

Antifeedant and sublethal effects of imidacloprid on Asian citrus psyllid, *Diaphorina citri*

Dhana Raj Boina,^{a*} Ebenezer O Onagbola,^a Masoud Salyani^b and Lukasz L Stelinski^a

Abstract

BACKGROUND: Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, transmits the causal bacteria of the devastating citrus disease huanglongbing (HLB). Because of the variation in spatial and temporal uptake and systemic distribution of imidacloprid applied to citrus trees and its degradation over time in citrus trees, ACP adults and nymphs are exposed to concentrations that may not cause immediate mortality but rather sublethal effects. The objective of this laboratory study was to determine the effects of sublethal concentrations of imidacloprid on ACP life stages.

RESULTS: Feeding by ACP adults and nymphs on plants treated daily with a sublethal concentration ($0.1 \mu\text{g mL}^{-1}$) of imidacloprid significantly decreased adult longevity (8 days), fecundity (33%) and fertility (6%), as well as nymph survival (12%) and developmental rate compared with untreated controls. The magnitude of these negative effects was directly related to exposure duration and concentration. Furthermore, ACP adults that fed on citrus leaves treated systemically with lethal and sublethal concentrations of imidacloprid excreted significantly less honeydew (7–94%) compared with controls in a concentration-dependent manner suggesting antifeedant activity of imidacloprid.

CONCLUSIONS: Sublethal concentrations of imidacloprid negatively affect development, reproduction, survival and longevity of ACP, which likely contributes to population reductions over time. Also, reduced feeding by ACP adults on plants treated with sublethal concentrations of imidacloprid may potentially decrease the capacity of ACP to successfully acquire and transmit the HLB causal pathogen.

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Keywords: imidacloprid; *Diaphorina citri*; antifeedant activity; sublethal effects; huanglongbing; citrus greening disease

1 INTRODUCTION

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is one of the most important insect pests of citrus worldwide. During phloem sap feeding, ACP transmits three phloem-limited bacterial pathogens in the genus *Candidatus Liberibacter*, responsible for huanglongbing (HLB).^{1,2} ACP, a native pest to Asia, was introduced into the USA and was first discovered in Florida in 1998;³ since then it has established throughout the state. HLB was first confirmed in Florida in 2005, and by 2008 it had become established throughout the citrus-growing areas of Florida.⁴ HLB-infected citrus trees become unproductive and eventually die,^{5,6} threatening the survival of the \$US1.4 billion Florida citrus industry.⁷ HLB-infected citrus trees serve as reservoirs of the pathogen, which is efficiently vectored by the mobile ACP. Therefore, removal of diseased trees and suppression of ACP populations are the recommended management practices for slowing disease spread.⁸ In the absence of other effective methods, application of soil and foliar insecticides is the only current option for ACP suppression.

Several insecticides are registered or are under the process of registration for ACP control in citrus crops in Florida. Of these, the neonicotinoid compound imidacloprid is highly active against many plant-sap-sucking Hemipteran insect pests,

including ACP.^{9–12} Imidacloprid acts by selectively binding to insect nicotinic acetylcholine receptors, leading to hyperexcitation and irreversible toxic symptoms.¹³ Given its systemic properties, soil-applied imidacloprid has less impact on natural enemies of ACP than many foliar contact insecticides.^{14–17}

Soil applications of imidacloprid are made to citrus trees for control of ACP in Florida and for control of glassy-winged sharpshooter, *Homalodisca vitripennis* (Germer), in California.^{8,18} Such applications can remain persistently effective for 2–3 months after application,^{8,18} which results in exposure of ACP nymphs and adults to concentrations ranging from sublethal to lethal. ACP feeding on plant tissues containing lethal concentrations of imida-

* Correspondence to: Dhana Raj Boina, Entomology and Nematology Department, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA.
E-mail: drboina@ufl.edu

a Entomology and Nematology Department, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA

b Agricultural and Biological Engineering Department, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA

cloprid causes immediate insect mortality by neuroexcitation. ACP feeding on plant tissues with sublethal concentrations, however, may lead to various physiological and behavioral changes, which have not been determined previously.

The physiological changes caused by exposure to sublethal concentrations of insecticides can result in reductions in developmental rate, longevity, fecundity and fertility, which decrease fitness.¹⁹ Alternatively, exposure to sublethal concentrations may increase fecundity by stimulating reproduction through hormoligosis or trophobiosis, which increases fitness.²⁰ Previous authors have reported that exposure to or feeding on sublethal concentrations of imidacloprid reduces growth, survival and reproduction in many Hemipteran insect pests.^{21–30} In contrast, exposure to or feeding on plants treated with imidacloprid increased the fecundity of a predatory mite, *Amblyseius victoriensis* Womersley,³¹ increased fecundity and longevity of the two-spotted spider mite, *Tetranychus urticae* Koch,³² and increased fecundity, larval weight and juvenile hormone III titer of the rice borer, *Tryporyza incertulas* (Walker).³³

Behavioral changes caused by exposure to or feeding on sublethal concentrations of an insecticide can also result in reduced feeding or searching behavior.¹⁹ For example, imidacloprid acts as an antifeedant for many Hemipteran insects, including aphids, whiteflies and green leafhoppers, at sublethal concentrations^{21–26,28} and reduces acquisition and/or transmission of potato leafroll virus by green peach aphid, *Myzus persicae* (Sulzer).²⁸

Soil application of imidacloprid to citrus trees results in lethal and sublethal concentrations in tree phloem to *H. vitripennis*.¹⁸ Similarly, ACP populations may be exposed to such concentrations throughout the season in commercially managed citrus where soil application of imidacloprid is practiced. Therefore, the present study was undertaken to determine the effects of sublethal concentrations of imidacloprid on ACP under two possible scenarios of feeding (short versus long duration for adults and constant versus decreasing concentration for nymphs) on systemically treated plants. In addition, the effect of different concentrations of imidacloprid on feeding activity of ACP adults was determined using a leaf systemic bioassay.

2 MATERIALS AND METHODS

2.1 Insects

Psyllids used in experiments were obtained from a greenhouse colony that was collected in 2005 from a 'Valencia' orange grove near Lake Alfred, Florida, and maintained thereafter without exposure to pesticides, as described in Wenninger *et al.*³⁴

2.2 Sublethal concentration of imidacloprid

The objective of this experiment was to determine the concentration of imidacloprid that is sublethal to ACP adults for subsequent tests on ACP reproduction and growth using a plant systemic bioassay. To this end, 1–2-month-old citrus seedlings, *Citrus aurantiifolia* (Christm.) cv. Swingle, grown in a greenhouse were uprooted and washed under tap water to remove potting soil from roots. Plant roots were completely immersed for 48 h in 30 mL glass vials containing aqueous solutions of imidacloprid (0.001, 0.01, 0.1, 1.0, 10 and 100 µg AI mL⁻¹) prepared by serial dilution of a commercial imidacloprid 240 g L⁻¹ SC (Admire 2F®; Bayer CropScience LP, Research Triangle Park, NC) in tap water. Plants that were immersed similarly in tap water for the same period served as controls. After 48 h of immersion, plants were

transferred to new vials with tap water, and 10 ACP adults were released onto each plant, including controls. Plants with insects were confined within 1 L transparent plastic cups (11.5 cm top diameter, 14 cm height) with perforations at the top for aeration and to prevent water condensation. The entire set-up was placed on a bench top in a walk-in growth room at 25 ± 1 °C and 50 ± 5% RH with a 14 : 10 h light : dark photoperiod. Each treatment, including the control, was replicated 3 times using three plants. Mortality counts were taken at 24, 48 and 72 h after release of adults. Insects that dropped off plants or clung to plants and did not move when prodded with a needle were scored as dead. The entire experiment was repeated 2 times on different dates. The sublethal concentration for subsequent experiments was determined on the basis of log-probit analysis of concentration–mortality data.

2.3 Effect on adult longevity, fecundity and fertility

The objective of this experiment was to determine the effect of the sublethal concentration of imidacloprid estimated in Section 2.2 on ACP adult longevity and reproduction after short and long durations of feeding using a slightly modified version of the plant systemic bioassay described in Section 2.2. To this end, 2–3-month-old citrus plants (cv. Swingle) with new flush were pretreated systemically (by soaking in 0.1 µg mL⁻¹ of imidacloprid for 48 h) and were repotted individually within 1 L plastic pots (11.5 cm top diameter, 12 cm height), and five pairs (five male and five female) of newly emerged (0–1-day-old) adults were released onto each plant. Plants were covered with 1 L transparent plastic cups as described above and placed on a bench top in a walk-in growth room at 25 ± 1 °C and 50 ± 5% RH with a 14 : 10 h light : dark photoperiod. Following this, two types of imidacloprid treatments were established. For the long-duration feeding treatment (LDF), plants were watered daily with 30 mL of an aqueous solution of imidacloprid (0.1 µg mL⁻¹) over 5 day periods throughout the adults' lifetime. After each 5 day period, adults were transferred onto a new set of imidacloprid-treated plants that were watered daily with the same imidacloprid solution. For the short-duration feeding treatment (SDF), plants were watered daily with 30 mL of an aqueous solution of imidacloprid (0.1 µg mL⁻¹) only during the first 5 day period. Thereafter, adults were transferred to new sets of untreated plants after each subsequent 5 day period that were treated daily with 30 mL of water only. Control plants were pretreated with water and subsequently watered daily with 30 mL of water only during each 5 day period, and adults were transferred between different sets of untreated plants. This ACP transfer procedure facilitated collection of eggs and provided new flush for oviposition throughout the fecundity observation (5–25 days). The total number of eggs laid by females on each plant per 5 day period (6–10, 11–15, 16–20 and 21–25 days) was counted under a stereomicroscope on days 10, 15, 20 and 25 after initiating the experiment. Fecundity counts were made only through day 25 of the experiment because fecundity is greatest during this interval of the female adults' life.³⁵ Excised new flush along with eggs was transferred into 90 mm plastic petri dishes with moistened filter paper over an agar bed (1.5%) to prevent desiccation of eggs. Petri dishes with eggs were maintained at the same environmental conditions as described above for nymph hatching. The number of fertile eggs, based on nymph hatching, was recorded 7 days after transfer to petri dishes for each batch of eggs collected. Adults were monitored at 3 day intervals for mortality until all of the test insects were dead. The entire experiment was repeated 3 times on different dates.

2.4 Effect on nymph developmental time and survival

The purpose of this experiment was to compare the impact of a constant versus a continuously decreasing sublethal concentration of imidacloprid on ACP nymph survival and developmental time using the plant systemic bioassay described above. The experimental procedures were similar to that described in Section 2.3, with slight modifications. Here, 2–3-month-old citrus plants (cv. Swingle) with new flush, pretreated systemically (as in Section 2.3), were repotted individually in 1 L plastic pots and placed within a Plexiglass sleeve cage (40 × 40 × 40 cm). Approximately 200 adult ACP of mixed age were released into each cage for 24 h to allow for oviposition. Thereafter, adults were removed by aspirating, and plants with 0–24-h-old eggs were transferred into a growth chamber set at 27 ± 1 °C and $80 \pm 5\%$ RH with a 14:10 h light:dark photoperiod. Following this initial procedure, two types of imidacloprid treatments were established. For the constant-concentration feeding treatment (CCF), a set of three imidacloprid-treated plants was watered daily with 30 mL of $0.1 \mu\text{g mL}^{-1}$ imidacloprid solution. For the decreasing-concentration feeding treatment (DCF), a set of three plants that had been pretreated with imidacloprid as described above was watered daily with tap water (30 mL). Control plants were pretreated with water and watered daily with 30 mL of water only. Five days after eggs were oviposited, the number of hatched nymphs on each plant was counted and recorded, and the remaining unhatched eggs were carefully removed. Nymphs were observed throughout their development to record the total duration until adult emergence, as well as survival. Nymph mortality was calculated as the difference between the total number of nymphs on initiation of the experiment and the total number of emerging adults. The entire experiment was repeated 3 times on different dates.

2.5 Effect on adult feeding

The objective of this experiment was to determine whether imidacloprid induces feeding deterrence against ACP adults in a leaf systemic bioassay. The petioles of freshly excised citrus leaves were immersed in aqueous solutions of imidacloprid (0.001 – $100 \mu\text{g mL}^{-1}$) in 30 mL glass vials for 24 h. Leaves soaked identically but in tap water served as the controls. After 24 h of immersion, 60 mm diameter leaf discs were excised and placed on 1.5% agar beds in 60 mm diameter plastic disposable petri dishes. Ten ACP adults were released into each dish, which was subsequently closed with a lid lined with 60 mm Whatman filter paper (Whatman International Ltd, Maidstone, UK). Petri dishes were maintained at 25 ± 1 °C and $50 \pm 5\%$ RH with a 14:10 h light:dark photoperiod in a walk-in growth room, and placed upside down to collect excreted honeydew droplets onto the filter papers. Each treatment was replicated 3 times (three dishes). At 24 h after insect release, filter papers were collected and subjected to a ninhydrin (Sigma-Aldrich, St Louis, MO) test by soaking filter paper for approximately 3 min in 1% (w/v) ninhydrin solution in acetone.²³ Dipping of filter papers with honeydew in ninhydrin solution turned honeydew droplets on filter paper discs into a dark purple color. The number of dark purple spots was counted on both control and treatment filter papers and was used to calculate the concentration of imidacloprid for 50% reduction in honeydew excretion (EC_{50}). The entire experiment was repeated 3 times on three different dates.

2.6 Effect of starvation on adult ACP survival

The objective of this experiment was to determine the longevity of ACP adults under simulated starvation conditions. This experiment

was conducted for comparison with the results of the feeding deterrence study (Section 2.5 above). A laboratory and a field strain of ACP were tested in this experiment. Ten ACP adults from each strain were released into separate 30 mm diameter petri dishes with agar, agar + a 30 mm diameter citrus leaf disc (excised from a freshly collected 'Valencia' orange leaf) or without agar and leaf material. Each treatment was replicated within three separate dishes, and the entire experiment was repeated twice on different dates. Adult mortality counts were taken at 1, 2, 3, 5 and 8 days after release of adults. ACP that did not move on prodding with a needle were scored as dead.

2.7 Calculations and statistical analyses

Adult ACP mortality data from two replicate experiments were pooled for each concentration tested and subjected to log-probit regression analysis (PROC PROBIT program)³⁶ after correcting for control mortality to calculate the concentration for sublethal effects. The mean (\pm SEM; $n = 9$) number of eggs laid per female per 5 days for each treatment was calculated from three replicate experiments. The mean (\pm SEM; $n = 9$) percentage of fertile eggs and mean adult survival time (in days) were calculated for each treatment. Similarly, the mean (\pm SEM; $n = 9$) nymph developmental time and survival were calculated for each treatment. Honeydew excretion data for each concentration were pooled from three replicate experiments and converted to the mean (\pm SEM; $n = 9$) percentage of the control. The above mean data were subjected to one-way analysis of variance (ANOVA; PROC GLM program)³⁶ for significant differences between treatment means, and means were separated using Fisher's least significant difference (LSD) test at an $\alpha = 0.05$ significance level. Using a log-probit regression analysis (PROC PROBIT program),³⁶ the concentration required for 50% reduction in honeydew excretion (EC_{50}) was calculated with 95% confidence intervals. Similarly, the time for 50% mortality (LT_{50}) in adults of both field and laboratory strains under starvation was calculated.

3 RESULTS

3.1 Sublethal concentration of imidacloprid

Log-probit regression analysis of concentration–mortality data revealed that the concentrations of imidacloprid required to cause mortality in 50% (LC_{50}) and 10% (LC_{10}) of test populations after 24 h feeding on treated plants were 24.79 and $0.14 \mu\text{g mL}^{-1}$ respectively. The LC_{50} value decreased sevenfold and 27-fold after 48 and 72 h feeding on treated plants, respectively, compared with 24 h feeding. However, the decrease in the LC_{10} value was minimal after 48 h (1.75-fold) and 72 h (3.5-fold) feeding. Therefore, $0.1 \mu\text{g mL}^{-1}$ ($\sim\text{LC}_{10}$) was selected for subsequent experiments to assess sublethal effects of imidacloprid on ACP adults and nymphs.

3.2 Effect on adult longevity, fecundity and fertility

The longevity, fecundity and fertility of ACP adults that fed continuously on plants treated daily with $0.1 \mu\text{g mL}^{-1}$ of imidacloprid (LDF) were affected more than in adults that fed for only 5 days on treated plants and were subsequently transferred to untreated plants (SDF). Adults that fed on plants in the LDF treatment began dying 6 days earlier than those that fed on plants treated with water alone (control) (Fig. 1a). Furthermore, all adults that fed on plants in the LDF treatment died by day 49 of the experiment, while death occurred on day 58 for adults that fed on control plants. There was a significant decrease in mean adult longevity in

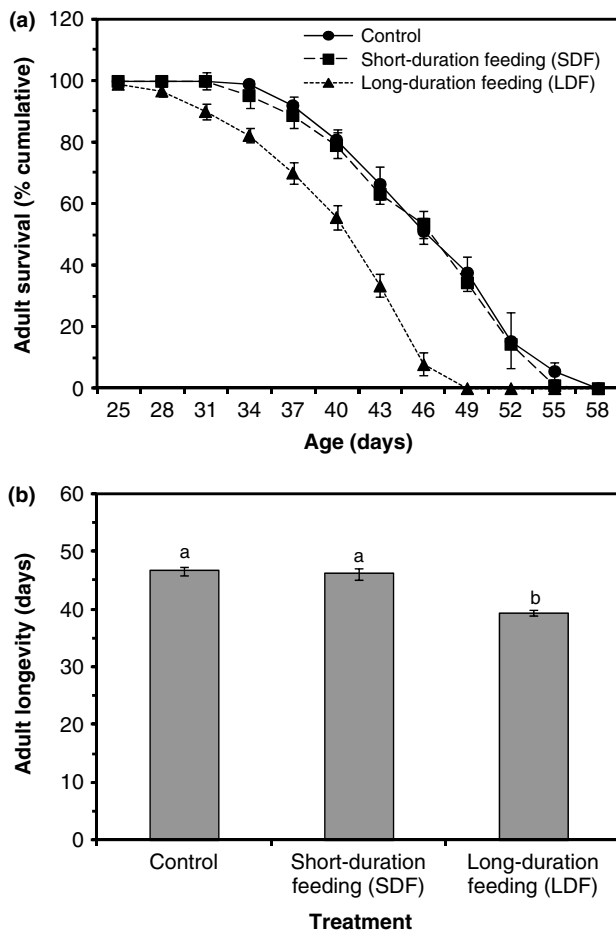


Figure 1. Cumulative (a) and mean (\pm SEM; $n = 9$) (b) survival of *Diaphorina citri* adults reared on plants in long- and short-duration feeding treatments compared with the untreated control. Long-duration feeding (LDF): adults were allowed to feed continuously throughout their lifespan over successive 5 day periods on plants treated systemically with $0.1 \mu\text{g mL}^{-1}$ imidacloprid solution. Short-duration feeding (SDF): adults were allowed to feed for 5 days on plants treated as in long-duration exposure and then transferred for successive 5 day periods onto untreated plants that were watered daily with 30 mL of tap water. Control: adults were allowed to feed continuously throughout their lifespan over 5 day periods on plants treated with tap water. Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$).

the LDF treatment compared with the control ($P < 0.05$) (Figs 1a and b). Also, adults that fed on plants in the SDF treatment began dying 3 days earlier than those that fed on the control plants; however, this effect did not last throughout the experiment, and no significant difference was observed in mean adult longevity between the SDF treatment and the control (Figs 1a and b).

At the sublethal concentration tested, imidacloprid also decreased the fecundity of ACP. In the LDF treatment, the number of eggs laid by females between days 6 and 25 of the experiment was lower than that observed in the SDF or control treatments (Fig. 2a). ACP fecundity decreased with increasing female age until day 20 in the LDF treatment. In the LDF treatment, 23, 31, 34 and 33% fewer eggs were laid by adults during the 6–10, 11–15, 16–20 and 21–25 day periods, respectively, compared with the control. In the SDF treatment, adults laid 6, 16, 3 and 5% fewer eggs during the 6–10, 11–15, 16–20 and 21–25 day periods, respectively, compared with the control (Fig. 2a). The mean number of eggs

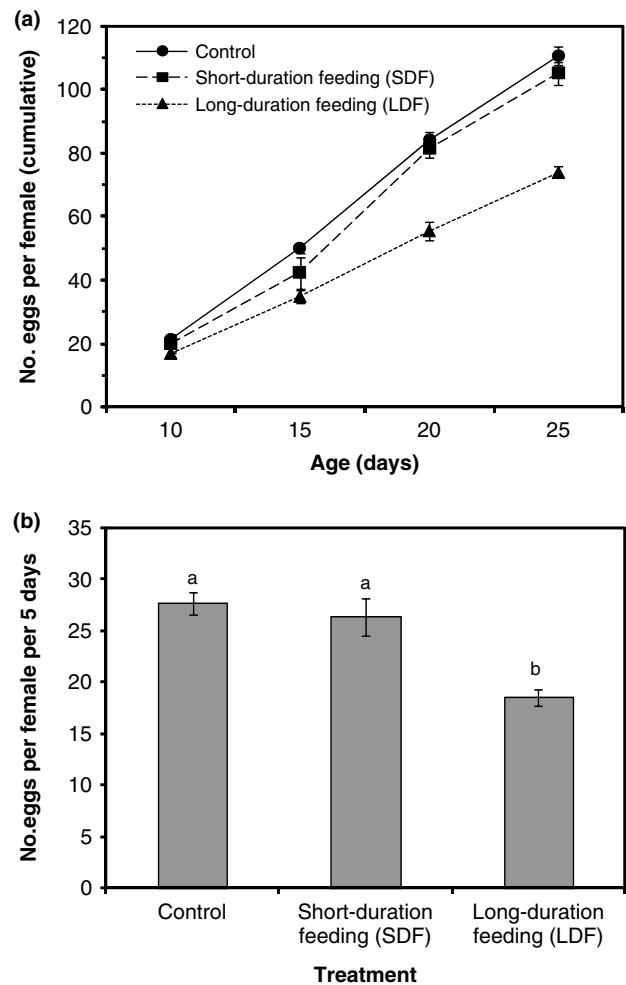


Figure 2. Cumulative (a) and mean (\pm SEM; $n = 9$) (b) fecundity of *Diaphorina citri* adults reared on plants in long- and short-duration feeding treatments compared with the untreated control. Long-duration feeding (LDF), short-duration feeding (SDF) and control as in Fig. 1. Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$).

laid per female per 5 days in the LDF treatment was significantly lower (18.5) than that in the SDF (26.3) or control (27.6) treatments ($P < 0.05$) (Fig. 2b).

As observed with adult longevity and fecundity, there was a significant reduction (11.0%) in the fertility of eggs laid by ACP adults that fed on treated plants in the LDF compared with that in the SDF (6.5%) or control (5.7%) treatments ($P < 0.05$) (Fig. 3). Although a slight reduction was observed in the percentage of eggs that hatched into nymphs in the SDF treatment, it was not significantly different from the control (Fig. 3).

3.3 Effect on nymph developmental time and survival

Developmental time of nymphs that fed on plants treated daily with $0.1 \mu\text{g mL}^{-1}$ of imidacloprid throughout their lifetime (CCF) was significantly delayed compared with those that fed on plants treated with $0.1 \mu\text{g mL}^{-1}$ for only 48 h (DCF) (Fig. 4a). Development of nymphs into adults was delayed by 3 days in the CCF treatment compared with the control (Fig. 4a). Also, adult emergence in the CCF treatment continued for 2 days after it was complete in the control treatment. In the DCF treatment,

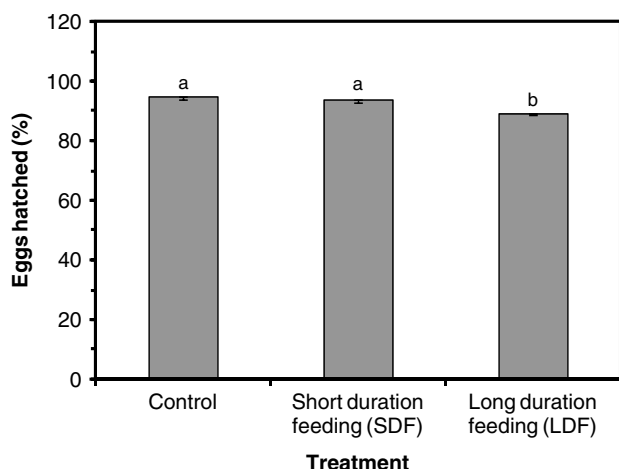


Figure 3. Fertility of *Diaphorina citri* adults reared on plants in long- and short-duration feeding treatments compared with the untreated control. Bars are means with SEM ($n = 9$). Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$). Long-duration feeding (LDF), short-duration feeding (SDF) and control as in Fig. 1.

there was a 2 day delay in the initiation of adult emergence compared with the control treatment. The mean duration for development of nymphs into adults was significantly longer in the CCF than in the DCF or control treatments ($P < 0.05$) (Fig. 4b). Furthermore, survival of nymphs that fed on plants in the CCF treatment decreased (12%) significantly compared with the control ($P < 0.05$) (Fig. 5). However, there was no significant difference in the survival of nymphs between the CCF and DCF treatments or between the DCF and control treatments.

3.4 Effect on adult feeding

There was a concentration-dependent antifeedant effect of imidacloprid on ACP adults as measured by a decrease in honeydew excretion (Fig. 6). With the exception of the lowest concentration tested, all of the treatments significantly reduced the amount of honeydew excreted compared with the control treatment (Fig. 6). Honeydew excretion decreased by >90% at the higher concentrations (10 or 100 $\mu\text{g mL}^{-1}$) tested compared with the control (Fig. 6). The calculated effective concentration for 50% reduction (EC_{50}) in honeydew excretion was 0.17 $\mu\text{g mL}^{-1}$ (0.11–0.26 $\mu\text{g mL}^{-1}$, 95% confidence intervals), which was almost 145-fold lower than that required for 50% mortality in the plant systemic bioassay following the same duration of exposure.

3.5 Effect of starvation on adult ACP survival

When no food was provided in petri dishes, 50% of adults from both the laboratory and field strains died within 2 days (Table 1). In petri dishes with solidified agar, mortality in 50% of adults from both strains occurred after approximately 5 days (Table 1). In contrast, no adult mortality occurred during the 8 day observation period in petri dishes with agar + a citrus leaf.

4 DISCUSSION

The purpose of this investigation was to gain insight into the potential impacts of sublethal concentrations of imidacloprid on ACP physiology and behavior. Given the prevalent use of imidacloprid in citrus production, a complete understanding of its

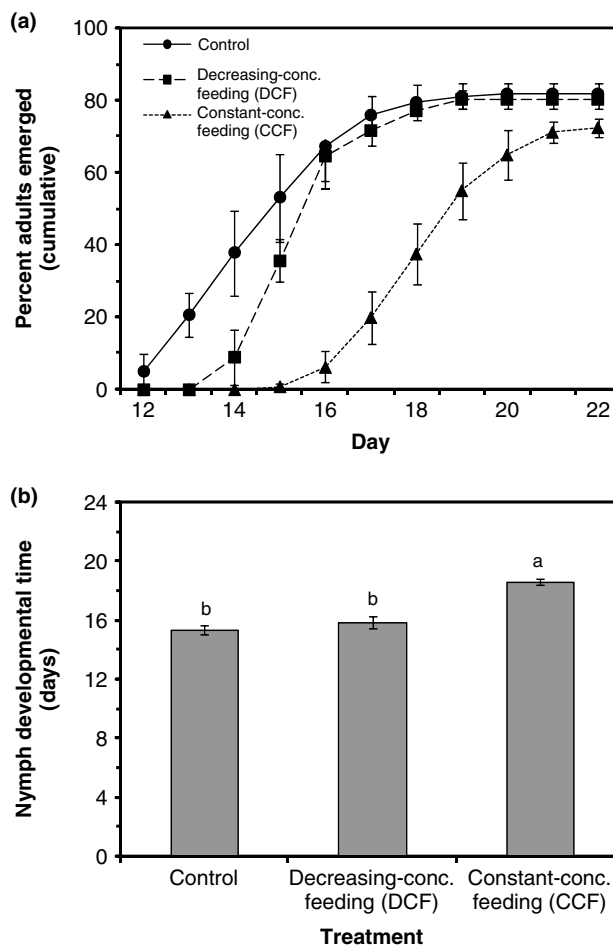


Figure 4. Cumulative (a) and mean (\pm SEM; $n = 9$) (b) developmental time of *Diaphorina citri* nymphs reared on plants in constant- and decreasing-concentration feeding treatments compared with the untreated control. Constant-concentration feeding treatment (CCF): nymphs were allowed to feed continuously throughout their lifespan on plants treated systemically with 0.1 $\mu\text{g mL}^{-1}$ of imidacloprid for 48 h and then watered daily with 30 mL of the same solution. Decreasing-concentration feeding treatment (DCF): nymphs were allowed to feed continuously throughout their lifespan on plants treated systemically with 0.1 $\mu\text{g mL}^{-1}$ of imidacloprid for 48 h and then watered daily with 30 mL of tap water. Control: adults were allowed to feed continuously throughout their lifespan on plants treated with tap water for 48 h and then watered daily with 30 mL of tap water. Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$).

Strain	Food	Number of insects	LT ₅₀ ^a (95% CL) ^b
Lab	No food	60	1.93 (–) ^c
	Agar	60	4.63 (4.28–5.00)
	Agar + citrus leaf	60	– ^d
Field	No food	60	1.70 (1.55–1.84)
	Agar	60	5.10 (4.69–5.56)
	Agar+ citrus leaf	60	– ^d

^a Time in days for 50% mortality in test population.
^b 95% confidence limits for LT₅₀.
^c 95% confidence limits could not be calculated.
^d No adult mortality was observed over 8 day period.

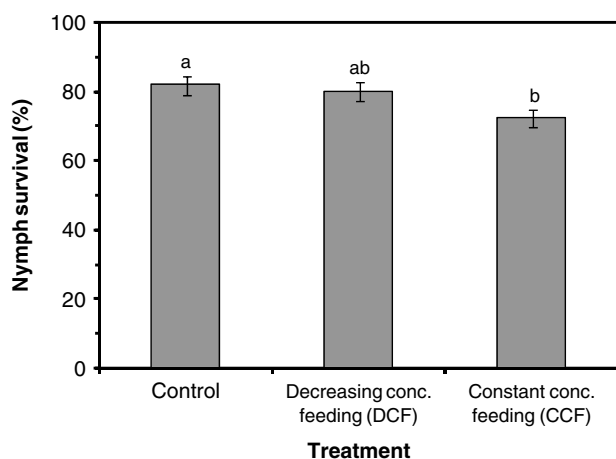


Figure 5. Survival of *Diaphorina citri* nymphs reared on plants in constant- and decreasing-concentration feeding treatments compared with the untreated control. Bars are means with SEM ($n = 9$). Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$). Constant-concentration feeding treatment (CCF), decreasing-concentration feeding treatment (DCF) and control as in Fig. 4.

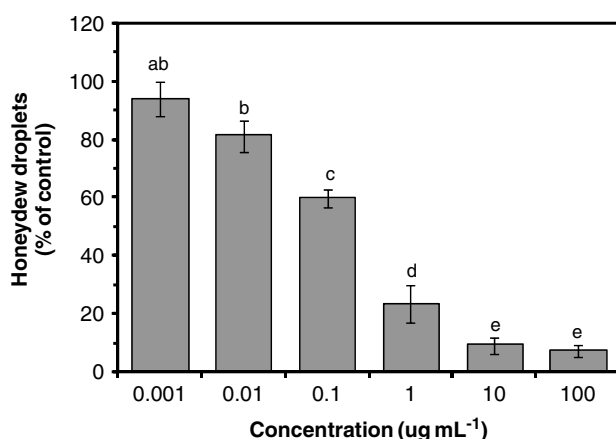


Figure 6. Antifeedant activity of imidacloprid at lethal and sublethal concentrations against *Diaphorina citri* adults. Each bar represents the percentage of control honeydew droplets excreted. Bars are means with SEM ($n = 9$). Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$).

activity against ACP is necessary. A concentration of $0.1 \mu\text{g mL}^{-1}$, which is equal to $\sim\text{LC}_{10}$ from the log-probit regression analysis of concentration–mortality data, was selected for investigation of sublethal physiological effects. On 1–2-month-old plants, $0.1 \mu\text{g mL}^{-1}$ caused mortality in $\leq 15\%$ of adult ACP after 72 h of feeding. However, this same concentration did not induce mortality for up to 20 days on 2–3-month-old plants, which also continuously produced new flush required for nymphal feeding and egg laying. Therefore, 2–3-month-old citrus plants were used in this investigation, allowing ACP to complete their life cycle on imidacloprid-treated plants. Normal performance (biological parameters) of ACP adults and nymphs on control plants suggested that the soaking procedure [uprooting, soaking in imidacloprid solution (or water) and repotting] performed in the present study did not have any adverse effects on growth and vigor of plants. Therefore, the observed results on ACP adults and nymphs from

feeding on imidacloprid-treated plants were due to the treatment effects only.

In the present study, feeding on a sublethal concentration ($0.1 \mu\text{g mL}^{-1}$) of imidacloprid adversely affected the longevity and reproductive potential of adults, as well as the developmental time and survival of nymphs. The magnitude of these negative effects, however, was dependent on the duration of feeding for adults and on the concentration of imidacloprid in plants for nymphs. Feeding by adults and nymphs on treated plants may have resulted in a duration-dependent accumulation of imidacloprid increasing deleterious sublethal effects over time. Although the authors did not determine the imidacloprid titers in treated plant parts, a similar accumulation of imidacloprid could have occurred in plants treated daily. Adult feeding on treated plants for a limited period of 5 days resulted in transient sublethal effects. Also, nymph feeding on treated plants with a decreasing concentration of imidacloprid ($< 0.1 \mu\text{g mL}^{-1}$) resulted in short-lived deleterious effects that subsided over time. The decrease in cumulative fecundity of adults in the short-duration feeding treatment and the delay in cumulative adult emergence in the decreasing-concentration feeding treatment observed initially compared with the control support the above conclusions.

ACP adults that fed on treated plants in the long-duration feeding treatment laid 33% fewer eggs than observed in the control. Reduced fecundity of ACP after feeding on citrus plants treated with a sublethal concentration of imidacloprid is consistent with previous studies investigating other Hemipteran insects. The fecundities of two aphid species [*Myzus persicae* and *M. nicotianae* (Blackman)], two green leafhopper species [*Nephotettix virescens* (Distant) and *N. cincticeps* Uhler] and a brown planthopper species [*Nilaparvata lugens* Stål] were reduced by more than 50% after feeding on plants/leaves treated with sublethal concentrations of imidacloprid.^{22,27,37} Similarly, Lashkari *et al.*²⁹ reported that the oviposition rate, longevity and growth of cabbage aphid, *Brevicoryne brassicae* L., were negatively affected by sublethal concentrations of imidacloprid. The present study also indicated that ACP adult lifespan was reduced by 8 days and nymph survival by 12%. However, the results suggested no evidence for a stimulatory effect of imidacloprid on ACP reproduction as has been observed with certain insect and mite species.^{31–33}

Systemically applied imidacloprid reduces feeding in aphids (*M. persicae* and *M. nicotianae*) and whiteflies (*Bemisia tabaci* Gennadius) at sublethal concentrations.^{21,23} In the present study, imidacloprid exhibited a concentration-dependent antifeedant effect on ACP adults, as measured by suppression of honeydew excretion. Minimal honeydew excretion by adult ACP at the higher concentrations ($\geq 1 \mu\text{g mL}^{-1}$) tested could have been the direct result of ACP mortality. However, reduced honeydew excretion at the lower concentrations ($\leq 0.1 \mu\text{g mL}^{-1}$) tested was likely the result of feeding deterrence, given that minimal or no adult mortality was observed at these concentrations.

In the present study, deleterious effects of imidacloprid on ACP at sublethal concentrations were not likely the result of feeding deterrence and subsequent starvation. This hypothesis was supported by a starvation test, which indicated 50% ACP mortality within 2 days in the absence of food (Table 1). In contrast, ACP adults that fed on plants treated with $0.1 \mu\text{g mL}^{-1}$ of imidacloprid survived for at least 20 days (Fig. 1a). A leaf systemic bioassay was used for determining the antifeedant effect of imidacloprid, whereas a plant systemic bioassay was employed for measuring other sublethal effects on ACP. In the plant systemic bioassay, it is conceivable that imidacloprid may have been

unevenly distributed among plant parts at concentrations below $0.1 \mu\text{g mL}^{-1}$. This may have caused ACP adults and nymphs to seek out feeding sites on plant parts containing concentrations of imidacloprid below $0.1 \mu\text{g mL}^{-1}$ that may not induce strong feeding deterrence. Similarly, *M. persicae* and *M. nicotianae* were starved on cabbage leaves treated with sublethal concentrations of imidacloprid owing to strong feeding deterrence, but resumed normal feeding when transferred to untreated leaves.^{21,25}

In commercially managed citrus, where imidacloprid is applied to the tree root zone and foliage, ACP populations are often exposed to sublethal concentrations of this insecticide owing to (a) variation in the spatial and temporal patterns of imidacloprid uptake and systemic distribution within trees,¹⁸ (b) a time lag between application and the attainment of lethal concentrations within trees,¹⁸ (c) metabolic degradation over time within trees¹⁸ and (d) short residual life due to physical degradation on plant surfaces. Imidacloprid, therefore, exerts a concentration-dependent three-pronged effect spatially and temporally in the field for reducing ACP populations capable of transmitting the HLB pathogen. Feeding by ACP on plant parts with lethal concentrations results in mortality. Feeding on plant parts with sublethal concentrations sufficient to induce strong feeding deterrence may interfere with successful acquisition and/or transmission of the HLB pathogen. Finally, feeding on plant parts with sublethal concentrations below that which induces strong feeding deterrence likely reduces ACP fitness by negatively affecting growth, reproduction and survival, which ultimately influences population dynamics over time.

Vector feeding deterrence is one of the effective methods for reducing spread of insect-borne disease because of unsuccessful acquisition and/or transmission of the causal pathogens.^{27,28} ACP adults require at least 30 min of continuous feeding on a diseased plant to acquire the HLB pathogen and 5–7 h of feeding for subsequent transmission to a healthy plant.^{38,39} Therefore, feeding deterrence induced by sublethal concentrations ($\leq 0.1 \mu\text{g mL}^{-1}$) of imidacloprid may influence ACP behavior in such a way that reduces the capacity of ACP to vector HLB pathogen. Future research will be focused on employing electrical penetration graph (EPG) analyses coupled with plant histological studies for monitoring the feeding behavior of ACP on citrus plant parts with sublethal concentrations of imidacloprid and its impact on pathogen acquisition and/or transmission by ACP.

The whole-plant systemic bioassay used in the present study for determining the effects of sublethal concentrations of imidacloprid on ACP could be used for developing baseline susceptibility data of ACP populations to available systemic insecticides and to monitor resistance development over time. Such monitoring is highly important given the dramatic increase in insecticide use against ACP in Florida citrus after the discovery of HLB. Furthermore, the sublethal effects measured in the present study could be used for monitoring subtle changes in susceptibility to systemic insecticides that are undetectable at $\geq \text{LC}/\text{LD}_{50}$ values owing to a shorter exposure period and measurement of one endpoint: mortality/survival.¹⁹

5 CONCLUSIONS

The presence of sublethal concentrations of imidacloprid within citrus tree tissues, resulting from uneven systemic distribution or metabolic degradation over time, likely contributes to HLB disease management because of several deleterious effects on the ACP vector. Feeding by ACP adults and nymphs on plant

parts containing sublethal concentrations ($\leq 0.1 \mu\text{g mL}^{-1}$) for a prolonged period negatively affects growth, development, reproduction and survival, which collectively reduces ACP fitness. This may reduce ACP populations over time. Furthermore, ingestion of sublethal concentrations ($\sim 0.1 \mu\text{g mL}^{-1}$) strongly deters adult ACP feeding, which likely interferes with successful acquisition and/or transmission of the HLB causal pathogen. However, testing this hypothesis will require further research.

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