



# Behavioral and hormetic effects of the butenolide insecticide, flupyradifurone, on Asian citrus psyllid, *Diaphorina citri*



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## ABSTRACT

Huanglongbing (HLB) or citrus greening is an economically important disease of citrus associated with the bacterium '*Candidatus Liberibacter asiaticus*'. The pathogen is transmitted by the Asian citrus psyllid, *Diaphorina citri* Kuwayama. Currently, insecticide management of *D. citri* and removal of infected trees are the only means of decreasing HLB infections. Therefore, with continuous use of insecticides, resistance is one of the most important problems facing citrus production. Flupyradifurone is a new generation butenolide insecticide that has been shown to deter insect feeding and reduce pathogen transmission. We determined the concentration-mortality response of *D. citri* to flupyradifurone. We also quantified sublethal effects of flupyradifurone on *D. citri* feeding, settling, flight behaviors, as well as, fecundity of females. We found that after 24 and 48 h of exposure, the LC<sub>50</sub> was 0.39 ng/μl (0.16–0.82) in a bottle bioassay and 10.43 ng/μl (2.07–55.87) in a leaf dip bioassay, respectively. *D. citri* that fed on citrus leaves treated with 0.001, 0.02, 0.39, 8.25 and 130.13 ng/μl solution of flupyradifurone excreted significantly less honeydew (8–83%) compared with a negative control; feeding inhibition occurred in a concentration dependent manner suggesting an antifeedant effect of flupyradifurone. There was no effect of flupyradifurone on *D. citri* settling behavior at 24 and 48 h after leaf treatment. However, there were significantly fewer adults on treated leaves at 72 h after treatment compared to untreated leaves. *D. citri* exposed to sublethal concentrations of flupyradifurone initiated flight at greater frequency than negative controls. *D. citri* exposed to sublethal concentrations (LC<sub>10</sub> and LC<sub>25</sub>) of flupyradifurone laid more eggs than those treated with the negative control. Exposure to lethal and sublethal concentrations of flupyradifurone induced hormesis in *D. citri* with respect to fecundity. Our data indicate that flupyradifurone may be a useful tool for managing *D. citri*; however, unexpected sublethal effects must be considered when integrating this chemistry into management programs.

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## 1. Introduction

Huanglongbing (HLB) is an important disease of citrus in Florida and has caused over \$3.6 billion of loss in the past five years (Hodges and Spreen, 2012). HLB infected trees show various symptoms of decline including fruit drop, off season bloom, and twig dieback. Fruit from infected trees is often diminished in size, misshapen, and bitter tasting (Halbert and Manjunath, 2004). Currently, vigorous vector management that relies heavily on the use of conventional insecticides targeted against *D. citri* is the only effective means to manage HLB (Grafton Cardwell et al., 2013).

Repeated use of the same insecticide or insecticide of similar mode of action has not only proven to be economically challenging but has led to development of resistance in some populations of *D. citri* in Florida (Tiwari et al., 2011; Kanga et al., 2016). Therefore, the need for newer, safer and effective chemistries remains critical for *D. citri* management.

The butenolide insecticide, flupyradifurone, was discovered in 2012. It is an agonist targeting nicotinic acetylcholine receptors (nAChRs); this is a novel mode of action for insects (Nauen, 1995; Jeschke et al., 2015). It acts selectively on the insect central nervous systems as a partial agonist of postsynaptic nAChRs and binds to acetylcholine (ACh) (Nauen et al., 2014; Jeschke et al., 2015). Flupyradifurone has systemic properties and therefore may be important for future management of *D. citri* to prevent and delay insecticide resistance (Coy et al., 2016). Studies using a leaf dip

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bioassays indicated that flupyradifurone shows little or no cross resistance among strains of greenhouse whiteflies, *Trialeurodes vaporariorum* (Westwood) and green peach aphids, *Myzus persicae* (Sulzer) (Nauen et al., 2014) metabolically resistant to imidacloprid. It may be a new resistance management tool for use in insecticide rotational program for sustainable pest control in the future (Smith and Giurcanu, 2013; Jeschke et al., 2015).

Sublethal effects that induce hormesis or change insect behavior may occur after exposure to an insecticide and have been documented for several pests such as diamondback moth, *Plutella xylostella* (Linnaeus) (Fujiwara et al., 2002; Chen and Nakasuji, 2004); spirea aphid, *Aphis citricola* (Neubauer et al., 1983); green peach aphid *Myzus persicae* (Sulzer) (Yu et al., 2010); and Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) (Vilca Mallqui et al., 2014). Behavioral changes or induced hormesis from sublethal exposure to insecticides may include both stimulation and inhibition. These include increased reproduction, alterations in the number of feeding and foraging events, changes in dispersal patterns, and avoidance of insecticide residues (Desneux et al., 2007). Evaluation of effects on behaviors and reproduction caused by sublethal insecticide exposures provides greater overall understanding of the impact of an insecticide for long-term management of agricultural pests with next generation insecticides (Chen and Nakasuji, 2004). The aim of the present investigation was to determine the effect of a new generation insecticide, flupyradifurone, on fecundity and behavior of *D. citri*.

## 2. Materials and methods

### 2.1. Insects and plants

*D. citri* were reared in a greenhouse at the Citrus Research and Education Center, University of Florida, Lake Alfred, FL. The culture originated from adults collected in 2000 from citrus in Polk County, FL. The culture was maintained on sweet orange [*Citrus sinensis* (L.) Osbeck] in a greenhouse with lights controlled on a 14:10 (light: dark) cycle. 'Kuharski Cariizo' [*C. sinensis* (L.) Osbeck] variety citrus plants 25–35 cm in height were planted in plastic pots (10 cm width and 14 cm height) and grown in a greenhouse isolated from *D. citri* under the same environmental conditions as insect rearing. These were used for settling and fecundity experiments. In all experiments, adults of mixed gender were used. It has been previously determined that the sex ratio of adults is 1:1 (Nava et al., 2007).

### 2.2. Insecticide

Analytical grade flupyradifurone (99.5%) was obtained from Chem Service Inc (West Chester, PA, USA). The flupyradifurone was dissolved in acetone to prepare a stock solution. Concentrations ranging between 0.001 and 1000 ng/μl of flupyradifurone were prepared in acetone for toxicity bottle assays described below. Solutions used for the leaf dip bioassay, feeding and settling behavior experiments, as well as, fecundity measurements were diluted in tap water from the stock solution.

### 2.3. Concentration-mortality of flupyradifurone to *D. citri* using bottle and leaf dip bioassays

For the bottle bioassay, 20 ml glass scintillation bottles (Wheaton Industries Inc., Millville, NJ) with a total inner surface of 46.7 cm<sup>2</sup> and measuring 4.7 cm in height and 2.5 cm in diameter were filled with 150 μl flupyradifurone/acetone dilution or acetone alone as a control. The bottles were then rotated on a mechanized roller for 30 min to achieve a uniform coat of insecticide on the

inside of the bottle. Ten 2–10 d old *D. citri* adults of mixed gender were placed into each vial and the cap was secured loosely. Five to seven concentrations of flupyradifurone were tested. Each concentration was replicated five times in a completely randomized design.

For the leaf dip bioassay, the test concentrations of flupyradifurone were prepared on the day of testing. Thirty five mm diameter Petri dishes (Fisher brand, Thermo Fisher Scientific, Waltham, MA) were used containing a 1.5% agar bed to maintain turgor of the citrus leaves. Citrus leaves were collected from a 'Valencia' orange grove that was not treated with insecticide, and 35 mm diameter leaf discs were excised. Excised leaf discs were dipped in test solutions for 30 s and were allowed to dry in a fume hood for 1 h. Leaf discs dipped in water alone served as a control. Treated leaf discs were placed on agar beds and ten 2–10 d old adult *D. citri* of mixed gender were transferred to each dish using a camel hair brush after a brief anesthetization with CO<sub>2</sub> for approximately 1 s. Each concentration of flupyradifurone was replicated five times. Each experiment was replicated three times. All experiments were placed in a growth chamber at 25 ± 1 °C and 60% ± 1% RH with a 14:10 light: dark photoperiod for 24 h. Mortality of *D. citri* was assessed 48 h after transfer into the growth chamber. Insects were considered dead when found on their side or back and unable to move when probed with a camel hair brush.

### 2.4. Feeding behavior of *D. citri*

The objective of this experiment was to quantify the effect of flupyradifurone treatment to citrus leaves on feeding of *D. citri* adults. The petioles of freshly excised citrus leaves were immersed in aqueous solutions of flupyradifurone (0.001, 0.02, 0.39, 8.25 and 130.13 ng/μl) in 20 ml glass vial for 30s and allowed to air dry in a fume hood 30 min prior to use in the bioassay. After 30 min, they were transferred to 35 mm diameter plastic disposable Petri dishes. Ten mixed gender *D. citri* were released into each dish, which was subsequently closed with a lid lined with 35 mm Whatman filter paper (Whatman International Ltd, Maidstone, UK). Petri dishes were sealed with parafilm, turned upside down and transferred into temperature controlled grower chambers. Petri dishes were maintained at 25 ± 1 °C and 50 ± 5% RH with a 14: 10 h light: dark photoperiod. Each treatment was replicated five times and water was used as a control treatment. At 48 h after insect release, filter papers 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 and then air-dried (Boina et al., 2009; Tiwari et al., 2012). The number of dark purple spots was counted on both control and treatment filter papers. The entire experiment was repeated three times.

### 2.5. Flight behavior of *D. citri*

Flight behavior was measured using an insect flight mill that has been thoroughly described in Martini et al. (2014). Briefly, the flight mill apparatus was composed of a 13 cm fiber optic strand forming the horizontal axis. The fiber optic was threaded through the eye of a sewing needle. The needle was positioned vertically between two magnets 2.5 cm apart. At the each end of optic fiber, two smaller pieces of optic fiber were glued in a vertical orientation. Six to seven d old *D. citri* adults that had been on flupyradifurone-treated plants (LC<sub>50</sub> concentration) for 5 d were used in this experiment. Controls were similarly exposed to trees pre-treated with a water spray control as described above. After exposure, psyllids were immobilized on an ice block and attached to one end of the fiber optic strand using nontoxic, washable glue (Elmer's products, Columbus, OH). A small piece of paper was attached to the other end as a

counterbalance and also to activate the sensor used to quantify revolutions. *D. citri* flight behavior was recorded for 2 h after attachment. All flight data were automatically recorded in a computer DATAQ data logger. After recording, the flight number (n), total flight duration (s), average and maximum flight speed (m/s), total flight distance (m), and time elapsed to flight initiation were calculated. Differences in flight behavior of psyllids (N = 20) exposed to the LC<sub>50</sub> concentration of flupyradifurone versus control insects exposed to water-treated leaves were compared. All the experiments were performed in an air conditioned room between 9:00 and 14:00 h at 27 ± 1 °C and 40% ± 10% RH.

### 2.6. Settling behavior of *D. citri*

The objective of this experiment was to evaluate the settling behavior of *D. citri* on flupyradifurone-treated citrus. Adult *D. citri* were subjected to either water treated 'Swingle' citrumelo [*Citrus paradise* MacFaden × *Poncirus trifoliata* (L) Raf] citrus plants or similar plants treated with one of five concentrations of flupyradifurone. Citrus plants (5–6 cm in height and with approximately the same amount of new leaf flush) were sprayed with 0.001, 0.02, 0.39, 8.25 and 130.13 ng/μl of flupyradifurone dissolved in water or water alone until run off using a hand atomizer (The Bottle Crew, West Bloomfield, MI) for a total volume of 5 ml per plant. After treatment, citrus plants were placed in a Plexiglass sleeve cage (40 cm × 40 cm X 40 cm) within 20 ml glass scintillation bottles in water. There were four replicate cages. One hundred *D. citri* adults were released into the center of each cage. The cages were housed under temperature-controlled conditions of 25 ± 2 °C and 50 ± 5% RH with 14:10 light: dark photoperiod. The total number of *D. citri* settling on each plant was recorded 24, 48 and 72 h after release.

### 2.7. Effect of flupyradifurone on development of *D. citri*

Potted 'Swingle' *Citrus aurantiifolia* (Chrism) plants (one year old) with new leaf flush were used for this experiment. The experiment was set up in a randomized complete block design comprised of six treatments with four replicates. The six treatments consisted of five concentrations (0.001, 0.02, 0.39, 8.25 and 130.13 ng/μl) of flupyradifurone dissolved in water and a water control. Treatments were applied with a handheld atomizer until runoff for a volume of 5 ml per plant. Plants were allowed to air dry and then exposed to four male and four female virgin adult *D. citri* for mating and oviposition. Each plant was covered with ventilated mesh and maintained at 25 ± 2 °C and 50 ± 5% RH with a 14:10 h light: dark photoperiod for 5 d. Thereafter, adults were removed from each plant and the number of eggs per plant was recorded under a stereomicroscope. After 3 d, the number of eggs that hatched per plant was recorded by counting the number of first instar nymphs per plant. Plants were observed every five d until adult emergence was complete. The population growth rate (PGR) was calculated using the equation  $PGR = \ln(N_f/N_0)/\Delta T$ , where  $N_f$  was final number of psyllids,  $N_0$  was initial number of psyllids and  $\Delta T$  was the total number of days for the experiment. Solving for PGR results in a rate of population growth similar to that obtained by the intrinsic rate of increase ( $r_m$ ) (Stark et al., 1997; Chen et al., 2010). Positive values of PGR indicate an increase, PGR = 0 indicates a stable population while negative PGR values indicate a population decline.

### 2.8. Statistical analysis

All statistical analyses were conducted using SAS (SAS, 2013). Log-log link function with probit procedure analysis was used to calculate LC<sub>50</sub> values with SAS. For feeding, settling behavior, and

fecundity experiments, observations were compared using one way analysis of variance (ANOVA) followed by Tukey's HSD test at each observation interval. The duration of flight and flight speed was analyzed using a Mann-Whitney rank sum test.

## 3. Results

### 3.1. Toxicity of flupyradifurone to *D. citri*

The LC<sub>50</sub> values obtained for *D. citri* with the bottle bioassay and leaf dip assay were 0.39 and 10.43 ng/μl at 24 and 48 h after exposure, respectively (Table 1). The toxicity using the bottle bioassay was 2744 times higher than with the leaf dip bioassay at the LC<sub>50</sub>, although the probit model for the leaf dip bioassay should be interpreted with caution since a non-homogenous response of mortality vs. concentration was observed ( $P < 0.05$  for goodness-of-fit test). The LC<sub>90</sub> values obtained for *D. citri* with the bottle bioassay and leaf dip assay were 130.13 and 665.64 ng/μl at 24 and 48 h after exposure, respectively. The toxicity with the bottle bioassay was 5.12 times higher than with the leaf dip bioassay at the LC<sub>90</sub>.

### 3.2. Effect of flupyradifurone on *D. citri* feeding

There was a concentration-dependent antifeedant effect of flupyradifurone on *D. citri* as measured by a decrease in honeydew excretion. With the exception of the lowest concentration tested, all of the treatments significantly reduced the amount of honeydew excreted (Fig. 1). There was a reduction of 90% at the highest concentration tested compared with the control ( $df = 5, 84$ ;  $F = 50.26$ ;  $P < 0.001$ ) (Fig. 1).

### 3.3. Effect of flupyradifurone on settling behavior of *D. citri*

Settling behavior of *D. citri* adults was not significantly different between the various concentrations of flupyradifurone tested and control at 24 h ( $df = 5, 24$ ;  $F = 1.35$ ;  $P = 0.279$ ) and 48 h ( $df = 5, 24$ ;  $F = 1.35$ ;  $p = 0.279$ ) after release; however, significant differences were found at 72 h ( $df = 5, 24$ ;  $F = 4.37$ ;  $P = 0.006$ ) after release (Fig. 2). After 72 h, more *D. citri* adults were observed on control plants than on any of the flupyradifurone treatments (Fig. 2).

### 3.4. Effect of flupyradifurone on flight behavior of *D. citri*

*D. citri* adults exposed to flupyradifurone at the LC<sub>50</sub> initiated flight more frequently and sooner than non-exposed controls (Table 2). Percentage of flyers exposed to the LC<sub>50</sub> concentration was 88.9% compared to 60.0% for the control (Table 2). The number of flights by *D. citri* treated with the LC<sub>50</sub> of flupyradifurone was over 5 times greater than that of controls (Table 2). There was a significant difference between control psyllids and those exposed to flupyradifurone.

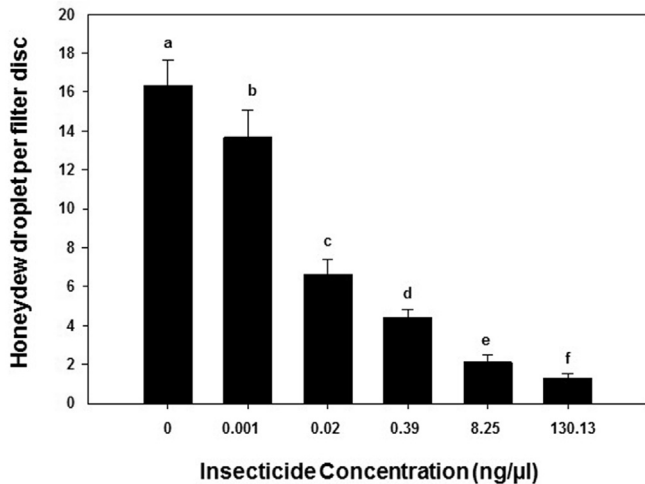
### 3.5. Effect of flupyradifurone on development of *D. citri*

The number of eggs ( $df = 5, 18$ ;  $F = 68.55$ ,  $P < 0.001$ ) laid per plant was significantly higher for *D. citri* exposed to the LC<sub>10</sub> and LD<sub>25</sub> treatments as compared with the control (Table 3). The number of eggs laid per plant was significantly lower than the control for the LC<sub>50</sub>, LC<sub>75</sub> and LC<sub>90</sub> treatments (Table 3). There were no significant differences in population growth rate (PGR) between treatments and the control with the exceptions of exposure to the LC<sub>75</sub> and LC<sub>90</sub> treatments where mortality did not allow calculation of PGRs (Table 3).

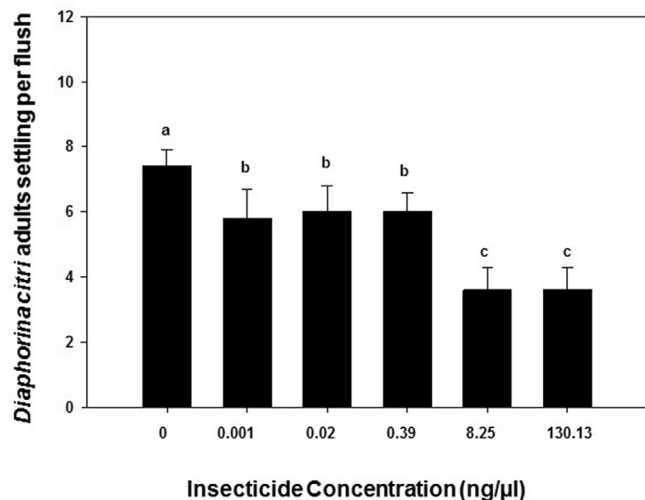
**Table 1**  
Toxicity of flupyradifurone to adult *Diaphorina citri* in bottle and leaf dip bioassays.

Bioassay	N	Slope	$\chi^2$ (df)	p value	LC <sub>50</sub> (ng/ $\mu$ L) 95% FL	LC <sub>90</sub> (ng/ $\mu$ L) 95% FL
Bottle	400	0.21 $\pm$ 0.08	3.37 (4)	0.497	0.39 (0.16–0.82)	130.13 (44.51–599.62)
Leaf dip	900	0.71 $\pm$ 0.49	19.6 (3) <sup>a</sup>	0.002	10.43 (2.07–55.87)	665.65 (103.68–71422)

<sup>a</sup> Significantly different from expected ( $P < 0.05$ ).



**Fig. 1.** Effect of flupyradifurone on *D. citri* adult feeding as measured by number of honeydew droplets produced. Citrus leaf discs were treated with various concentration of flupyradifurone or water. Different letters above bars indicate significant differences among means according to Tukey's test ( $P < 0.001$ ).



**Fig. 2.** Settling preference of *D. citri* adults on citrus plants treated with various concentrations of flupyradifurone or water 72 h after release of adults. Bars within a panel not labeled by same letter indicate significant differences among means according to Tukey's test ( $P < 0.05$ ).

#### 4. Discussion

Lethal concentrations of insecticides suppress arthropod populations by acting through a primary mode of action that causes mortality; however, sub-lethal concentrations may exhibit a secondary mode of action which may compromise homeostasis and

cause changes in behavior and reproduction (Desneux et al., 2007; Guedes et al., 2016; Qu et al., 2015; Tan et al., 2012). Discovering the relationship between insect behaviors, hormesis and insecticide activity are important components of integrated pest management, where changes in behavior and physiology may impact population control (Desneux et al., 2007). Insecticide-induced stress in pests and changes in behavioral responses are of growing importance as next generation insecticides continue to become more target specific (Guedes et al., 2016). Furthermore, insecticide exposure may interfere not only with conspecific behavioral interactions, but also with hetero-specific behavioral interactions. This is a potentially unrealized consequence to pest management and may increase the risk of pest damage (Barbieri et al., 2013; Ndiath et al., 2014).

The phloem limited bacterium, *Candidatus Liberibacter asiaticus*, that causes HLB in citrus is rapidly and effectively transmitted by *D. citri* (Pelz-Stelinski et al., 2010). We found an antifeedant effect at higher concentration of flupyradifurone tested based on reduced honeydew excretion and where minimal adult mortality was observed. Our results indicate that further studies are warranted utilizing techniques such as electrical penetration graph (EPG) monitoring to more precisely characterize the feeding behavior of *D. citri* on flupyradifurone-treated foliage.

Sublethal effects of flupyradifurone were observed by comparing *D. citri* settling behavior on treated versus control plants. During the first 48 h of following psyllid exposure to this insecticide, there was no clear trend; however, at 72 h fewer adults settled on plants treated with flupyradifurone than on the control plants. Reduced settling of *D. citri* adults on flupyradifurone-treated trees should not only reduce direct damage, but also reduce pathogen acquisition and perhaps inoculation. Several insecticides reduce settling behavior of insect vectors of plant pathogens (Ioriatti et al., 2009). Deltamethrin, fenvalerate, pirimicarb and methamidophos reduce settling by green peach aphid, *Myzus persicae* (Sulzer), and cause them to move to untreated leaf surfaces (Lowery and Boiteau, 1988). Joost and Riley (2005) demonstrated *Frankliniella fusca* (Hinds) preferred to settle on leaves of non-treated plants compared with those treated with imidacloprid. Host avoidance by *D. citri* caused by flupyradifurone treatment may contribute to HLB management and this hypothesis warrants further testing.

Hormesis is a biphasic dose response to an environmental agent characterized by low dose stimulation and a complementary inhibitory or toxic effect at a high dose (Calabrese and Baldwin, 2003). Our results indicate hormesis with *D. citri* following exposure to flupyradifurone. At the LC<sub>10</sub> and LC<sub>25</sub> exposure, the number of eggs per plant increased significantly as compared with the control. However, following exposure to the LC<sub>50</sub>, LC<sub>75</sub>, and LC<sub>90</sub> treatments, egg production was reduced as compared with the control.

Sub-lethal dosages of insecticides may have varying effects on the behavior and physiology of targeted insects. For example, Tomé et al. (2014) reported stimulus-dependent impairment of swimming speed and wriggling movement, thought to cause refuge seeking and escape responses in larvae of the yellow fever

**Table 2**  
Comparison of flight capabilities of *D. citri* adults, as measured by a flight mill apparatus, following exposure to flupyradifurone-treated (LC<sub>50</sub>) versus non-treated control leaf surfaces.

Flight parameters	No Psyllids	Mean ± SE		F	t <sup>a</sup>	P
		Control	Treatment (Treated LC <sub>50</sub> )			
Flight number (n)	20	1.10 ± 0.39a	5.78 ± 2.12b	29.036	−2.288	0.035*
Total flying duration (s)	20	8.37 ± 3.61a	29.16 ± 12.82a	6.003	−1.561	0.157
Average flight speed (m/s)	20	0.53 ± 0.10a	1.26 ± 0.32a	1.742	−1.916	0.079
Maximum flight speed (m/s)	20	0.65 ± 0.17a	2.20 ± 0.62b	12.592	−2.417	0.042*
Total flight distance (m)	20	2.13 ± 0.75a	9.56 ± 3.81a	11.159	−1.914	0.094
Time to elapsed to the 1st flight (s)	20	1781 ± 1069a	122.8 ± 38.1a	27.634	1.550	0.172
Fly %		60	88.9	—	—	—

\*P < 0.05.

<sup>a</sup> t-test in Statistics 22.0.

**Table 3**  
Effect of flupyradifurone on development of *D. citri*.

Insecticide Concentration (ng/μl)	No of females and males per replicate	Number of Egg per plant	F <sub>1</sub> Adult	Population Growth Rate
Control	4 × 4 <sup>a</sup>	63.00 ± 4.41a <sup>b</sup>	1.80 ± 0.18cb	−0.06 ± 0.00a
0.001	4 × 4	85.00 ± 8.10b	2.60 ± 0.16ab	−0.05 ± 0.02a
0.02	4 × 4	86.00 ± 3.08b	2.60 ± 0.79ab	−0.05 ± 0.01a
0.39	4 × 4	42.00 ± 5.31c	4.31 ± 0.83a	−0.03 ± 0.00a
8.25	4 × 4	3.50 ± 0.87d	0.54 ± 0.21cb	—
130.13	4 × 4	3.25 ± 0.48d	0.00 ± 0.00c	—

<sup>a</sup> Each treatment was replication 4 times.

<sup>b</sup> Data are expressed as the mean ± SE; means within the same column followed by the same letter are not significantly different (ANOVA and Tukey's HSD test, P = 0.05).

mosquito, *Aedes aegypti* (Linnaeus), as sublethal effects of three different insecticides (deltamethrin, imidacloprid and spinosad). Yin et al. (2008) demonstrated that soluble sugars and fat content of brown planthopper, *Nilaparvata lugens* (Stål), adults feeding on rice plants treated with insecticides were higher than those of controls. They reported that *N. lugens* exposed to insecticide accumulate more energy for their migration and dispersal and their flight capacities are enhanced. Our current results suggest a similar effect in that *D. citri* exposed to sublethal concentrations (LC<sub>50</sub>) of flupyradifurone exhibited enhanced flight capabilities as compared with non-treated controls. Enhanced dispersal of *D. citri* following exposure to sub-lethal concentrations of flupyradifurone could have unintended negative consequences by potentially increasing the spread of the HLB causal pathogen. This warrants further investigation and illustrates how an understanding of sub-lethal effects is needed when evaluating a possible toxicant for management of a pathogen vector.

## 5. Conclusion

Flupyradifurone was toxic to *D. citri* adults and exhibited greater toxicity in the bottle bioassay than in the leaf dip bioassay following feeding on treated leaf surfaces. Exposure of *D. citri* to flupyradifurone at sub-lethal concentrations reduced feeding, host settling and the number of eggs laid by *D. citri*, but increased flight behaviors. Therefore, integration of flupyradifurone as a management tool for suppression of *D. citri* will require further field-based investigations, including monitoring and visual inspections to determine how sub-lethal effects may contribute to population growth and vector movement. Importantly, the ultimate impact of an insecticide on a population of a target insect should not be assessed exclusively based on direct lethal effects, but also take the multitude of possible sub-lethal effects into account. Our laboratory investigation defines a number of sub-lethal effects that will require investigation in field tests.

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