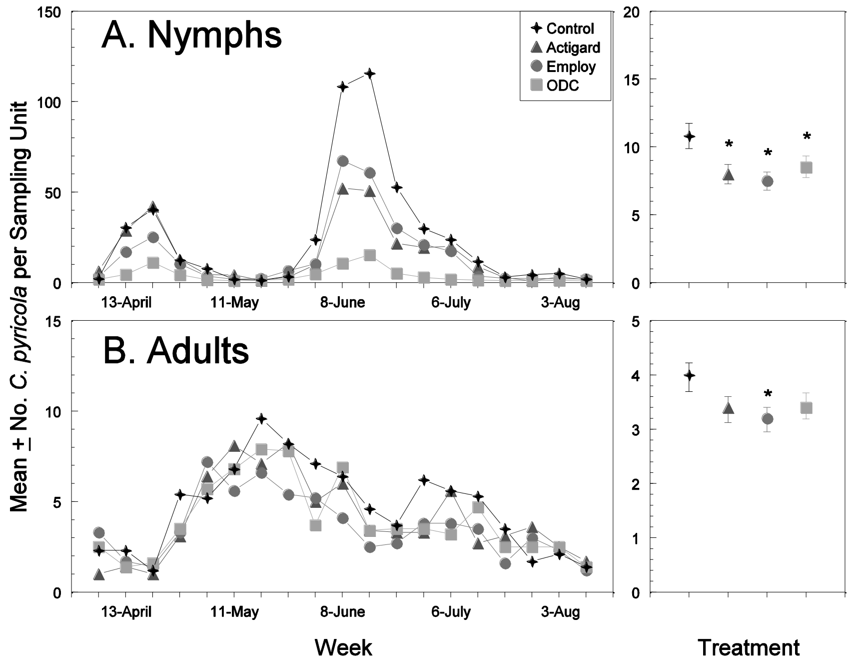

Figure 3. Mean number of pear psylla nymphs per shoot (A) and adults per beat sheet sample (B) in 2016. Dates provided on the x-axis indicate days on which foliar applications were applied. Figures on the right show the overall effects of treatment regardless of sampling week. Error bars denote standard errors and asterisks indicate that values are significantly different from the untreated control treatment.

Objective 2. Test the effects of defense elicitors on recruitment of natural enemies.

We did not observe any consistent effects of defense elicitors on densities of natural enemies.

Objective 3. Test the combined effects of potassium and magnesium fertilization on induced defenses against pear psylla.

Greenhouse assays confirmed our previous results that Actigard treatments reduce pear psylla numbers (Figure 4). Results also revealed that foliar application of magnesium sulfate by itself also reduced pear psylla numbers (Figure 4), which is consistent with anecdotal reports on aphids. Adding magnesium sulfate to the Actigard treatment did not improve plant protection provided by Actigard alone (Figure 4). We found no evidence that potassium fertilization influences pear psylla numbers.

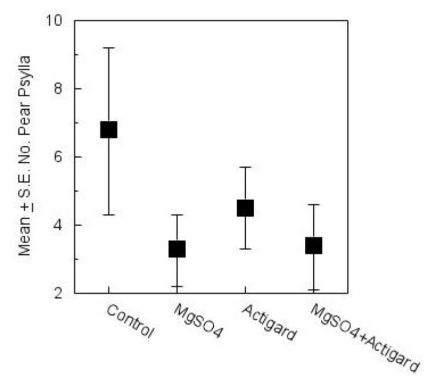

Figure 4. Effects of Magnesium sulfate and Actigard applications on pear psylla performance

Objective 4. Test the effects of defense elicitors on the obligate bacterial symbiont of pear psylla.

We first developed methods to compare populations of the obligate symbiont of pear psylla, *Carsonella*, among different insects. One method uses fluorescence *in situ* hybridization (FISH) to visually detect *Carsonella* in bacteriocytes, specialized insect cells which harbor the bacteria. This method was largely based on our FISH assay to detect *Liberibacter* in specific tissues of potato psyllid (Cooper et al. 2014). Using FISH, we labeled *Carsonella* with a fluorescent probe and measured the intensity of fluorescence to estimate relative bacteria densities in individual bacteriocytes (Figure 5A inset). Our second method relies on quantitative real time PCR (qPCR) to estimate bacteria densities in whole insects. Using these methods, we showed that *Carsonella* was more abundant in females than in males (Figure 5). These results confirmed that our methods are suitable for comparing *Carsonella* among pear psylla, and showed that insect sex should be controlled in our future studies.

Because *Carsonella* varied between sexes, only females were used to examine the effects of defense elicitors on endosymbiont titers. *Carsonella* titers in whole insects were not altered by plant defenses activated by Actigard (Figure 6A). However, *Carsonella* titers in individual bacteriocytes were reduced in psylla exposed to trees treated with Actigard compared with those on control trees (Figure 6B). Results suggest that plant defenses against pathogens may reduce the obligate endosymbiont of psylla, which may explain how psylla are reduced on induced trees. However, it is not possible to discern whether *Carsonella* is directly altered by plant defense compounds, or if declining health of psylla by plant defenses leads to reductions in *Carsonella*.

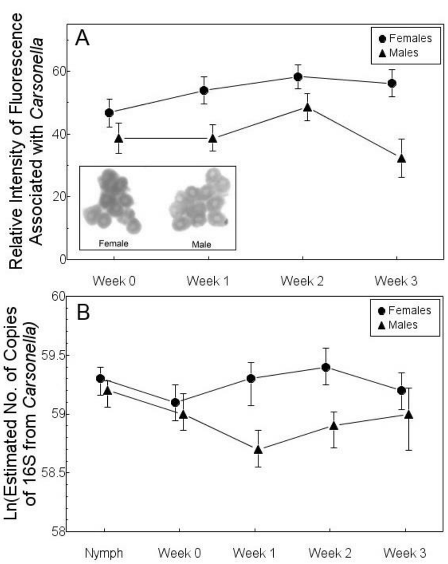

Figure 5. Comparison of *Carsonella* densities among females and males using FISH (A) and qPCR (B). Inset shows samples of bacteriocytes containing *Carsonella* labeled with a fluorescent probe; the darker cells indicate a greater density of *Carsonella*.

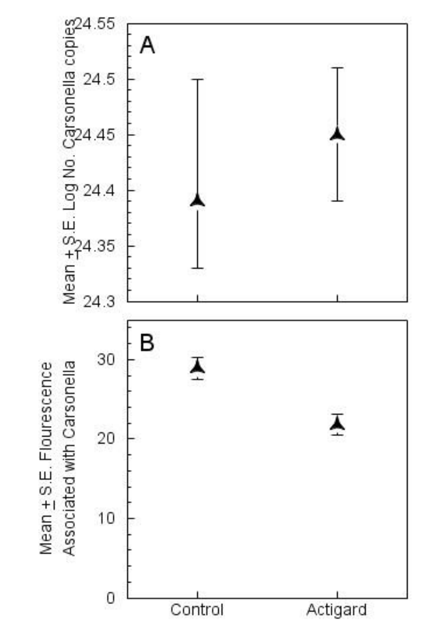

Figure 6. Effects of defense elicitors on *Carsonella* titers.

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Cooper W. R., and D. R. Horton. 2015. Effects of elicitors of host plant defenses on pear psylla, *Cacopsylla pyricola*. Entomol. Exp. Appl. 157: 300-306.

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EXECUTIVE SUMMARY

Defense elicitors are products that activate acquired defense responses in plants, thus making plants less susceptible to attack by a broad range of pests. We previously demonstrated under laboratory conditions that foliar applications of the defense elicitors Actigard (acibenzolar-S-methyl), Employ (harpin protein), or ODC (chitosan) to potted pear trees (*Pyrus communis* L.) each caused an increase in mortality of pear psylla nymphs, and altered the settling and oviposition behavior of the adults. The objective of the current study was to determine whether the use of defense elicitors in pear orchards reduces wild populations of pear psylla, and to determine whether these defense responses, which are primarily associated with defense against plant pathogens, may suppress psylla by reducing titers of the obligate bacterial endosymbiont, *Carsonella*.

Summary of Findings

We monitored psylla populations over a 3-year period on orchard-grown trees treated with water (untreated control), Actigard, Employ, or ODC. Fewer nymphs were observed on trees treated with elicitors compared with untreated trees in both 2014 and 2016. A similar but statistically non-significant pattern was observed in 2015 when nearly 30% fewer nymphs were observed on trees treated with elicitors versus untreated controls. Observed reductions in psyllid numbers by defense elicitors were modest, and do not warrant the use of these products alone for managing pear psylla. However, these products are often used for management of fire blight, and our observations that elicitors also reduce pear psylla populations may be useful for integrated (disease + insect) pest management approaches.

We developed two methods to estimate relative abundance of *Carsonella* in bacteriocytes and whole bodies of psyllids: fluorescence in situ hybridization and qPCR, respectively. We first compared *Carsonella* populations between female and male insects, to determine if our elicitor trials must consider sex of the psyllid specimen in the analysis. Estimations using fluorescence in situ hybridization indicated that *Carsonella* was more abundant in bacteriocytes of female psylla than in those of males. Analyses by qPCR using whole-body specimens indicated *Carsonella* was more abundant in females than in males. Thus, our study indicates that female psyllids harbor greater populations of *Carsonella* than do males, and that sex of specimens should be considered in studies which require estimations of *Carsonella* populations. Psyllid age (0 to 3 weeks after adult eclosion) had no effects on estimates of *Carsonella* numbers. *Carsonella* was observed in ovarioles of newly emerged females, and formed an aggregation in the posterior end of mature oocytes. Based on these results, we controlled for insect sex when evaluating effects of defense elicitors on *Caronella* titers. In the elicitor studies, we observed reductions in *Carsonella* numbers in psylla collected from trees treated with Actigard compared with psyllids from control trees, providing evidence that defense responses may act indirectly on psylla fitness by reducing titers of the obligate endosymbiont.

Peer-Reviewed Publications

Cooper W. R., and D. R. Horton. 2015. Effects of elicitors of host plant defenses on pear psylla, *Cacopsylla pyricola*. Entomol. Exp. Appl. 157: 300-306.

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., and D. R. Horton. In Review. Elicitors of host plant defenses partially suppress pear psylla (*Cacopsylla pyricola*, Hemiptera: Psyllidae) populations under field conditions. Submitted to J. Insect Sci. on 5-December 2016.