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**Effects of cyantraniliprole, a novel anthranilic diamide insecticide, against Asian citrus  
psyllid under laboratory and field conditions**

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## Abstract

**BACKGROUND:** The Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), is the most destructive pest of citrus in Florida. The development of insecticide resistance in several populations of *D. citri* has been documented. There is an urgent need to develop and integrate novel tools for the successful management of *D. citri* and also to prevent the development of insecticide resistance.

**RESULTS:** We investigated the effects of a relatively newer chemistry, cyantraniliprole, against *D. citri*. The contact toxicity of cyantraniliprole was 297 fold higher against *D. citri* than its primary parasitoid, *Tamarixia radiata* (Hymenoptera: Eulophidae). *D. citri* settled and fed less on cyantraniliprole-treated plants than controls at concentrations as low as 0.025 and 0.125  $\mu\text{g AI mL}^{-1}$ , respectively. *D. citri* egg production, first instar emergence and adult emergence were significantly reduced on plants treated with 0.25, 0.02 and 0.25  $\mu\text{g AI mL}^{-1}$  of cyantraniliprole, respectively, when compared with control plants. Under field conditions, foliar and drench treatments with cyantraniliprole (1436.08  $\text{gH}^{-1}$ ) reduced numbers of *D. citri* adults and nymphs, as well as, of a secondary pest, citrus leafminer, *Phyllocnist citrella* (Lepidoptera: Gracillariidae), more than a standard insecticide.

**CONCLUSIONS:** These results suggest that cyantraniliprole should be a valuable new tool for rotation into *D. citri* management programs. For insecticide resistance management, cyantraniliprole may be particularly useful for rotation with neonicotinoids. In addition, cyantraniliprole was much less toxic to *T. radiata* than to *D. citri* and thus may have less impact on biological control than other currently used broad spectrum insecticides, such as organophosphates and pyrethroids.

**KEYWORDS:** *Diaphorina citri*, insecticide resistance, *Phyllocnistiscitrella*, sub-lethal effects, *Tamarixia radiata*, toxicology.

## 1 INTRODUCTION

Cyantraniliprole is a second-generation anthranilic diamide insecticide discovered by DuPont Crop Protection. This insecticide is currently registered under the active ingredient trade name Cyzapyr™. Anthranilic diamides have a unique mode of action that involves activating ryanodine receptors (RyR), which play a critical role in muscle function.<sup>1-3</sup> Cyantraniliprole binds to the RyR, causing uncontrolled release and depletion of calcium from muscle cells, thus preventing further muscle contraction and ultimately leading to death.<sup>4</sup> Cyantraniliprole is a reduced-risk insecticide, with a very low toxicity to vertebrates and non-target organisms. It has root systemic and translaminar activity against a broad spectrum of sucking and chewing insects.<sup>4</sup> Cyantraniliprole is currently not yet registered for application in certain fruit crops or vegetables in the United States; however, such registrations are pending. The first generation anthranilic diamide insecticide, chlorantraniliprole, has shown promising results in the management of lepidopteran, hemipteran and coleopteran pests.<sup>1-3,5-10</sup>

Asian citrus psyllid, *Diaphorina citri*, is currently the most important insect pest of citrus throughout the world.<sup>11-14</sup> This insect transmits huanglongbing (HLB) in citrus, which causes severe decline in trees and possible death. *D. citri* is currently managed with insecticides, which involves multiple applications of limited available modes of action during a growing season.<sup>15-17</sup> Current reliance on aggressive application of insecticides has not only proven to be economically challenging, but also has led to the development of resistance in some populations of *D. citri* in Florida.<sup>12</sup> Therefore, the need for newer, safer, and effective chemistries remains critical for HLB management and insecticide resistance management programs for *D. citri*. New modes of action offer two advantages: they serve as candidates for insecticide rotation programs and can be supplemented with augmentative releases of natural enemies within integrated pest

management programs. The objective of the present study was to evaluate the efficacy of cyantraniliprole against *D. citri* through a series of laboratory, greenhouse, and field studies. In addition, cyantraniliprole was evaluated against citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), and *Tamarixia radiata*, an ectoparasitoid of *D. citri*, under available circumstances.

## 2 MATERIALS AND METHODS

### 2.1 Insect cultures

The culture of *D. citri* is continuously reared at the Citrus Research and Education Center (CREC), University of Florida, Lake Alfred, FL. The culture was established in 2000 using field populations collected in Polk Co., FL, USA (28.0° N, 81.9° W) prior to the discovery of HLB in the state. The culture is maintained on 'sour orange' (*Citrus aurantium* L.) seedlings without exposure to insecticides in a greenhouse at 27-28°C, 60-65% R.H. and a 14:10 (L: D) photoperiod. One-three day old *T. radiata* adults (male : female = 50 : 50) were obtained from Eric Rohrig, Division of Plant Industry, Florida. *T. radiata* adults were reared on *D. citri* and orange jasmine, *Murraya paniculata*, under greenhouse conditions. Upon receiving *T. radiata*, adults were released into cages with a strip of honey and used for bioassays on the following day.

### 2.2 Insecticides

All toxicity bioassays with *D. citri* and *T. radiata* were conducted with analytical grade insecticide provided by DuPont Crop Protection, Newark-Stine-Haskell Lab, Wilmington, DE. Field, laboratory and greenhouse experiments were conducted using preregistered formulated material (HGW 10SE and HGW 20SC) provided by DuPont Crop Protection. Commercially available

formulations of fenpropathrin (Danitol 2.4EC; Valent USA Corp., Walnut Creek, CA) and thiamethoxam (Platinum; Syngenta Crop Protection, LLC, Greensboro, NC) were used as positive controls in field experiments.

### 2.3 Toxicity of cyantraniliprole to *D. citri* and *T. radiata*

The glass vial method described by Snodgrass<sup>18</sup> was used to evaluate direct toxicity of cyantraniliprole to *D. citri* and *T. radiata* adults. Twenty-ml glass scintillation vials (Wheaton Industries Inc., Millville, NJ) measuring 6.1 cm in height and 2.8 cm in diameter were used in the assays. One ml of technical grade cyantraniliprole dissolved in acetone or acetone alone was applied to each vial. The vials were then rotated on a mechanized roller (Microplate Technology Specialist, Vienna, VA) without heat for 30 min for a uniform coat of insecticide on the inner surface of the vial and for acetone to evaporate. To each glass vial, 20-30 *D. citri* or *T. radiata* adults were inserted after the insecticide solution dried or acetone evaporated. Six concentrations of cyantraniliprole were tested, based on preliminary data collected for each insect species. Each concentration was replicated 5-6 times. Glass vials with insects were held upright in a growth chamber set at  $25 \pm 2$  °C,  $50 \pm 5\%$  RH and a 14:10 (L:D) photoperiod for 24 h. Mortality of *D. citri* or *T. radiata* was assessed 24 h after transfer into the growth chamber. Insects found on their side or back that were unable to move when probed with a camel hair brush were considered dead. Mortality data were corrected for control mortality (< 5%) using Abbott's formula.<sup>19</sup> Mortality data were analyzed separately for *D. citri* and *T. radiata*. Mortality data were pooled for each concentration and subjected to probit regression analysis to calculate the LC<sub>50</sub> with corresponding 95% confidence intervals and slopes of regression lines.<sup>20</sup>

## 2.4 Effect of cyantraniliprole on *D. citri* feeding

Feeding activity of *D. citri* was measured indirectly by quantifying honeydew excretion of adults during exposure to leaf surfaces treated with various concentrations of cyantraniliprole. The experiment was arranged as a randomized complete block design with seven treatments and each treatment was replicated at least six times. Seven treatments consisted of six concentrations (0.13, 0.63, 1.25, 2.50, 5.00 and 10.00  $\mu\text{g AI mL}^{-1}$ ) of cyantraniliprole (HGW 10SE) dissolved in water and a control (water only). Bioassay arenas consisted of agar (Fisher Scientific, Fair Lawn, NJ) coated 60-mm plastic disposable Petri dishes (Thermo Fisher Scientific, Waltham, MA). Fresh citrus leaves collected from insecticide-free Valencia orange trees maintained in a greenhouse were used for all bioassays. Leaf disks 60 mm in diameter were excised, dipped in the treatment (insecticide or water) solutions for 30 s, and allowed to air-dry in a fume hood for 1 h prior to use in the bioassays. After 1 h, the leaf discs were placed in Petri dishes and five adults of mixed gender were transferred into each dish using a camel hair brush. The Petri dish was sealed with a lid lined with 60 mm Whatman filter paper (Whatman International Ltd, Kent, UK). Petri dishes were wrapped with parafilm, turned upside down and transferred into temperature-controlled growth chambers (Percival Scientific, Inc., Perry, IA) set at  $24 \pm 1^\circ\text{C}$ ,  $50 \pm 5\%$  RH and a 14:10 (L:D) photoperiod. After 48 h, filter papers were collected and subjected to a ninhydrin (Sigma-Aldrich, St Louis, MO) test to count honeydew droplets.<sup>21</sup> The number of honeydew droplets was compared among concentrations of cyantraniliprole by one-way ANOVA, followed by Fisher's protected LSD mean separation test (PROC GLM).<sup>20</sup>

## 2.5 Effect of cyantraniliprole on settling behavior of *D. citri*

To evaluate settling behavior of *D. citri* on cyantraniliprole-treated versus non-treated citrus, adults were subjected to untreated citrus 'Swingle' *Citrus aurantiifolia* (Christm.) and similar plants treated with one of the five concentrations of cyantraniliprole. Citrus plants were sprayed with 0.02, 0.25, 0.50, 2.50, or 5.00  $\mu\text{g AI mL}^{-1}$  of cyantraniliprole dissolved in water or water alone until run-off using a handheld atomizer (The Bottle Crew, West Bloomfield, MI). Water alone served as the control. Plants were allowed to air dry before moving into cages. After treatment, citrus plants of similar age (14–16 week old) and vigor were placed randomly into a plexiglass cage (40 cm  $\times$  40 cm  $\times$  40 cm). All six treatments were randomly arranged within each cage as a choice test. Plexiglass cages contained fine mesh sleeves for easy access of plants and insects. There were six cages, with each cage representing a single replicate. Fifty *D. citri* adults were released into the center of each cage. The cages were housed under temperature-controlled conditions of  $25 \pm 2^\circ\text{C}$ ,  $50 \pm 5\%$  RH and a 14:10 (L:D) photoperiod. The total number of *D. citri* settling on each plant was recorded 24, 48 and 72 h after release. The number of adults found on each plant was compared among various treatments using one-way ANOVA followed by Fisher's Protected LSD test at each observation interval.<sup>20</sup>

## 2.6 Effect of cyantraniliprole on developmental stages of *D. citri*

Potted 'Swingle' *Citrus aurantiifolia* (Christm.) plants (two-three months old) with new flush, as defined by Hall and Albrigo,<sup>22</sup> were used for this experiment. The experiment was set up in a randomized complete block design comprised of six treatments and each treatment replicated 3–6 times. The entire experiment was repeated twice. Six treatments consisted of five concentrations (0.02, 0.25, 0.50, 2.50, or 5.00  $\mu\text{g AI mL}^{-1}$ ) of cyantraniliprole (HGW 10SE) dissolved in water and a control (water only). Each plant was sprayed with one of the five



concentrations of cyantraniliprole water until runoff using a handheld atomizer (The Bottle Crew, West Bloomfield, MI). Water alone served as the control. Plants were allowed to air dry and then were exposed to five pairs of adult *D. citri* for mating and oviposition. Each plant was covered with a ventilated cover and maintained at  $25 \pm 2$  °C,  $50 \pm 5\%$  RH and a 14:10 (L:D) photoperiod for 72 h. Thereafter, adults were removed from each plant and the number of eggs per plant was recorded under a stereomicroscope. Three days after egg counts, the number of eggs that hatched per plant was recorded by counting the number of first instar nymphs per plant. Plants were observed on a weekly basis until total adult emergence occurred. Plants were observed for signs of phytotoxicity throughout the experiment. Numbers of eggs, first instar nymphs and adults per plant across treatments were tested for homogeneity of variance and normality to ensure that the assumptions of ANOVA were met. Separate one-way ANOVAs and Fisher's protected LSD mean separation tests were performed to compare the mean numbers of eggs, first instar nymphs, and adults per plant among the various concentrations of cyantraniliprole by pooling data from both experiments (PROC GLM).<sup>20</sup>

## 2.7 Effect of cyantraniliprole treatment under field conditions

The effect of formulated cyantraniliprole treatments was evaluated against *D. citri* under field conditions to complement laboratory experiments. Given the coinciding occurrence of the secondary citrus pest, citrus leafminer, (*P. citrella*), data were also collected for this insect. Two separate experiments were conducted during the summer of 2012 at a grove in Winter Garden (N 28°28.451'; W 81°38.498') (Orange County), FL, to investigate the effects of foliar and drench applications of cyantraniliprole on *D. citri* and *P. citrella*. Both experiments were conducted in a 5-year-old block of 'Navel' citrus trees planted at a tree spacing of 3.8 x 6.1 m. The

experiments were arranged as a randomized complete block design with four replicates. The foliar application experiment consisted of the following three treatments: cyantraniliprole (HGW10SE) at the rate of 1.5 L h<sup>-1</sup>, fenpropathrin (Danitol 2.4EC) at the rate of 1.2 L h<sup>-1</sup> (positive control), and an untreated (negative) control. The drench application experiment consisted of the following five treatments: cyantraniliprole (HGW 20SC) at the rates of 1.1, 1.5 and 2.2 L h<sup>-1</sup>; thiamethoxam (Platinum) at the rate of 0.8 L h<sup>-1</sup> (positive control) and untreated (negative) control. Each treatment was replicated four times for each experiment. Each replicate was comprised of four trees and replicate blocks were separated by two rows of trees. Foliar sprays were made with a truck mounted hand gun sprayer with the pump set to 200 psi delivering 0.3 gallons of finished spray per tree. The spray caused visible leaf runoff. Drench applications were made using a vehicle mounted Admire application system (Chemical Containers, Inc, Lake Wales, FL) set to deliver 0.26 L per tree. For drench applications, each delivery was aimed within the drip-line of a standard ground irrigation system. Sampling of adult and immature stages of *D. citri* and *P. citrella* was conducted weekly up to 8-9 weeks after the application of treatments, with the first sample taken three days after foliar or drench application. *D. citri* nymphs were sampled on 10 randomly collected feather flush<sup>22</sup> from each replicate and ranked on a scale of 0-3 (0 for none, 1 for 1-5 nymphs, 2 for 6-10 nymphs and 3 for more than 10 nymphs). For *D. citri* adults, the stem tap sampling method was used.<sup>23</sup> Ten stem tap samples were collected from each replicate, with a total of forty tap samples for each treatment. Likewise, sampling for *P. citrella* was performed on ten randomly collected feather flush from each replicate. The number of *P. citrella* larvae and pupae per flush was counted. Four separate ANOVAs were performed to compare the number of *D. citri* adults per tap, *D. citri* nymph ranking, *P. citrella* larvae and pupae among the treatments, followed by Fishers Protected LSD

tests for each experiment (PROC MIXED).<sup>20</sup> For each ANOVA, insecticide treatment and date of sample collection served as main effects.

### 3 RESULTS

#### 3.1 Toxicity of cyantraniliprole to *D. citri* and *T. radiata*

The LC<sub>50</sub> values obtained with *D. citri* were significantly lower than with *T. radiata* (Table 1). Based on non-overlapping confidence intervals, the LC<sub>50</sub> value for *T. radiata* was 297 fold higher than that obtained for *D. citri*.

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

One-way ANOVA indicated a highly significant effect of cyantraniliprole concentration ( $F = 11.72$ ;  $df = 6, 59$ ;  $P < 0.0001$ ) on the number of honeydew droplets recorded per filter paper disc. The number of honeydew droplets recorded per filter paper disc was significantly reduced at all concentrations of cyantraniliprole tested when compared with the control treatment (Fig. 1).

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

Settling behavior of *D. citri* adults differed significantly among the various concentrations of cyantraniliprole tested and the control at 72 h ( $F = 8.26$ ;  $df = 5, 30$ ;  $P < 0.0001$ ) after release; however, no differences were found at 24 ( $F = 0.56$ ;  $df = 5, 30$ ;  $P = 0.7262$ ) and 48 ( $F = 1.28$ ;  $df = 5, 30$ ;  $P = 0.2992$ ) h after release (Fig. 2). After 72 h, more psyllids were observed alighting on control plants than on any of the cyantraniliprole treatments (Fig. 2).

#### 3.4 Effect of cyantraniliprole on developmental stages of *D. citri*

The number of eggs ( $F = 4.73$ ;  $df = 5, 48$ ;  $P = 0.0014$ ), first instar nymphs ( $F = 5.07$ ;  $df = 5, 48$ ;  $P = 0.0008$ ) and adults ( $F = 3.03$ ;  $df = 5, 48$ ;  $P = 0.0185$ ) produced per plant differed significantly between the treatments tested. *D. citri* egg production was significantly reduced at  $0.25 \mu\text{g AI mL}^{-1}$  or higher concentrations of cyantraniliprole, whereas first instar nymph production was significantly reduced at  $0.02 \mu\text{g AI mL}^{-1}$  or higher concentrations of cyantraniliprole as compared with the control (Fig. 3). Adult emergence was significantly reduced at  $0.25 \mu\text{g AI mL}^{-1}$  or higher concentrations of cyantraniliprole as compared with the control (Fig. 3).

### 3.5 Effect of cyantraniliprole treatment under field conditions

For the foliar application experiment, *D. citri* nymph ranking per flush was significantly affected by insecticide treatment ( $F = 185.46$ ;  $df = 2, 752$ ;  $P < 0.0001$ ), observation day ( $F = 29.54$ ;  $df = 6, 752$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 21.15$ ;  $df = 12, 752$ ;  $P < 0.0001$ ). Likewise, the number of *D. citri* adults per tap was significantly affected by insecticide treatment ( $F = 23.57$ ;  $df = 2, 936$ ;  $P < 0.0001$ ), observation day ( $F = 21.21$ ;  $df = 7, 936$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 3.66$ ;  $df = 14, 936$ ;  $P < 0.0001$ ). Both *D. citri* nymph ranking per flush and adults counted per tap were significantly lower in plots treated with cyantraniliprole than in the control and fenpropathrin treatments (Table 2). The number of *P. citrellae* larvae per flush was significantly affected by insecticide treatment ( $F = 33.84$ ;  $df = 2, 752$ ;  $P < 0.0001$ ), observation day ( $F = 20.17$ ;  $df = 6, 752$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 2.84$ ;  $df = 12, 752$ ;  $P = 0.0008$ ). Likewise, the number of *P. citrellae* pupae per flush was significantly affected by insecticide treatment ( $F = 19.75$ ;  $df = 2, 751$ ;  $P < 0.0001$ ),

observation day ( $F = 7.19$ ;  $df = 6, 751$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 2.65$ ;  $df = 12, 751$ ;  $P = 0.0017$ ). Both, *P. citrellalarvae* and pupae per flush were significantly lower in cyantraniliprole treated plots than in the control and fenprothrin treatments (Table 3).

For the drench application experiment, *D. citrinymph* ranking per flush was significantly affected by insecticide treatment ( $F = 218.61$ ;  $df = 4, 1627$ ;  $P < 0.0001$ ), observation day ( $F = 78.49$ ;  $df = 8, 1627$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 15.01$ ;  $df = 32, 1627$ ;  $P < 0.0001$ ). Likewise, the number of *D. citri* adults per tap was significantly affected by insecticide treatment ( $F = 62.88$ ;  $df = 4, 1755$ ;  $P < 0.0001$ ), observation day ( $F = 11.58$ ;  $df = 8, 1755$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 4.79$ ;  $df = 32, 1755$ ;  $P < 0.0001$ ). The *D. citri* nymph ranking per flush was significantly lower in plots treated with cyantraniliprole than in the control and thiamethoxam treatments (Table 4). The number of *D. citri* adults per tap was significantly lower in plots treated with the highest rate of cyantraniliprole than in the control and thiamethoxam treatments (Table 4). The number of *P. citrellalarvae* per flush was significantly affected by insecticide treatment ( $F = 126.96$ ;  $df = 4, 1318$ ;  $P < 0.0001$ ), observation day ( $F = 14.30$ ;  $df = 6, 1318$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 11.73$ ;  $df = 24, 1318$ ;  $P < 0.0001$ ). Likewise, the number of *P. citrellapupae* per flush was significantly affected by insecticide treatment ( $F = 49.42$ ;  $df = 4, 1318$ ;  $P = 0.0003$ ), observation day ( $F = 4.32$ ;  $df = 6, 1318$ ;  $P < 0.0001$ ) and interactions between main effects ( $F = 5.03$ ;  $df = 24, 1318$ ;  $P = 0.0017$ ). Both *P. citrellalarvae* and pupae per flush were significantly lower in cyantraniliprole-treated plots than in the control and thiamethoxam treatments (Table 5).

#### 4 DISCUSSION

Insecticide resistance is a possible risk to successful management programs for *D. citri*. Resistance management programs are currently being developed by incorporating safer and newer chemistries and insecticide rotation modules. A component of resistance management for *D. citri* is relaxing use of those chemistries to which resistance is already developing and integrating new and unique modes of action. Cyantraniliprole is one such novel class of insecticide with a unique mode of action that has potential for effective inclusion into current *D. citri* management programs. This is the first report to combine laboratory and field evaluations of this new mode of action against *D. citri*.

Cyantraniliprole is effective against a range of insect pests of various host plants.<sup>5,24,25</sup> This insecticide has not only been effective in the management of insect pests, but also may have less impact on natural enemies than traditional broad spectrum chemistries. *T. radiata* is an effective parasitoid of *D. citri*, causing significant mortality of the pest under laboratory and field conditions.<sup>26,27</sup> In a field study, *D. citri* colonies exposed to biotic factors including *T. radiata* resulted in significantly reduced population growth when compared to colonies that were protected from attack by the third trophic level.<sup>27</sup> Our data indicate that *T. radiata* is relatively much less susceptible to cyantraniliprole than *D. citri*. Likewise, cyantraniliprole is nontoxic to *T. triozae*, a parasitoid of potato/tomato psyllid, *Bactericera cockerelli*.<sup>28</sup> Spraying cyantraniliprole during times of the year when these parasitoids are active (such as mid-summer), may spare these biological control agents as compared with other broad spectrum chemistries. However, effects of cyantraniliprole on other active biological control agents of *D. citri*, such as beetles, lacewings and spiders will require further investigation.

Our results indicate that cyantraniliprole reduces feeding by *D. citri* adults at a rate as low as  $0.125 \mu\text{g AI mL}^{-1}$ . This may impact transmission of the causal pathogen of HLB; however, direct feeding and transmission experiments are needed to verify this hypothesis.

Cyantraniliprole and chlorantraniliprole have been shown to reduce feeding of other insect pests.<sup>29,30</sup> Feeding cessation of *Spodoptera exigua*, *Helicoverpa zea*, *Trichoplusia ni* and *Plutella xylostella* occurs within 25.3, 20.3, 23.4 and 15.4 min, respectively, of exposure of larvae to leaf-discs treated with chlorantraniliprole at the rate of  $167 \text{ mg AI L}^{-1}$ .<sup>29</sup> Feeding by *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) is also reduced after exposure to plants treated with cyantraniliprole at the rate of  $4.41 \text{ mg AI per plant}$ .<sup>30</sup> Up to a 50% reduction in *D. citri* feeding was recorded in this study following cyantraniliprole treatment; however, further investigation is necessary to determine how this may affect pathogen spread by *D. citri*.

Sub-lethal effects of cyantraniliprole were observed by comparing *D. citri* settling behavior on treated vs. control plants. During the first 48 h of the experiment, there was no clear trend; however, at 72 h fewer adults settled on plants treated at the  $0.025 \mu\text{g AI mL}^{-1}$  rate than on control plants. Reduced settling of *D. citri* adults on cyantraniliprole-treated trees should not only reduce direct damage, but also reduce pathogen acquisition and perhaps inoculation. Several insecticides reduce settling behavior of vectors of plant pathogens.<sup>31</sup> Deltamethrin, fenvalerate, pirimicarb and methamidophos reduce settling by green peach aphid and cause them to move to untreated leaf surfaces.<sup>31</sup> Host avoidance by *D. citri* due to cyantraniliprole treatment may contribute to HLB management and this hypothesis warrants further testing.

Further sub-lethal effects of cyantraniliprole on *D. citri* included reduced egg deposition, egg hatch, and adult emergence. Anthranilic diamides have caused similar effects in other insects

investigated to date.<sup>6,32</sup> In addition to being highly toxic to neonate larvae of *Spodoptera exigua* and *Lobesia botrana*,<sup>6,32</sup> chlorantraniliprole caused more than 20% egg mortality in *L. botrana*.<sup>32</sup> Our results indicate that cyantraniliprole may impact *D. citri* populations by reducing egg hatch. *Candidatus Liberibacter asiaticus* is transmitted transovarially from parent to offspring at a rate of 2–6%,<sup>33,34</sup> thus, reduced egg hatch may reduce vertical transmission of the pathogen, in addition to general population reduction as a result of lower psyllid fecundity.

In the field experiments, foliar and drench applications of cyantraniliprole were effective in reducing populations of *D. citri*, as well as, an important secondary pest of citrus, *P. citrella*. These results underscore the potential broad spectrum activity of cyantraniliprole in citrus pest management. Cyantraniliprole also demonstrated a long-lasting residual effect; the effect lasting for more than a month against both pests. Efficacy of cyantraniliprole when applied as a soil drench was longer (one week) than that observed with the foliar application. The field results indicate broad spectrum activity of this chemistry in citrus.

Cyantraniliprole was highly effective against *D. citri* under both laboratory and field conditions and exhibited numerous additional sub-lethal effects. It also exhibited significant efficacy against a secondary lepidopteran pest of citrus (*P. citrella*), while being much less toxic to the primary parasitoid of *D. citri* (*T. radiata*). The toxicity data reported here will provide a baseline for selecting screening doses for monitoring shifts in sensitivity among populations of *D. citri* in Florida and elsewhere for the purpose of resistance monitoring, once cyantraniliprole becomes commercially available to citrus growers in the U.S.A. A proactive approach to maintaining the effectiveness of cyantraniliprole and preventing evolution of resistance to this new tool will be important. Also, this new mode of action should play a large role in the stewardship of existing modes of action, which are still effective for *D. citri* management.



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Table 1: Log-dose probit mortality data for laboratory susceptible *Diaphorina citri* and *Tamarixia radiata* when exposed to cyantraniliprole using a glass vial method.

Insect	n	LC <sub>50</sub> (µg AI ml <sup>-1</sup> )	CI 95%		Slope	SEM	$\chi^2$ (P-value)
<i>D. citri</i>	624	0.2704	0.1759	0.4279	0.6861	0.0586	2.8971 (0.4078)
<i>T. radiata</i>	604	80.2262	11.1042	14900	0.2162	0.0544	0.8935 (0.6397)

Table 2. Efficacy of cyantraniliprole (HGW 10SE) against *Diaphorina citri* adults and nymphs following a foliar application.

Treatment	Overall mean <sup>1</sup> ( $\pm$ SEM) <i>D. citri</i> nymph ranking
HGW 10SE @ 1.5 L H <sup>-1</sup>	0.35 $\pm$ 0.04c
Fenpropathrin (Danitol 2.4EC) @ 1.2 L H <sup>-1</sup>	0.96 $\pm$ 0.06b
Untreated Check	1.59 $\pm$ 0.07a
	Overall mean <sup>2</sup> ( $\pm$ SEM) <i>D. citri</i> adults per tap
HGW 10SE @ 1.5 L H <sup>-1</sup>	0.30 $\pm$ 0.05c
Fenpropathrin (Danitol 2.4EC) @ 1.2 L H <sup>-1</sup>	0.99 $\pm$ 0.15b
Untreated Check	1.32 $\pm$ 0.13a

<sup>1</sup>Overall ANOVA model was significant:  $F = 40.10$ ;  $df = 20, 752$ ;  $P < 0.0001$ .

<sup>2</sup>Overall ANOVA model was significant:  $F = 10.73$ ;  $df = 23, 936$ ;  $P < 0.0001$ .

Values followed by a different letter within a column and developmental stage are significantly different according to ANOVA followed by Fishers Protected LSD test ( $P < 0.05$ ).



Table 3. Efficacy of cyantraniliprole (HGW 10SE) against citrus leafminer, *Phyllocnistiscitrella*, larvae and pupae following a foliar application.

Treatment	Overall mean <sup>1</sup> ( $\pm$ SEM) larvae per flush
HGW 10SE @ 1.5 L H <sup>-1</sup>	1.16 $\pm$ 0.17b
Fenpropathrin (Danitol 2.4EC) @ 1.2 L H <sup>-1</sup>	3.48 $\pm$ 0.29a
Untreated Check	3.29 $\pm$ 0.24a
Overall mean <sup>2</sup> ( $\pm$ SEM) pupae per flush	
HGW 10SE @ 1.5 L H <sup>-1</sup>	0.03 $\pm$ 0.01c
Fenpropathrin (Danitol 2.4EC) @ 1.2 L H <sup>-1</sup>	0.40 $\pm$ 0.07a
Untreated Check	0.25 $\pm$ 0.04b

<sup>1</sup>Overall ANOVA model was significant:  $F = 11.14$ ;  $df = 20, 752$ ;  $P < 0.0001$ .

<sup>2</sup>Overall ANOVA model was significant:  $F = 5.72$ ;  $df = 20, 751$ ;  $P < 0.0001$ .

Values followed by a different letter within a column and developmental stage are significantly different according to ANOVA followed by Fishers Protected LSD test ( $P < 0.05$ ).

Table 4. Efficacy of cyantraniliprole (HGW 20SC) against *Diaphorina citri* adults and nymphs following a drench application.

Treatment	Overall mean <sup>1</sup> ( $\pm$ SEM) <i>D. citri</i> nymph ranking
HGW 20SC @ 1.1 L H <sup>-1</sup>	1.13 $\pm$ 0.06b
HGW 20SC @ 1.5 L H <sup>-1</sup>	0.83 $\pm$ 0.06c
HGW 20SC @ 2.2 L H <sup>-1</sup>	0.44 $\pm$ 0.05e
Thiamethoxam (Platinum) @ 0.8 L H <sup>-1</sup>	0.64 $\pm$ 0.06d
Untreated Check	2.21 $\pm$ 0.06a
Mean <sup>2</sup> ( $\pm$ SEM) <i>D. citri</i> adults per tap	
HGW 20SC @ 1.1 L H <sup>-1</sup>	0.34 $\pm$ 0.05b
HGW 20SC @ 1.5 L H <sup>-1</sup>	0.41 $\pm$ 0.05b
HGW 20SC @ 2.2 L H <sup>-1</sup>	0.09 $\pm$ 0.02c
Thiamethoxam (Platinum) @ 0.8 L H <sup>-1</sup>	0.30 $\pm$ 0.06b
Untreated Check	1.54 $\pm$ 0.14a

<sup>1</sup>Overall ANOVA model was significant:  $F = 45.06$ ;  $df = 44, 1627$ ;  $P < 0.0001$ .

<sup>2</sup>Overall ANOVA model was significant:  $F = 11.31$ ;  $df = 44, 1755$ ;  $P < 0.0001$ .

Values followed by a different letter within a column and developmental stage are significantly different according to ANOVA followed by Fishers Protected LSD test ( $P < 0.05$ ).

Table 5. Efficacy of cyantraniliprole (HGW 20SC) against citrus leafminer, *Phyllocnistiscitrella*, larvae and pupae following a drench application.

Treatment	Overall mean <sup>1</sup> ( $\pm$ SEM) larvae per flush
HGW 20SC @ 1.1 L H <sup>-1</sup>	0.35 $\pm$ 0.08c
HGW 20SC @ 1.5 L H <sup>-1</sup>	0.09 $\pm$ 0.03c
HGW 20SC @ 2.2 L H <sup>-1</sup>	0.03 $\pm$ 0.01c
Thiamethoxam (Platinum) @ 0.8 L H <sup>-1</sup>	3.44 $\pm$ 0.38b
Untreated Check	7.71 $\pm$ 0.61a
Overall mean <sup>2</sup> ( $\pm$ SEM) pupae per flush	
HGW 20SC @ 1.1 L H <sup>-1</sup>	0.04 $\pm$ 0.01c
HGW 20SC @ 1.5 L H <sup>-1</sup>	0.02 $\pm$ 0.01c
HGW 20SC @ 2.2 L H <sup>-1</sup>	0.01 $\pm$ 0.00c
Thiamethoxam (Platinum) @ 0.8 L H <sup>-1</sup>	0.24 $\pm$ 0.04b
Untreated Check	0.68 $\pm$ 0.08a

<sup>1</sup>Overall ANOVA model was significant:  $F = 26.34$ ;  $df = 34, 1318$ ;  $P < 0.0001$ .

<sup>2</sup>Overall ANOVA model was significant:  $F = 9.98$ ;  $df = 34, 1318$ ;  $P < 0.0001$ .

Values followed by a different letter within a column and developmental stage are significantly different according to ANOVA followed by Fishers Protected LSD test ( $P < 0.05$ ).

## Figure Legend

Figure 1. Effect of cyantraniliprole on *D. citri* adult feeding as measured by the number of honeydew droplets produced. Citrus leaf discs treated by various concentrations of cyantraniliprole or water were exposed to five *D. citri* adults. Bars not labeled by the same letter are significantly different from one another according to the Fisher's protected LSD ( $P < 0.05$ ).

Figure 2. Settling preference of *D. citri* adults on citrus plants treated with various concentrations of cyantraniliprole or water 24 (A), 48 (B), and 72 (C) h after release of adults. Bars within a panel not labeled by the same letter are significantly different from one another according to the Fisher's protected LSD ( $P < 0.05$ ).

Figure 3. Effect of cyantraniliprole on various developmental stages of *D. citri*. Mean number of eggs deposited per plant (A), mean number of first instar nymphs eclosing on plants (B), and mean number of adults eclosing on plants (C) sprayed with various concentrations of cyantraniliprole. Bars within a panel not labeled by the same letter are significantly different from one another according to the Fisher's protected LSD ( $P < 0.05$ ).

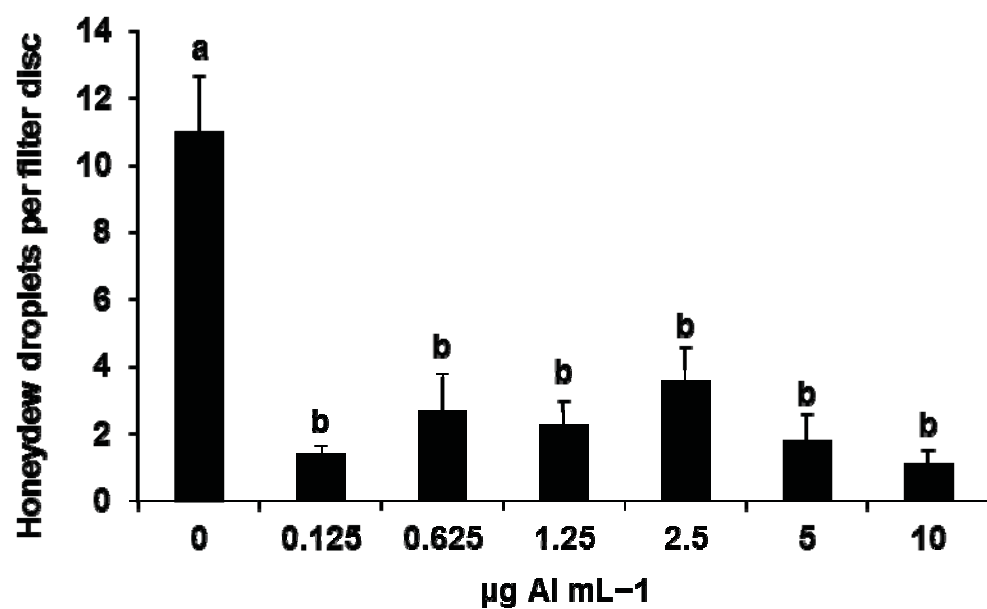


Fig. 1.

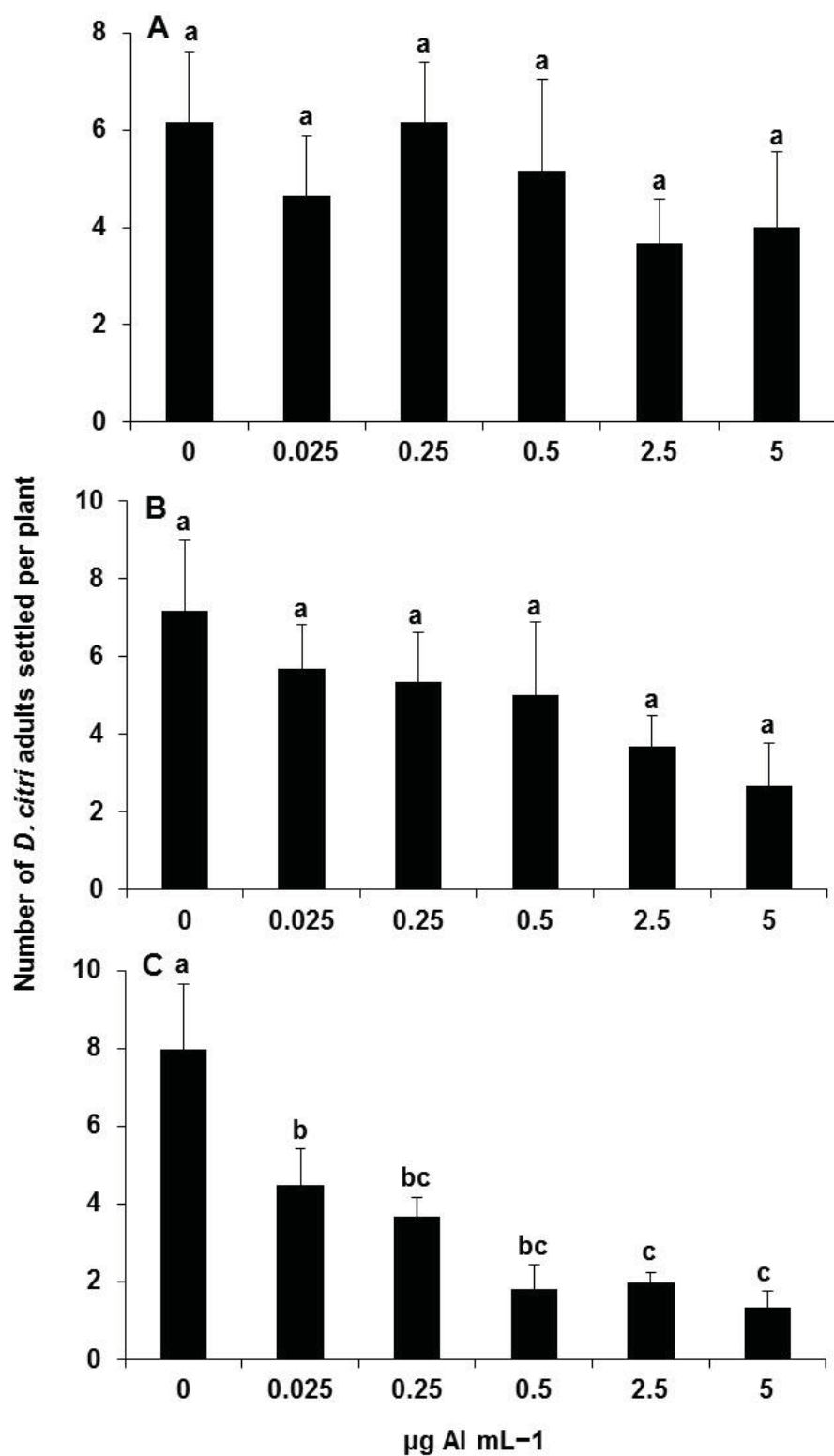


Fig. 2.

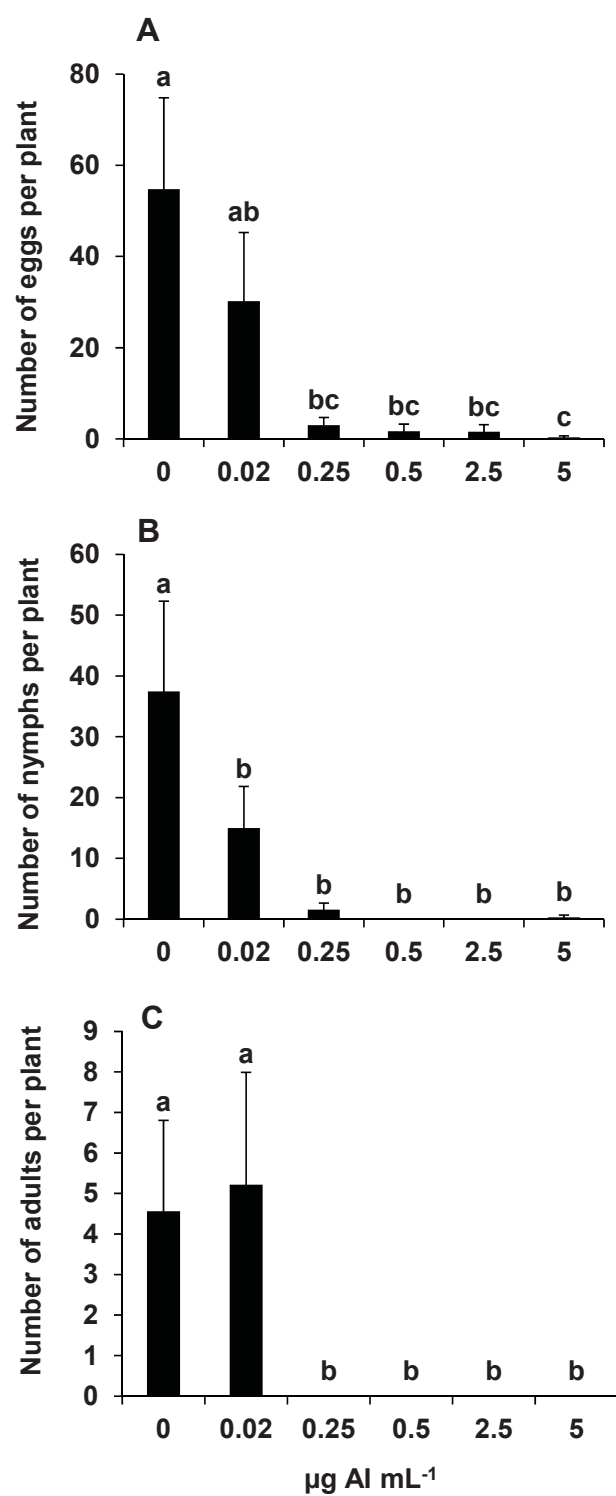


Fig. 3.