Chemical Control of the Asian Citrus Psyllid, *Diaphorina citri* Kuwayama

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The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is an important pest of citrus crops worldwide. It is a vector of bacteria in the genus *Candidatus* Liberibacter, the presumable causative agents of huanglongbing (greening) disease in citrus. A series of laboratory investigations was conducted to determine the effects of spray droplet size and temperature on the toxicity of insecticides against ACP as well as effects of a sub-lethal concentration of imidacloprid. In general, ACP mortality increased as spray droplet size decreased regardless of ACP life stage and insecticide (fenpropathrin) discharge rate. Toxicity of the two organophosphate insecticides, chlorpyrifos and dimethoate, increased with increasing temperature from 17 to 37 °C, while the trend was reversed for two synthetic pyrethroids, fenpropathrin and lambda-cyhalothrin. Feeding by ACP adults and nymphs on plants with a sub-lethal concentration of imidacloprid adversely affected adult longevity, fecundity, and fertility as well as nymph survival and development time. Possible improvements to current ACP management are discussed based on the above results.

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is a cosmopolitan pest of citrus crops (Anonymous, 2000). Mature female adults lay eggs on immature vegetative flush, and after hatching, nymphs feed on new flush and pass through five instars (Tsai and Liu, 2000). Both adults and nymphs are prolific feeders on plant phloem sap. As a result they excrete large amounts of honeydew on which sooty mold develops. The most important economic loss associated with this pest results from its capacity as a vector of bacteria in the genus, *Candidatus* Liberibacter (Bové, 2006; Catling, 1970; Halbert and Manjunath, 2004). These bacteria are presumably responsible for huanglongbing (HLB) disease in citrus (Bové, 2006). HLB is one of the most destructive diseases of citrus because diseased trees become unproductive and eventually die (Bové, 2006). Due to the strong dispersal capabilities of ACP (Boina et al., 2009a), it has become established throughout the citrus growing regions of Florida. Currently, HLB infected citrus trees have been detected in 33 citrus growing counties of Florida, threatening the existence of the US$1.4 billion Florida citrus industry (Anonymous, 2008, 2009)

Suppression of ACP populations using multiple sprays of foliar-applied insecticides coupled with one or two soil-applied systemic insecticides is a widely followed practice in Florida (Rogers et al., 2008; Srinivasan et al., 2008) for minimizing the spread of HLB. However, toxicity of insecticides against the target pest is influenced by environmental conditions such as temperature (Scott, 1995; Boina et al., 2009b). Therefore, determining temperature-toxicity relationships for different insecticides would facilitate the optimum use of insecticides. In addition, insecticides need to be applied at optimum droplet size to maximize pest control (Omar and Mathews, 1991; Womac et al., 1994) resulting in increased efficiency of spray applications. Furthermore, when soil applications of systemic insecticides such as imidacloprid are made, ACP populations can be exposed to sub-lethal concentrations due to variation in temporal/spatial uptake and distribution within a citrus tree (Castle et al., 2005). Determining the effect of these sub-lethal concentrations of imidacloprid on biology and reproduction of ACP is important to understand the full impact of this widely used insecticide. Herein, we review the results of investigations on the effects of 1) post-treatment temperature on the toxicity of selected insecticides against ACP; 2) spray droplet size and discharge rate on mortality of ACP life stages; and 3) a sub-lethal concentration of imidacloprid on ACP adults and nymphs.

Materials and Methods

Temperature–toxicity relationships of insecticides against ACP adults. Effect of temperature on the toxicity of selected organophosphate (OP) (chlorpyrifos and dimethoate) and synthetic pyrethroid (SP) (fenpropathrin and lambda-cyhalothrin) insecticides against ACP was tested using a petri dish bioassay (Prabhaker et al., 2006) at 17, 27, and 37 °C. At each temperature, five to six concentrations of each insecticide were tested. Excised citrus (‘Valencia’ orange) leaf discs 60 mm in diameter were dipped in test solutions prepared in tap water for 30 s and were allowed to dry in a fume hood for 1 h. Leaf discs dipped in tap water alone served as controls. Treated leaf discs were placed on agar beds in 60-mm diam. Petri dishes and 10 adult ACP were

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transferred to each dish. Each concentration was replicated three times and the whole study was repeated twice. Petri dishes with insects were transferred to a growth chamber (Percival Scientific, Inc., Perry, IA) set at 17 ± 1, 27 ± 1, or 37 ± 1 °C and 80 ± 5% RH with a 14 : 10 (L : D) h photoperiod. Mortality counts of insects were taken 48 h after transfer into the growth chambers. Insects that remained on their lateral side or could not right themselves when a petri dish was tapped gently were considered dead and included in mortality counts. Mortality data were corrected for control mortality, which was always <5% using Abbott's formula (Abbott, 1925). Mortality data for each concentration were pooled from both studies and subjected to probit regression analysis to calculate the LC_{50} values (the lethal concentration required to cause mortality of 50% of the population) at each exposure temperature with corresponding 95% confidence intervals (CIs) (PROC PROBIT program, SAS Institute, 2005).

Effect of spray droplet size and discharge rate on mortality of ACP. In this investigation, fenpropathrin was tested at one-half (169 g AI/ha) and three-quarter (253 g AI/ha) field recommended rate (338 g AI/ha), which resulted in 0.38 and 0.58 mg AI/min spray discharge rates, respectively. Using a vibrating orifice droplet generator (Model 3050, Thermo-Systems Inc., St. Paul, MN) equipped with piezo-electric nozzles, droplets of 40-, 52-, 101-, 148-, 174-, and 265-μm length mean diameters were generated as described by Salyani et al. (1987). The output from the generator was applied as a swath onto the target plant moving at 7.2 $cm \cdot s^{-1}$ on a conveyer belt. Spray targets consisted of 'Swingle citrumelo'seedlings that were 15 to 20 cm high and infested with ACP eggs, nymphs, and adults. Water-sensitive papers were used to assess the droplet density and spray distribution uniformity during the experiment. Each treatment was replicated with four plants and four unsprayed plants served as controls. Pre- and posttreatment counts of eggs and nymphs were taken 1 d before and 7 d after spray, respectively, on three randomly chosen new flushes per plant and over the entire plant for adults. The data were arcsine transformed before analysis. The effect of droplet size on ACP mortality at a given spray discharge rate was determined by an analysis of variance (ANOVA) at $\alpha = 0.05$ (PROC GLM program, SAS Institute, 2005). Also, the slopes of linear regression lines of percent mortality versus droplet size were compared by *t*-tests at $\alpha = 0.05$ for significant differences between spray discharge rates (PROC TTEST Program, SAS Institute, 2005).

Effects of a sub-lethal concentration of imidacloprid on ACP adults and nymphs. For observations on adults, 2- to 3-month-old 'Swingle' seedlings were systemically treated by soaking bare-rooted root systems in 0.1 ppm (0.1 $mg \cdot L^{-1}$) of imidacloprid (sub-lethal concentration based on preliminary study results) or tap water for 48 h. This concentration is approximately 1800-fold lower than the recommended label rate for soil drench (179.25 mg/ft^3 container when mixed in 1 L of water and applied). Seedlings were repotted individually in 1-L plastic pots of peat-based soilless media. Following this procedure, five pairs of newly emerged male and female adults (10 total ACP) were confined on each plant within 1-L transparent plastic cups on a bench top in a walk-in growth room at 25 ± 1 °C, 50 ± 5% RH and 14 : 10 (L : D) photoperiod (Boina et al., 2009c). Control plants were watered daily with 30 mL tap water, whereas treatment plants were watered daily with 30 mL 0.1 ppm imidacloprid solution. After each 5-d interval, adults were transferred onto a new set of similarly treated plants throughout the experiment. The number of eggs laid by females on each plant per 5-d interval was counted on the 10th, 15th, 20th, and 25th days after first confinement of the adults on plants using a stereomicroscope. Excised new growth flush samples with eggs were transferred into 90-mm plastic petri dishes with moistened filter paper over an agar bed (1.5%) and maintained at the same environmental conditions as described above to monitor nymph hatching. The total number of nymphs that hatched from eggs was recorded 7 d after transfer to petri dishes for each batch of eggs collected. Adults were monitored at 3-d intervals for mortality until all of the test insects were dead.

For observations on nymphs, the experimental procedures were similar to that described above for adults with slight modifications. Systemically bare-root treated and repotted 'Swingle' seedlings were placed within a plexiglass sleeve cage (40 × 40 × 40 cm). Approximately 200 adult ACP of mixed ages were released into each cage for 24 h to allow for oviposition. Thereafter, adults were removed by aspirating and plants with zero- to 24-h-old eggs were transferred into a growth chamber set at 27 ± 1 ºC and 80 ± 5% RH and on a 14 : 10 (L : D) photoperiod. Control plants were watered daily with tap water and treatment plants were watered daily with 0.1 ppm imidacloprid solution. Hatched nymph development was monitored until the adult stage to determine survival and developmental time. All the treatments were replicated three times and the whole experiment was repeated three times. Significant differences between treatments were determined by paired *t*-tests at $\alpha = 0.05$ (PROC TTEST program, SAS Institute, 2005) after arcsine transformation of data.

Results and Discussion

Temperature-toxicity relationships of insecticides against ACP adults. In general, toxicity of the two OP insecticides increased, as shown by the decrease in the LC_{50}, as the temperature

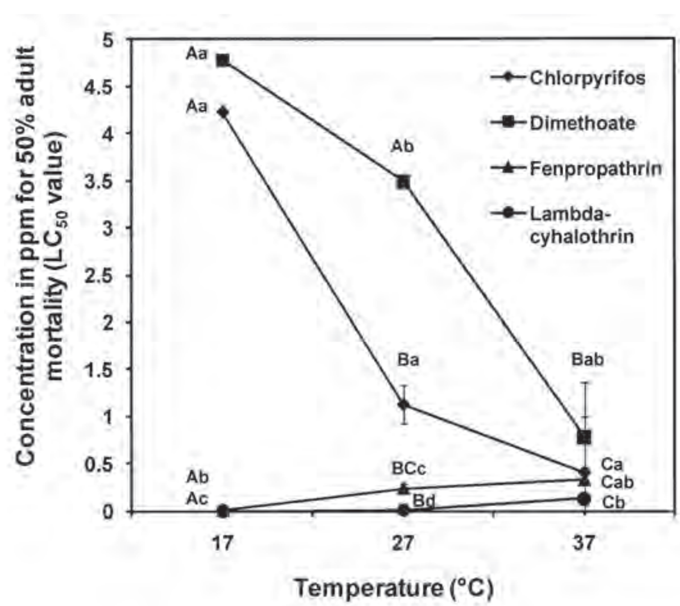

Fig. 1. Temperature–toxicity relationships for selected organophosphate and synthetic pyrethroid insecticides against the Asian citrus psyllid adults. Error bars represent 95% confidence intervals (CIs). Large 95% CIs are not displayed for clarity of the graph. Symbols with different lower case letter at a given temperature are significantly different based on non-overlap of 95% CIs. Symbols with different upper case letter for a given insecticide are significantly different based on non-overlap of 95% CIs.

increased from 17 to 37 °C (Fig. 1). There was an overall 10-fold increase in toxicity of chlorpyrifos when temperature was increased from 17 to 37 °C, while the increase was 6-fold for dimethoate for the same increase in temperature (Fig. 1). The opposite trend (though not significant at 37 °C) was observed for the two SPs (Fig. 1). The toxicity of fenpropathrin and lambda-cyhalothrin decreased significantly as the temperature increased from 17 to 37 °C. The overall decrease in toxicity of fenpropathrin was 39-fold, while decrease in toxicity was 65-fold for lambda-cyhalothrin at the tested temperature range (17 to 37 °C) (Fig. 1). The possible reasons for the observed temperature-dependent toxicity of OP and SP insecticides against ACP adults have been outlined by Boina et al. (2009b). These results indicate that when cooler temperatures (~17 °C) prevail in the field, application of the tested SPs may provide greater control of ACP populations than at higher temperatures. On the other hand, when warmer ambient temperatures (~37 °C) prevail, application of tested OPs may result in greater control of ACP populations than at lower temperatures. Similarly, aerial application of some insecticides in cooler months of the year resulted in greater control of the European corn borer in sweet corn than during summer months (Musser and Shelton, 2005). Also, efficacy of lambda-cyhalothrin against soybean aphid is greater during cooler temperatures than warmer temperatures, whereas performance of chlorpyrifos is greater during higher than lower temperatures (Rice et al., 2005).

Effect of spray droplet size and discharge rate on mortality of ACP. In general, the density of spray droplets per area on targets increased as droplet size decreased. Spraying as 40-, 52-, 101-, 148-, 174-, and 265-μm diameter droplets resulted in 327, 303, 210, 166, 123, and 81 droplets/cm², respectively (Table 1). Irrespective of the ACP life stage and fenpropathrin discharge rate, mortality of ACP decreased as droplet size increased from 40 to 265 μm along with the decrease in droplet area density. At the 0.38 mg AI/min discharge rate, the two smaller droplet sizes (40 and 52 μm) resulted in significantly greater mortality of eggs than the remaining four larger droplet sizes (Table 1). At the 0.58-mg AI/min discharge rate, the three smaller droplet sizes resulted in equivalent egg mortality, which was significantly greater than the remaining three larger droplet sizes (Table 1). Comparison of slopes of linear regression lines of egg mortality versus droplet size for the two spray discharge rates indicated a highly significant difference (Table 1). At the 0.38-mg AI/min discharge rate, the three smaller droplet sizes resulted in significantly greater nymph morality than the three larger droplet sizes (Table 2). At the 0.58-mg AI/min discharge rate, the three smaller droplet sizes caused significantly greater mortality than the two larger droplet sizes (174 and 265 μm). Comparison of slopes of linear regression lines of nymph mortality versus droplet size for the two spray discharge rates indicated a highly significant difference (Table 2).

At the 0.38-mg AI/min discharge rate, the two smaller droplet sizes resulted in significantly greater morality of adults than the three larger droplet sizes (Table 3). At the 0.58-mg AI/min discharge rate, the three smaller droplet sizes caused significantly greater mortality than the two larger droplet sizes (174 and 265 μm). Comparison of slopes of linear regression lines of adult mortality vs. droplet size for the two spray discharge rates indicated

Table 1. Effect of spray droplet size and spray discharge rate on efficacy of fenpropathrin against the Asian citrus psyllid eggs at 81–327 droplets/cm².

Spray droplet diam (μm)	Droplet density	Mean percent mortality at 7 d posttreatment[z] 0.38 mg AI/min[y]	0.58 mg AI/min[x]
40	327.2 ± 12.5	98.5 ± 1.5 ab	100.0 ± 0.0 a
52	303.4 ± 10.9	99.3 ± 0.6 a	100.0 ± 0.0 a
101	209.9 ± 14.3	79.1 ± 3.7 c	100.0 ± 0.0 a
148	165.8 ± 11.7	83.5 ± 5.7 bc	93.0 ± 3.0 b
174	123.1 ± 7.4	50.2 ± 4.3 d	97.4 ± 1.5 b
265	81.3 ± 10.8	33.9 ± 9.3 e	83.3 ± 2.1 c
F-statistic (df)		27.45 (5)	16.89 (5)

[z]Compared to pretreatment counts.
[y]Spray discharge rate on target plants at ½ recommended field rate.
[x]Spray discharge rate on target plants at ¾ recommended field rate.
Means (±SE; $n = 4$) not labeled by the same lower case letter in a column are significantly different using LSD tests ($P < 0.05$).
A highly significant difference (t = 8.04; $P < 0.0001$) between linear regression line slope values of 0.38 mg AI/min and 0.58 mg AI/min was observed when percent mortality was regressed on droplet size.

Table 2. Effect of spray droplet size and spray discharge rate on efficacy of fenpropathrin against the Asian citrus psyllid nymphs at 81–327 droplets/cm²

Spray droplet diam (μm)	Mean percent mortality at 7 d posttreatment[z] 0.38 mg AI/min[y]	0.58 mg AI/min[x]
40	97.3 ± 1.6 a	100.0 ± 0.0 a
52	100.0 ± 0.0 a	100.0 ± 0.0 a
101	84.5 ± 4.1 a	100.0 ± 0.0 a
148	67.9 ± 6.9 b	99.2 ± 0.8 ab
174	61.9 ± 3.5 bc	91.2 ± 5.2 bc
265	47.7 ± 9.4 c	88.6 ± 4.3 c
F-statistic (df)	15.38 (5)	3.50 (5)

[z]Compared to pretreatment counts.
[y]Spray discharge rate on target plants at ½ recommended field rate.
[x]Spray discharge rate on target plants at ¾ recommended field rate.
Means (±SE; $n = 4$) not labeled by the same lower case letter in a column are significantly different using LSD tests ($P < 0.05$).
A highly significant difference (t = 5.06; $P < 0.0001$) between linear regression line slope values of 0.38 mg AI/min and 0.58 mg AI/min was observed when percent mortality was regressed on droplet size.

Table 3. Effect of spray droplet size and spray discharge rate on efficacy of fenpropathrin against the Asian citrus psyllid adults at 81–327 droplets/cm² density.

Spray droplet diam (μm)	Mean percent mortality at 7 d posttreatment[z] 0.38 mg AI/min[y]	0.58 mg AI/min[x]
40	84.9 ± 2.3 a	92.4 ± 3.4 ab
52	83.4 ± 5.8 a	100.0 ± 0.0 a
101	68.2 ± 9.9 ab	91.1 ± 3.4 ab
148	55.5 ± 5.8 bc	82.2 ± 9.6 bc
174	56.5 ± 4.9 bc	75.0 ± 4.1 c
265	36.4 ± 3.9 c	72.1 ± 6.3 c
F-statistic(df)	6.39 (5)	4.09 (5)

[z]Compared to pretreatment counts.
[y]Spray discharge rate on target plants at ½ recommended field rate.
[x]Spray discharge rate on target plants at ¾ recommended field rate.
Means (±SE; $n = 4$) not labeled by the same lower case letter in a column are significantly different using LSD tests ($P < 0.05$).
A nonsignificant difference ($t = 1.91$; $P < 0.05$) between linear regression line slope values of 0.38 mg AI/min and 0.58 mg AI/min was observed when percent mortality was regressed on droplet size.

a nonsignificant difference (Table 3). These findings suggest that when sprayed as smaller size droplets (40–50 μm), fenpropathrin causes significantly greater morality of ACP than when sprayed as larger size droplets (174–265 μm) at both discharge rates. Furthermore, comparable mortality of ACP eggs, nymphs, and adults could be obtained at both discharge rates when delivered as smaller droplets.

Effects of a sub-lethal concentration of imidacloprid on ACP adults and nymphs. ACP adults that fed on plants with the sub-lethal concentration of imidacloprid (0.1 ppm) laid significantly fewer eggs and viability of these eggs was significantly lower than eggs laid by adults fed on the control plants (Table 4). The life span of adults was also significantly reduced compared with the control adults (Table 4). Nymphs that fed on the treated plants took significantly longer to reach the adult stage compared with the control nymphs (Table 4). Similarly, when nymphs were fed on plants containing the sub-lethal concentration of imidacloprid, a significantly lower percentage of nymphs survived into the adult stage compared with the control nymphs (Table 4).

When soil applications of imidacloprid are made in the field, it may lead to the sub-lethal concentrations in the tree due to 1) variation in uptake by the plants with time (temporal), 2) variation in systemic distribution within the plant (spatial), and 3) degradation of imidacloprid in the plant over time (Castle et al., 2005). Therefore, both ACP adults and nymphs are exposed to these sub-lethal concentrations of imidacloprid in citrus tree at various periods over their life. Our results suggest that longevity, fecundity and egg viability of adults as well as survival and developmental time of nymphs could be adversely affected when they feed continuously on plants containing a sub-lethal concentration of imidacloprid. This suggests that even after the systemic concentration of imidacloprid within citrus decreases below that which causes acute mortality, continuous feeding by ACP on such concentrations adversely affects their biology and reproduction. This reduces their fitness, which may result in a reduction of ACP populations over time. However, such continuous exposure of ACP to sub-lethal concentrations may lead to resistance development in the survivors.

Overall, during cooler months of the year (November–March), when ACP adults overwinter, application of broad-spectrum SPs such as fenpropathrin and lambda-cyhalothrin effectively suppresses ACP populations (Boina et al., 2009b). This may help in reducing the numbers of ACP adults at the beginning spring, when citrus trees produce new flush and ACP start laying eggs. The subsequent build up of ACP populations in summer months (May–August) could be effectively controlled by spraying broad spectrum OP insecticides such as chlorpyrifos and dimethoate.

Fenpropathrin sprayed at the smaller droplet sizes of 40–50 μm and higher area density, provides better control of ACP populations compared to spraying at larger droplets of 174–265 μm. However, there are problems associated with spraying small droplets (40–50 μm) such as potential spray drift. Therefore, applications of fenpropathrin at 100–150 μm droplet size may provide an acceptable level of ACP control in the field. This may also minimize the problems of spray drift associated with smaller (40–50 μm) and spray run off from the target associated with larger (174–265 μm) droplet sizes.

Although imidacloprid exhibits sub-lethal effects at low concentrations, application of broad spectrum insecticides during the periods between soil applications, when concentrations of imidacloprid are low within trees, could help mitigate spread of HLB by ACP.

Table 4. Effect of a sub-lethal concentration of imidacloprid on the Asian citrus psyllid adults and nymphs.

Parameter	Control	Imidacloprid (0.1 ppm)	*t*-statistic (df)	*P* value
Adult lifespan (days)	47	39	13.51 (8)	<0.0001
Adult fecundity (eggs/female/day)	5.5	3.7	5.83 (8)	0.0004
Adult fertility (%)	95	88	10.36 (8)	<0.0001
Nymph survival (%)	82	72	7.12 (8)	0.0001
Nymph development (days)	15.3	18.6	15.91 (8)	<0.0001

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