

Effect of *Candidatus Liberibacter asiaticus* infection on susceptibility of Asian citrus psyllid, *Diaphorina citri*, to selected insecticides

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Abstract

BACKGROUND: In the present investigation, the effect of *Candidatus Liberibacter asiaticus* (Las), a bacterium considered to be responsible for causing Huanglongbing (HLB) disease in citrus, on the physiology of its vector, the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama, was determined. Specifically, the effects of Las infection on the susceptibility of ACP to selected insecticides were determined. Furthermore, total protein content and general esterase activity were quantified in Las-infected and uninfected ACP to gain insight into the possible mechanism(s) responsible for altered susceptibility to insecticides owing to Las infection.

RESULTS: LC₅₀ values were significantly lower in Las-infected than in uninfected ACP adults for chlorpyrifos and spinetoram. Furthermore, there was a general trend towards lower LC₅₀ values for three other insecticides for Las-infected ACP; however, the differences were not statistically significant. Total protein content ($\mu\text{g mL}^{-1}$) was significantly lower in Las-infected (23.5 ± 1.3 in head + thorax; 27.7 ± 1.9 in abdomen) than in uninfected (29.7 ± 2.1 in head + thorax; 35.0 ± 2.3 in abdomen) ACP. Likewise, general esterase enzyme activity ($\text{nmol min}^{-1} \text{mg}^{-1}$ protein) was significantly lower in Las-infected (111.6 ± 4.5 in head + thorax; 109.5 ± 3.7 in abdomen) than in uninfected (135.9 ± 7.5 in head + thorax; 206.1 ± 23.7 in abdomen) ACP.

CONCLUSION: Susceptibility of ACP to selected insecticides from five major chemistries was greater in Las-infected than in uninfected ACP. The lower total protein content and reduced general esterase activity in Las-infected than in uninfected ACP may partly explain the observed higher insecticide susceptibility of Las-infected ACP.

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Keywords: Asian citrus psyllid; *Diaphorina citri*; *Candidatus Liberibacter asiaticus*; citrus greening; general esterase activity; Huanglongbing; insecticide toxicity; total protein content

1 INTRODUCTION

Huanglongbing (HLB) is one of the most destructive and economically important diseases of citrus throughout the world.^{1,2} HLB is associated with either of three species of a fastidious phloem-inhabiting gram-negative bacterium, *Candidatus Liberibacter asiaticus* (Las), *Ca. L. americanus* (Lam) or *Ca. L. africanus* (Laf).^{3,4} The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), vectors both Las and Lam in Asia and the Americas, and the South African citrus psyllid, *Trioza erytrea* (Del Guercio) (Hemiptera: Psyllidae), vectors Laf in Africa. Although limited success in culturing Las and Lam was recently achieved (four or five single-colony transfers),⁵ techniques for sustained growth of pure bacteria are yet to be developed. HLB reduces yield by causing premature fruit drop and increases fruit bitterness.¹ HLB-infected trees typically decline within 5–8 years of infection, and currently there is no available cure to treat diseased trees.¹ Current practices for HLB management include the use of disease-free planting material, removal of infected trees and suppression of the ACP vector.²

The susceptibility of Las-free (uninfected) ACP to insecticides from several classes has been previously investigated under field and laboratory conditions.^{6–9} However, this has not been investigated for Las-infected ACP. The presence of bacteria and

yeast is known to alter the susceptibility of host insects to toxins.^{10–14} For example, infection of *Culex pipiens* L. (Diptera: Culicidae) by the bacterium *Wolbachia* increases the fitness cost of resistance to insecticides.¹² It is possible that *Wolbachia* infection adversely affects the host, which may increase the cost of resistance, rendering insects more susceptible to insecticides.¹² Furthermore, infection of whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), with *Rickettsia* increases susceptibility to acetamiprid, thiamethoxam, spiromesifen and pyriproxyfen.¹³ It was suggested that increased susceptibility of *B. tabaci* to insecticides is a result of significantly reduced host fitness owing to the presence of *Rickettsia*.¹³

Given the intense use of insecticides for ACP management in areas infected with HLB,⁸ it is important to determine whether Las infection alters the susceptibility of ACP to insecticides. Therefore, the present study was conducted to test the hypothesis that

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Las infection affects the susceptibility of ACP to insecticides of various modes of action. In addition, total protein content and general esterase activity between Las-infected and uninfected controls were compared as an initial measure of the potential mechanism(s) that may alter ACP susceptibility to insecticides owing to Las infection.

2 MATERIALS AND METHODS

2.1 Asian citrus psyllid cultures

Uninfected and Las-infected ACP used in insecticide bioassays and for quantifying total protein content and general esterase activity were drawn from cultures continuously reared at the Citrus Research and Education Center (CREC), University of Florida, Lake Alfred, Florida. The uninfected culture was established in 2000 using field populations collected in Polk Co., Florida (28.0° N, 81.9° W), prior to the discovery of HLB in the state, and was maintained on sour orange (*Citrus aurantium* L.) seedlings without exposure to insecticides in a greenhouse at 27–28 °C, 60–65% RH and 14:10 h light:dark photoperiod. The Las-infected culture was established in 2009 from the uninfected laboratory population by rearing ACP on Las-infected sour orange seedlings in a separate greenhouse approved for rearing Las-infected citrus plants and ACP under the environmental conditions described above.

2.2 Insecticides

Uninfected and Las-infected ACP adults were tested for susceptibility to commercial formulations of five insecticides from different insecticide chemistry classes and modes of action (Table 1). Each insecticide was tested at 5–6 concentrations prepared in distilled water on the day of testing.

2.3 Petri dish bioassay

The susceptibility of uninfected and Las-infected ACP adults was determined using a petri dish bioassay method.^{9,15} Bioassay arenas were prepared by pouring 3–5 mL of a 1.5% agar solution into 60 mm diameter plastic disposable petri dishes (Fisherbrand; Thermo Fisher Scientific, Waltham, MA) to form a solidified bed. Fresh citrus leaves collected from Valencia orange trees maintained in a CREC greenhouse were used in bioassays. Leaf discs (60 mm diameter) were excised, dipped in test aqueous insecticide dilutions for 30 s and allowed to air dry in a fume hood for 1 h prior to bioassays. For the control treatment, leaf discs were dipped in distilled water alone. After 1 h, leaf discs were placed on agar beds, and 10–15 adult ACP were transferred into each dish using a camel hair brush following a brief anesthetization with

CO₂ to facilitate handling and transfer.⁹ Petri dishes were wrapped with parafilm (Pechiney Plastic Packaging, Chicago, IL) to prevent escape of adults. Sealed petri dishes with ACP were transferred into a growth chamber (Percival Scientific, Inc., Perry, IA) set at 25 ± 1 °C, 50 ± 5% RH and 14:10 h light:dark photoperiod. Each concentration of an insecticide was replicated 3 times ($n = 30–45$ ACP per concentration). Two sets of bioassays were conducted simultaneously, one using uninfected and the other using Las-infected ACP adults. Bioassays with uninfected ACP were repeated twice on different days, while bioassays with Las-infected ACP were repeated several (5–6) times. This was because not all ACP adults collected from the infected ACP culture tested positive for the presence of Las. The percentage of Las-infected ACP adults ranged from 30 to 80%; therefore, experiments were repeated until an n of 30–45 Las-infected ACP was obtained per insecticide concentration. All ACP found negative for Las from the infected culture were excluded from the analysis.

Mortality of ACP was assessed 48 h after transfer into the growth chamber. ACP found on their side or back that were unable to move when probed with a camel hair brush were considered dead. Mortality data were corrected for control mortality (<5%) using Abbott's formula.¹⁶ Mortality data were analyzed separately for uninfected and Las-infected ACP. Mortality data for uninfected and Las-infected ACP were pooled for each concentration and subjected to probit regression analysis to calculate the LC₅₀ for each insecticide with corresponding 95% confidence intervals and slopes of regression lines.¹⁷ The LC₅₀ values between Las-infected and uninfected ACP were considered significantly different ($P < 0.05$) if their 95% confidence intervals did not overlap. After the mortality data were recorded for bioassays using Las-infected ACP, each dead ACP was transferred and stored individually in a sterile 1.5 mL microcentrifuge tube (Fisher Scientific Co., Pittsburg, PA) containing 80% ethanol at –20 °C until DNA was extracted to confirm the presence of Las by quantitative real-time polymerase chain reaction (qPCR). However, in bioassays using uninfected ACP, ten dead adults were randomly chosen for each insecticide concentration and stored as described above for use in qPCR assays to confirm the absence of Las (method described below).

2.4 Sample preparation and enzyme extraction for general esterase activity

Only freshly emerged ACP adults from both Las-infected and uninfected ACP cultures, as described earlier, were used for enzyme extraction and total protein content quantification. Adults were not separated on the basis of sex because there was no difference

Table 1. Insecticides tested against *Diaphorina citri*

Common name/formulation	Trade name	Class	Mode of action	Manufacturer/supplier
Carbaryl 480 g L ⁻¹ SC	Sevin XLR Plus	Carbamate	Acetylcholinesterase inhibitor	Bayer CropScience LP, Research Triangle Park, NC
Chlorpyrifos 480 g L ⁻¹ EC	Lorsban 4E	Organophosphate	Acetylcholinesterase inhibitor	Dow AgroSciences, LLC, Indianapolis, IN
Fenpropathrin 288 g L ⁻¹ EC	Danitol 2.4EC	Synthetic pyrethroid	Sodium channel modulator	Valent USA Corp., Walnut Creek, CA
Imidacloprid 192 g L ⁻¹ SC	Provado 1.6F	Neonicotinoid	Nicotinic acetylcholine receptor agonist/antagonist	Bayer CropScience LP, Research Triangle Park, NC
Spinetoram 250 g kg ⁻¹ WG	Delegate WG	Microbial	Nicotinic acetylcholine receptor and GABA agonist/antagonist	Dow AgroSciences, LLC, Indianapolis, IN

in the total protein content between male and female ACP adults (data not shown). General esterase activity was measured separately for uninfected and Las-infected ACP adults. ACP adults reared on Las-infected host plants and presumed to be infected with Las were cut into two halves with a sterile blade. One half comprised the head + thorax, and the other half comprised the abdomen of an adult ACP. Initially, the head + thorax section was used to quantify general esterase activity and total protein content levels, and the abdomen was used in qPCR assays to determine Las infection. This was repeated a second time, where the abdomen section was used for determining general esterase activity and total protein content levels and the head + thorax section was used in qPCR assays. The same number of samples for each section of the ACP was used in qPCR assays as well as general esterase activity and total protein content assays.

Each half-section of an ACP adult used in general esterase activity and total protein content assays was homogenized using a handheld homogenizer with a plastic pestle (Fisher Scientific Co., Pittsburg, PA) in ice-cold phosphate buffer (0.1 M; pH 7.5; 180 μ L) containing 3 mL L⁻¹ Triton X-100 (Sigma Aldrich, St Louis, MO) in a 1.5 mL microcentrifuge tube. Microcentrifuge tubes were centrifuged at 12 600 rpm (Eppendorf Centrifuge 5415R; Fisher Scientific Co., Pittsburg, PA) for 15 min at 4 °C. Following centrifugation, 80 μ L of the supernatant was transferred into a clean microcentrifuge tube and mixed with phosphate buffer (0.1 M; pH 7.5; 80 μ L) and placed on ice until use in assays.

2.5 General esterase enzyme activity

General esterase activity was measured following a protocol^{18–20} based on the amount of naphthol produced from the hydrolysis of naphtholic ester. Four aliquots of 15 μ L of the enzyme solution were pipetted into separate wells of a 96-well microplate (NUNC PolySorp; Fisher Scientific Co., Pittsburg, PA). In addition, 135 μ L of 0.3 mM α -naphthyl acetate (Sigma Aldrich, St Louis, MO) with a final concentration of 0.27 mM was added to each well. The plate was covered with aluminum foil and incubated for 30 min at 37 °C. Following incubation, 50 μ L of Fast Blue B Salt in 5% SDS solution was added to each well to stop the reaction. The mixture was set aside at room temperature for 15 min to develop color. General esterase activity was determined by measuring the amount of α -naphthol as a final product at 595 nm using a microplate reader (Spectramax 250; Sunnyvale, CA) at 25 °C. The amount of α -naphthol produced was calculated on the basis of the optical density value obtained from the α -naphthol standard curve. Mean general esterase activity was calculated and standardized per mg of protein measured for each ACP (see below). General esterase activity was estimated on samples obtained from 50 confirmed Las-infected and uninfected ACP adults. In each case, 50 total samples consisted of 25 head + thorax sections and the same number of abdomens. Two-way analysis of variance (ANOVA) followed by Fisher's protected LSD mean separation tests was performed to determine differences in general esterase activity between uninfected and Las-infected ACP, using ACP body section (head + thorax or abdomen) and infection type (uninfected or Las-infected) as main effects (SAS, PROC GLM).¹⁷

2.6 Total protein content estimation

The total protein content in the enzyme preparations was estimated using bovine serum albumin (BSA) (Sigma Aldrich, St Louis, MO).²¹ Four 20 μ L aliquots of enzyme preparation were pipetted into separate wells of the 96-well microplate.

Bicinchoninic acid (180 μ L) in 4% cupric sulfate solution (Sigma Aldrich, St Louis, MO) was added to each well. The plate was covered with aluminum foil and incubated for 30 min at 37 °C. Following incubation, the plate was set aside at room temperature for 5 min to develop color and read at 562 nm using a microplate reader (Spectramax 250; Sunnyvale, CA) at 25 °C. Total protein content in the enzyme extraction was estimated on the basis of the standard curve generated from serial dilutions of BSA. Total protein content was estimated separately for 50 confirmed Las-infected and uninfected ACP adults. Two-way ANOVA followed by Fisher's protected LSD mean separation tests was used to determine differences in total protein content between uninfected and Las-infected ACP, using ACP body section (head + thorax or abdomen) and infection type (uninfected or Las-infected) as main effects (PROC GLM).¹⁷

2.7 DNA extraction

Individual adult ACP from insecticide and biochemical assays were homogenized in a buffer solution (Qiagen, Valencia, CA) using a sterile mortar and lysed overnight at 56 °C in a hybridization oven (Model 136 400; Boekel Scientific, Feasterville, PA) prior to extraction of total DNA. Samples used for DNA extractions from insecticide and biochemical assays consisted of whole (intact) ACP and half-sections (head + thorax or abdomen) respectively. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol, with modifications for extraction of bacterial DNA from arthropods. Samples were eluted in 35 μ L buffer AE and stored in sterile 1.5 mL microcentrifuge tubes at -20 °C for use in qPCR assays.

2.8 Quantitative real-time PCR

All qPCR assays were performed in an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) using a multiplex TaqMan qPCR assay developed for detection of *Ca. Liberibacter asiaticus*.²² qPCR was performed in 25.5 μ L reaction volumes using 96-well MicroAmp[®] reaction plates (Applied Biosystems, Foster City, CA). Reactions, conducted in duplicate, contained the following: 1 μ L template DNA, 12.5 μ L TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 235 nM each of target (LasF, 5'-TCGAGCGCGTATGCAATACG-3'; LasR, 5'-GCGTTATCCCGTAGAAAAAGGTAG-3') (GenBank accession number L22532)²² and internal control primers specific to the *wingless* (*wg*) gene (GenBank accession number AF231365) (WgF, 5'-GCTCTCAAAGATCGGTTTGACGG-3'; WgR, 5'-GCTGCCACGAACGTTACCTTC-3')²³ and 118 nM of each probe (WGp, JOE-5'TTACTGACCATCACTCTGGACGC3'-BHQ2);²⁴ HLBp, FAM-5'AGACGGGTGAGTAACGCG-BHQ1)²² (Integrated DNA Technologies, Inc., Coralville, IA). qPCR reactions consisted of 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Each 96-well plate contained a no DNA template control (NTC), a positive control (containing Las DNA in DNA extractions from ACP) and a negative control (no Las DNA in DNA extractions from ACP). Samples were considered positive for *wg* gene or Las if the cycle quantification (C_q) value determined by the ABI 7500 Real-Time software (v.1.4; Applied Biosystems) was 35 or less.

3 RESULTS

3.1 Insecticide bioassay

Las qPCR assays of Las-positive ACP extracts and positive control reactions resulted in mean (\pm SEM) C_q values of 28.26 ± 0.21

and 28.52 ± 1.38 respectively. Similarly, the mean C_q values of the *wg* assay in positive controls and test samples were 25.66 ± 0.04 and 24.47 ± 0.24 respectively. The LC_{50} values obtained with Las-infected ACP were numerically lower than with uninfected counterparts for all five insecticides tested (Table 2). Based on the non-overlapping confidence intervals at 95%, significant differences between the LC_{50} values of Las-infected and uninfected ACP were observed for chlorpyrifos and spinetoram. Overall, the greatest difference in susceptibility (3.1-fold) between Las-infected and uninfected ACP adults was to the neonicotinoid imidacloprid. Las-infected ACP were 2.8-fold more susceptible to the organophosphate chlorpyrifos, than uninfected counterparts. The differences in susceptibility between Las-infected and uninfected ACP for the microbial insecticide spinetoram, the carbamate carbaryl and the synthetic pyrethroid fenpropathrin ranged between 1.2- and 2.4-fold (Table 2). In general, insecticides acting on acetylcholine/nicotinic acetylcholine receptors were more toxic to Las-infected ACP adults than the synthetic pyrethroid targeting sodium channels.

3.2 General esterase enzyme activity and total protein content

Las qPCR assays of Las-positive ACP extract and positive control reactions resulted in mean (\pm SEM) C_q values of 28.81 ± 0.63 and 32.87 ± 4.08 respectively. Similarly, the mean C_q values of the *wg* assay in positive controls and test samples were 26.51 ± 0.08 and 24.48 ± 0.17 respectively. For total protein content, the infection type (uninfected or Las-infected) ($F = 22.4$; $df = 1, 96$; $P < 0.0001$), ACP body section (head + thorax or abdomen) ($F = 7.1$; $df = 1, 96$; $P = 0.009$) and the interaction between the main effects ($F = 8.0$; $df = 1, 96$; $P = 0.006$) were significant. For both head + thorax and abdomen sections, total protein content was significantly higher in Las-infected than in uninfected ACP (Fig. 1A). For general esterase activity, the infection type ($F = 12.7$; $df = 1, 96$; $P = 0.0006$) and ACP body section ($F = 6.3$; $df = 1, 96$; $P = 0.01$) were significant effects, whereas the interaction between the main effects was not significant ($F = 0.08$; $df = 1, 96$; $P = 0.8$). For both head + thorax and abdomen sections, general esterase activity was significantly higher in Las-infected than in uninfected ACP (Fig. 1B). The total protein content and general esterase activity was significantly

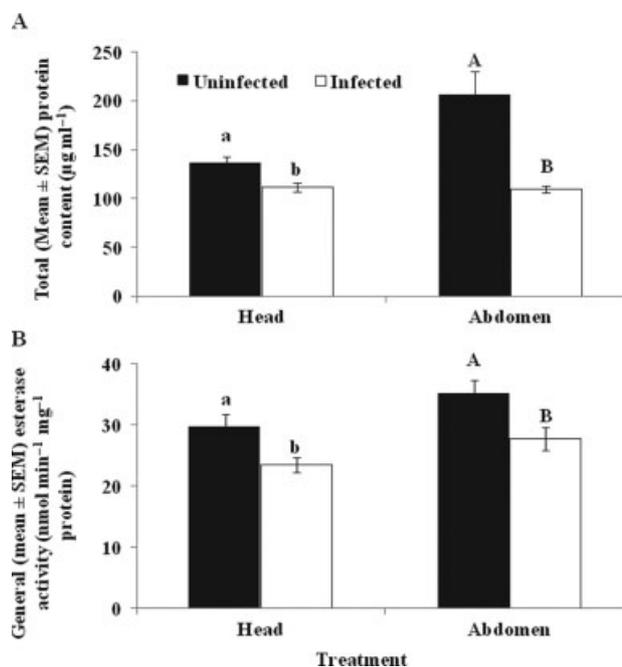


Figure 1. Comparison of total protein content (A) and general esterase activity (B) between head + thorax and abdomen sections of *Candidatus Liberibacter asiaticus*-infected ($n = 50$) versus uninfected ($n = 50$) *Diaphorina citri* adults.

higher in the abdominal section when compared with the head + thorax section in both Las-infected and uninfected ACP.

4 DISCUSSION

Asian citrus psyllid adults infected with *Candidatus Liberibacter asiaticus* were significantly more susceptible to two insecticides (chlorpyrifos and spinetoram) and exhibited a general trend of greater susceptibility to three others from various classes than that of uninfected counterparts. Greater susceptibility of Las-infected ACP owing to infection with this bacterial pathogen may be associated with a physiological cost to the host,

Table 2. Toxicity of various insecticides against *Candidatus Liberibacter asiaticus*-infected and uninfected *Diaphorina citri* adults

Insecticide	<i>D. citri</i>	N^a	LC_{50} (mg AI L ⁻¹)	95% CL	Slope (\pm SE)	χ^2 (df)
Carbaryl	Infected	164	10.72	2.67–26.74	5.38 (\pm 1.57)	14.85 ^c (3)
	Uninfected	180	13.55	12.65–14.40	10.46 (\pm 1.29)	1.90 (3)
Chlorpyrifos ^b	Infected	153	0.28	0.17–0.40	1.73 (\pm 0.35)	4.99 (3)
	Uninfected	180	0.78	0.53–1.09	1.70 (\pm 0.19)	4.74 (3)
Fenpropathrin	Infected	180	0.05	0.001–0.16	1.26 (\pm 0.31)	8.34 ^c (3)
	Uninfected	180	0.06	0.01–0.14	1.48 (\pm 0.27)	7.57 (3)
Imidacloprid	Infected	140	0.15	0.06–0.29	1.44 (\pm 0.30)	1.37 (3)
	Uninfected	180	0.47	0.27–0.72	1.07 (\pm 0.24)	8.96 ^c (3)
Spinetoram ^b	Infected	125	0.20	0.07–0.36	1.32 (\pm 0.31)	2.21 (3)
	Uninfected	180	0.48	0.37–0.60	2.30 (\pm 0.30)	0.92 (3)

^a Number of *Candidatus Liberibacter asiaticus*-infected or uninfected ACP adults used for calculating LC_{50} for each insecticide.

^b LC_{50} values significantly different between *Candidatus Liberibacter asiaticus*-infected or uninfected ACP adults based on non-overlap of 95% confidence intervals.

^c Significant χ^2 values ($P < 0.05$).

resulting in reduced fitness. Fitness may be affected by reduced production or inhibition of degrading or detoxifying enzymes. The presence of *Wolbachia* and *Rickettsia* imposes physiological costs on their respective hosts, *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae) and *B. tabaci*.^{13,25} Other microorganisms are also known to alter the susceptibility of insects to various toxic compounds, including both plant-derived and synthetic insecticides, by various mechanisms.^{10–14} The presence of a symbiotic yeast, *Symbiotaphrina kochii* Jurzitza ex Gams & v. Arx, reduces the susceptibility of cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), to several insecticides.¹⁰ This is due to the symbiont's broad-spectrum ability to detoxify insecticides. In another example, a bacterium, *Enterobacter agglomerans* (Beijerinck), which inhabits the apple maggot fly, *Rhagoletis pomonella* Walsh (Diptera; Tephritidae), is known to detoxify phloridzin.¹¹ Furthermore, another bacterium, *Klebsiella oxytoca* (Flügge) Lautrop, commonly found in the alimentary tract of *R. pomonella*, degrades purine and purine derivatives.²⁶ In addition, toxicities of insecticides with different modes of action, such as acetamiprid, thiamethoxam, spiromesifen and pyriproxyfen, are also altered in *B. tabaci* by the presence of *Rickettsia*.¹³ A strain of *B. tabaci* infected with two types of secondary symbiotic bacteria, *Wolbachia-Arsenophonus* and *Rickettsia-Arsenophonus*, is significantly more susceptible to thiamethoxam, imidacloprid, pyriproxyfen and spiromesifen than a strain infected with only one secondary symbiotic bacterium, *Arsenophonus*.¹⁴ Broadly, the above studies suggest that the presence of a bacterium or yeast alters the degrading and/or detoxifying mechanisms for toxic compounds in host insects; however, such alterations could be an advantage or disadvantage to the host. In the present study, increased susceptibility of Las-infected ACP adults to tested insecticides suggests that the altered mechanism for detoxification of insecticides is disadvantageous to the host insect.

Esterases are a fairly large group of enzymes responsible for degrading various exogenous and endogenous ester-linked compounds and have been directly linked with insecticide resistance in several insects.²⁷ Results from the present study indicated that greater susceptibility of Las-infected ACP adults to insecticides corresponded to lower general esterase activity, and vice versa for uninfected ACP. A similar relationship between insecticide susceptibility and general esterase activity was found in green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae).²⁰ The orange morphs of *M. persicae* exhibited higher esterase levels which correlated with lower insecticide susceptibility.²⁰ Lower levels of general esterase activity in Las-infected ACP than in uninfected counterparts may be associated with a lower capacity for degrading organophosphates. This might lead to accumulation of such compounds, resulting in higher mortality. Alternatively, lower general esterase activity in Las-infected ACP adults may be a result of reduced esterase production owing to bacterial infection, thus benefitting the infecting pathogen. Pathogen infection is known to alter host insects' esterase activity, which improves their growth and development. Higher RNA levels of a parasitic worm, *Wuchereria bancrofti* Cobbold, were found in *Culex quinquefasciatus* Say (Diptera: Culicidae) with lower esterase levels (insecticide-susceptible), whereas fewer *W. bancrofti* were found in mosquitoes with high levels of esterase (insecticide-resistant).²⁸ It was reported that high esterase levels are detrimental to the development of *W. bancrofti*, and insecticide-susceptible mosquitoes with low levels of esterase are therefore a better host for the development of *W. bancrofti*. Thus, reduced general esterase activity in Las-infected ACP adults

may be caused by Las to its own advantage, suggesting that the development of insecticide resistance and the increased ability of ACP to vector Las may not be mutually selected for. However, determining the effect of Las on other major enzymes involved in degrading or detoxifying other classes of insecticides is needed for further support of the above hypothesis.

Lower levels of total protein content in Las-infected ACP adults could be a result of protein uptake by the bacterium or reduced dietary intake of protein by infected ACP adults. Under both scenarios, lower levels of protein in Las-infected ACP could be considered as another physiological cost associated with bacterial infection. Similar results were found in other studies, where parasitism of insects affected the uptake of carbohydrate and protein.^{29,30} *Manduca sexta* L. (Lepidoptera, Sphingidae) larvae parasitized by *Cotesia congregata* (Say) (Hymenoptera: Braconidae) exhibited reduced utilization efficiency (the efficiency of conversion of ingested food to body mass) when compared with unparasitized counterparts.^{29,30} In addition, parasitized larvae had lower total protein and total free amino acid concentrations than unparasitized larvae.³⁰

Increased mortality, low protein content and reduced general esterase activity indicate a host fitness disadvantage for ACP infected by Las. Therefore, the results of the present study indicate that Las infection may be detrimental to ACP, suggesting a non-symbiotic relationship. A similar non-symbiotic relationship between *Rickettsia* and *B. tabaci* has been described, where the presence of *Rickettsia* imposed a fitness cost by lowering the insect's resistance to insecticides.¹³ Higher mortality of Las-infected than uninfected ACP suggests that Las-infected psyllids may be selected against under commercial ACP management practices relying on insecticides. Selection against Las-infected ACP may limit the spread of HLB. This hypothesis is consistent with the notion that insecticide resistance contributes to the spread of vector-borne disease.²⁸ However, further investigations are needed to examine the effects of Las infection of ACP on other groups of enzymes, which may explain the greater mortality of infected adults exposed to the other classes of insecticides evaluated here. Such investigations will also help elucidate the mechanisms of altered host physiology with respect to insecticide resistance management programs for ACP.

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