International Journal of Pest Management

ISSN: 0967-0874 (Print) 1366-5863 (Online) Journal homepage: http://www.tandfonline.com/loi/ttpm20

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To cite this article: Gurpreet S. Brar, Xavier Martini & Lukasz L. Stelinski (2017) Lethal and sub-lethal effects of a novel sulfoximine insecticide, sulfoxaflor, against Asian citrus psyllid and its primary parasitoid under laboratory and field conditions, International Journal of Pest Management, 63:4, 299-308, DOI: 10.1080/09670874.2016.1258501

To link to this article: http://dx.doi.org/10.1080/09670874.2016.1258501

INTERNATIONAL JOURNAL OF PEST MANAGEMENT, 2017
VOL. 63, NO. 4, 299–308
http://dx.doi.org/10.1080/09670874.2016.1258501

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Lethal and sub-lethal effects of a novel sulfoximine insecticide, sulfoxaflor, against Asian citrus psyllid and its primary parasitoid under laboratory and field conditions

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ABSTRACT

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is the most important international pest of citrus because it transmits the bacteria that cause huanglongbing (HLB). HLB limits citrus production globally. We evaluated the toxicity of sulfoxalor against *D. citri* and its parasitoid, *Tamarixia radiata* Waterston. Sulfoxaflor was as toxic as imidacloprid to adult *D. citri*. The LC_{50} values for sulfoxaflor and imidacloprid were 8.17 and 5.7 μg AI mL^{-1}, respectively. The LC_{50} of sulfoxaflor for *T. radiata* adults was 3.3 times greater than for *D. citri* adults. Treatment with sulfoxaflor resulted in reduced oviposition, development of nymphs, and emergence of adult *D. citri* on plants, as compared with controls. The lowest concentration that reduced adult emergence was 0.6 μg AI mL^{-1}. There was reduced feeding by *D. citri* adults on leaves treated with sulfoxaflor. The residual toxicity of sulfoxaflor was equivalent to imidacloprid. Under field conditions, formulated sulfoxaflor reduced populations of *D. citri* compared with untreated controls. Sulfoxaflor is a novel mode of action and is an effective tool for *D. citri* management.

ARTICLE HISTORY
Received 15 June 2016
Accepted 19 September 2016

KEYWORDS
Neonicotinoids; citrus greening disease; insecticide resistance; ecotoxicology; non-target insects; sub-lethal effects

1. Introduction

Sulfoxaflor is a recent insecticide from the relatively new class of sulfoxamines for control of phloem feeding insects that has a similar mode of action than neonicotinoids as it interacts with nicotinic acetycholine receptors (nAChRs) in the nervous system of insects. However, sulfoxaflor interacts with nAChRs differently than other nAChR-interacting insecticides such as neonicotinoids, nicotine spinosyns, and nereistoxin analogues (Yu et al. 2008; Babcock et al. 2011; Watson et al. 2011; Zhu et al. 2011). Sulfoxaflor is sufficiently different from the neonicotinoids (Group 4A, http://www.irac-online.org/modes-of-action) in structure and biochemistry to be categorized as a 4C mode of action by the Insecticide Resistance Action Committee (IRAC) (Nauen & Denholm 2005; Sparks et al. 2012, 2013). The formulated product of sulfoxaflor (21.1%), Closer® (Dow AgroSciences, Indianapolis, IN, USA), is currently registered for use in Florida citrus; however, this insecticide is becoming an important additional tool for management of other pests throughout the world.

Neonicotinoids are widely applied for control of plant sap-sucking pests internationally. However, extensive use has resulted in cases of resistance development on all continents where this has been investigated. The first report of resistance to imidacloprid, for example, was documented for *Bemisia tabaci* (Genniadus) in less than six years after its initial introduction (Cahill et al. 1996; Nauen & Denholm 2005; Roditakis et al. 2009). Since then, occurrences of resistance to imidacloprid and other neonicotinoids continue to be reported. There is apparent lack of cross-resistance between sulfoxaflor and traditional neonicotinoids (Babcock et al. 2011; Zhu et al. 2011), suggesting that it might be a useful tool for resistance management in cases where resistance to the neonicotinoids is an existing problem (Longhurst et al. 2013). For example, there was no cross-resistance observed between sulfoxaflor and imidacloprid for *Trialeurodes vaporariorus* Westwood and very low cross-resistance for *B. tabaci* (Longhurst et al. 2013). In many insect species, including *Nilaparvata lugens* (Stål) (Puinean et al. 2010; Watson et al. 2011), *Musa domestica* Linnaeus (Markussen & Kristensen 2010), *Myzus persicae* (Philippou et al. 2010), *B. tabaci* (Karunker et al. 2008, 2009), and *Diaphorina citri* Kuwayama (Tiwari et al. 2011), metabolism of imidacloprid by cytochrome P 450 monooxygenase enzymes has been implicated in development of resistance (Babcock et al. 2011; Zhu et al. 2011). It has been suggested that the lack of cross-resistance between sulfoxaflor and group 4A neonicotinoids may be due to the stability of this novel chemistry against monooxygenase enzyme-based digestion (Zhu et al. 2011).

The Asian citrus psyllid, *D. citri* Kuwayama (Hemiptera: Liviidae), is the most important pest of

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citrus crops worldwide. A gram-negative bacterium, "*Candidatus* Liberibacter asiaticus" (Las) transmitted by *D. citri*, causes the citrus disease huanglongbing (HLB) (Jagoueix et al. 1994; Halbert and Manjunath 2004; Grafton-Cardwell et al. 2013). HLB leads to heavy leaf drop, dieback of stems, premature fruit drop, irregular flushing and blossoming, and ultimate tree death within 5–10 years (Grafton-Cardwell et al. 2013). Infected trees produce small, distorted, and bitter tasting fruit (Halbert & Manjunath 2004). In the USA, *D. citri* was first reported in Florida in June 1998, and since then, it has spread to Alabama, Arizona, California, Georgia, Louisiana, Mississippi, and Texas (Hall et al. 2013). HLB has been reported in all citrus growing regions of Florida since its initial report in August 2005. Since 2005, HLB has reduced citrus acreage by 37%, production volume by 58%, and employment of farm labor by 17.5% (Hodges et al. 2014). This disease has similar devastating effects throughout citrus growing regions in Africa, Asia, and South America (Grafton-Cardwell et al. 2013).

Currently, *D. citri* is managed by aggressive applications of a limited number of insecticides of various modes of action during each growing season (Qureshi et al. 2014; Boina & Bloomquist 2015). The frequent and repeated application of insecticides with the limited available modes of action has resulted in the development of insecticide resistance in Florida populations of *D. citri* (Tiwari et al. 2011). There is immediate need for insecticides with different modes of action as additional tools for effective rotation when managing *D. citri*.

Tamarixia radiata (Waterston) is a eulophid parasitoid of *D. citri* originating from northwest India that has been popularly released as a part of classical and augmentation biological control programs against *D. citri* (Qureshi et al. 2009). The parasitoid has not been compatible with many types of insecticides used (Hall & Nguyen 2010; Beloti et al. 2015), and varying levels of effectiveness with biological control have been observed in certain situations (Qureshi & Stansly 2009; Grafton-Cardwell et al. 2013). The impact of newer chemistries on natural enemies also requires investigation.

The objectives of this study were to quantify: (1) the acute toxicity of sulfoxaflor to *D. citri* and the compatibility of sulfoxaflor with *T. radiata* adults; (2) the sublethal effects of sulfoxaflor on development, feeding, and settling behaviors of *D. citri*; (3) the residual toxicity of sulfoxaflor to *D. citri* adults; and (4) the efficacy of sulfoxaflor under field conditions.

2. Materials and methods

2.1. Insect culture and insecticides

D. citri adults were collected from a colony maintained in a greenhouse at the Citrus Research and Education Center, University of Florida in Lake Alfred, FL, USA. The colony was established in 2000 prior to the discovery of HLB in Florida. The culture was established from field populations collected in Polk County, FL, USA (28.0′ N, 81.9′ W). Thereafter, the colony has been maintained in greenhouse on sweet orange (*Citrus sinensis* (L.) Osbeck) plants at approximately 25–28 °C, 50%–65% RH and at 14:10 (L:D) photoperiod. Bi-monthly testing of randomly sampled nymphs, adults, and plants by quantitative polymerase chain reaction assays was conducted to confirm that psyllids and plants in this culture were uninfected by Las. *T. radiata* were procured from a colony maintained by the Florida Division of Plant Industry in Gainesville, FL, USA. *T. radiata* were released into Plexiglas® cages (40 cm × 40 cm × 40 cm) containing filter paper strips with honey as a food source and used for bioassays on the following day.

The formulated product of sulfoxaflor, Closer® (21.8% AI Dow AgroSciences, Indianapolis, IN, USA), was used for all laboratory and field experiments. Commercially available formulations of imidacloprid (Provado®, 17.4% AI, Bayer Crop Science, Research Triangle Park, NC, USA), fenpropathrin (Danitol® 2.4EC, 30.9%, Valent USA Corp., WalnutCreek, CA, USA) and spinetoram (Delegate®, 25% AI Dow AgroSciences, Indianapolis, IN, USA) were used in the field experiments for comparison. The insecticides were diluted in tap water for all treatments tested.

2.2. Toxicity of sulfoxaflor to D. citri and T. radiata adults

A leaf disk bioassay was conducted to assess the acute toxicity of sulfoxaflor to *D. citri* adults (Boina et al. 2009). Leaves collected from insecticide-free "Valencia" orange trees were washed, dried, and cut into 34 mm diameter discs. Six concentrations were tested based on preliminary data collected for each species (Brar, unpublished data). The leaf discs were subsequently dipped in either of six concentrations of sulfoxaflor-formulated product (0, 0.3, 1.0, 3.0, 9.0, 27.0, and 81.0 AI μg mL^{-1}) solutions or imidacloprid-formulated product (0, 0.3, 1.0, 3.0, 9.0, 27.0, and 81.0 AI μg mL^{-1}) for 30 s and then air-dried in a fume hood for 30 min. The treated leaf discs were then placed on 35 × 10 mm Petri dishes (Fisher Scientific, Pittsburg, PA, USA) with a bed of agar to maintain moisture for the leaf disc. Fifteen *D. citri* adults of mixed age and gender were added to each dish and the lids were sealed with Parafilm (Beemis Flexible Package, Neenah, WI, USA). The Petri dishes were placed upside down in an incubator (Thermo Scientific, Waltham, MA, USA) set at 25 ± 2 °C and 50% ± 5% RH with a 14:10 h L:D photoperiod. The experiment was repeated twice with four replicates in each trial.

A modified leaf disc bioassay was used to assess direct toxicity of sulfoxaflor to *T. radiata*. The treated leaf discs and Petri dishes were prepared as described earlier. On the inner side of the lid, five 4 μL drops of honey water solution (10% v/v) were dispensed to provide a food source for *T. radiata*. Ten adults of mixed age and gender were placed into each Petri dish. Sealed Petri dishes were placed upside down in the incubator set at 25 ± 2 °C and 50% ± 5% RH with a 14:10 h L: D photoperiod. Mortality of *D. citri* and *T. radiata* adults was assessed 24 h after treatment. Insects unable to move when prodded with a fine probe were considered dead. The experiment was repeated twice with four replicates conducted during each trial.

2.3. Feeding behavior of D. citri adults

Feeding by adult *D. citri* was assessed indirectly by quantifying honeydew excretion using a ninhydrin test (Boina et al. 2009). Fresh citrus leaves were excised from insecticide-free "Valencia" orange trees, washed, dried, and cut into 34 mm diameter discs. The leaf discs were subsequently dipped in solutions of sulfoxaflor-formulated product at various concentrations (0.1, 0.3, 0.9, 2.7, 8.1, and 27.3 AI μg mL^{-1}) or water alone for 30 s and then air-dried in a fume hood for 30 min. The treated leaf discs were then placed into 35 × 10 mm Petri dishes with a bed of agar to maintain moisture as described earlier. Whatman filter paper (Whatman International Ltd, Kent, UK) was used to line the upper lid. Five adults of mixed age and gender were placed into each Petri dish and the lids were sealed with Parafilm and held upside down in an incubator set at 25 ± 2 °C and 50% ± 5% RH with a 14:10 h L:D photoperiod. The filter papers were removed after 48 h, replaced, and then again collected after 72 h of treatment. The collected filter papers were subjected to the ninhydrin test and the honeydew droplets were counted (Boina et al. 2009). The experiment was arranged as a randomized complete block design with six replicates per treatment.

2.4. Settling behavior of D. citri adults

The objective of this experiment was to investigate settling behavior (Tiwari & Stelinski 2013) of adult *D. citri* on plants treated with sulfoxaflor versus untreated plants. "Swingle citrumelo" (*Citrus paradisi* MacFaden. X *Poncirus trifoliata* (L.) Raf.) plants of approximately the same age (14–16 weeks old), height, and vigor were sprayed until runoff with various concentrations of formulated sulfoxaflor product: (0.4, 1.2, 3.6, 10.2, and 30.6 AI μg mL^{-1}) diluted in tap water or water alone as the control. Plants were allowed to air dry for 2 h and were randomly placed into a Plexiglas cage (40 cm × 40 cm × 40 cm). There were six cages set up for the choice test, with each cage containing one plant from each treatment. The six treatments were randomly arranged within each cage as a choice test. The experiment was arranged as a randomized complete block design. Fifty *D. citri* adults of mixed age and gender were released in the middle of each cage. The cages were placed into a rearing room maintained at 25 ± 2 °C and 50% ± 5% RH and a 14:10 (L:D) photoperiod. The number of adults settling on each plant treated with a particular concentration of sulfoxaflor or the control was recorded after 24, 48, and 72 h post-treatment.

2.5. Effect of sulfoxaflor on developmental stages of D. citri

Potted "Swingle citrumelo" plants (5–6 months old) with new flush, as defined by Hall and Albrigo (2007), were used for this experiment. Plants were sprayed with a hand-held sprayer until runoff with sulfoxaflor-formulated product dissolved in water at various concentrations (0.6, 1.8, 5.4, 16.2, and 48.6 μg AI mL^{-1}). Water alone served as the control. There were five replicates per treatment. The experiment was set up in a randomized complete block design. Each plant was exposed to five male and five female *D. citri* adults of mixed ages. The adults were removed after three days and the number of eggs per plant was counted. Observations were subsequently recorded every three days to quantify the number of eggs, first to fifth instar nymphs, and adults. Plants were observed for possible phytotoxicity throughout the experiment.

2.6. Residual toxicity of sulfoxaflor to D. citri adults

The objective of this experiment was to assess the residual toxicity of sulfoxaflor to *D. citri* adults. Potted "Swingle citrumelo" plants (5–6 months old) were sprayed with a hand-held sprayer with sulfoxaflor [0.42 L ha^{-1}, (0.43 g L^{-1} AI)], imidacloprid [1.47 L ha^{-1} (1.21 g L^{-1} AI)], or water alone (control) until runoff. The treatments tested were the highest recommended foliar field spray rates for each insecticide. A total of 16 plants were sprayed with each treatment. Plants were then left to air dry outside the laboratory for 2 h. At 0, 8, 15, and 22 days after treatment (DAT), a batch of four plants from the 16 plants sprayed within each treatment were infested with 30 *D. citri* adults of mixed gender. Plants were then covered with individual screens to keep *D. citri* adults confined onto plants. Observations were recorded for the number of dead *D. citri* each day after infestation until seven days for each plant. Insects unable to move when prodded with a fine probe were considered dead.

2.7. Field trials of sulfoxaflor

Formulated sulfoxaflor was evaluated against *D. citri* under field conditions to complement laboratory

studies. Two independent experiments were conducted in 2011 and 2012 to evaluate efficacy. The first experiment was conducted in 2011 at the Citrus Research and Education Center in Polk County, FL, USA in a mixed planting of "Hamlin" and "Valencia" oranges. Trees were 2.4 × 3.6 m in height and were planted at 3 × 3.6 m from one another. Insecticide was applied on 15 September 2011. The applications were made with an LV-8 low volume applicator (Curtis Dyna-Fog LTD, Westfield, IN, USA) delivering approximately 9.46 L of spray per hectare. Applications were made during periods of thermal inversion, between 22 and 23 h. Average wind speed was measured at 2.2–6.1 km h^{-1} during applications and the forward speed of the applicator was between 8.0 and 11.5 km h^{-1}. The foliar application consisted of formulated sulfoxaflor (0.2 L ha^{-1}), formulated sulfoxaflor + oil (0.2 L ha^{-1} + 2% v/v), formulated sulfoxaflor + oil (0.425 L ha^{-1} + 2% v/v), fenpropathrin (Danitol) + oil (1.2 L ha^{-1} + 2% v/v), and spinetoram (Delegate) + oil (0.48 L ha^{-1} + 2% v/v). The oil used was IAP 435 crop oil (Integrated Agribusiness Professionals, Fresno, CA, USA). The control plots were left untreated. Single rows of trees acted as buffers separating plots. The experiment was a randomized complete block design with all treatments applied to four replicates of six-tree plots.

Pre-treatment assessments were conducted two days prior to treatment. Post-treatment sampling was conducted after three days and with weekly samples thereafter for 4–5 weeks. Ten terminal flush samples were collected from the interior portions of each replicate plot and were placed in 70% alcohol for transfer to the laboratory and inspection under a stereomicroscope. The flush was ranked using a 0–3 scale to ascertain efficacy of treatments against *D. citri* nymphs: (0 nymphs per flush was ranked 0, 1–5 nymphs per flush ranked 1, 6–10 nymphs per flush ranked 2, 11 or more nymphs per flush ranked 3). On each sampling date, tap sampling (Hall et al. 2007) was employed to measure densities of adult *D. citri*. For each tap sample, a tree branch was vigorously tapped with a PVC pipe directly over a horizontally placed 210 × 297 mm plastic white sheet. All *D. citri* adults found on the sheet following branch agitation were counted and recorded. Ten such samples were collected per replicate plot with 40 taps samples per treatment.

The second experiment was conducted in 2012 in Orange County, FL, USA in a planting of "Navel" oranges (N 28.473°, W 81.648°). Trees were 1.8–3.6 m in height and were planted on 3.0 × 6.0 m spacing. Foliar treatments were applied using a handgun spray applicator (H. D. Hudson GES-505) on 25 April. The pump was set at 200 psi delivering 1.13 L of finished spray per tree. The foliar application consisted of fenpropathrin (Danitol) (1.2 L ha^{-1}), formulated sulfoxaflor + oil (0.3 L ha^{-1} + 2% v/v), and formulated sulfoxaflor + oil (0.2 L ha^{-1} + 2% v/v). The control treatment was left untreated. The experiment was a randomized complete block design with single row buffers between blocks and all treatments were applied to six tree plots. The treatments were replicated four times. Pre-treatment counts were conducted two days prior to treatment. Post-treatment sampling was conducted at three days and then weekly for 5–6 weeks. Plants were sampled for both nymph and adult *D. citri* as described earlier. Ten terminal flush samples from the interior portions of each replicate plot were collected and placed in paper bags and transferred to the laboratory for inspection under a stereomicroscope.

2.8. Statistical analyses

All data were analyzed using SAS statistical software (SAS 2003). All data were tested for normal distribution, as well as homogeneity of variance between treatments. Data with non-normal distribution or significant different variances between treatments were log transformed, prior to analyses. Data were subjected to separate one-way analyses of variance (ANOVA) and Fisher's protected least significant difference (LSD) means separation tests. Probit regression analysis was conducted to calculate LC_{50} values and its corresponding 95% confidence intervals (CIs) for both *D. citri* and *T. radiata* adults.

3. Results

3.1. Toxicity of sulfoxaflor to D. citri and T. radiata adults

D. citri adult mortality following treatment with sulfoxaflor was equivalent to imidacloprid, based on overlapping CIs, at the LC_{50} level. The LC_{50} values for sulfoxaflor and imidacloprid were 8.17 and 5.7 μg AI mL^{-1}, respectively (Table 1). The LC_{50} value of sulfoxaflor for the parasitoid *T. radiata* was 3.3 times higher than that for *D. citri* adults (Table 1).

3.2. Feeding behavior of D. citri adults

There was significant reduction in feeding of *D. citri*, as measured by honeydew excretion, on surfaces treated with sulfoxaflor as compared with the control. The

Table 1. Toxicity of sulfoxaflor to *Diaphorina citri* and *Tamarixia radiata*.

Insecticide	Insect	*N*	LC_{50} (μg mL^{-1}) (95% CI)[a]	Slope (±SE)	X^2 (df)[b]
Sulfoxaflor	*Diaphorina citri*	805	8.17 (3.9–19.9)	1.20 ± 0.18	5.5 (4)
Imidacloprid	*Diaphorina citri*	840	5.7 (2.9–11.7)	1.40 ± 0.21	5.9 (4)
Sulfoxaflor	*Tamarixia radiata*	480	27.12 (12.7–60.7)	1.17 ± 0.29	4.17 (3)

Note: [a]95% confidence intervals for LC_{50}.
[b]Chi-square goodness of fit test and degrees of freedom.
SE standard error.

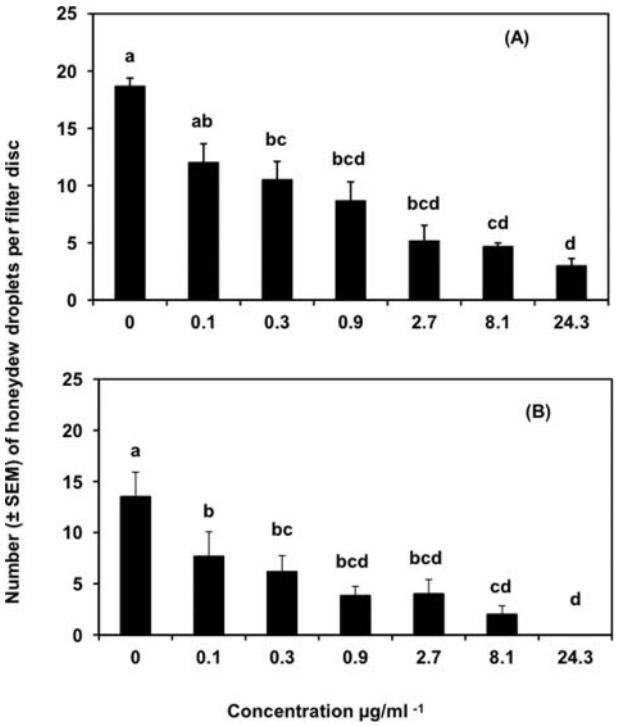

Figure 1. Effect of sulfoxaflor on feeding of *D. citri* adults at: (A) 0–48 h and (B) 49–72 h after exposure. The numbers of honeydew droplets per filter paper disc were counted for each time interval. Bars not labeled with same letter are significantly different from each other at the $P < 0.05$ level of confidence.

number of honeydew droplets recorded per filter paper for treatments with sulfoxaflor differed significantly after 0–48 ($F = 4.56$; df = 6, 35; $P = 0.002$) and 49–72 h ($F = 7.68$; df = 6, 35; $P < 0.001$) of feeding from the control. During the 0–48 and 49–72 h intervals of feeding, 0.1 and 0.3 AI µg mL^{-1} concentrations of sulfoxaflor were the lowest concentrations, respectively, to reduce feeding as compared with the control treatment (Figure 1).

3.3. *Settling behavior of* D. citri *adults*

There was no difference in the number of *D. citri* that settled on plants treated with the various concentrations of sulfoxaflor tested, as compared with untreated plants ($F = 2.5$; df = 5, 95; $P = 0.200$) after release onto treated plants. Also, time after application had no effect on the settling of *D. citri* adults ($F = 1.82$; df = 2, 95; $P = 0.160$).

3.4. *Effect of sulfoxaflor on development stages of* D. citri

There were fewer eggs ($F = 3.19$; df = 5, 24; $P = 0.021$), first instars nymphs ($F = 5.67$; df = 5,24; $P = 0.001$), second instar nymphs ($F = 6.98$; df = 5, 24; $P < 0.001$), third instars nymphs ($F = 7.38$; df = 5, 24; $P < 0.001$), fourth instar nymphs ($F = 4.77$; df = 5, 24; $P = 0.004$), fifth instar nymphs ($F = 7.01$; df = 5, 24; $P < 0.001$), and adult *D. citri* ($F = 5.06$; df = 5,

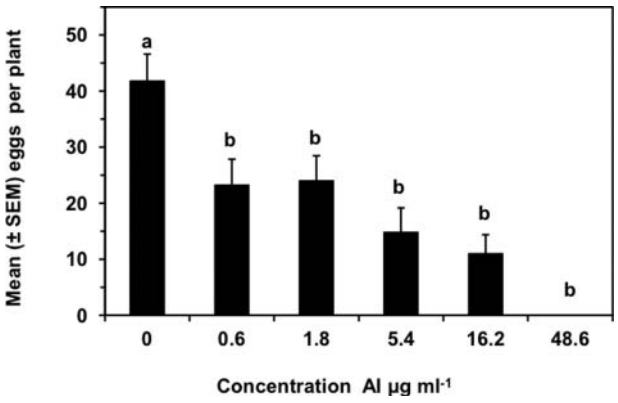

Figure 2. Mean number of eggs deposited per plant by *D. citri* adults treated with various concentrations of sulfoxaflor. Bars represent treatment means with standard error of the mean (SEM). The treatment bars not labeled with same letter are significantly different from one another at the $P < 0.05$ level of confidence.

24; $P = 0.003$) on plants treated with sulfoxaflor than on control treatments. The lowest concentration that reduced egg production was 0.6 AI µg mL^{-1} (Figure 2). The 0.6 AI µg mL^{-1} concentration of sulfoxaflor

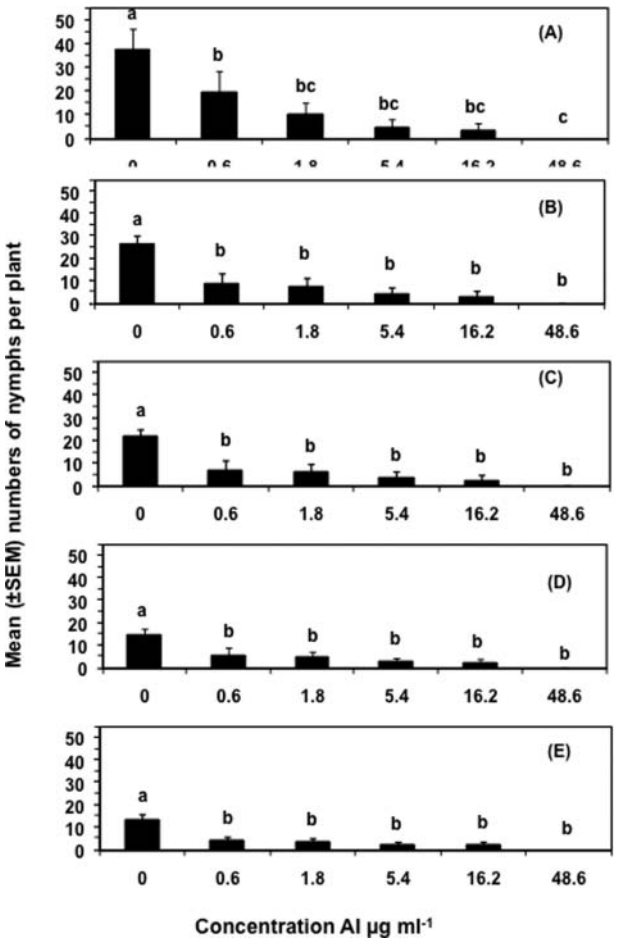

Figure 3. Mean number of *Diaphorina citri* (A) first instar, (B) second instar, (C) third instar, (D) fourth instar nymphs, and (E) fifth instar nymphs per plant on plants treated with various concentrations of sulfoxaflor. Bars represent treatment means with SEM. Treatments not labeled with same letter are significantly different from one another at the $P < 0.05$ level of confidence.

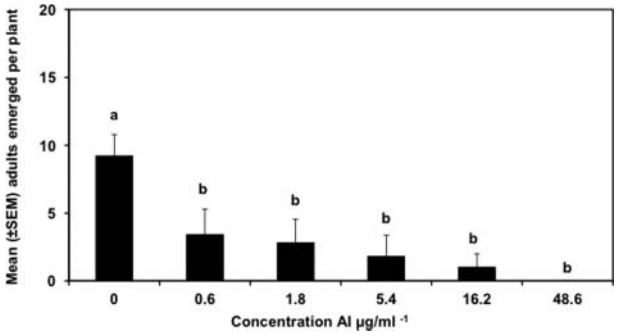

Figure 4. Mean number of *D. citri* adults that emerged on plants treated with various concentrations of sulfoxaflor. Bars represent treatment means with SEM. Treatments not labeled with same letter are significantly different from one another at the $P < 0.05$ level of confidence.

reduced survival of first to fifth instar nymphs, as well as, adults as compared with the control (Figures 3 and 4). There were no apparent phytotoxicity symptoms to plants observed after treatment applications.

3.5. Residual toxicity of sulfoxaflor to D. citri adults

Sulfoxaflor and imidacloprid were both toxic to *D. citri* adults as compared with the control 0–7 ($F = 35.16$; df = 2, 9; $P < 0.001$), 8–14 ($F = 13.67$; df = 2, 9; $P = 0.002$), 15–21 ($F = 29.01$; df = 2,9 $P < 0.001$), and 22–28 days ($F = 51.09$; df = 2,9; $P \leq 0.001$) after treatment (Figure 5). There was no difference between sulfoxaflor and imidacloprid toxicity for any data point (Figure 5).

3.6. Field trials of sulfoxaflor

In the first field trial conducted in 2011, lower populations of adults psyllids were observed for a week on trees treated with a low volume (0.2 L ha^{-1}) application of sulfoxaflor without oil until 8 DAT as compared with the control. Populations of adult *D. citri* were significantly reduced on plants treated with fenpropathrin approximately 15 DAT as compared with the control (Table 2).

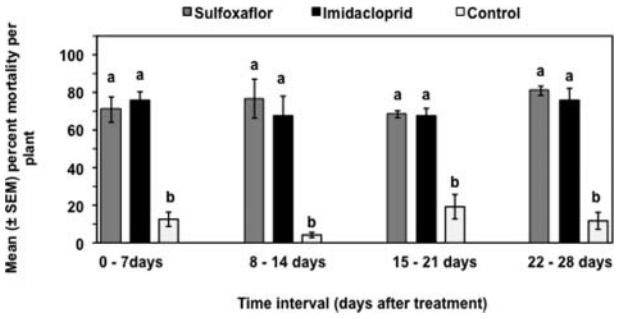

Figure 5. Mean percent mortality of *D. citri* adults on plants treated with sulfoxaflor, imidacloprid, or water (control) at various intervals following treatment. Treatments not labeled with same letter are significantly different from one another at the $P < 0.05$ level of confidence.

In the second field trial conducted in 2012, trees treated with high rate spray of formulated sulfoxaflor + horticultural petroleum oil (0.3 L ha^{-1} + 2% v/v) had lower populations of adult *D. citri* 2 and 8 DAT, whereas the lower rate spray of formulated sulfoxaflor + oil (0.2 L ha^{-1} + 2% v/v) had lower populations only at 8 DAT. The high rate spray of formulated sulfoxaflor had significantly lower nymph populations at 8 DAT. Fenpropathrin reduced adult *D. citri* populations 2, 8, and 22 DAT and nymph population 2, 15, 22, and 36 DAT (Table 3).

4. Discussion

The possibility of insecticide resistance in populations of *D. citri* has complicated management of this important pest globally. For example, frequent and repeated application of a limited number of modes of action has resulted in development of insecticide resistance in Florida (Tiwari et al. 2011). Insecticide resistance may be managed for *D. citri* by integrating different modes of action to existing protocols (Grafton-Cardwell et al. 2013). Sulfoxaflor was specifically developed against sap-sucking insects and currently there is little to no evidence of cross-resistance with neonicotinoids (Yu et al. 2008; Babcock et al. 2011; Watson et al. 2011; Zhu et al. 2011; Sparks et al. 2012). The objective of this investigation was to evaluate sulfoxaflor against *D. citri* and its compatibility with *T. radiata* under laboratory conditions, and efficacy of sulfoxaflor against *D. citri* under field conditions.

In laboratory evaluations, sulfoxaflor was as active as imidacloprid against *D. citri* adults. Our results are congruent with earlier studies conducted with sap-sucking insects. Sulfoxaflor was as toxic as imidacloprid when tested against *M. persicae*, *B. tabaci*, *N. lugens*, *Nephotettix cincticeps* Uhler, and *Lygus hesperus* Knight (Wen et al. 2009; Babcock et al. 2011; Watson et al. 2011; Zhu et al. 2011). However, sulfoxaflor was more toxic than imidacloprid when tested against *Aphis gossypii* Glover (Babcock et al. 2011; Zhu et al. 2011). Mortality of *D. citri* was similar on leaves treated with sulfoxaflor at residue ages of 0–7, 8–14, 15–21, and 22–28 DAT, as compared with imidacloprid. These results differ with another investigation, where sulfoxaflor was significantly more stable than imidacloprid to UV photolysis and its residues were more effective in controlling *M. persicae* than imidacloprid (Zhu et al. 2011).

Also, sub-lethal effects of sulfoxaflor on *D. citri* resulted in reduced oviposition and survival of first to fifth instar nymphs, as well as, reduced adult emergence. The lowest concentration tested (0.6 AI µg mL^{-1}) reduced egg, larval, and adult emergence, as compared with the control. In a similar investigation, cyantraniliprole reduced the development of *D. citri* at a 0.25 AI µg mL^{-1} concentration, which was the

Table 2. Mean (±SEM) number of adult *Diaphorina citri* and nymph rating for infestation on citrus flush in Polk County, FL, USA, 2011.

		3 DBT[a]		4 DAT[b]		8 DAT[b]		15 DAT[b]		21 DAT[b]		26 DAT[b]	
Treatment	Rate/hectare	Adults[c]	Nymphs[d]	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
Control	N/A	0.7 a[e] (0.21)	No Flush	0.6 a (1.01)	No Flush	1.0 a (0.3)	0.2 a (0.2)	0.3 a (0.08)	1.67 a (0.53)	0.15 a (0.07)	0.91 a (0.21)	0.18 a (0.07)	2.33 a (0.28)
Fenpropathrin (Danitol 2.4 EC) + oil	1.2 L ha^{-1} + 2% v/v	0.45 a (0.14)	No Flush	0.03 b (0.03)	No Flush	0.13 b (0.05)	0.53 a (0.16)	0.13 b (0.05)	0.70 ab (0.30)	0.1 a (0.06)	1.16 a (0.34)	0.03 a (0.03)	0.7 a (0.3)
Spinetoram (Delegate WG) + oil	0.3 L ha^{-1} + 2% v/v	0.38 a (0.15)	No Flush	0.25 b (0.09)	No Flush	0.1 b (0.05)	0.92 a (0.22)	0.08 b (0.04)	0.15 b (0.09)	0.13 a (0.06)	1.55 a (0.28)	0.05 a (0.03)	2.06 a (0.19)
Sulfoxaflor	0.2 L ha^{-1}	0.5 a (0.15)	No Flush	0.25 b (0.10)	No Flush	0.13 b (0.07)	1.4 a (0.37)	0.3 ab (0.09)	3.0 a (0.0)	0.25 a (0.13)	2.74 a (0.15)	0.2 a (0.11)	2.0 a (1.41)
Sulfoxaflor + oil	0.2 L ha^{-1} + 2% v/v	0.4 a (0.11)	No Flush	0.53 ab (0.13)	No Flush	0.48 ab (0.13)	1.0 a (0.23)	0.3 ab (0.11)	1.27 a (0.32)	0.38 a (0.16)	3.0 a (0.0)	0.3 a (0.09)	1.33 a (0.37)
Sulfoxaflor + oil	0.4 L ha^{-1} + 2% v/v	0.2 a (0.07)	No Flush	1.05 a (0.17)	No Flush	1.1 a (0.31)	2.0 a (0.37)	0.83 a (0.16)	2.0 a (1.11)	0.5 a (0.14)	2.0 a (0.58)	0.18 a (0.07)	1.17 a (0.40)
		$P > 0.05$	N/A	$P \leq 0.05$	N/A	$P \leq 0.05$	$P > 0.05$	$P \leq 0.05$	$P \leq 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Note: [a]Days before treatment.
[b]Days after treatment.
[c]Mean number of adults per 10 tap samples per replicate.
[d]Mean nymph rating based on assessment of 10 flush terminals per replicate.
[e]Means within a column followed by the same letter are not significantly different (ANOVA followed by LSD, $P \leq 0.05$). Data were subjected to $\ln(x + 1)$ transformation to normalize the distributions and homogenize variance prior to analyses. Untransformed means are presented in the table.

Table 3. Mean (± SEM) number of adult *D. citri* and nymph rating for infestation on "Navel" orange flush in Orange County, FL, USA, 2012.

		3 DBT[a]		2 DAT[b]		8 DAT		15 DAT		22 DAT		29 DAT		36 DAT	
Treatment	Rate/hectare	Adult[c]	Nymph[d]	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph
Control	N/A	0.98 a[c] (0.37)	1.27 a (0.15)	0.5 a (0.14)	0.63 a (0.14)	1.1 a (0.31)	1.27 a (0.19)	0.33 a (0.11)	2.41 a (0.14)	0.8 a (0.17)	2.45 a (0.13)	1.6 b (0.39)	2.44 a (0.14)	2.83 a (0.51)	1.0 a (0.11)
Sulfoxaflor + oil	0.3 L ha^{-1} + 2% v/v	0.7 a (0.19)	1.42 a (0.15)	0.18 b (0.06)	0.39 a (0.14)	0.3 b (0.11)	0.47 b (0.13)	0.25 a (0.10)	1.25 ab (0.19)	0.35 ab (0.10)	2.25 a (0.16)	5.7 a (2.72)	1.85 a (0.14)	2.23 a (0.39)	1.14 a (0.13)
Sulfoxaflor + oil	0.2 L ha^{-1} + 2% v/v	0.43 a (0.11)	1.54 a (0.17)	0.3 a (0.10)	0.62 a (0.19)	0.33 b (0.10)	1.47 a (0.20)	0.53 a (0.14)	1.74 a (0.18)	0.55 ab (0.13)	2.31 a (0.14)	3.9 ab (1.04)	2.13 a (0.13)	4.5 a (0.69)	1.28 a (0.16)
Fenpropathrin (Danitol 2.4 EC)	1.2 L ha^{-1}	1.4 a (0.37)	1.43 a (0.15)	0.0 b (0.0)	0.09 b (0.05)	0.08 b (0.04)	0.65 ab (0.16)	0.18 a (0.07)	0.56 b (0.10)	0.13 b (0.06)	1.33 b (0.18)	1.1 b (0.31)	1.6 a (0.18)	2.05 a (0.29)	0.46 b (0.08)
		$P > 0.05$	$P > 0.05$	$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.05$	$P > 0.05$	$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.05$	$P > 0.05$	$P > 0.05$	$P \leq 0.05$

Note: [a]Days before treatment.
[b]Days after treatment.
[c]Mean number of adults per 10 tap samples per replicate.
[d]Mean nymph rating based on assessment of 10 flush terminals per replicate.
[e]Means within a column followed by the same letter are not significantly different (ANOVA followed by LSD, $P \leq 0.05$). Data were subjected to $\ln(x + 1)$ transformation to normalize the distributions and homogenize variance prior to analyses. Untransformed means are presented in the table.

lowest to reduce oviposition and adult emergence (Tiwari & Stelinski 2013). Also, *D. citri* maintained on plants treated with an aqueous solution of imidacloprid (0.1 AI μg mL^{-1}) exhibited reduced fertility, fecundity, and nymph survival, as compared with controls (Boina et al. 2009). Sub-lethal effects against *D. citri* have also been observed with pyriproxyfen, buprofezin, and diflubenzuron and caused reduced development of eggs, nymphs, and adults (Boina et al. 2010; Tiwari et al. 2012). The present investigation suggests that sulfoxaflor has potential to reduce development of *D. citri* at a comparatively lower concentration than other effective insecticides, which may reduce spread of the pathogen causing HLB. Sub-lethal effects may be an additional important component for managing the vector in addition to direct mortality, especially when, like in the case of sulfoxaflor, the residual activity of the insecticide is extended.

We also found that at a concentration above 0.1 AI μg mL^{-1}, sulfoxaflor reduced feeding by *D. citri* as indirectly measured by honeydew excretion. Likewise, 0.125 AI μg mL^{-1} of cyantraniliprole reduced feeding of *D. citri* (Tiwari & Stelinski 2013). Similarly, imidacloprid (Boina et al. 2009; Serikawa et al. 2012) and pyriproxyfen (Boina et al. 2010) reduced feeding of *D. citri* under laboratory conditions. Feeding reduction is critical as *D. citri* acquires and transmits the Las pathogen during feeding, and is considered as an efficient vector. Previous investigations have shown that continuous feeding of approximately 30 min is required on infected trees to successfully acquire the pathogen, and 5–7 h of feeding is required on healthy plants to transmit the pathogen (summarized in Grafton-Cardwell et al. 2013). Therefore, feeding deterrence to *D. citri* on citrus plants may reduce spread of HLB. Finally, it is also possible that deterring settling by *D. citri* onto host plants may reduce spread of HLB (Chiyaka et al. 2012). In the present investigation, settling behavior of *D. citri* was not affected by sulfoxaflor, suggesting that it is not a repellent. Other insecticides, such as cyantraniliprole affected the settling behavior of *D. citri* 72 h after treatment (Tiwari & Stelinski 2013).

Low volume application of sulfoxaflor reduced populations of *D. citri* in the field at rates of 0.2 L ha^{-1} soon after treatments were applied. Similarly higher volume applications at the rate of 0.3 L ha^{-1} effectively reduced *D. citri* populations soon after application. However, the field residual activity of sulfoxaflor was only approximately one week with both types of application methods, which was three times shorter than the residual activity observed under laboratory conditions. Sulfoxaflor is effective against *N. lugens* Stål at rate of 100 and 75 g ai ha^{-1} under field conditions for a period of 15 DAT (Ghosh et al. 2013). The current field results suggest that sulfoxaflor is effective in reducing *D. citri* population; however, there is only a brief period of residual activity. These results suggest that foliar applications of this insecticide should be more effective when used on an area-wide scale as compared with spot applications in order to prevent re-infestation by migrating adult *D. citri*.

Interestingly, the LC_{50} value of sulfoxaflor for *T. radiata* was more than three times greater than for *D. citri*. Therefore, sulfoxaflor might be less toxic to *T. radiata* than the Group 4A neonicotinoids (Hall & Nguyen 2010); however, future field studies are needed to evaluate its overall toxicity to beneficial insects occurring within citrus agro-ecosystems. Similarly, cyantraniliprole, which is a novel anthranilic diamide insecticide, has an LD_{50} value for *T. radiata* 297-fold higher than for *D. citri* (Tiwari & Stelinski 2013). Integration of biological control in addition to the current insecticide program is a remaining challenge for citrus industry in Florida (Monzo et al. 2014). Repeated applications of insecticides have been found to reduce the population of natural enemies in the groves, especially during the critical period of spring flushing (Monzo et al. 2014). Insecticides commonly used in citrus groves reduce between 35% and 79% of *D. citri* nymphs parasitized by *T. radiata* under laboratory conditions (Beloti et al. 2015). Therefore, it is critical to identify and use insecticides that are the less toxic to natural enemies of *D. citri*.

Sulfoxaflor was effective against *D. citri* with its active and residual toxicity comparable to currently used neonicotinoids. Sub-lethal effects of sulfoxaflor resulted in reduced development and feeding by *D. citri* adults. The toxicity data reported here suggest that this should be an effective tool against *D. citri*, and also provide a baseline for future resistance monitoring efforts for this new chemistry. Given its high toxicity to sap-sucking insects and apparent lack of cross-resistance to other nAChR-acting insecticides, sulfoxaflor should be a likely candidate for integration into management rotations with other insecticides for management of *D. citri*.

Acknowledgments

We thank K. Addison, B. Holliday, T. Addison, A. Hoyte, and I. Jackson for *D. citri* collection, maintenance, and technical assistance. We acknowledge Dr Eric Rohrig, Florida Division of Plant Industry, for providing *T. radiata*. This work was supported by the Citrus Research and Development Foundation [Grant number 603].

Disclosure statement

The authors declare that they have no conflicts of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

Citrus Research and Development Foundation [grant number 603].

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