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## BASELINE SUSCEPTIBILITY OF *BEMISIA TABACI*, BIOTYPE B (HEMIPTERA: ALEYRODIDAE) TO CHLORANTRANILIPROLE IN SOUTHERN FLORIDA

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### ABSTRACT

Chlorantraniliprole 200 mg ai L<sup>-1</sup> (Rynaxypyr® 200 SC) is the first xylem systemic insecticide in the new chemistry class, anthranilic diamides. A laboratory systemic bioassay using cut stems of cotton seedlings was developed to quantify the baseline susceptibility of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) biotype B, to chlorantraniliprole. Bioassays were conducted for a susceptible laboratory colony and for 11 field populations collected in 2008 and 2009 in Southern Florida. Baseline data of the susceptible colony (targeting first instar nymphs with initial exposure at the egg stage) for chlorantraniliprole in 2008 and 2009, revealed a pooled LC<sub>50</sub> and slope values of 0.033 mg ai L<sup>-1</sup> and 1.186, correspondingly. With the implementation of the stabilization period in the bioassay method in 2009, the susceptible colony generated LC<sub>50</sub> and slope values of 0.182 mg ai L<sup>-1</sup> and 0.972, respectively. LC<sub>50</sub> and slope values of field collected populations (targeting nymphs as above) ranged from 0.016 to 0.046 mg ai L<sup>-1</sup> and 0.889 to 1.595, respectively, in 2008 and 2009. Resistance ratio values at 50% mortality (RR<sub>50</sub>) on nymphs of field colonies ranged from 0.496 to 1.377. LC<sub>50</sub> and slope values of the last 3 field collected populations of 2009, using the stabilization period, ranged from 0.117 to 0.251 mg ai L<sup>-1</sup> and 0.885 to 1.395, respectively, and RR<sub>50</sub> values ranging from 0.645 to 1.381. The overlapping of the fiducial limits of the LC<sub>50</sub> values, the low RR<sub>50</sub> values, and no significant differences in the slopes of the probit lines between the laboratory and field colonies, indicate that *B. tabaci* populations collected in Florida in 2008 and 2009 were highly susceptible to chlorantraniliprole. This anthranilic diamide insecticide is a promising tool in integrated pest management programs for *B. tabaci*, particularly where field populations have developed resistance to other insecticide groups. The baseline information developed in the present study confirmed the susceptibility of field populations in Florida and represents the basis for future susceptibility monitoring programs to help ensure the continued viability of chlorantraniliprole for *B. tabaci* management.

Key Words: Rynaxypyr®; DPX-E2Y45; anthranilic diamides; sweetpotato whitefly; pest management; pesticide resistance

### RESUMEN

Chlorantraniliprole 200 mg ia L<sup>-1</sup> (Rynaxypyr® 200 SC) es el primer insecticida xilema-sistémico en la nueva clase química, diamidas anthranílicas. Un bioensayo sistémico de laboratorio, utilizando plántulas de algodón con el sistema radicular cortado, fue desarrollado para cuantificar la susceptibilidad de la mosca blanca de la batata, *Bemisia tabaci* (Gennadius) biotipo B, a chlorantraniliprole. Los bioensayos fueron conducidos en una colonia susceptible de laboratorio y en 11 poblaciones de campo recolectadas en 2008 y 2009 en el sur-este de Florida. Datos de susceptibilidad de ninfas en primer ínstar (con exposición inicial en estado de huevo) de la colonia susceptible revelaron para chlorantraniliprole en 2008 y 2009, LC<sub>50</sub> y pendiente promedio de 0.033 mg ia L<sup>-1</sup> y 1.186, respectivamente. Con la implementación del periodo de estabilización en el bioensayo en 2009, la colonia susceptible generó una LC<sub>50</sub> y una pendiente de 0.182 mg ia L<sup>-1</sup> y 0.972, correspondientemente. Los valores de LC<sub>50</sub> y pendiente de poblaciones recolectadas en el campo (para ninfas de primer ínstar, como anteriormente) oscilaron entre 0.016 a 0.046 mg ia L<sup>-1</sup> y 0.889 a 1.595, respectivamente, en 2008 y 2009. La tasa de resistencia al 50% de mortalidad (RR<sub>50</sub>) de ninfas de colonias de campo fluctuaron entre 0.496 a 1.377. Los valores de LC<sub>50</sub> y pendiente de las tres últimas poblaciones de campo recolectadas en 2009, utilizando el periodo de estabilización, se extendieron de 0.117 a 0.251 mg ia L<sup>-1</sup> y 0.885 a 1.395, respectivamente, y valores de RR<sub>50</sub> de 0.645 a 1.381. El traslape de los límites fiduciales de las LC<sub>50</sub>, los valores bajos de RR<sub>50</sub>, y la falta de diferencias significativas de las pendientes de las líneas próbit entre las colonias de laboratorio y campo, indica que las poblaciones de *B. tabaci* recolectadas en Florida eran altamente susceptible a chlorantraniliprole. Este insecticida en las diamidas anthranílicas es una herramienta prometedoras en programas de manejo integrado de plagas para *B. tabaci*, particularmente donde poblaciones de campo han desarrollado resistencia a

otros grupos de insecticidas. La información desarrollada en el presente estudio confirma la susceptibilidad de las poblaciones de campo en Florida y representa la base para programas futuros de monitoreo de susceptibilidad que ayuden a asegurar la viabilidad continua de chlorantraniliprole en el manejo de *B. tabaci*.

Palabras Clave: Rynaxypyr®; DPX-E2Y45; diamidas anthranílicas; mosca blanca de la batata; manejo de plagas; resistencia a insecticidas

Since the introduction of the exotic biotype B of the sweetpotato whitefly [*Bemisia tabaci* (Genadius)] in 1987 in Florida and the begomovirus Tomato Yellow Leaf Curl Virus (TYLCV) that it vectors in 1997, both have persisted as the major phytosanitary problem for tomato growers (Price 1987; Schuster et al. 1990; Kring et al. 1991; Brown et al. 1995; Polston et al. 1999). *B. tabaci* also reduces fruit quality and yield production by direct feeding on tomato leaves and also by inducing irregular ripening of tomato fruits (Schuster et al. 1989, 1990). Florida is one of the main tomato growers, contributing to US tomato production with \$ 620.2 and 564.7 million in 2010 and 2011, respectively, most of which consisted of fresh market tomatoes (USDA-NASS 2012). To keep this level of production, insecticides are used as the main mean for controlling the vector and reducing the virus incidence and severity in tomato crops. As a consequence, *B. tabaci* has developed resistance to all the insecticide chemistries used to control it (Cahill et al. 1996a, b, c; Palumbo et al. 2001; Toscano et al. 2001; Li et al. 2003; Horowitz et al. 2007). The discovery of new insecticidal compounds with unique modes of action and target sites is critical to the ongoing success of tomato crop protection.

Chlorantraniliprole (Rynaxypyr®, DPX-E2Y45, Coragen®, DuPont Crop Protection, Wilmington, Delaware) is a xylem systemic insecticide with a new mode of action in the new anthranilic diamide chemical class (DuPont 2008). The diamides belong to IRAC Group 28, the ryanodine receptor modulators (IRAC 2012). Insecticides in this class bind to and activate the ryanodine receptors in insect muscle cells, stimulating calcium release from the internal stores and causing impaired regulation, paralysis and death (Lahm et al. 2005, 2007 2009; Cordova et al. 2006, 2007; Legocki et al. 2008; Wilks et al. 2008). Chlorantraniliprole has demonstrated efficacy in the field against biotype B of *B. tabaci*, especially when applied to the root zone (Portillo et al. 2008; Schuster et al. 2008), and has been shown to be safe to non-target arthropods, including pollinators, numerous beneficial insects and predatory mites (Dinter et al. 2008; DuPont 2008; Preetha et al. 2009; Brugger et al. 2010; Shaw & Wallis 2010; Gradish et al. 2010, 2011).

Because of the potential for the development of resistance of *B. tabaci* to insecticides, a resistance management program was initiated in Florida in 2000 (Schuster & Thompson 2001). An integral

part of that program included resistance monitoring of field populations, which initially focused on the neonicotinoid, imidacloprid, but was later expanded to include other neonicotinoids such as thiamethoxam, acetamiprid and dinotefuran; the pyrethroid bifenthrin; the organochlorine endosulfan; and the insect growth regulator buprofezin (Schuster & Thompson 2001, 2004; Schuster et al. 2002, 2003, 2006; Schuster 2007). As new products are developed for use in managing *B. tabaci*, it is necessary to develop a baseline susceptibility database prior to the registration and commercial use of the product that can be used as reference for future resistance monitoring efforts. Chlorantraniliprole (Rynaxypyr®) was registered as Coragen® in Florida in Aug 2008. Therefore, the objectives of the present investigation were to develop a bioassay in 2008 for estimating the susceptibility of *B. tabaci* to chlorantraniliprole and to use the bioassay to establish the baseline susceptibility of field-collected populations in Southern Florida. This method has since been published as the IRAC-approved method for testing chlorantraniliprole against *B. tabaci*.

## MATERIALS AND METHODS

### Host Plant

Cotton (*Gossypium hirsutum* L., var. 'Deltapine 491'; Malvales: Malvaceae) was selected as the host plant to be used in the bioassays since it had been successfully used in previous systemic bioassays (Schuster et al. 2010; Caballero et al. 2013). Seedlings were grown inside organdy-covered cages within an isolated greenhouse to ensure they were non-infested. Plants were used when the first true leaf had a diam of 2 cm.

### Susceptible Whitefly Colony

A strain of biotype B of *B. tabaci* that had been maintained on tomato plants (*Solanum lycopersicum* L., var. 'Lanai'; Solanales: Solanaceae) in the laboratory for almost 20 yr without exposure to insecticides and without reintroduction of whiteflies from the field was utilized for comparing the relative susceptibility of field populations to chlorantraniliprole. In this study, adults from the original susceptible colony were used to establish a new colony on cotton plants, since cotton was a more convenient host plant for the bioassays.

### Field Populations

To establish field populations of *B. tabaci*, nymph-infested foliage was collected from commercial tomato fields in Southern Florida. Five populations were collected in the 2008 spring crop season, and 6 additional populations were collected during the spring of 2009. Collected foliage was placed in cages of 60 × 60 × 60 cm (BioQuip cat. 1450NS) with non-infested cotton plants maintained in a growth chamber at 26–28 °C with a photoperiod of 12:12 h L:D. The leaf samples were left in the cages for several days to allow as many adults as possible to emerge and settle. These emerged adults were considered the F<sub>1</sub> generation. The whitefly populations were maintained on cotton plants for the duration of the testing. When insufficient adults in the F<sub>1</sub> generation were available to conduct bioassays, the field populations were reared to the F<sub>2</sub>–F<sub>4</sub> generations until sufficient adults were available.

### Systemic Bioassay

The bioassay is a modification of the EARML method developed for the insect growth regulator buprofezin and was the same used to develop baseline susceptibility data for anthranilic diamides (Cahill et al. 1996c; Li et al. 2012; Caballero et al. 2013). Ten to 14 whitefly adults of unknown age and gender were aspirated and transferred to clip-cages (2 cm diam, 1 cm high) on the abaxial surface of the true leaf. Adults were allowed to lay eggs for 24 h then removed and the eggs were counted under a dissecting stereoscope. The sample size was uniformly adjusted to 25 eggs per leaf to avoid first instar competition and migration from leaves. In order to ensure homogeneous insecticide uptake, the height of the seedling stems was standardized to 15.25 cm from the terminal growth point by cutting each stem at its base. It was found that cutting the stems at a diagonal angle with a clean, disinfected scalpel on a cutting board significantly reduced problems with sudden death of seedlings during the bioassay due to contamination. For the last 3 field populations collected in 2009, as well as for the laboratory colony, a ‘stabilization period’ was included as an additional upgrade to the bioassay. This ‘stabilization period’ consisted of placing the cut stems in water for 24 h during the oviposition period prior to placing them in insecticide solution to ensure uniform saturation with water and, therefore, to ensure equal insecticide absorption. A dilution series of 4, 1, 0.25, 0.0625, 0.003906, and 0.000976 mg ai L<sup>-1</sup> of chlorantraniliprole (Coragen® 200 SC, DuPont Crop Protection, Wilmington, Delaware) was prepared using double de-ionized water. An untreated control with water only was included. The cut stems were placed in the respective insecticide solutions in 13 mm diam × 60 mm long

vials with a total volume of 8.5 cc (Fisher cat. 0333925C). The cut stems in vials were placed inside cages and these were maintained in a growth chamber at 26–28 °C at 12:12 h L:D for 14 days for hatching of eggs and for full development of second instar nymphs. Mortality of eggs and first instar nymphs was assessed by subtracting the number of surviving second instar nymphs from the initial egg counts.

### Statistical Analyses

Dose-response data were analyzed by standard probit analysis, estimating LC<sub>50</sub> values and the respective fiducial limits, slope and standard error (SE) of the regression line and the chi square value ( $\chi^2$ ) (SAS Institute 1994). The resistance ratios at 50% mortality (RR<sub>50</sub>) were calculated by dividing the LC<sub>50</sub> value of each field population by that of the susceptible colony. Each experiment consisted of 4 replicates with sample size (*N*) of 309–642 *B. tabaci* eggs, not including those used for untreated controls. To assess and validate the consistency of the systemic bioassay, the entire experiment was repeated 3 times on different dates over a period of a month with the susceptible colony. Once the bioassay method was validated as described above, a single experiment with 4 replicates was conducted per field population. Comparisons of the fiducial limits of the LC<sub>50</sub> values were used to determine significant differences between the laboratory colony and the field colonies as well among the field colonies. The slopes of the laboratory colony and the field populations were also compared to test for differences among populations within years using analyses of covariance (Proc ANCOVA) (SAS Institute 1994).

## RESULTS

The systemic bioassay for chlorantraniliprole timed at the egg stage and using the susceptible colony provided repeatable results in 3 experiments in 2008 with LC<sub>50</sub> values ranging from 0.0173 to 0.0522 mg ai L<sup>-1</sup>, and slopes from 1.154 to 1.373. The data, therefore, were pooled yielding a pooled LC<sub>50</sub> and slope of 0.033 mg ai L<sup>-1</sup> and 1.186, respectively (Table 1). In 2009, the susceptible colony, using the stabilization period, generated a LC<sub>50</sub> and slope of 0.182 and 0.972, correspondingly.

Probit analyses of the mortality data of field populations collected from 5 commercial tomato farms in Southern Florida during the spring of 2008 and 3 during the spring of 2009 (GCC-EV-Tom, GCC #4, and GCC-BW) indicated no difference in susceptibility compared with the laboratory colony (Table 1). LC<sub>50</sub> and slope values ranged from 0.016 to 0.046 mg ai L<sup>-1</sup>, and 0.889 to 1.595, respectively. The fiducial limits of the LC<sub>50</sub> val-

TABLE 1. BASELINE SUSCEPTIBILITY OF *BEMISIA TABACI* FIELD-COLLECTED POPULATIONS FROM SOUTHERN FLORIDA TO CHLORANTRANILIPROLE USING A CUT COTTON STEM, SYSTEMIC UPTAKE BIOASSAY TIMED AT THE EGG STAGE IN THE LABORATORY IN 2008 AND 2009.

Site <sup>1</sup>	Crop	County	N	LC <sub>50</sub> mg ai L <sup>-1</sup> (95%FL)	Slope (±SE)	χ <sup>2</sup>	P value	RR <sub>90</sub>
<b>2008</b>								
Laboratory <sup>2</sup>	<i>G. hirsutum</i>	Hillsborough	1,345	0.033 (0.019 - 0.053)	1.186 (±0.096)	10.481	0.033	—
Immokalee (F <sub>2</sub> )	<i>S. lycopersicum</i>	Dade	473	0.016 (0.010 - 0.025)	0.957 (±0.083)	2.887	0.577	0.496
Clewiston (F <sub>2</sub> )	<i>S. lycopersicum</i>	Collier	463	0.033 (0.020 - 0.050)	0.968 (±0.090)	6.552	0.161	0.997
Homestead (F <sub>2,3</sub> )	<i>S. lycopersicum</i>	Hendry	371	0.046 (0.012 - 0.123)	1.077 (±0.184)	11.437	0.022	1.377
Parrish (F <sub>2</sub> )	<i>S. lycopersicum</i>	Manatee	466	0.032 (0.021 - 0.048)	0.964 (±0.084)	2.847	0.584	0.980
Mayakka City (F <sub>2</sub> )	<i>S. lycopersicum</i>	Manatee	309	0.036 (0.021 - 0.056)	1.238 (±0.150)	2.848	0.583	1.086
<b>2009</b>								
Laboratory <sup>2</sup>	<i>G. hirsutum</i>	Hillsborough	1,345	0.033 (0.019 - 0.053)	1.186 (±0.096)	10.481	0.033	—
GCC-EV-Tom (F <sub>2</sub> )	<i>S. lycopersicum</i>	Hendry	600	0.028 (0.008 - 0.072)	0.889 (±0.126)	13.600	0.009	0.856
GCC #4 (F <sub>2,3</sub> )	<i>S. lycopersicum</i>	Collier	600	0.019 (0.003 - 0.063)	1.320 (±0.266)	29.541	<0.001	0.585
GCC-BW (F <sub>2,3</sub> )	<i>S. lycopersicum</i>	Collier	607	0.017 (0.001 - 0.082)	1.595 (±0.437)	45.915	<0.001	0.509
<b>2009<sup>3</sup></b>								
Laboratory	<i>G. hirsutum</i>	Hillsborough	583	0.182 (0.121 - 0.259)	0.972 (±0.110)	6.014	0.198	—
Homestead #1 (F <sub>1</sub> )	<i>S. lycopersicum</i>	Dade	466	0.117 (0.075 - 0.178)	0.855 (±0.083)	2.352	0.671	0.645
Devil's Garden (F <sub>3</sub> )	<i>S. lycopersicum</i>	Hendry	583	0.231 (0.157 - 0.323)	0.977 (±0.107)	7.433	0.115	1.269
GCC #2 (F <sub>3</sub> )	<i>S. lycopersicum</i>	Collier	642	0.251 (0.082 - 0.555)	1.395 (±0.274)	17.336	0.002	1.381

<sup>1</sup>The F<sub>1</sub> generation included adults emerging in the laboratory from field collected foliage. Subsequent generations were reared on cotton to obtain enough adults for the bioassay.

<sup>2</sup>Pooled data of 3 experiments on the susceptible laboratory colony.

<sup>3</sup>Cut seedlings were placed in de-ionized water for 24 h (stabilization period) prior to being placed in the insecticide test solutions.



ues of the laboratory colony and those of the field colonies overlapped, indicating no significant difference. The  $RR_{50}$  values of field colonies ranged from 0.496 to 1.377, also indicating no differences (Table 1). The slopes also did not differ significantly ( $F_{8,62} = 0.01$ ;  $P = 1.00$ ).

The last 3 field populations collected during the spring of 2009 (Devil's Garden, Homestead #1, and GCC #2) were evaluated following a stabilization period (cut stems allowed to stay in water for 24 h prior to introducing the insecticide) and compared to the laboratory colony that was similarly evaluated. The  $LC_{50}$  value for the laboratory colony for a single bioassay was higher than when a stabilization period was not used. The  $LC_{50}$  values for these field populations are also higher than those not receiving the stabilization period, ranging from 0.117 to 0.251 mg ai L<sup>-1</sup> although the slope ranged from 0.885 to 1.395, which was comparable to the slope of the populations treated with no stabilization period (Table 1). We speculate that the stabilization period causes plants to be uniformly saturated with water, thus reducing insecticide uptake in the plants and increasing the  $LC_{50}$  values as a response. Nevertheless, the probit analyses indicated no difference in susceptibility of the field populations compared to the laboratory colony. The fiducial limits of the  $LC_{50}$  values of the laboratory and field colonies overlapped supporting this conclusion. The  $RR_{50}$  values of the field populations were also low, ranging from 0.645 to 1.381 (Table 1). In addition, the slopes did not differ significantly among the laboratory and field populations ( $F_{3,27} = 0.04$ ;  $P = 0.99$ ).

#### DISCUSSION

The systemic bioassay for estimating susceptibility of *B. tabaci* to chlorantraniliprole proved to be consistent in several experiments with the susceptible laboratory colony. The susceptible colony has been in laboratory culture for almost 20 yr with no insecticide contact and without field introduction of whiteflies. Thus, the baseline results for this laboratory colony will be a key element for monitoring changes in susceptibility to chlorantraniliprole in field populations in time and space. Likewise, the analyses of the results of field populations demonstrated similar susceptibility to the laboratory population, which indicates that chlorantraniliprole has no cross-resistance to insecticides currently being used for whitefly control in Florida. Although chlorantraniliprole was registered in Aug 2008 and the field populations were collected from Apr to Jun in 2008 and in the spring of 2009, they showed high level of susceptibility to this insecticide. The lower mortality values in the last 3 populations analyzed in 2009 is attributed to the implementation of the stabilization period, causing less insecticide uptake by the cut seedlings. This step is considered key and a

methodology upgrade that was incorporated later in this study to standardize amount of insecticide absorbed by each of the plants in the experiment. Similar results were found, using the same bioassay method, in a parallel study carried out at the University of Arizona, which was part of the team to develop baseline susceptibility data to chlorantraniliprole and cyantraniliprole (Li et al. 2012). The  $LC_{50}$  value in the present study, using the stabilization period, for the susceptible laboratory colony was of 0.182 mg ai L<sup>-1</sup> compared to 0.179 mg ai L<sup>-1</sup> reported by University of Arizona (Li et al. 2012) (Table 1).

Chlorantraniliprole has demonstrated excellent control of a broad-spectrum of Lepidopteran crop pests, including the families Crambidae (Ghidui et al. 2009), Gelechiidae (Astor & Scals 2009), Noctuidae (Hardke et al. 2011), Pieridae (Dhawan 2010), Plutellidae (Wang et al. 2010), Psychidae (Rhaind & Sadof 2009), Pyralidae (Yang et al. 2010), and Tortricidae (Loriatti et al. 2009). It has also shown evidence of excellent control on insects of other orders such as Diptera [Tephritidae (Teixeira et al. 2008), Tipulidae (Peck et al. 2008), and Agromyzidae (Conroy et al. 2008)]; Coleoptera [Chrysomelidae (Tang et al. 2009), Curculionidae (Reding & Ranger 2011), and Scarabaeidae (Koppenhöfer & Fuzy 2008)]; Homoptera [Delphacidae (Wang et al. 2009) and Pseudococcidae (Dhawan et al. 2008)]; and Isoptera [Rhinotermitidae (Spomer & Kamble 2011)]. In addition, chlorantraniliprole has exhibited activity against whitefly populations (Homoptera: Aleyrodidae) in laboratory and in the field as well as suppression of transmission of the begomovirus TYLCV (Portillo et al. 2008; Schuster et al. 2008). Chlorantraniliprole also has shown safety to non-target arthropods, including pollinators, parasitoids and predatory insects and mites, and synergistic effects with an entomopathogenic nematode for controlling white grubs (Dinter et al. 2008; DuPont 2008; Koppenhöfer & Fuzy 2008; Preetha et al. 2009; Brugger et al. 2010; Shaw & Wallis 2010; Gradish et al. 2010, 2011). Thus, chlorantraniliprole is a promising tool as part of an integrated pest management program for *B. tabaci*, particularly where whiteflies have already developed resistance to other insecticide groups (Cahill et al. 1996a, b, c; Palumbo et al. 2001; Toscano et al. 2001; Li et al. 2003; Horowitz et al. 2007). The baseline information developed in the present study will be an essential component of a resistance management program and will help ensure the continued viability of chlorantraniliprole for *B. tabaci* management.

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