Research Article

Received: 3 October 2011 Revised: 21 February 2012 Accepted article published: 19 March 2012 Published online in Wiley Online Library: 31 May 2012

(wileyonlinelibrary.com) DOI 10.1002/ps.3323

Effects of buprofezin and diflubenzuron on various developmental stages of Asian citrus psyllid, *Diaphorina citri*

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Abstract

BACKGROUND: *Diaphorina citri* populations in Florida are developing resistance to commonly used neurotoxic insecticides. Alternatives to neurotoxins, such as insect growth regulators, are needed to control this season-long subtropical pest to prevent or delay development of insecticide resistance. In the present investigation, two insect growth regulators (IGRs), buprofezin and diflubenzuron, were evaluated against various developmental stages of *D. citri*.

RESULTS: The 0–1-day-old *D. citri* eggs were more susceptible to buprofezin and diflubenzuron than the 3–4-day-old eggs. Adult emergence was completely suppressed by treating first- or third-instar nymphs with buprofezin or diflubenzuron at 30–240 or 23–184 μg mL^{-1} rates respectively. Treatment of fifth-instar nymphs with diflubenzuron at a rate of 184 μg mL^{-1} and with buprofezin at 30–240 μg mL^{-1} rates resulted in approximately 20 and 15–80% reductions in adult emergence respectively. The mean number of eggs per plant was reduced at 5 days after topical treatment with diflubenzuron. Mean egg hatch per plant was reduced at 5 and 6–15 days after topical treatments with buprofezin and diflubenzuron respectively.

CONCLUSION: Buprofezin and diflubenzuron effectively suppressed *D. citri* adult emergence. *D. citri* were more susceptible as early (first–third-instar) than late (fifth-instar) nymphs. Both IGRs inhibited egg production and egg hatch. Reduction in the number of subsequent offspring suggests reduced vertical transmission of *Candidatus* Liberibacter asiaticus, the pathogen thought to cause citrus greening disease. The present results indicate that both IGRs tested here should be effective tools for rotation in insecticide-based *D. citri* management programs.

Keywords: biorational insecticide; chitin synthesis inhibitor; insect growth regulator; citrus greening; huanglongbing; insecticide toxicity; integrated pest management; ovicide

1 INTRODUCTION

Citrus greening, also known as huanglongbing, is one of the most destructive and economically important diseases of citrus throughout the world. Produce loss due to premature fruit drop, increased fruit bitterness and distorted fruit shape causes economic losses.[1,2] Trees infected with greening eventually die within 5–10 years. Infected trees left in managed or abandoned citrus groves can be sources of pathogen inoculum, increasing spread of the disease.[3] Currently, the greening infection rate in central Florida is estimated to be between 1.4 and 3.6%, and may be up to 100% in the southern and eastern parts of Florida.[3,4] The severe impact of greening on citriculture has led to implementation of rigorous management programs for suppression of the vector (Asian citrus psyllid, *Diaphorina citri* Kuwayama) that transmits the putative greening pathogen *Candidatus* Liberibacter asiaticus.

D. citri that are infected with *Candidatus* Liberibacter asiaticus are less susceptible to insecticides than uninfected counterparts.[5,6] Currently, *D. citri* populations are suppressed with foliar (6–8 year^{-1}) and soil (1–2 year^{-1}) applications of insecticides.[5,7] In some cases, repeated use of the same insecticide or insecticides with a similar mode of action (MOA) has led to the development of varying levels of resistance in *D. citri* populations throughout Florida.[7] Therefore, sustainable *D. citri* management programs will require integration of several management tactics and insecticide classes.

Insect growth regulators (IGRs) are useful components of integrated pest management programs owing to their low vertebrate toxicity, selectivity and unique mode of action.[8] IGRs act by disrupting the molting process, or cuticle formation, during specific developmental stages in insects.[8] Buprofezin and diflubenzuron inhibit chitin synthesis, resulting in abnormal deposition of endocuticle and molting disruption, ultimately causing premature death.[9,10] Buprofezin (2-*tert*-butylimino-3-isopropyl-5-phenyl-3,4,5,6-tetrahydro-2-thiadiazine-4-one) is effective against a wide range of hemipteran pests, including *Aonidiella aurantii, Bemisia tabaci, Nilaparvata lugens* and *Trialeurodes vaporariorum*.[11–16] Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea] is effective in the management of *Cacopsylla melanoneura, C. pyri, Lambdina*

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fiscellaria fiscellaria, Lymantria dispar and *Spodoptera littoralis*.[17–21] IGRs are effective against several phloem-feeding and sap-sucking insects and thus are suitable candidates for integration into *D. citri* management programs.

Given the extensive use of neurotoxins and existing resistance levels to the neonicotinoid imidacloprid in Florida,[7] the direct and indirect effects of insecticides with alternative modes of action, such as IGRs, require investigation. The objective of this laboratory study was to quantify the effects of two IGRs, buprofezin and diflubenzuron, on various developmental stages of *D. citri.* The lethal and sublethal effects of these two IGRs on the development of *D. citri* nymphs and female fertility/fecundity were investigated.

2 MATERIALS AND METHODS

2.1 *D. citri* culture and insecticides

D. citri adults and nymphs were drawn from a laboratory culture maintained in a greenhouse at the Citrus Research and Education Center, University of Florida. The culture was established in 2000 from field populations in Polk County, Florida (28.0′ N, 81.9′ W), prior to the discovery of greening in the state.[22] The culture is maintained on sweet orange [*Citrus sinensis* (L.) Osbeck] seedlings without exposure to insecticides at 27–28 °C, 60–65% RH and a 14 : 10 (L : D) photoperiod. Analytical-grade buprofezin (purity 99.1%) and diflubenzuron (purity 99.5%) were obtained from Chemservice (West Chester, PA). Various concentrations of each IGR were diluted in tap water for spray application treatments or in acetone for topical application treatments, described below.

2.2 Effect of buprofezin and diflubenzuron on *D. citri* egg mortality via topical spray

Four potted 'Swingle' *Citrus aurantiifolia* (Christm.) plants (2–3 months old) with new flush, as defined by Hall and Albrigo,[23] were placed into each of 36 plexiglass cages (40 cm × 40 cm × 40 cm) with fine mesh sleeves for easy access. Plants were exposed to 200–300 adult *D. citri* of mixed gender for mating and oviposition for 48 h at 25 ± 2 °C, 50 ± 5% RH and a 14 : 10 (L : D) photoperiod. Thereafter, adults were removed from each cage, and the number of eggs per flush was recorded under a stereomicroscope. From each cage, two plants were used to obtain 0–1-day-old eggs, and the remaining two plants were used to obtain 3–4-day-old eggs. Plants designated for 0–1-day-old eggs were immediately sprayed with one of five concentrations (treatments) of either IGR (buprofezin: 10, 20, 40, 80 and 160 μg mL^{-1}; diflubenzuron: 10, 20, 40, 80 and 160 μg mL^{-1}) dissolved in water until run-off using a handheld atomizer (The Bottle Crew, West Bloomfield, MI). Water alone served as the control. Concentrations of each IGR were selected on the basis of preliminary results from tests with concentrations causing 5–95% mortality. Each treatment was replicated 6–8 times. In some cases, eggs in discrete groups on a single plant were used as replicates for each treatment. Each IGR was tested individually with separate control treatments. Five days after treatment, eggs were examined under a stereomicroscope, and the number of eggs that hatched per plant was recorded. Plants designated for the 3–4-day-old egg treatments were held for another 48 h before spray applications were made using the previously described concentration treatments. Plants designated for 0–1-day-old eggs were also visually inspected for the presence of eggs older than 1 day and, if found, were removed. Likewise, plants designated for 3–4-day-old eggs were inspected for younger eggs and, if found, were removed. The 0–1-day-old and 3–4-day-old eggs were distinguished from each other on the basis of their coloration: 0–1-day-old eggs were characterized by a pale dull yellow color, and 3–4-day-old eggs appeared bright solid yellow.

The percentage egg hatch was calculated using the total number of eggs recorded before treatment and the number of hatched eggs recorded 5 days post-treatment. The percentage egg hatch across treatments was tested to ensure that the assumptions of homogeneity of variance and normality were met before data were analyzed. Four separate one-way analyses of variance (ANOVAs) and Fisher's protected LSD mean separation tests (PROC GLM)[24] were performed to compare the mean percentage egg hatch among the various concentrations of each IGR tested for each egg age. Lethal concentrations of each IGR, causing 50% egg hatch inhibition (LC_{50}) with corresponding 95% confidence intervals (CIs) for 0–1- and 3–4-day-old eggs, were determined using probit analysis (PROC PROBIT).[24]

2.3 Effect of buprofezin and diflubenzuron on *D. citri* nymphs and adult emergence

The above-described plexiglass cages were used to evaluate the effects of buprofezin and diflubenzuron on *D. citri* nymph development and adult emergence. Each plexiglass cage contained four potted flushing 'Swingle' plants (2–3 months old). A total of 200–300 newly emerged *D. citri* females and males (∼1:1 ratio) were released into each cage. Adults were allowed to mate and oviposit for 48 h on flush. After 5 days, the number of emerged nymphs per plant was recorded using a stereomicroscope. Remaining unhatched eggs were removed. Plants containing newly emerged nymphs were divided into three sets: each set comprised seven cages designated for *D. citri* development into first-, third- or fifth-instar nymphs prior to treatment. Plants within each nymph age were sprayed with one of the four concentrations (treatments) of each IGR (buprofezin: 30, 60, 120 and 240 μg mL^{-1}; diflubenzuron: 23, 46, 92 and 184 μg mL^{-1}) dissolved in water or water alone (control) until run-off. Each treatment was replicated 5–6 times within each set of plants, and the experiment was repeated 2 times. Each IGR was evaluated separately with corresponding control treatments. In some cases, nymphs of similar age in discrete groups on a single plant were used as replicates for each treatment. Treated plants with nymphs were maintained under controlled conditions until adult emergence, as described in Section 2.2. The number of nymphs per plant or per branch for each instar was counted prior to treatment application. Subsequently, the number of nymphs molting into subsequent instars and also adult emergence were quantified for each treatment. One-way ANOVAs and Fisher's protected LSD mean separation tests (PROC GLM) were performed to compare mean percentage survivorship between two successive developmental stages among various concentrations of IGR and control.[24] The lethal concentration of each IGR resulting in 50% mortality (LC_{50}) for each instar was calculated using probit analysis (PROC PROBIT) with corresponding 95% CIs.[24]

2.4 Effect of buprofezin and diflubenzuron on *D. citri* oviposition and egg hatch

Adults obtained from plants treated with various concentrations of IGR or a water control during the fifth-instar nymph (as described in Section 2.3) were used to evaluate the effects of buprofezin and diflubenzuron on the fecundity and fertility of emerging *D. citri* females. The sex of these adults was determined, and three pairs of males and females emerging from each treatment were transferred

onto an untreated flushing plant. Each plant with three pairs of adults comprised a replicate, and each treatment was replicated 4 times in a plexiglass cage. Adults were allowed to feed, mate and oviposit on plants under controlled conditions within plexiglass cages, as described earlier. After 2 days, the number of eggs per plant was recorded, and, after 5 days, the number of hatched eggs was counted. One-way ANOVA followed by Fisher's protected LSD mean separation (PROC GLM)[24] was performed to compare the mean number of eggs that were oviposited or that hatched for each treatment.

The effects of buprofezin and diflubenzuron on fecundity and fertility of *D. citri* were also quantified after treating newly emerged males and females from the laboratory culture. Adults were treated using a topical application method.[7] Two concentrations (treatments) of each IGR (buprofezin: 120 and 240 μg mL^{-1}; diflubenzuron: 92 and 184 μg mL^{-1}) diluted in acetone versus a control (only acetone) were compared by topical application to the abdomen of *D. citri*. Treatments were applied using a 10 μL Hamilton syringe (Hamilton County, Reno, NV) with 0.2 μL of total solution per adult. Treatments were replicated 4 times, and each replicate consisted of three pairs of male and female *D. citri*. Treated adults were allowed to mate and oviposit on an untreated flushing citrus plant. Plants were maintained under controlled conditions as described in Section 2.2. *D. citri* adults were removed from plants at 1, 5 and 10 days after treatment, and the number of eggs per plant was counted. The number of hatched eggs or first-instar nymphs per plant was also counted 6, 10 and 15 days after treatment. The percentage egg hatch across treatments was tested to ensure that the assumptions of homogeneity of variance and normality were met before data were analyzed. The mean number of eggs and the percentage egg hatch per plant were compared between treatments using ANOVA followed by Fisher's protected LSD mean separation (PROC GLM)[24] for each observation day within each IGR treatment.

3 RESULTS

3.1 Effect of buprofezin and diflubenzuron on *D. citri* egg mortality

The higher concentrations of both buprofezin and diflubenzuron tested caused a reduction in egg hatch when compared with the controls. The concentration of buprofezin was a significant factor affecting mean percentage egg hatch of both 0–1-day-old eggs ($F = 233.50$; df = 5, 38; $P < 0.0001$) (Fig. 1A) and 3–4-day-old eggs ($F = 48.53$; df = 5, 45; $P < 0.0001$) (Fig. 1B). Likewise, the concentration of diflubenzuron significantly affected mean percentage egg hatch of both 0–1-day-old eggs ($F = 15.44$; df = 5, 12; $P < 0.0001$) (Fig. 1C) and 3–4-day-old eggs ($F = 16.42$; df = 5, 12; $P < 0.0001$) (Fig. 1D). Buprofezin application at 20 μg mL^{-1} or higher rates caused a significant reduction in egg hatch in both 0–1- and 3–4-day-old *D. citri* eggs. For example, buprofezin caused 88 and 66% reductions in egg hatch of 0–1- and 3–4-day-old eggs, respectively, when applied at a rate of 160 μg mL^{-1}. The lowest concentration of diflubenzuron to reduce

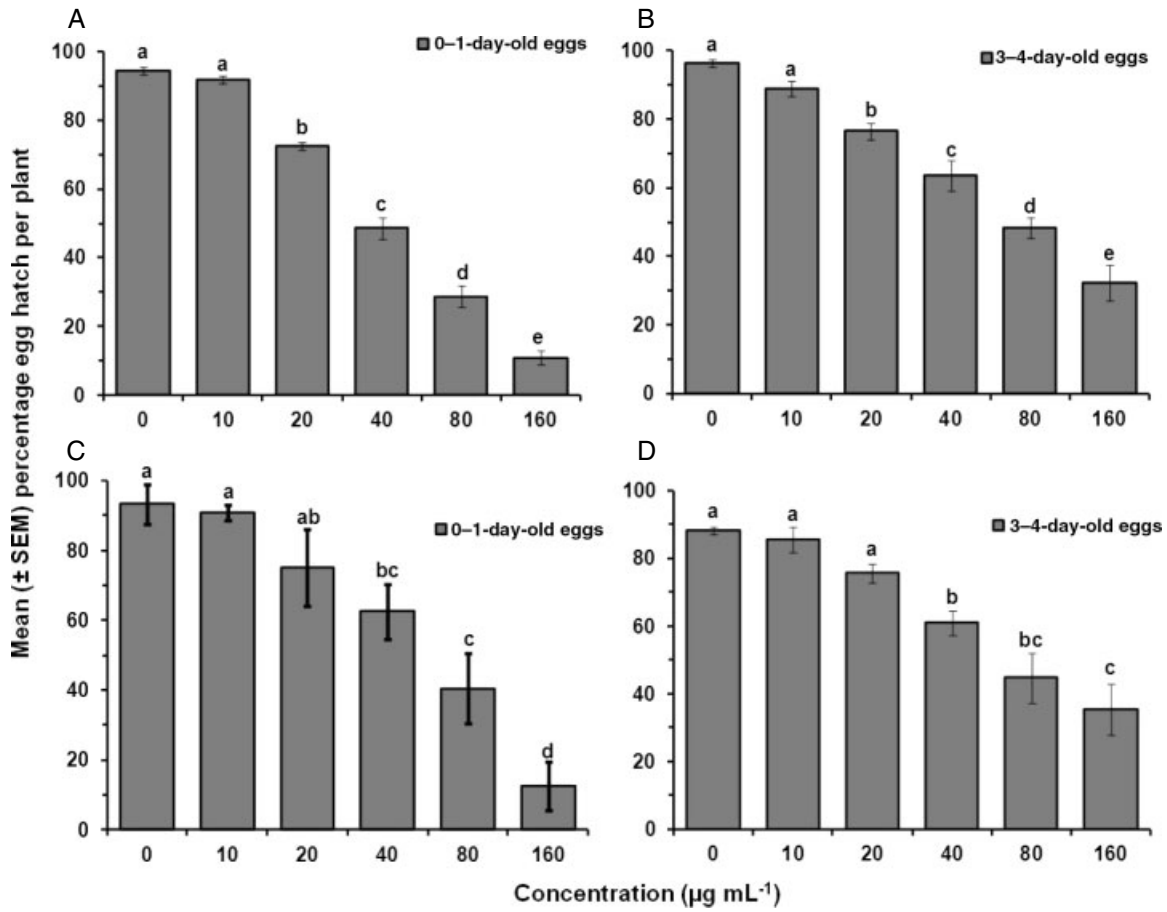

Figure 1. Mean percentage hatch of *D. citri* eggs treated with various concentrations of buprofezin (A and B) and diflubenzuron (C and D). Bars not labeled by the same letters within each egg age and insecticide treatment are significantly different from one another ($P < 0.05$).

Table 1. Toxicity of buprofezin and diflubenzuron to various developmental stages[a] of *Diaphorina citri*

Developmental stage	*n*	LC_{50} (mg L^{-1}) (95% CI)	LC_{90} (mg L^{-1}) (95% CI)	Slope ± SEM	χ^2
Buprofezin					
0–1-day-old egg[b]	1218	41.09 (37.64–44.89)	166.09 (142.15–199.89)	2.11 ± 0.11	2.37
3–4-day-old egg[b]	1755	65.39 (51.93–82.64)	443.67 (299.42–780.94)	1.54 ± 0.11	10.70
First-instar nymph[c]	456	21.25 (10.19–31.40)	387.46 (228.42–1120.00)	1.01 ± 0.19	4.40
Second-instar nymph[c]	137	33.85 (29.16–38.55)	62.35 (51.50–92.31)	4.83 ± 0.96	0.14
Diflubenzuron					
0–1-day-old egg[b]	738	56.48 (50.57–63.76)	226.59 (178.99–309.45)	2.12 ± 0.16	3.58
3–4-day-old egg[b]	636	69.17 (57.01–87.36)	596.49 (378.46–1137.00)	1.37 ± 0.13	0.06
First-instar nymph[c]	640	28.33 (19.60–36.38)	429.56 (265.91–974.47)	1.08 ± 0.15	2.43
Second-instar nymph[c]	106	20.47 (11.63–26.76)	56.16 (42.37–105.82)	2.92 ± 0.74	0.27

[a] The LC_{50} values for subsequent instars were not calculated because of the lack of concentration ranges resulting in 10–90% mortality for those instars.
[b] Percentage egg hatch was calculated using the total number of eggs recorded before treatment and the number of hatched eggs recorded 5 days post-treatment.
[c] Mean percentage mortality between two successive nymphal instars among various concentrations of IGR and the control was used to calculate the lethal concentration of each IGR resulting in 50% mortality (LC_{50}) for each instar using probit analysis (PROC PROBIT) with corresponding 95% CIs.[24]

egg hatch was 40 µg mL^{-1} for both 0–1- and 3–4-day-old eggs. Furthermore, diflubenzuron caused 86 and 60% reductions in egg hatch in 0–1- and 3–4-day-old eggs, respectively, when applied at a rate of 160 µg mL^{-1}. The 0–1-day-old eggs were significantly more susceptible than the 3–4-day-old eggs to buprofezin, based on non-overlapping confidence intervals at 95% of associated LC_{50} values (Table 1). However, egg susceptibility to diflubenzuron did not differ between 0–1- and 3–4-day-old eggs (Table 1).

3.2 Effect of buprofezin and diflubenzuron on *D. citri* nymphs and adult emergence

Buprofezin reduced the survival of molting instars and adult emergence when applied to various stages of *D. citri* development. Treatment of first-instar nymphs with each concentration of buprofezin significantly reduced the emergence of second-instar nymphs (mean percentage reduction = 57.04 ± 14.84) (F = 118.03; df = 4, 46; $P < 0.0001$), third-instar nymphs (mean percentage reduction = 76.61 ± 17.82) (F = 409.66; df = 4, 46; $P < 0.0001$), fourth-instar nymphs (mean percentage reduction = 81.57 ± 18.19) (F = 1715.42; df = 4, 46; $P < 0.0001$) and fifth-instar nymphs (mean percentage reduction = 82.53 ± 17.40) (F = 2079.10; df = 4, 46; $P < 0.0001$). In addition, treatment of first-instar nymphs with various concentrations of buprofezin significantly reduced adult emergence (F = 1605.37; df = 4, 46; $P < 0.0001$) (Fig. 2A). Treatment of first-instar nymphs with the 240 µg mL^{-1} concentration resulted in 13.5, 0.7, 0.0, 0.0 and 0.0% survival of second, third, fourth and fifth instars and adult stages respectively. Likewise, treatment of third-instar nymphs with various concentrations of buprofezin significantly reduced the emergence of fourth-instar nymphs (mean percentage

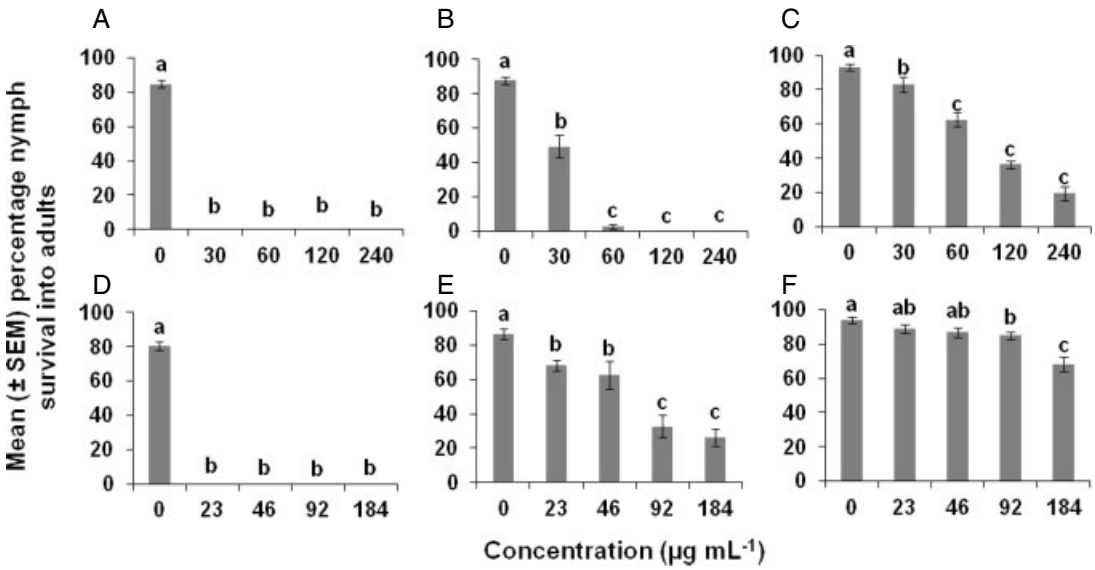

Figure 2. Mean percentage survival of *D. citri* nymphs into adults after exposure to various concentrations of buprofezin (A, B and C) and diflubenzuron (D, E and F) when treated as first-instar nymphs (A and D), third-instar nymphs (B and E) and fifth-instar nymphs (C and F). Bars not labeled by the same letter are significantly different from one another ($P < 0.05$).

reduction = 12.71 ± 3.82) ($F = 9.19$; df = 4, 52; $P < 0.0001$) and fifth-instar nymphs (mean percentage reduction = 60.25 ± 18.47) ($F = 246.93$; df = 4, 52; $P < 0.0001$). Treatment of third-instar nymphs with each concentration of buprofezin significantly reduced adult emergence ($F = 156.67$; df = 4, 52; $P < 0.0001$), and no adults emerged after treatment with the two highest concentrations tested (Fig. 2B). Treatment of fifth-instar nymphs with various concentrations of buprofezin also significantly reduced adult emergence ($F = 80.43$; df = 4, 45; $P < 0.0001$) (Fig. 2C). The lethal concentration of buprofezin resulting in 50% mortality (LC_{50}) was not significantly different between first and second instars, based on overlapping confidence intervals at 95% (Table 1). The LC_{50} values for subsequent instars were not calculated owing to the lack of concentration ranges resulting in 10–90% mortality.

Diflubenzuron application caused similar effects to buprofezin, reducing survival of molting instars and adult emergence when applied to various stages of development. Treatment of first-instar nymphs with various concentrations of diflubenzuron significantly reduced emergence of second-instar nymphs (mean percentage reduction = 56.08 ± 14.10) ($F = 39.88$; df = 4, 47; $P < 0.0001$), third-instar nymphs (mean percentage reduction = 76.93±16.94) ($F = 198.22$; df = 4, 47; $P < 0.0001$), fourth-instar nymphs (mean percentage reduction = 82.90 ± 16.79) ($F = 866.84$; df = 4, 47; $P < 0.0001$) and fifth-instar nymphs (mean percentage reduction = 83.92 ± 16.08) ($F = 1113.36$; df = 4, 47; $P < 0.0001$). In addition, treatment of first-instar nymphs with various concentrations of diflubenzuron eliminated adult emergence ($F = 1246.91$; df = 4, 47; $P < 0.0001$) (Fig. 2D). Treatment of first-instar nymphs with the 184 μg mL^{-1} concentration resulted in 18.0, 0.8, 0.0, 0.0 and 0.0% survival of second, third, fourth and fifth instars and adult stages respectively. Likewise, treatment of third-instar nymphs with each concentration of diflubenzuron tested significantly reduced emergence of fourth-instar nymphs (mean percentage reduction = 19.88 ± 6.83) ($F = 23.01$; df = 4, 45; $P < 0.0001$) and fifth-instar nymphs (mean percentage reduction = 32.46 ± 9.21) ($F = 20.38$; df = 4, 45; $P < 0.0001$). In addition, treatment of third-instar nymphs with various concentrations of diflubenzuron significantly reduced adult emergence ($F = 20.70$; df = 4, 45; $P < 0.0001$) (Fig. 2E). Treatment of fifth-instar nymphs with various concentrations of diflubenzuron also significantly reduced adult emergence ($F = 11.91$; df = 4, 45; $P < 0.0001$) (Fig. 2F), and the effect was greatest at the two highest concentrations tested. The LC_{50} for diflubenzuron was not significantly different between first- and second-instar nymphs, based on overlapping 95% confidence intervals (Table 1). The LC_{50} values for subsequent instars were not calculated because of lack of concentration ranges resulting in 10–90% mortality.

3.3 Effect of buprofezin and diflubenzuron on *D. citri* oviposition and egg hatch

Neither IGR tested affected the total number of eggs oviposited in comparison with controls when applied to fifth-instar nymphs; however, percentage egg hatch was significantly reduced at the highest concentrations of both IGRs tested. The concentration of buprofezin did not significantly affect the number of eggs oviposited ($F = 1.87$; df = 4, 15; $P = 0.1679$) (Fig. 3A), but did negatively affect subsequent egg hatch ($F = 5.28$; df = 4, 15; $P = 0.0074$) (Fig. 3B) of females emerging from fifth-instar nymphs. The two highest concentrations of buprofezin reduced egg viability by 15 and 23%, respectively, when compared with the control treatment. The mean number of eggs oviposited was not significantly reduced in females emerging from fifth-instar nymphs treated with various concentrations of diflubenzuron in comparison with the control ($F = 1.66$; df = 4, 15; $P = 0.2117$) (Fig. 3C). Mean percentage egg hatch per plant was significantly reduced ($F = 3.12$; df = 4, 15; $P = 0.0468$) (Fig. 3D) in females emerging from fifth-instar nymphs treated with various concentrations of diflubenzuron in comparison with the control. The highest concentration of diflubenzuron tested reduced the viability of eggs by 24% when compared with the control.

The concentrations of buprofezin tested did not reduce numbers of eggs oviposited in comparison with the control (day 1: $F = 3.72$; df = 2, 9; $P = 0.0665$; day 5: $F = 1.91$; df = 2, 9; $P = 0.2037$; day 10: $F = 2.18$; df = 2, 9; $P = 0.1686$) (Fig. 4A) when applied to newly emerged adults. Furthermore, percentage egg hatch was not reduced in comparison with the control at 6 days after application ($F = 4.14$; df = 2, 9; $P = 0.530$) and at 15 days after application ($F = 3.88$; df = 2, 9; $P = 0.0611$). However, mean percentage egg hatch 10 days after treatment was significantly reduced for buprofezin-treated adults in comparison with the control ($F = 16.87$; df = 2, 9; $P = 0.0009$) (Fig. 4B). Treating female *D. citri* with diflubenzuron did not consistently affect the number of eggs oviposited per female. The mean numbers of eggs oviposited 1 day after treatment ($F = 2.41$; df = 2, 9; $P = 0.1449$) and 10 days after treatment ($F = 0.96$; df = 2, 9; $P = 0.4192$) was not affected by diflubenzuron at any of the dosages tested. However, the mean number of eggs recorded per plant 5 days after treatment was significantly reduced in comparison with the control ($F = 4.96$; df = 2, 9; $P = 0.0353$) (Fig. 4C). Mean percentage egg hatch 6 days after treatment ($F = 6.84$; df = 2, 9; $P = 0.0156$), 10 days after treatment ($F = 12.99$; df = 2, 9; $P = 0.0022$) and 15 days after treatment ($F = 16.17$; df = 2, 9; $P = 0.0010$) with diflubenzuron was significantly reduced with the various concentrations tested in comparison with the control (Fig. 4D).

4 DISCUSSION

Citrus greening is a major threat to citriculture in Florida and other citrus-growing regions. Currently, growers depend on insecticides to reduce populations of *D. citri* in order to control the disease. This dependence has resulted in changes in insecticide susceptibility levels in several populations of *D. citri* throughout Florida.[7] Therefore, combining insecticides with diverse modes of action into comprehensive management programs is needed for sustainable management of *D. citri*. In the present work, the lethal and sublethal effects of two insect growth regulators against various developmental stages of *D. citri* were investigated. The results indicate that both buprofezin and diflubenzuron should be effective for suppression of *D. citri* populations. Currently, the labeled rate of diflubenzuron (Micromite 80WGS) against *D. citri* is 0.44 kg ha^{-1}, which is equivalent to 312.05 μg mL^{-1}. Likewise, the labeled rate of buprofezin (Applaud 70 WS) against citrus whitefly is 0.84 kg ha^{-1}, which is equivalent to 599.13 μg mL^{-1}.

Both buprofezin and diflubenzuron reduced the progression of *D. citri* through each life stage. In addition, both IGRs decreased fecundity of *D. citri* females and decreased egg viability. The percentage egg hatch was reduced when eggs were exposed to 20 μg mL^{-1} or higher concentrations of buprofezin and 40 μg mL^{-1} or higher concentrations of diflubenzuron. However, when nymphs were exposed, the percentage egg hatch was reduced only at 120 μg mL^{-1} or higher concentrations of

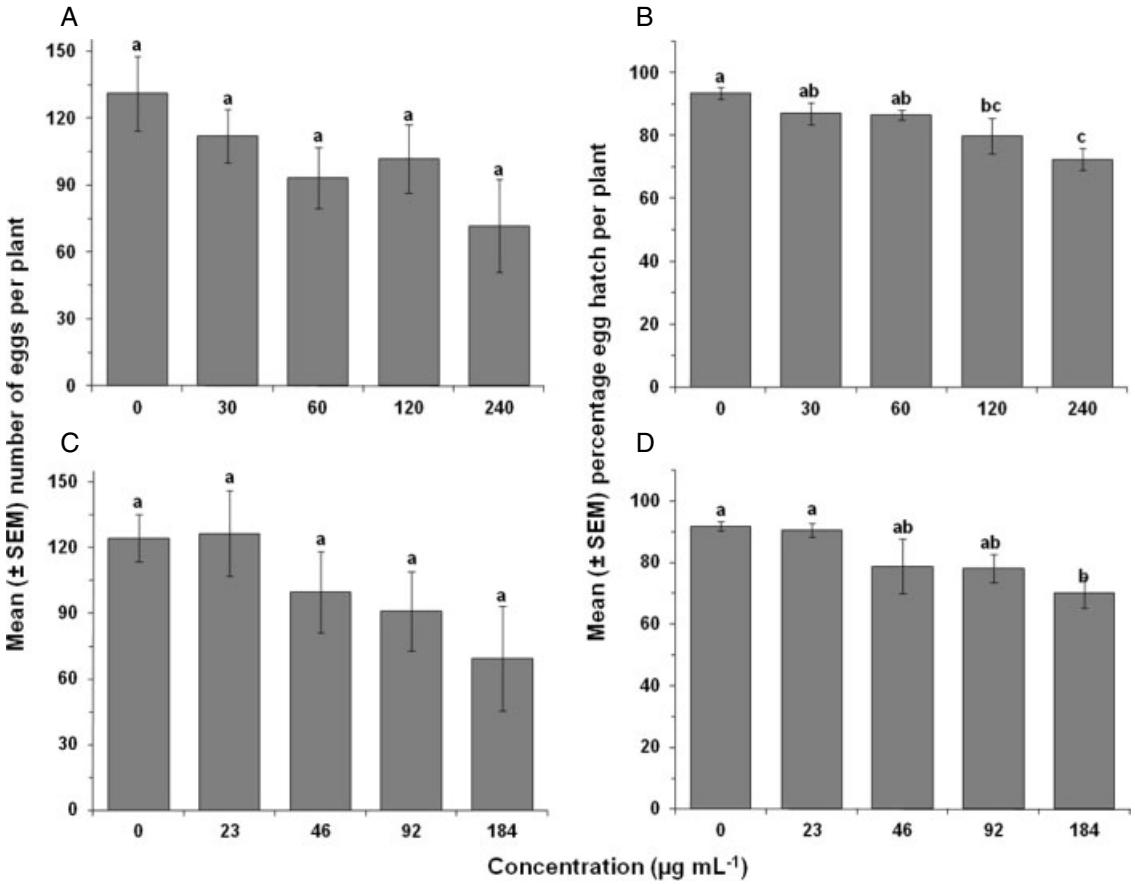

Figure 3. Mean number of eggs (A and C) and mean percentage egg hatch (B and D) per plant from *D. citri* females emerging from fifth-instar nymphs treated with various concentrations of buprofezin (A and B) and diflubenzuron (C and D). Bars not labeled by the same letter are significantly different from one another ($P < 0.05$).

buprofezin and at 184 µg mL^{-1} of diflubenzuron. However, field studies are needed with formulated materials of these compounds to confirm the laboratory results. In keeping with the present results, diflubenzuron reduces egg hatch of citrus root weevil, *Diaprepes abbreviates*, by 98%,[25] while buprofezin reduces egg hatch of *Bemisia tabaci* by 13–26%.[26] The 0–1-day-old *D. citri* eggs were more susceptible than 3–4-day-old eggs to each IGR tested. Less mature eggs (24–30 h old) have been characterized by a thinner embryonic membrane with greater permeability than mature eggs (48–54 h old) for *Diabrotica undecimpunctata howardi*.[27] Therefore, younger eggs may be more permeable to IGR treatments than older eggs.

Both IGRs tested also inhibited molting of *D. citri* nymphs. Molting of first-instar nymphs into subsequent instars was inhibited by 30–240 and 23–184 µg mL^{-1} of buprofezin and diflubenzuron respectively. In addition, first-instar *D. citri* nymphs were more susceptible to each IGR tested than eggs. The present findings are consistent with results from earlier investigations, in which IGR treatments caused greater reduction in survival rates of earlier- than later-instar nymphs of various insect species.[28–32] The greater susceptibility of earlier than later instars to each IGR tested here suggests possible age-dependent differences between detoxifying mechanisms in various developmental stages. Levels of detoxifying enzymes have been reported to vary among developmental stages of *D. citri*.[33] Future experiments are needed fully to evaluate the timing of IGR treatment relative to oviposition, as well as the effects of weathering on residue longevity.

The present results indicate that buprofezin and diflubenzuron cause sublethal effects with respect to female fertility when applied to fifth-instar nymphs and adult females. Egg hatch was negatively affected by each IGR tested here. Egg hatch was reduced in a concentration-dependent manner by both buprofezin and diflubenzuron. Egg hatch was reduced up to 15 days after treatment with diflubenzuron, and up to 10 days after treatment with buprofezin, suggesting 1–2 weeks of possible field efficacy. The present results with *D. citri* are consistent with previous investigations of the effects of IGRs. Similar results were observed with *Cydia pomonella, Leptinotarsa decemlineata*, and *Tribolium castaneum* when treated with novaluron,[34–36] a chitin synthesis inhibitor, and for *B. tabaci* when treated with buprofezin.[13]

Both buprofezin and diflubenzuron reduced female fertility, whether they were applied directly to adult females or fifth-instar nymphs prior to adult emergence. Buprofezin and diflubenzuron can suppress prostaglandin formation and vitellogenesis during embryogenesis.[37,38] Likewise, pyriproxyfen reduced *D. citri* egg development by affecting the growth of ovaries.[28] As *Candidatus* Liberibacter asiaticus is transmitted transovarially from parent to offspring at a rate of 2–6%,[39,40] reduced egg hatch of 0–1-day-old eggs after treatment with the higher dosages tested here should reduce vertical transmission of the citrus greening causal agent.

Direct mortality of eggs and nymphs as a result of treatment with buprofezin or with diflubenzuron and also sublethal effects

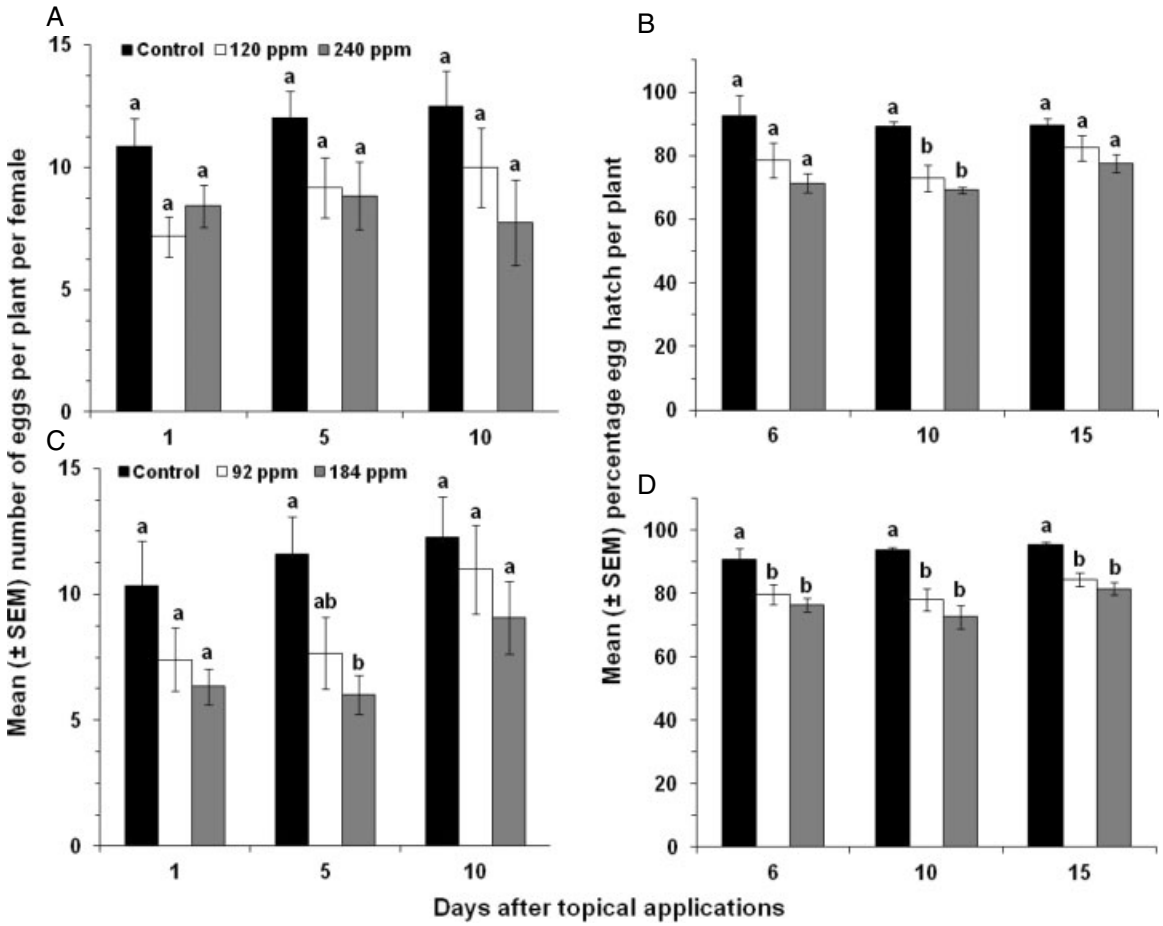

Figure 4. Mean number of eggs (A and C) and mean percentage egg hatch (B and D) per plant per *D. citri* female from adults treated topically with two concentrations of buprofezin (A and B) and diflubenzuron (C and D) in comparison with acetone (control). Bars not labeled by the same letter within each day of observation are significantly different from one another ($P < 0.05$).

on females suggest that these insecticides should be useful components of a management strategy for *D. citri*. Early instars are more susceptible than later instars, and thus applications of each IGR may be more effective when directly coinciding with the availability of citrus leaf flush for *D. citri* egg laying. IGRs have been reported as a relatively safe alternative to broad-spectrum insecticides owing to their specific mode of action.[31,41] The present results suggest that the inclusion of buprofezin and diflubenzuron with other insecticides into current management programs for *D. citri* should be useful for population suppression and may help delay the onset of resistance to broad-spectrum neurotoxins for populations of this pest. However, IGRs are slower acting than conventional insecticides[29] and are stage specific, and therefore effective timing of application will likely be more important than that needed with neurotoxins.[13] The present laboratory results indicate the efficacy of buprofezin and diflubenzuron against *D. citri*; however, further field testing is needed.

ACKNOWLEDGEMENTS

This project was supported by a grant from the Citrus Research and Development Foundation to LLS. The authors are grateful to A Hoyte, I Jackson and M Flores for *D. citri* collections and technical assistance. They thank Wendy Meyer for reviewing earlier versions of the manuscript. They also thank the three anonymous reviewers for improving previous versions of the manuscript.

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