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BASELINE TOXICITY OF SPIROMESIFEN TO BIOTYPE B OF *BEMISIA TABACI* IN FLORIDA

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ABSTRACT

Biotype B of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is a worldwide pest of many agronomic and horticultural crops, including tomato. Extensive pesticide use targeting this pest has led to the development of resistance to every major class of insecticides. The baseline toxicity of spiromesifen was established for laboratory susceptible and field collected *B. tabaci* biotype B, populations in Florida in 2005 and 2006, respectively, using a leaf dip bioassay method for 2nd instar. LC₅₀ values for field populations of *B. tabaci* ranged from 0.63 mg[AI] l⁻¹ to 0.86 mg[AI] l⁻¹ in 2005 and from 0.46 mg[AI] l⁻¹ to 2.08 mg[AI] l⁻¹ in 2006. No population had a RR₅₀ value over 3.5 in either year and the fiducial limits of the LC₅₀ values for the laboratory and field populations overlapped, indicating no differences among them. The laboratory and field baseline toxicity data generated in this study confirmed the susceptibility of field populations of *B. tabaci* to spiromesifen and will be useful in documenting any future changes in the susceptibility of the whitefly to the insecticide.

Key Words: Spiromesifen, Insecticide control, Insecticide resistance, *Bemisia*, Sweetpotato whitefly

RESUMEN

Biotipo B de la mosca blanca del camote, *Bemisia tabaci* (Genn.), es una plaga mundial de muchos cultivos agronómicos y horticolas, incluyendo el tomate. El uso de pesticidas dirigidos a esta plaga ha llevado al desarrollo de resistencia a todas las principales clases de insecticidas. Se estableció la base de referencia para la toxicidad de espiromesifeno para cuatro y ocho poblaciones de *B. tabaci* en el campo en Florida en el 2005 y el 2006, respectivamente, utilizando un método de bioensayo de sumergir las hojas para las ninfas del segundo estadio. Los valores de CL₅₀ para las poblaciones del campo de *B. tabaci* varían entre 0.63 mg[AI] l⁻¹ a 0.86 mg[AI] l⁻¹ en el 2005 y de mg[AI] l⁻¹ a 2.08 mg[AI] l⁻¹ en el 2006. Ninguna población tenía un valor RR₅₀ de más de 3.5 en cualquier de los dos años, y el límite de fiabilidad de los valores de CL₅₀ para las poblaciones de laboratorio y de campo se superpusieron, lo que indica no hay diferencias entre ellos. Los datos para la base de referencia de toxicidad para los estudios del laboratorio y de campo generados en este estudio confirma la susceptibilidad de las poblaciones de campo de *B. tabaci* a espiromesifeno y será útil para documentar cualquier cambio futuro en la susceptibilidad de la mosca blanca a los insecticidas.

Biotype B of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), has become one of the most serious agricultural pests worldwide due to its tremendous potential to cause direct crop damage and to transmit plant viruses. Although the whitefly induces an irregular ripening disorder of tomato fruit, most damage to tomato results from the transmission of viruses, particularly Tomato yellow leaf curl virus (TYLCV) (Schuster et al. 1995; Varma & Malathi 2003). Numerous applications of neonicotinoids, pyrethroids, organophosphates and endosulfan are applied from planting to harvesting to control whiteflies directly and TYLCV indirectly in Florida and elsewhere. Because of extensive insecticide applications, *B.*

tabaci has developed resistance to almost all major chemical groups, including the neonicotinoids and the insect growth regulators buprofezin and pyriproxyfen (Palumbo et al. 2001; Horowitz et al. 2007). Cases of reduced susceptibility of *B. tabaci* to insecticides have also been reported from Florida (Stansly et al. 1991; Schuster et al. 2003). Although no resistance has been detected in *B. tabaci* biotype B against imidacloprid and structurally similar compounds, such as thiamethoxam and acetamiprid, under field conditions in Florida, cases of reduced susceptibility of adults to imidacloprid and thiamethoxam have been detected (Schuster et al. 2010). Thus, new insecticides that act on selective biochemical sites

present in specific insect groups are needed for resistance management of the whitefly.

Spiromesifen is a spirocyclic phenyl-substituted tetrone acid derivative with broad spectrum insecticidal and acaricidal activity against whiteflies (*Bemisia* and *Trialeurodes* spp.) and spider mites (*Tetranychus* and *Panonychus* spp.) in vegetable and field crops (Nauen et al. 2005). This compound has a novel mode of action of lipid biosynthesis inhibition, resulting in reduced fecundity of adults and in the inability of the younger insect growth stages to develop. The predominant mode of intoxication of whiteflies is by both contact and direct feeding. Nymphal stages of whiteflies are affected more rapidly than the adults and the nymphs treated with spiromesifen did not molt properly and failed to reach adulthood (Nauen et al. 2005). To date active ingredient has not been reported to show cross resistance with any insecticide for which resistant mite or whitefly field populations have been identified (Palumbo 2004). The objective of the current study was to establish baseline susceptibility of laboratory and field collected populations of *B. tabaci* biotype B to spiromesifen in Florida.

MATERIALS AND METHODS

Bemisia tabaci Whitefly Samples

Field populations of *B. tabaci* were sampled by collecting nymph-infested foliage from tomato fields from different locations in Florida in 2005 and 2006. For rearing, the leaves bearing nymphs and puparia were brought to the laboratory and were placed with cotton plants in insect rearing cages at $24 \pm 1^\circ\text{C}$ under a standard photoperiod of 12:12 h L:D. The cotton plants were raised free of insects under greenhouse conditions. Whitefly adults that had emerged from the tomato leaves and settled on the cotton plants were used in the bioassays. Whitefly adults used for calculating resistance ratios were obtained from a susceptible laboratory colony that had been in culture for more than 15 years on tomato plants without any exposure to insecticides and without reintroduction of whiteflies from the field. Susceptibility to spiromesifen was evaluated in whiteflies collected from 4 tomato fields in 2005 and 8 fields in 2006. None of the fields was exposed to spiromesifen in 2005 while 3 fields (Hendry 1, Hendry 2, Hendry 3) were treated with spiromesifen once before collection of nymphs in 2006. All the populations of 2006 were determined to be biotype B through mitochondrial cytochrome oxidase I sequence analysis and micro satellite marker identification (McKenzie et al. 2009)

Bemisia tabaci Nymph Bioassay

Bioassays were conducted with a commercial formulation of spiromesifen (Oberon® 2SC,

Bayer Crop Science, USA). Cotton plants (40 cm high, 4 to 6 nodes) grown in the greenhouse were used for all the bioassays. Nymphal-dip bioassay protocols with slight modifications were similar to those published by Cahill et al. (1996) and Nauen et al. (2005). Ten *B. tabaci* female adults were confined in a clip cage (2 cm diameter, 1 cm high) on a cotton leaf for 24 h for oviposition. Bioassays were performed on cotton leaves to allow easy attachment of clip cages and counting of eggs and nymphal instars. After 24 h, females were removed and the plants were kept under controlled conditions (temperature 22-25 °C and 70-75% RH) for hatching of eggs and development of the nymphs. When the second instar nymphs were predominant (10-12 d old), first and third instar nymphs were removed from the infested leaves with a camel's hair brush and second instar nymphs were counted. The infested leaves were dipped for 10 s in 7 serial dilutions (6, 3, 1.5, 0.75, 0.375, 0.1875, 0.0937 mg[AI]l⁻¹ and control) of spiromesifen prepared with de-ionized water on the d of the bioassays. Only de-ionized water was used for the control treatment. Plants were shifted to controlled conditions after air drying the leaves at room temperature for 1 h. The mortality was recorded 10-12 d after treatment when adults started emerging from the pupae. Nymphs that were desiccated or detached from the leaf surface and late instar nymphs from which no adults emerged after 7 d were considered dead. There were 3 to 4 replicates in all the bioassays, depending upon the availability of whitefly adults. Each replicate comprised 1 treated leaf per plant.

Data Analyses

A standard probit analysis was used to estimate the slopes and the LC₅₀ values for the laboratory colony and each of the field populations (SAS 2003). The relative susceptibility at the 50% mortality level (RR₅₀) was calculated by dividing the LC₅₀ of the field population by the LC₅₀ value of the laboratory colony. New baseline LC₅₀ values for the laboratory colony were obtained for each of the persons for each of the yr to ameliorate any bias among the persons conducting bioassays.

RESULTS

Spiromesifen showed excellent toxicity to the susceptible laboratory and field-collected *B. tabaci* nymphs in 2005 prior to the field use of spiromesifen in Florida. All whitefly populations had estimated LC₅₀ values between 0.53 mg[AI]l⁻¹ and 0.86 mg[AI]l⁻¹, with the lower value corresponding to the susceptible laboratory colony (Table 1). No colony had a RR₅₀ (resistance ratio) value above 2.0 and the fiducial limits of the LC₅₀ values for the laboratory and field populations overlapped, indicating no differences among

TABLE 1. MONITORING THE SUSCEPTIBILITY OF SWEETPOTATO WHITEFLY NYMPHS FROM FIELD POPULATIONS TO SPIROMESIFEN USING A LEAF DIP BIOASSAY METHOD DURING 2005.

Site	County	Crop	Slope	n	χ^2	P	LC ₅₀ (mg [AI] L ⁻¹)	Fiducial Limits	RR ₅₀
Lab colony	—	Cotton	1.19	315	0.36	0.99	0.53	0.39 0.72	—
Ruskin	Hillsborough	Tomato	1.68	298	2.95	0.57	0.78	0.61 1.02	1.47
Parrish1	Manatee	Tomato	1.81	558	26.6	<0.001	0.86	0.46 1.98	1.62
Myakka	Manatee	Tomato	1.51	1088	24.7	<0.001	0.63	0.35 1.03	1.19
Parrish2	Manatee	Tomato	1.37	402	8.39	0.08	0.70	0.40 1.15	1.32

them. The average LC₅₀ and RR₅₀ values for all of the field-collected populations were 0.74 mg [AI] L⁻¹ and 1.4, respectively.

Bioassays conducted in 2006 (Table 2) confirmed susceptibility of *B. tabaci* to spiromesifen. The laboratory colony had an estimated LC₅₀ value of 0.59 mg [AI] L⁻¹. LC₅₀ values of field collected populations ranged from 0.56 mg [AI] L⁻¹ (Homestead 2) to 2.08 mg [AI] L⁻¹ (Hendry3). None of the colonies had RR₅₀ values above 3.5. The average of the LC₅₀ values of the field-collected *B. tabaci* populations of 2006 was slightly higher than the average LC₅₀ value of 2005; however, as in 2005, the fiducial limits of the LC₅₀ values for the laboratory and field populations overlapped, indicating no differences among them. The average LC₅₀ and RR₅₀ values for 2006 were 1.06 mg [AI] L⁻¹ and 1.79, respectively.

DISCUSSION

Baseline susceptibility bioassays in 2005 confirmed the full susceptibility of *B. tabaci* nymphs of the laboratory and field populations collected in 4 locations in Florida in the absence of spiromesifen exposure. The susceptibility was retained in 2006 when 3 of the tested populations were exposed to spiromesifen before collection of nymphs. The LC₅₀ values for field populations over both 2005 and 2006 ranged from 0.46 to 2.08 mg [AI] L⁻¹, which was 4.5-fold difference in susceptibility. This contrasts with results in Arizona and California where a 29-fold difference in susceptibility

of field populations collected in 2005-2006 was observed (0.21 to 6.08 mg [AI] L⁻¹) (Prabhaker et al. 2008).

No populations in the present study indicated RR₅₀ values over 3.5 in either 2005 or 2006. Although the average RR₅₀ value for the year 2006 was slightly higher than the RR₅₀ value 2005, this may be attributed to the differences in persons who conducted the bioassays or to the variability in the field populations. Six of the 8 populations tested in 2006 against spiromesifen showed reduced susceptibility (RR₅₀ values ≥ 10) to imidacloprid and thiamethoxam (Schuster et al. 2010), thus indicating the absence of any cross resistance between spiromesifen and these neonicotinoids. Likewise, no cross resistance was detected in a laboratory strain selected for imidacloprid-resistance (Prabhaker et al. 2008). The RR₅₀ values obtained in the present study also were within the range of RR₅₀ values obtained elsewhere for *B. tabaci* biotypes B and Q, including neonicotinoid and pyriproxyfen resistant strains (Nauen et al. 2005). Although no resistance was observed to spiromesifen under laboratory and field conditions, up to 15 fold resistance to spirodiclofen (another lipid biosynthesis inhibitor) in *Tetranychus urticae* Koch under selection pressure has been demonstrated (Nauen and Konanz 2005).

Spiromesifen has been reported to be safe to key beneficial arthropods (Kavitha et al. 2006; Lakshmi et al. 2006; Irigaray et al. 2007; Bielza et al. 2009). Toxicity of spiromesifen to *B. tabaci* in the absence of cross resistance to neonicotinoids,

TABLE 2. MONITORING THE SUSCEPTIBILITY OF SWEETPOTATO WHITEFLY NYMPHS FROM FIELD POPULATIONS TO SPIROMESIFEN USING A LEAF DIP BIOASSAY METHOD DURING 2006.

Site	County	Crop	Slope	n	χ^2	P	LC ₅₀ (mg [AI] L ⁻¹)	Fiducial Limits	RR ₅₀
Lab colony	—	Cotton	1.01	1928	7.32	0.12	0.59	0.41 0.78	—
Hendry1	Hendry	Tomato	1.65	1434	57.1	<0.001	1.64	0.50 4.45	2.78
Hendry3	Hendry	Tomato	2.63	780	42.6	<0.001	2.08	0.02 3.76	3.51
Hendry4	Hendry	Tomato	1.05	605	3.86	0.43	1.03	0.66 1.43	1.75
Homestead2	Dade	Bean	1.28	1876	9.87	0.08	0.56	0.43 0.72	0.95
Labelle	Hendry	Tomato	1.08	2181	5.58	0.35	0.87	0.76 1.00	1.47
Myakka	Manatee	Tomato	1.11	897	1.59	0.81	0.95	0.66 1.25	1.59
Wimauma	Manatee	Tomato	0.85	1061	7.80	0.10	0.88	0.27 1.63	1.49
Parrish	Manatee	Tomato	0.82	1103	9.43	0.05	0.46	0.08 1.03	0.78

pyriproxyfen and conventional insecticides suggest that spiromesifen can be a valuable tool in management of insecticide resistance in *B. tabaci* in vegetable crops. The possibility of rapid development of resistance in *B. tabaci* to almost all classes of insecticides demands continuous resistance monitoring for spiromesifen along with its judicious use in the field.

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