Research Article

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Effect of pyriproxyfen, a juvenile hormone mimic, on egg hatch, nymph development, adult emergence and reproduction of the Asian citrus psyllid, *Diaphorina citri* Kuwayama

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Abstract

BACKGROUND: The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is a vector of bacteria presumably responsible for huanglongbing (HLB) disease in citrus. In this laboratory study, an investigation was made of the activity of pyriproxyfen, a juvenile hormone mimic, on ACP eggs, nymphs and adults to evaluate its potential as a biorational insecticide for inclusion in an integrated pest management (IPM) program for ACP.

RESULTS: Irrespective of egg age, timing or method of treatment, a significantly lower percentage of eggs (5–29%) hatched after exposure to 64 and 128 μg mL^{-1} of pyriproxyfen. Only 0–36% of early instars (first, second and third) and 25–74% of late instars (fourth and fifth) survived to adults following exposure to 16, 32 and 64 μg mL^{-1} of pyriproxyfen. However, 15–20% of adults that emerged following treatment as late instars exhibited morphological abnormalities. Furthermore, pyriproxyfen adversely affected reproduction (fecundity and fertility) of adults that emerged from treated fifth instars or that were treated topically with 0.04 μg as adults.

CONCLUSIONS: Application of pyriproxyfen at 64 μg mL^{-1} resulted in greater inhibition of egg hatch and suppression of adult emergence compared with lower rates. Pyriproxyfen also markedly reduced female fecundity and egg viability for adults that were exposed either as fifth instars or as newly emerged adults. The ovicidal, larvicidal and reproductive effects against ACP suggest that pyriproxyfen is suitable for integration into an IPM program for ACP.

Keywords: Asian citrus psyllid; pyriproxyfen; morphological abnormalities; ovicidal activity; reproductive effects; huanglongbing

1 INTRODUCTION

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is a worldwide pest of citrus plants including cultivated orange, lemon and lime.[1] In the USA, ACP was first noticed in June 1998 in Florida[2] and has subsequently spread to Alabama, Arizona, California, Georgia, Louisiana, Mississippi and Texas,[3] aided by its strong dispersal capabilities.[4] Both adults and nymphs ingest plant sap from phloem tissues and excrete copious amounts of honeydew, on which sooty mold grows which may affect photosynthesis. Females oviposit on new flush. On hatching, nymphs go through five instars before emerging as adults in 11–15 days.[5] Nymphs are sluggish and move slowly when disturbed. The greatest economic impact caused by this pest results from its ability to transmit three phloem-limited bacteria in the genus *Candidatus* Liberibacter.[6–8] These bacteria are presumably responsible for huanglongbing (HLB) disease, also known as citrus greening.[7–9] HLB is considered one of the most destructive diseases of citrus in the world, and all known commercial cultivars of citrus are considered to be susceptible. Diseased trees may live for 5–10 years with symptoms of twig dieback and severe fruit drop. In addition, they produce small, lopsided and bitter-tasting fruit, rendering them unfit for consumption.[8] The presence of HLB has been confirmed in citrus-growing areas worldwide, except for the Mediterranean basin, Western Asia, Australia and the Pacific Ocean islands.[8] HLB was first confirmed in Florida in August 2005 and has subsequently spread to all citrus-growing areas of the state[10] owing to rapid vectoring by ACP which was already established.

The approaches currently used for reducing the rate of HLB spread within Florida are quick removal of diseased trees and suppression of ACP populations. Suppression of ACP populations requires several applications of foliar insecticides (6–8 per year), coupled with one or two soil applications of a systemic insecticide, imidacloprid or aldicarb.[11,12] However, repeated use of available chemistries with few modes of action may lead to evolution of

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insecticide resistance in ACP populations. Therefore, it is important to utilize insecticides with different modes of action. This may reduce selection pressure for resistance evolution. Evaluating the efficacy of potential candidate insecticides with unique modes of action against ACP is necessary to determine their utility in ACP management. One potential component of an integrated pest management (IPM) program for ACP is the use of insect growth regulators (IGRs). IGRs are a unique class of insecticides that act selectively on various life stages of insects in many orders.[13] While conventional insecticides target the nervous system, IGRs target chemical and hormonal cascades that govern molting and metamorphosis.[13,14] Owing to their unique mode of action, IGRs have also been considered less harmful to beneficial insects[15,16] by comparison with conventional neurotoxicants.

Pyriproxyfen, 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether, is a juvenile hormone mimic (JHM). It was first registered in Japan in 1991 for controlling mosquitoes.[17] External application of pyriproxyfen causes imbalance in JH levels inside the insect, which results in strong suppression of embryogenesis, metamorphosis, adult formation and/or sterility in many insect pests.[18–29] The following study was conducted to evaluate the potential of pyriproxyfen as an IPM tool for suppression of ACP populations. The objectives of the present study were to determine the effects of pyriproxyfen on (1) egg hatch, nymph development and adult emergence and (2) fecundity and egg viability of adults treated directly (by topical application) or indirectly (emerging from treated fifth instars).

2 MATERIALS AND METHODS

2.1 Insect culture and insecticide

Adult and immature ACP used in experiments were obtained from a greenhouse colony initiated from ACP adults collected from a 'Valencia' orange grove in 2005 and maintained on 'Valencia' orange without exposure to insecticides at the Citrus Research and Education Center, Lake Alfred, FL, as described in Wenninger *et al.*[30] A 103 g AI L^{-1} emulsifiable concentrate (EC) commercial formulation of pyriproxyfen (Knack 0.86 EC; Valent USA Corp., Walnut Creek, CA) was used at 4, 8, 16, 32, 64 and 128 µg AI mL^{-1} in various experiments, equivalent to 0.06, 0.12, 0.25, 0.5, 1.0 and 2.0× the labeled field rate respectively. The labeled field rate for Homopteran scale insects in citrus is 121 g AI ha^{-1} in 1890 L of water.

2.2 Effect on egg hatch

The objective of this experiment was to determine the direct ovicidal activity of pyriproxyfen against ACP eggs laid either before treatment or after treatment by leaf dip or residual contact bioassays respectively. Plant material used in experiments consisted of 2–3-month-old citrus plants ['Swingle', *Citrus aurantiifolia* (Christm.)] with new leaf flush, as defined by Hall and Albrigo.[31] Plants were taken from a greenhouse and placed in plexiglass cages (40 by 40 by 40 cm) with sleeves for easy access. Mated females along with males (200–300) were released into each cage, and females were allowed to oviposit for 48 h. Adults were manually aspirated from cages. New flushes with eggs were excised either from plants after 48 h to obtain 0–48-h-old eggs (younger eggs) or from plants that were maintained in the same cages for another 48 h to obtain 49–96-h-old eggs (older eggs). The number of eggs per individual excised leaf flush was counted under a stereomicroscope, and they were then dipped in various concentrations of pyriproxyfen prepared in tap water or tap water alone (control) for 15 s. Treated flush samples with eggs were allowed to air dry under a fume hood for 1–2 h and then placed individually into petri dishes containing 1.5% agar beds and moistened filter papers to prevent desiccation of eggs. Each treatment, including the control, was replicated with 12–15 flush samples, and each flush contained 7–121 eggs. Petri dishes with eggs were placed in a growth chamber set at 25 ± 1 °C and 50 ± 5% RH with a 14 : 10 h light : dark photocycle. ACP eggs usually hatch within 4–5 days. Eggs were observed under a stereomicroscope 7 days after being placed in the growth chamber, and the number of hatched/unhatched eggs per flush was recorded.

In a separate experiment designed to assess residual ovicidal activity, citrus plants with new flush as described above were sprayed until run-off using a handheld atomizer (The Bottle Crew, West Bloomfield, MI) with various concentrations of pyriproxyfen, as described above, or tap water (control). Treated plants were air dried for 1–2 h and then placed in plexiglass cages. Mated females along with males (200–300) were released into each cage for oviposition, as in the previous experiment. In this manner, 0–48-h-old eggs (younger eggs) were obtained. Each treatment was replicated with nine plants, and each plant contained 12–56 eggs. Plants were maintained at 25 ± 2 °C and 50 ± 5% RH in an insectary under a 14 : 10 h light : dark photocycle. Plants were watered as needed. Egg hatch counts were taken as described above using a 10× hand lens.

2.3 Effect on nymphs and adult emergence

The objective of this experiment was to determine the susceptibility of various ACP instars to pyriproxyfen. Mated females were allowed to lay eggs on new flush of citrus plants placed in plexiglass cages for 24 h, as described in Section 2.2. Five days after egg laying, unhatched eggs were removed using a camel hair brush. Nymphs were either treated as first instars or allowed to develop into second, third, fourth or fifth instars. The number of nymphs present on each plant was counted before applying treatments. Plants with first, second, third, fourth or fifth instars were sprayed until run-off with one of the concentrations (8, 16, 32 or 64 µg mL^{-1}) in tap water prepared freshly on the day of testing or tap water alone (control). Each treatment was replicated with 11–15 plants, and each plant contained 6–61 nymphs. Treated plants with nymphs were maintained at 25 ± 2 °C and 50 ± 5% RH in an insectary under a 14 : 10 h light : dark photocycle. Nymphs were followed through adult emergence by recording the number of nymphs that entered into the subsequent instar. Survival at each concentration was determined by taking the ratio of adults emerged to the total number of nymphs treated for each instar. The number of nymphs molting successfully into the next instar for recently treated instars was also recorded at 48 h after treatment to determine acute toxicity.

2.4 Effect on reproduction

The objective of this experiment was to determine the effects of pyriproxyfen on reproduction of adults that emerged from treated fifth instars as described in Section 2.3 or newly emerged adults from the greenhouse colony that were treated topically. Effects on reproduction were evaluated by measuring the fecundity and viability of eggs. Adults that emerged from treated fifth instars for each concentration (0, 8, 16, 32 or 64 µg mL^{-1}) were sexed, and three pairs (male : female in 1 : 1) were transferred onto an untreated plant with new flush for feeding, mating and subsequent egg laying. Similarly, three pairs of adults that emerged from

control plants were sexed and transferred to untreated plants. Each treatment was replicated 5 times. Plants were maintained at 25 ± 2 °C and 50 ± 5% RH in an insectary under a 14 : 10 h light : dark photocycle. Adults were continuously transferred to untreated plants at 3 day intervals for 12 days. The number of eggs laid on each plant was counted using a 10× hand lens. Each batch of plants with eggs was kept for 7 days, and the number of eggs that hatched was counted.

Effects of pyriproxyfen on adult reproduction were also quantified following topical application to adults. Topical applications were made with a 10 µL Hamilton syringe (Hamilton Co., Reno, NV) by treating the abdomens of newly emerged male and female ACP (greenhouse colony) with 0.04 µg (0.4 µL of a 100 µg mL^{-1} solution prepared in acetone) of pyriproxyfen. Adults treated identically with acetone alone served as controls. Three pairs of treated females and males (1 : 1 ratio) were released per untreated citrus plant with new flush for oviposition. The treatment and control were replicated with five plants. Plants were maintained in an insectary at 25 ± 2 °C and 50 ± 5% RH under a 14 : 10 h light : dark photocycle. Adults were transferred to a new set of untreated plants at 3 day intervals for 12 days. The number of eggs laid on each plant was recorded after adult transfer, whereas the number of eggs hatching was recorded 7 days after adult removal.

2.5 Statistical analyses

The percentage data from various experiments were subjected to arcsine square root transformation, which normalized distributions and homogenized variances across treatments. The transformed data were analyzed either by an analysis of variance (ANOVA) or by *t*-tests. The mean (± SEM; $n = 12–15$) percentage egg hatch inhibition data for younger and older eggs were subjected to one-way ANOVA (PROC GLM program[32]). Significant differences between treatment means were separated by Fisher's least significant difference (LSD) tests at $\alpha = 0.05$. Also, the pooled egg hatch inhibition data at each concentration for younger or older eggs were subjected to probit analysis (PROC PROBIT program[32]), and the concentration for 50% egg hatch inhibition (LC_{50}) with corresponding 95% confidence intervals (CIs) was calculated. The PROC PROBIT program estimates the LC_{50} values after correcting for control mortality. The mean (± SEM; $n = 11–15$) percentage nymph survival to adults for each instar tested was calculated at each concentration and subjected to one-way ANOVA (PROC GLM program[32]), followed by LSD tests for mean separation at $\alpha = 0.05$. The LC_{50} values for each instar were calculated from pooled nymph mortality data (48 h) at each concentration after correcting for control mortality. The mean (± SEM; $n = 4–6$) number of eggs laid per female per 3 days was calculated at each concentration and subjected to one-way ANOVA (PROC GLM program[32]), followed by LSD mean separation at $\alpha = 0.05$. Similarly, significant differences between the mean percentage of egg hatching were determined by one-way ANOVA, followed by LSD mean separation tests at $\alpha = 0.05$. Similar data for topically treated adults were subjected to independent sample *t*-tests (PROC TTEST program[32]).

3 RESULTS

3.1 Effect on egg hatch

In the leaf dip bioassay, pyriproxyfen exhibited concentration-dependent ovicidal activity against younger and older ACP eggs laid before treatment (Figs 1a and b). With the exception of 4 µg mL^{-1}, each concentration of pyriproxyfen significantly

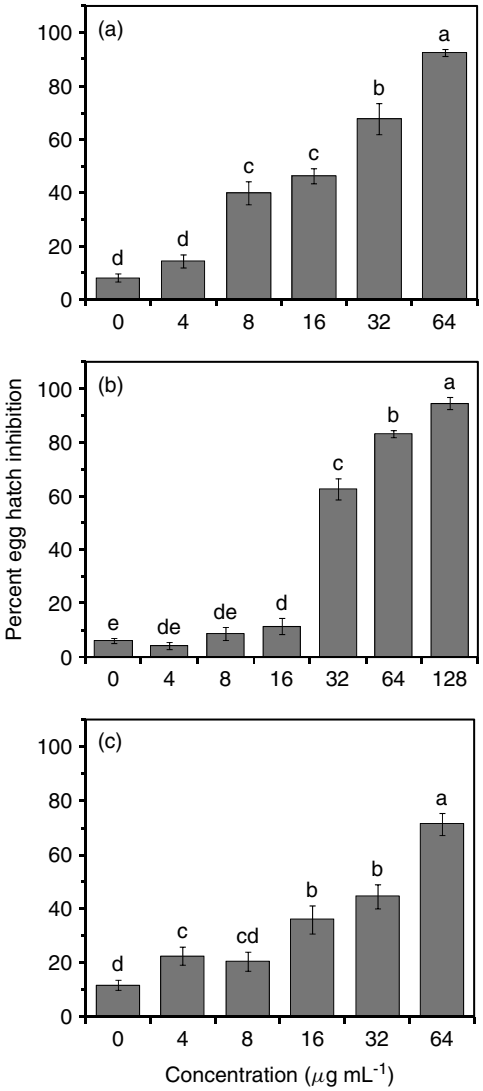

Figure 1. Percentage ACP egg hatch inhibition after exposure to various concentrations of pyriproxyfen tested in leaf dip or residual contact bioassay: (a) younger eggs (0–48 h) laid before treatment application by leaf dip method; (b) older eggs (49–96 h) laid before treatment application by leaf dip method; (c) younger eggs laid after treatment application to citrus plants by residual contact method. Bars are means with SEM ($n = 12–15$). Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$).

inhibited the hatching of younger eggs compared with the control (Fig. 1a) ($F = 88.56$; df = 5, 75; $P < 0.0001$). Although a similar trend in the activity of pyriproxyfen was observed against older eggs, their sensitivity was lower compared with that of younger eggs. For older eggs, the two lower concentrations (4 and 8 µg mL^{-1}) did not affect egg hatch and were comparable with the control (Fig. 1b). In contrast, the highest concentration (128 µg mL^{-1}) suppressed egg hatch in >90% of older eggs, which was significantly different from the remaining treatments and the control (Fig. 1b) ($F = 138.65$; df = 6, 79; $P < 0.0001$). For eggs laid after the treatment (residual contact), egg hatch was inhibited by each treatment in a concentration-dependent manner, with the exception of the 8 µg mL^{-1} treatment which did not significantly affect egg hatch compared with the control. The highest concentration (64 µg mL^{-1}) resulted in nearly 70% egg hatch inhibition, which was significantly higher than the

Table 1. Ovicidal activity of pyriproxyfen on *Diaphorina citri*

Application timing	Egg age (h)	(*n*)	LC_{50}[a] (95% CI)[b]	Slope (± SE)	χ^2 (df)[c]
After egg laying[d]	0–48	2041	18.45 (12.10–28.38)	1.50 (±0.19)	17.69 (3)
After egg laying[d]	49–96	2653	37.03 (21.71–58.87)	2.97 (±0.64	98.92 (4)
Prior to egg laying[e]	0–48	1332	32.43 (16.38–228.43)	1.17 (±0.26)	20.63 (3)

[a] Concentration in μg mL^{-1} for 50% egg hatch inhibition.
[b] 95% confidence intervals for LC_{50}.
[c] Chi-square goodness-of-fit statistic and degrees of freedom.
[d] Determined by leaf dip bioassay method.
[e] Determined by residual contact bioassay method.

remaining treatments and the control (Fig. 1c) ($F = 30.53$; df = 5, 48; $P < 0.0001$). Although differences in susceptibility of eggs to pyriproxyfen were observed owing to age, timing and method of treatment, overlapping of 95% CIs for the LC_{50} values indicated that these differences were not significant (Table 1).

3.2 Effect on nymphs and adult emergence

There was a positive relationship between pyriproxyfen concentration and mortality for ACP nymphs ($r = 0.8$; $P < 0.0001$). Pyriproxyfen caused acute mortality of all instars at each concentration, which was more pronounced in early instars (first, second and third) than late instars (fourth and fifth). Those nymphs that survived to molt into the next instar were either affected in subsequent instars or completed development and emerged as adults, depending on the concentration and nymphal instar. When first instars were treated, all concentrations of pyriproxyfen significantly decreased survival compared with the control (Fig. 2a) ($F = 1353.43$; df = 4, 70; $P < 0.0001$), and none of the first instars treated with the 16, 32 and 64 μg mL^{-1} reached the adult stage (Fig. 2a). Similarly, when second instars were treated, all concentrations reduced survival compared with the control (Fig. 2b) ($F = 159.16$; df = 4, 47; $P < 0.0001$), with no nymphs surviving at the two higher concentrations (Fig. 2b). All concentrations of pyriproxyfen significantly suppressed adult emergence, compared with the control, when third instars were treated (Fig. 2c) ($F = 361.10$; df = 4, 55; $P < 0.0001$), with only a negligible percentage (1%) emerging as adults at the two higher concentrations (Fig. 2c). Even the lowest concentration significantly reduced the emergence of adults, although this concentration did not affect fourth and fifth instar nymphs, >90% of which emerged successfully (Figs 2d and e).

In contrast to early instars, 23–56% of treated fourth and fifth instars emerged as adults at the two higher concentrations, although survival was significantly lower than in the remaining concentrations and the controls (Figs 2d and e) ($F = 61.26$ and 34.86; df = 4, 50; $P < 0.0001$). Nonetheless, overlapping of 95% CIs for the LC_{50} values calculated based on acute mortality (48 h mortality) indicated no significant difference among instars in susceptibility to pyriproxyfen (Table 2).

Pyriproxyfen caused morphological abnormalities in 15–20% of adults emerging from treated fourth and fifth instars at the two higher concentrations (Fig. 3). Affected individuals were characterized by abnormalities such as a wider abdomen, thicker antennae (nymphal characters), twisted wings and, at times, darker body coloration (Fig. 3). Also, emergence of a portion (10%) of these adults was affected. Completely developed adults were found with their legs and abdomen attached to the exuvia; these were unable to emerge completely for up to 3 days and eventually died (Fig. 3).

3.3 Effect on reproduction

Adults emerging from fifth instars that were treated with the two higher concentrations laid consistently fewer eggs 0–12 days after emergence compared with the controls (Figs 4a to d). However, fecundity of these adults was significantly lower than the control only during the initial 6 days after emergence (Figs 4a and b). At the two higher concentrations, females laid only 61 and 58% of the eggs laid in the controls 0–3 days after emergence, which were significant reductions compared with the controls (Fig. 4a) ($F = 4.27$; df = 4, 21; $P = 0.01$). Similarly, at the two higher concentrations, significantly fewer eggs (only 64–67% of the controls) were laid compared with the remaining concentrations and the controls 4–6 days after emergence (Fig. 4b) ($F = 5.36$; df = 4, 21; $P = 0.004$). At 7–9 and 10–12 days after emergence, fecundity of adults treated with pyriproxyfen in the fifth instar was not different from the controls (Figs 4c and d) ($F = 1.24$ and 1.33; df = 4, 21; $P = 0.32$ and 0.29).

The two higher concentrations significantly reduced the viability of eggs laid 0–6 days after emergence compared with the controls (Figs 5a and b). Only 69–79% of the eggs laid 0–3 days after emergence hatched at the two higher concentrations, which was significantly lower than the controls (Fig. 5a) ($F = 3.67$; df = 4, 21; $P = 0.02$). Viability of eggs laid 4–6 days after emergence for the two higher concentrations was lower than for those laid 0–3 days after emergence (Figs 5a and b). Only 59% of eggs hatched at the highest concentration, which was significantly lower than the remaining treatments and the controls (Fig. 5b) ($F = 11.75$; df = 4, 21; $P < 0.0001$). Eggs laid 7–9 days after emergence had viability similar to the controls in all treatments (Fig. 5c) ($F = 1.48$; df = 4, 21; $P = 0.24$). However, only 83% of eggs hatched at the highest concentration, which was significantly lower than at the lowest concentration (Fig. 5c). The viability of eggs laid 10–12 days after emergence in all treatments was comparable with the controls (Fig. 5d) ($F = 1.41$; df = 4, 21; $P = 0.26$).

Although topical application of pyriproxyfen to newly emerged adults at the 100 μg mL^{-1} concentration did not result in acute lethal effects, it reduced fecundity and egg viability. However, the manifestation of the effect on fecundity was delayed, and its magnitude was lower in topically treated adults than in adults emerging from treated fifth instars. There was no significant difference between treated and control adults in the number of eggs laid 0–3 days after emergence (Fig. 6a) ($t = 0.39$; df = 4; $P = 0.71$). However, the viability of eggs laid by treated females during this period was significantly lower compared with the

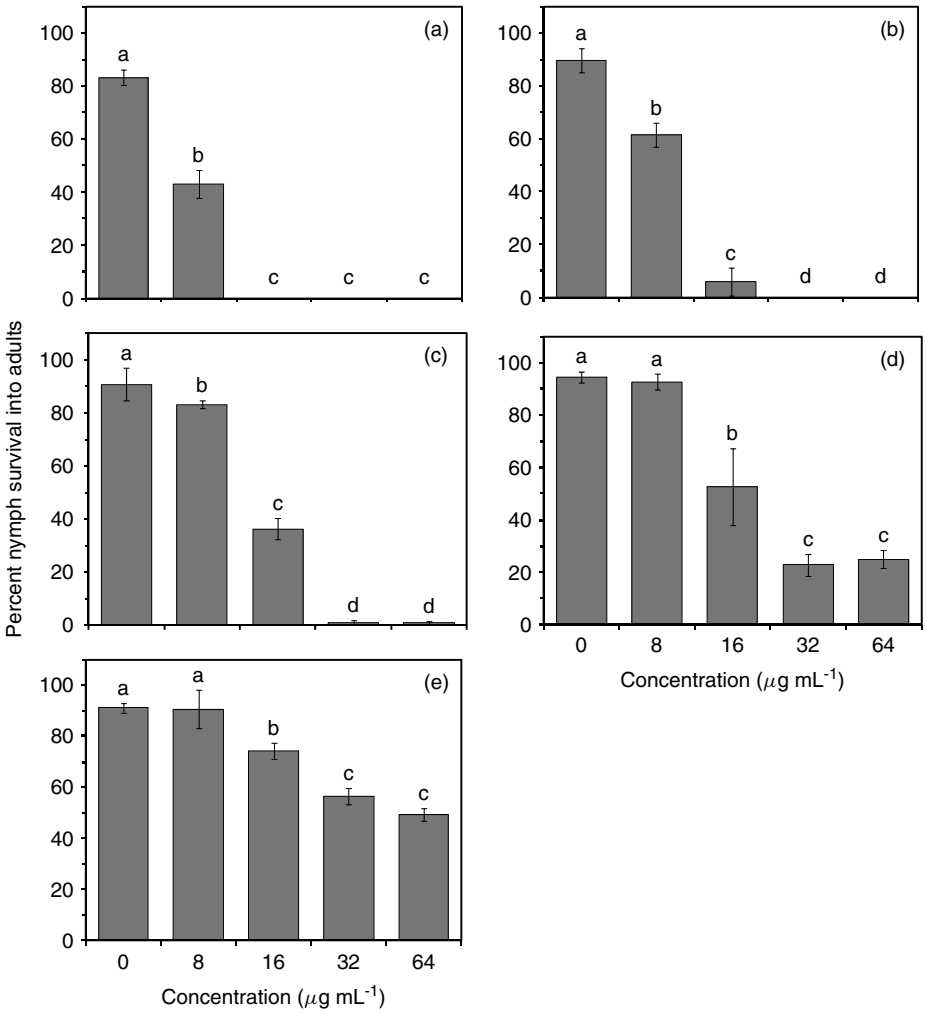

Figure 2. Percentage survival of ACP nymphs into adults after exposure to various concentrations of pyriproxyfen when treated at (a) first, (b) second, (c) third, (d) fourth and (e) fifth instar. Bars are means with SEM ($n = 11–15$). Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$). No bar indicates 100% mortality.

Table 2. Effect of pyriproxyfen on developmental stages of *Diaphorina citri*

Stage treated	Stage evaluated[a]	(*n*)	LC_{50}[b] (95% CI)[c]	Slope (± SE)	χ^2 (df)[d]
Instar I	Instar II	1992	17.62 (1.43–44.34)	1.82 (±0.37)	26.24 (2)
Instar II	Instar III	1237	21.55 (8.77–45.57)	2.40 (±0.42)	16.68 (2)
Instar III	Instar IV	1142	25.25 (15.12–46.30)	2.22 (±0.32)	9.04 (2)
Instar IV	Instar V	980	44.27 (22.17–3581)	1.97 (±0.44)	14.89 (2)
Instar V	Instar V	906	51.29 (42.74–65.26)	1.51 (±0.15)	4.58 (2)

[a] Mortality counts were taken 48 h after treating the nymphs.
[b] Concentration in μg mL^{-1} for 50% mortality in treated nymphal instar.
[c] 95% confidence intervals for LC_{50}.
[d] Chi-square goodness-of-fit statistic and degrees of freedom.

controls (Fig. 6b) ($t = 5.66$; df = 4; $P = 0.004$). Topically treated females laid significantly fewer eggs than the controls 4–6 days after emergence (Fig. 6a) ($t = 2.77$; df = 4; $P = 0.05$), and the viability of these eggs was significantly lower (Fig. 6b) ($t = 5.81$; df = 4; $P = 0.004$). The fecundity and viability of eggs laid 7–9 and 10–12 days after emergence by females treated topically with 100 μg mL^{-1} pyriproxyfen was comparable with the controls (Figs 6a and b) ($t = 1.27$ and 0.69; df = 4; $P = 0.27$ and 0.52 for fecundity; $t = 1.97$ and 1.26; df = 4; $P = 0.11$ and 0.27 for egg viability).

4 DISCUSSION

The present investigation showed that pyriproxyfen is lethal to ACP eggs and nymphs but not to adults. However, adults treated topically with pyriproxyfen or adults emerging from treated fifth

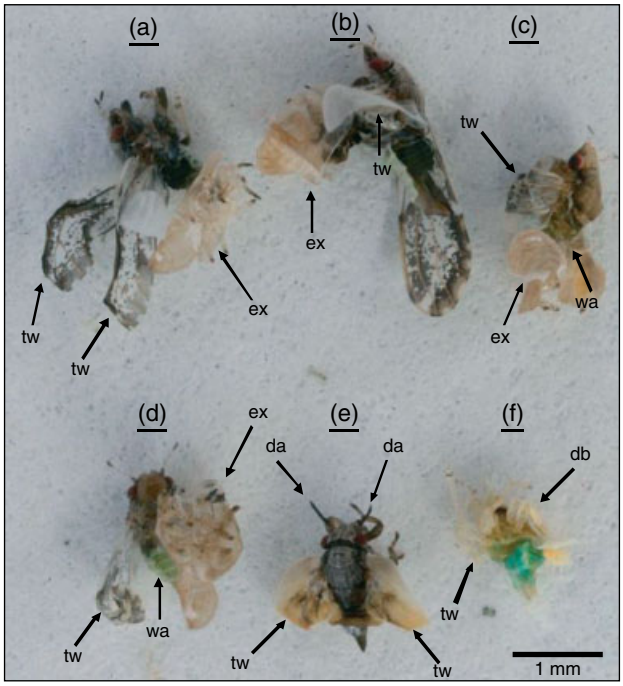

Figure 3. Morphological abnormalities observed in ACP adults caused by exposure of late instars (fourth and fifth) to the two higher concentrations of pyriproxyfen (32 and 64 μg mL^{-1}). a–f, twisted wings (tw); a–d, unable to emerge completely from exuvia (ex); c and d, wide abdomen (wa); e, deformed (thicker) antennae (da); f, deformed body (db).

instars exhibited sublethal effects in the form of reduced fecundity and egg viability. The strong ovicidal activity of pyriproxyfen against ACP was age and concentration dependent. In general, percentage egg hatch inhibition increased with concentration regardless of egg age, timing or method of treatment. Although pyriproxyfen inhibited egg hatch in both younger and older eggs, the former were more susceptible. These findings concur with those obtained with glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar),[23] in which suppression of egg hatch by pyriproxyfen was more pronounced in younger eggs (24–48 h; LC_{50} 20 μg mL^{-1}) than in older eggs (49–120 h; only 30% egg hatch inhibition at 94 μg mL^{-1}). When compared with 49–120-h-old *H. vitripennis* eggs (only 30% egg hatch inhibition at 94 μg mL^{-1}), 49–96-h-old ACP eggs seemed more susceptible to pyriproxyfen (LC_{50} = 37.03 μg mL^{-1}). The inclusion of 97–120-h-old *H. vitripennis* eggs by Prabhaker and Toscano[23] may explain some of the discrepancy between their study and the present one. JHMs exhibit juvenoidal action without structural similarity to JH, and, when applied before blastokinesis, they disrupt embryogenesis.[33,34] This could be the prime reason for higher susceptibility of younger eggs compared with older ones in both modes of exposure.

In the present study, pyriproxyfen exhibited both acute and delayed mortality against ACP nymphs. Acute mortality was more pronounced in early than in late ACP instars at the three higher pyriproxyfen concentrations, whereas delayed mortality was more pronounced in early than in late instars at the lowest concentration. Collectively, the results indicate that pyriproxyfen should be most effective against early ACP nymphal stages because the LC_{50} value for fifth instars was nearly threefold higher than that for first instars. Kawada *et al.*[35] reported that exposure of last-instar German cockroach, *Blatella germanica* (L.), nymphs to pyriproxyfen suppressed adult emergence, reduced reproductive capacity and caused morphological abnormalities in those adults that did emerge. Similarly, pyriproxyfen affected emergence of ACP adults from treated late instars at the two higher concentrations tested in the present study. Morphological abnormalities observed in ACP adults were similar to those

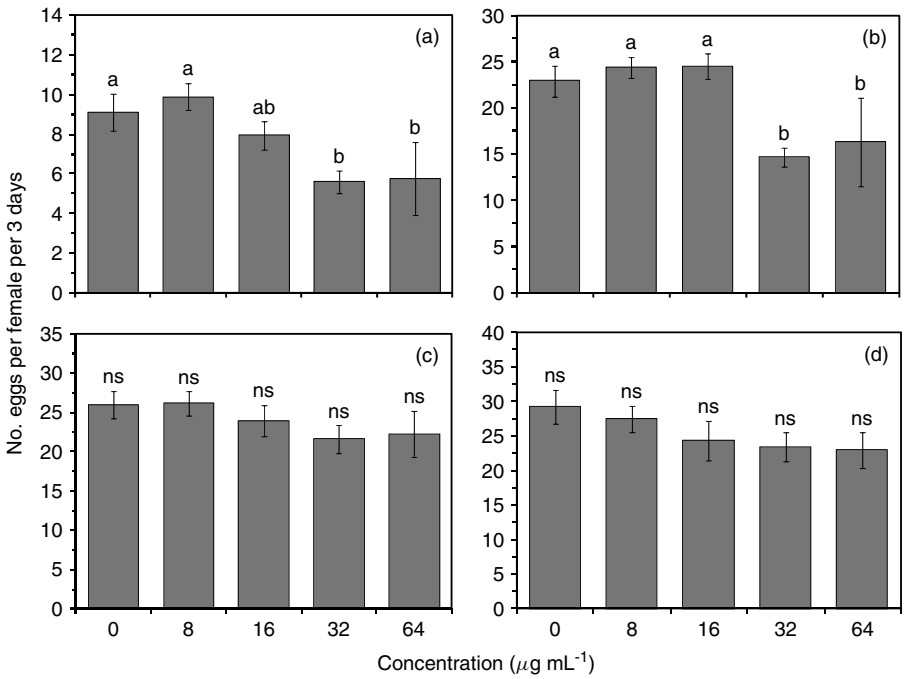

Figure 4. Fecundity of ACP females emerging from fifth instars treated with various concentrations of pyriproxyfen, observed during (a) 0–3, (b) 4–6, (c) 7–9 and (d) 10–12 days after emergence. Bars are means with SEM (n = 4–6). Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$), and bars labeled with 'ns' indicate no significant difference between treatments.

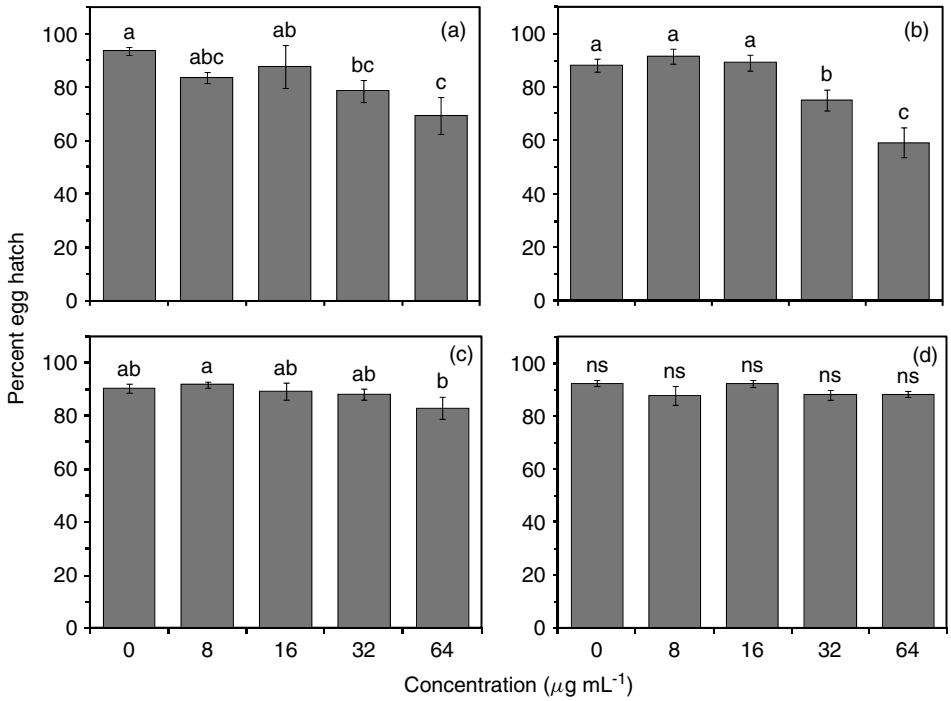

Figure 5. Viability of eggs laid by ACP females emerging from fifth instars treated with various concentrations of pyriproxyfen, observed during (a) 0–3, (b) 4–6, (c) 7–9 and (d) 10–12 days after emergence. Bars are means with SEM ($n = 4–6$). Bars not labeled by the same letter are significantly different from one another according to LSD ($P < 0.05$), and bars labeled with 'ns' indicate no significant difference between treatments.

observed with *B. germanica* after such exposure.[36] However, the authors did not notice development of supernumerary instars in treated ACP nymphs as was observed with some aphid species [*Aphis glycines* Matsamura and *Lipaphis erysimi* (Kaltenbach)].[21,24] Although a small proportion of treated ACP nymphs developed into normal adults, they were unable to emerge completely from exuviae and eventually died.

When applied at higher concentrations to late instars, pyriproxyfen appeared to affect normal development of ovaries and/or eggs of emerging female adults. Similarly, it appeared to affect egg development when applied topically to newly emerged adults. Congruent with the present results, pyriproxyfen affected the growth of ovaries in *B. germanica* when applied to female adults, resulting in reduced egg viability.[37] The negative effects of pyriproxyfen on ACP reproduction persisted for 6 days after emergence and subsided thereafter, possibly owing to the restoration of normal JH titers following degradation of this externally applied IGR. In the present study, pyriproxyfen did not cause mortality when 0.04 μg was applied topically to adults.

The strong ovicidal and larvicidal activity of pyriproxyfen at 32 μg mL^{-1} (half the field rate) or at 64 μg mL^{-1} (the full field rate) against younger eggs and early instars of ACP, respectively, indicates that application of pyriproxyfen targeting freshly laid eggs and freshly hatched nymphs would maximize efficacy in the field. Furthermore, residual activity of pyriproxyfen at these rates on younger eggs provides a short window of flexibility in field applications. The deleterious effects of pyriproxyfen application at the above rates on the development of late instars to the adult stage and on reproduction would contribute to population reductions over time as the number of viable breeding adults as well as their fecundity and egg viability are reduced. Future studies are needed to determine the duration of effective residual activity for pyriproxyfen at 32 or 64 μg mL^{-1} against eggs and nymphs under field conditions. As ACP oviposit only on the new flush, information on residual activity at the above rates in the field would be helpful in determining the possibility of split applications at half field rates for protection of new flush for an extended period.

Overall, the present study demonstrates that pyriproxyfen has potential for ACP management. Current management practices for ACP are dominated by broad-spectrum insecticides including organophosphates and synthetic pyrethroids. Inclusion of IGRs for ACP management as part of an IPM program would diversify the current limited modes of action that are predominantly used for managing this pest. Use of new chemistries with unique modes of action would also aid in insecticide resistance management. Several studies have reported that pyriproxyfen has no adverse effects on beneficial insects,[38–41] while others have reported negative impacts.[42,43] Therefore, the effect of pyriproxyfen on natural enemies of ACP in citrus requires evaluation to determine its compatibility with biological control.

5 CONCLUSIONS

In laboratory studies, pyriproxyfen exhibited marked activity against younger eggs and early instars in a concentration-dependent manner, but did not cause mortality in adults. Pyriproxyfen significantly suppressed adult emergence and resulted in morphological abnormalities. Furthermore, treatment of fifth instars and newly emerged adults resulted in significant reduction in female fecundity and egg viability. The direct and indirect effects of pyriproxyfen against ACP eggs, nymphs and adults may render it a useful component of an IPM program for ACP. Further field-scale testing is needed to determine how best to incorporate pyriproxyfen into an IPM program for ACP.

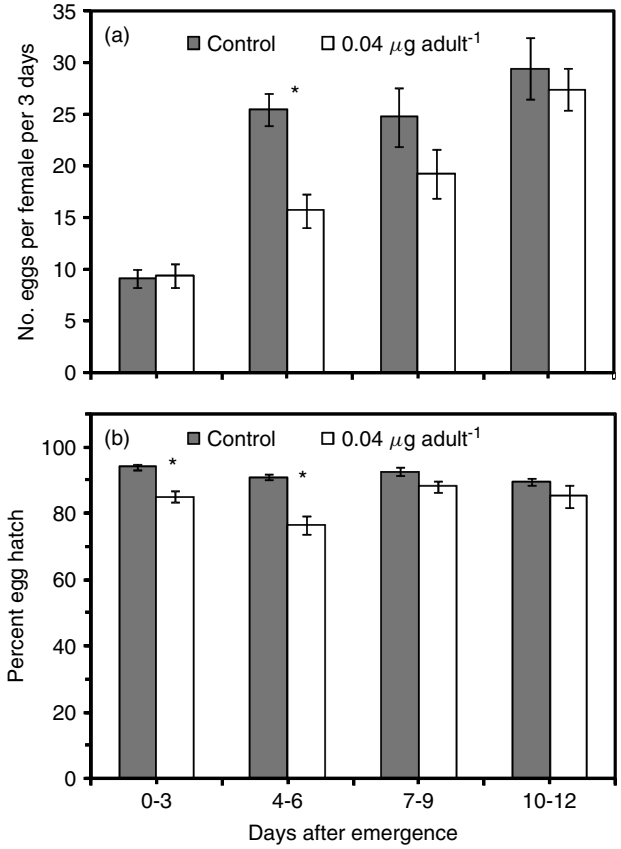

Figure 6. Fecundity (a) and viability of eggs (b) of ACP adult females treated topically with 0.04 μg of pyriproxyfen per adult. Treated female fecundity/egg viability was measured at 0–3, 4–6, 7–9 and 10–12 days after emergence. Bars are means with SEM ($n = 5$). Pair of bars with an asterisk at a given day after emergence (x axis) indicates significant difference between treatment and the control according to independent sample t-tests ($P < 0.05$).

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