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RESIDUAL EFFECTS OF NEW INSECTICIDES ON EGG AND NYMPH DENSITIES OF *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE)

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ABSTRACT

The residual effects of 4 new insecticides (cyazypyr, flupyradifurone, pyrifluquinazon, and sulfoxaflor) and 2 registered insecticides (pymetrozine and a combination of zeta-cypermethrin and bifenthrin) on egg and nymph densities of *Bemisia tabaci* biotype B were evaluated at 3, 7 or 14 days after treatment (DAT) of the tomato seedlings with insecticides. Whole plant egg and nymph counts were taken weekly for 3 wk after the introduction of whitefly adults. The study was repeated 3 times. Egg densities tended to be statistically higher at 14 DAT than 3 or 7 DAT in the zeta-cypermethrin/bifenthrin and pymetrozine treatments. The other materials demonstrated greater efficacy than zeta-cypermethrin/bifenthrin and pymetrozine on 14 DAT. Egg densities were very low in all insecticide treatments compared to untreated plants at 3 and 7 DAT. Egg densities on plants treated with cyazypyr, flupyradifurone, pyrifluquinazon, and sulfoxaflor were statistically similar within a given DAT interval for most trials. Treatment effects on nymph densities were similar to treatment effects on egg densities. Cyazypyr, flupyradifurone, pyrifluquinazon, and sulfoxaflor represent distinct modes of action, and should contribute to future integrated pest management and integrated resistance management plans for *B. tabaci* on tomato.

Key Words: *Bemisia tabaci*, cyazypyr, flupyradifurone, pyrifluquinazon, sulfoxaflor

RESUMEN

Se evaluaron los efectos residuales de 4 nuevos insecticidas (cyazypyr, flupyradifurone, pyrifluquinazon, y sulfoxaflor) y 2 insecticidas registrados (pimetrozina y una combinación de zeta-cipermetrina y bifentrina) sobre la densidad de huevos y ninfas de *Bemisia tabaci* biotipo B sobre plántulas de tomate a los 3, 7 o 14 días después del tratamiento (DAT, en inglés) con insecticidas. Se contó el numero de huevos y ninfas sobre las plantas enteras semanalmente durante las 3 semanas después de la introducción de los adultos de mosca blanca. Se repitió el estudio 3 veces. La densidad de huevos tendió a ser estadísticamente mayor a los 14 DAT que a los 3 o 7 DAT en los tratamientos con zeta-cypermethrin/bifenthrin y pimetrozina. Los otros materiales demostraron una mayor eficacia que zeta-cypermethrin/bifenthrin pimetrozina y los 14 DAT. La densidad de huevos fue mas baja en todos los tratamientos de insecticidas en comparación con las plantas no tratadas a los 3 y 7 DAT. La densidad de huevos sobre las plantas tratadas con cyazypyr, flupyradifurone, pyrifluquinazon, y sulfoxaflor fueron estadísticamente similares dentro de un intervalo dado de DAT para la mayoría de las pruebas. El efecto de los tratamientos sobre la densidad de ninfas fue similar al efecto de los tratamientos sobre la densidad de huevos. Cyazypyr, flupyradifurone, pyrifluquinazon y sulfoxaflor representan distintos modos de acción, y deben contribuir en los planes futuros para el manejo integrado de plagas y de manejo de resistencia de *Bemisa tabaci*.

Palabras Clave: *Bemisia tabaci*, cyazypyr, flupyradifurone, pyrifluquinazon, sulfoxaflor

The silverleaf whitefly, *Bemisia tabaci* biotype B (Gennadius) (Hemiptera: Aleyrodidae) vectors many economically important crop viruses, including Tomato yellow leaf curl virus (TYLCV), a begomovirus in the family Geminiviridae. TY-LCV impacts tomato (*Lycopersicon esculentum* Mill.; Solanales: Solanaceae) production globally (Czosnek & Ghanim 2011), and is the primary constraint to production in Florida (Mossler et al. 2009), one of the foremost producers of fresh market tomatoes in the United States (USDA NASS 2011). Tomato varieties resistant to TYLCV are available. However it is common in Florida for growers to plant susceptible varieties and man-

age *B. tabaci* and TYLCV with a combination of at-planting, drip injected and foliar insecticide applications. Intensive insecticide use has led to the development of resistance to key insecticides. Decreased susceptibility to the neonicotinoid insecticides imidacloprid and thiamethoxam has been documented in Florida (Schuster et al. 2009) and China (Wang et al. 2010.). Resistance to pyrethroids, the feeding inhibitor pymetrozine, the juvenile hormone mimic pyriproxifen and other insecticides has been documented in *B. tabaci* populations from different regions of the globe in recent years (Castle et al. 2010; Gorman et al. 2010; Ma et al. 2010). Endosulfan is an insecticide in the cyclodiene group that many growers in the United States include in their insecticide programs for whitefly management. The registration for endosulfan is being withdrawn by the Environmental Protection Agency for use in tomato as of Dec 2014. The combination of insecticide resistance and loss of registrations has stimulated the need for new insecticides to suppress *B. tabaci* and TYLCV in the United States and other tomato growing regions.

Insecticides that are nearing registration and that have shown promise for management of *B. tabaci* include cyazypyr® (E.I. du Pont de Nemours and Co.), flupyradifurone® (Bayer Crop Science), pyrifluquinazon® (Nichino Corp.) and sulfoxaflor® (Dow Chemical Co.) (Tokumaru & Hayashida 2010; Wiles et al. 2011; Zhu et al. 2011). Cyazypyr, also referred to cyantraniliprole, is a ryanodine receptor modulator. Sulfoxaflor is a nicotinic acetylcholine receptor agonist within the sulfoximine insecticide class. Flupyradifurone is also nicotinic acetylcholine receptor agonist. The mode of action of pyrifluquinazon is unknown. Cyazypyr, flupyradifurone and sulfoxaflor have systemic activity; pyrifluquinazon operates primarily as a contact material.

Chemical suppression of *B. tabaci* over the long term requires the judicious use of insecticides possessing different modes of action to prevent the development of insecticide resistance. Information on the residual efficacy of insecticides is necessary for the development of effective insecticide rotations. Greenhouse studies were carried out to determine how cyazypyr, flupyridifurone, pyrifluquinazon, and sulfoxaflor affect egg densities produced by *B. tabaci* when adult whiteflies were confined on tomato seedlings 3, 7, or 14 days after the application of materials. Densities of *B. tabaci* nymphs were quantified to determine if any treatments affected egg viability. Two registered products, pymetrozine (Fulfill™, Syngenta Corp.) and zeta-cypermethrin/bifenthrin (Hero™, FMC Corp.), were included in the studies for comparison. Pymetrozine is a selective hemipteran feeding blocker which has been implicated in the suppression of *B. tabaci* and transmission of TYLCV (Polston & Sherwood 2003). It has both systemic and translaminar activity. Zeta-cypermethrin/bifenthrin is one of several pyrethroid insecticides used for management of *B. tabaci*. These 6 insecticides were compared to determine if there were significant differences in residual activity from the perspective of reducing the density of eggs laid. This information will be combined with other greenhouse and field studies to develop guidelines to enable growers to integrate new materials into their whitefly/TYLCV management plans.

METHODS

Tomato plants (var. Florida 47) were grown from seed in Fafard 3B potting mix (Conrad Fafard, Inc., Agawam, Massachusetts) in a greenhouse in Speedling (Speedling Inc., Ruskin, Florida 33570) trays cut to contain 32 cells. Plants were watered and fertilized as needed with 20- 20-20 water soluble plant food with micronutrients (J. R. Peters Inc., Allentown, Pennsylvania). Insecticide treatments were applied when seedlings had 4 true leaves, 4-5 wk after planting. All plants receiving insecticide treatments were treated on the same day for a given trial.

The maximum labeled rate per application was used for registered products, and the manufacturer's suggested per acre rate was used for products nearing registration. The per acre rates used for each material were - cyazypyr: 20.5 fl. oz (1.5 L/ ha; DuPont DPX-HGW86 10 SE - 0.320 g active ingredient/L); pymetrozine: 2.75 fl. oz (200 mL/ ha; Syngenta Fulfill Insecticide - 0.206 g active ingredient/L); zeta-cypermethrin/bifenthrin: 10.3 fl. oz (751 mL/ha; FMC Hero Insecticide - 0.059 g/L zeta-cypermethrin + 0.178 g/L bifenthrin); pyrifluquinazon: 3.2 fl. oz (233 mL/ha; Nichino NNI-0101 20SC - 0.108 g active ingredient/L); flupyridifurone: 14 fl. oz (1022 mL/ha; Bayer Sivanto 200SL- 0.437 g active ingredient/L); and sulfoxaflor: 5.6 fl. oz (408 mL/ha; Dow AgroSciences GF-2032 240SC - 0.210 g active ingredient/L). The per acre rate was converted to a per plant rate based on 3,630 plants/acre and multiplied by 360, the number of tomato seedlings treated per treatment (approximately 0.1 acre). Each insecticide was mixed in 5 gallons of water (18.927 L), equivalent to 50 gallons/acre (468 L/ha). This was applied to 360 tomato seedlings (12 trays of 30 plants) per treatment for 15 s, until run off. Applications were made at 60 psi with a 2.5 gal (9.46 L), hand-held CO_2 -powered sprayer outfitted with a single nozzle with a D-5 disk and #45 core (Spraying Systems Co., Glendale Heights, Illinois).

After insecticides were applied, each tray was placed on a food service tray inside a $33 \times 43 \times 23$ cm PVC-frame cage enclosed in an organdy mesh bag with a sealed Velcro opening. Treated seedlings were maintained in a greenhouse at 23-31 °C, 45-85% RH and natural light.

Each caged seedling tray received 100 whitefly adults on one of 3 inoculation dates. Adult whiteflies were collected from a colony that has been maintained on TYLCV-infected tomato plants at the University of Florida Gulf Coast Research and Education Center since 2005. Four cages from each treatment were inoculated with whitefly adults 3 days after the application of the insecticide treatment (DAT). Four cages from each treatment were inoculated with whitefly adults 7 DAT, and 4 cages from each treatment were inoculated 14 DAT.

Tomato seedling samples were collected from each cage 7, 14 and 21 d after whitefly adults were introduced. On each sample date, 4 seedlings were removed randomly from each cage. The underside of each leaf was examined beneath a stereo microscope for the presence of whitefly eggs and nymphs. The number of eggs and nymphs per plant was recorded. In total, twelve plants from each cage were sampled. The average of the egg or nymph densities from these twelve plants was used for analysis.

The purpose of this experiment was to evaluate how residual exposure to different materials impacted the density of eggs and nymphs produced by *Bemisia tabaci*. This experiment was repeated 3 times and lasted 5 wk from the application of insecticides to the collection of the final plant sample. Insecticides were applied on 3 Oct 2011 for Trial 1; on 8 Dec 2011 for Trial 2; and 20 Jan 2012 for Trial 3. A total of 84 cages were used for each trial.

Statistical Analysis

The response variables of interest were the average number of eggs or nymphs per tomato seedling, and the factors of interests were insecticide treatment, days after insecticide treatment (DAT) that whitefly adults were confined with tomato seedlings, and the interaction between the 2 factors. Egg and nymph data were log-transformed in order to obtain residuals that were approximately normally distributed. Confidence intervals for the treatment medians were constructed using Tukey's multiple pairwise comparisons test. The data analysis was performed using SAS/STAT and SAS/IML software, Version 9.3 of the SAS System for Windows (SAS 2011). The linear models were fit in PROC GLIMMIX and the goodness of fit analysis was performed in PROC UNIVARIATE. Non-transformed means are reported in the tables.

RESULTS

When egg densities from the 3 trials were pooled for a combined analysis, the treatment by experiment interaction for egg densities was sig-

nificant (F_{12} , _{377.5} = 3.13, $P < 0.01$). For this reason data from each trial will be discussed separately. Days after insecticide treatment (3, 7 or 14 DAT), insecticide treatment and the interaction between the 2 were significant for eggs and nymphs for each trial with the exception of the interaction for nymphs in trial 2 (Table 1).

Overall egg and Nymph Densities

Average egg densities $(\pm$ SEM) per plant in the untreated control ranged from 56.96 ± 7.9 (Trial) 2) to 184.15 ± 36.57 (Trial 3) (Table 2). The highest egg densities at 3 and 7 DAT in plants receiving insecticide treatments were in the pymetrozine treatment $(10.98 \pm 4.05 \text{ and } 9.63 \pm 2.94 \text{ in Trials})$ 1 and 2 respectively). The highest egg densities at 14 DAT were in the zeta-cypermethrin/bifenthrin treatment $(24. 46 \pm 10.72)$.

Average nymph densities in the untreated control ranged from 53.98 ± 10.63 (Trial 3) to 223.64 ± 63.61 (Trial 1) (Table 3). The highest nymph densities in plants treated with insecticide were in the pymetrozine treatments in Trial 1 (11.52 \pm 3.23 at 3 DAT; 11.98 ± 4.68 at 7 DAT; 20.40 ± 8.38 at 14 DAT).

Effect of Day after Treatment within Insecticide Trials

Egg densities 3, 7, and 14 DAT were not significantly different from each other in the untreated control in any trial (Table 4; see Table 2 for egg densities). Nymphs densities in the untreated control were likewise unaffected by day after treatment (Table 4). In each trial, in the zetacypermethrin/bifenthrin treatment egg densities at 14 DAT were significantly higher than at 3 and 7 DAT (Table 2). Nymph densities were higher at 14 DAT than 3 DAT in the zeta-cypermethrin/bifenthrin treatment in 2 trials. In the pymetrozine treatment, egg and nymph densities were higher at 14 DAT than 3 DAT in 2 trials.

Egg and nymph densities at 14 DAT were significantly higher than densities in earlier exposures for cyazypyr, pyrifluquinazon and sulfoxaflor in one trial each. Day after treatment did not affect egg densities in the flupyradifurone treatment in any trial, and affected nymph densities only in trial 2.

Insecticide Treatment Effects

Untreated. Egg densities were always significantly higher on plants in the untreated control than in any insecticide treatment at 3 and 7 DAT comparisons, and higher at 14 DAT in Trials 2 and 3 on untreated plants than plants receiving insecticide treatments. Egg densities at 14 DAT for zeta-cypermethrin/bifenthrin and pymetrozine were not significantly different from the un-

treated control in Trial 1. Nymph densities were significantly higher in the untreated control than other treatments in all studies except at 7 and 14 DAT for Trial 1. In Trial 1, nymph densities were not significantly different between the untreated control and the pymetrozine treatment at 7 DAT, or among the untreated control, pymetrozine and zeta-cypermethrin/bifenthrin at 14 DAT. Flupyradifurone. Egg densities in the flupyra-

difurone treatment were in the lowest groupings statistically at 3, 7 and 14 DAT for each trial. Nymph densities in the flupyradifurone treatment were in the lowest group or not statistically different from the lowest group of nymph densities at 3, 7 and 14 DAT for each trial except 7 DAT in Trial 2.

Pyrifluquinazon and sulfoxaflor. With the exception of one 14 DAT comparison, egg densities in the pyrifluquinazon and sulfoxaflor treatments were in the lowest grouping or not statistically different from the lowest grouping for each trial. Nymph densities in the pyrifluquinazon treatment were in the lowest group or not statistically different from the lowest group in 2 trials at 3 and 7 DAT. At 14 DAT, nymph densities in the pyrifluquinazon treatment were consistently in the lowest grouping. Nymph densities in the sulfoxaflor treatment were in the lowest groupings statistically in 6 of the 9 comparisons.

Zeta-cypermethrin/bifenthrin. Egg densities in the zeta-cypermethrin/bifenthrin treatment at 3 and 7 DAT were consistently in the lowest statistical groupings for each trial. Egg densities at 14 DAT for zeta-cypermethrin/bifenthrin were variable - not statistically different from pymetrozine in 2 trials, not different from the untreated control in one trial, and not different from any non-pymetrozine treatment in one trial. As with egg densities, nymph densities in the zeta-cypermethrin/bifenthrin treatment were in the lowest group or not different statistically from the lowest group at 3 and 7 DAT. At 14 DAT, nymph densities in the zeta-cypermethrin/bifenthrin treatment tended to be in the higher groupings and in trial 1 were not different from the untreated control.

Cyazypyr. Egg densities at 3 DAT were statistically higher in the cyazypyr treatment than the flupyradifurone and zeta-cypermethrin/bifenthrin treatments in Trial 2. However egg densities were not significantly different among cyazypyr, flupyradifurone, pyrifluquinazon, sulfoxaflor and zeta-cypermethrin/bifenthrin at 3 DAT in trials 1 and 3. Overall egg densities for each treatment in each trial were less than 1 egg per plant at 3 DAT, and so of limited concern from a pest management perspective. Egg densities were significantly higher at 7 DAT in the cyazypyr treatment than flupyradifurone, pyrifluquinazon and zeta-cypermethrin/bienthrin treatments in Trial

TABLE 2. AVERAGE WHOLE PLANT EGG DENSITIES ON TOMATO PLANTS CONFINED WITH 100 WHITEFIX ADULTS 3, 7 OR 14 DAYS AFTER THE APPLICATION OF INSECTIOIDE TREATMENTS. TABLE 2. AVERAGE WHOLE PLANT EGG DENSITIES ON TOMATO PLANTS CONFINED WITH 100 WHITEFLY ADULTS 3, 7 OR 14 DAYS AFTER THE APPLICATION OF INSECTICIDE TREATMENTS.

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P = 0.05).

*Means within the same insecticide treatment not followed by the same lower case letter at 3, 7 or 14 DAT are significantly different using Tukey's multiple pairwise comparisons test (

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*Means within the same insecticide treatment not followed by the same lower case letter at 3, 7 or 14 DAT are significantly different using Tukey's multiple pairwise comparisons test (

TABLE 4. STATISTICAL PARAMETERS DESCRIBING THE INTERACTION BETWEEN DAY AFTER TREATMENT (3, 7 OR 14) AND TREATMENT FOR EGG AND NYMPH DENSITIES FOR EACH INSECTICIDE.

††See Table 2 for egg densities and means separation.

**See Table 3 for nymph densities and means separation.

†Non-significance indicates that densities were not statistically different (P > 0.05) at 3, 7 or 14 DAT.

*Significance indicates that densities were statistically different ($P \le 0.05$) at 3, 7 or 14 DAT.

different between cyazypyr and other non-pymetrozine treatments at 7 DAT in the other 2 trials. Egg densities at 14 DAT in the cyazypyr treatment were variable across trials. Nymph densities in the cyazypyr treatment were in the lowest group or not statistically different from the lowest group at 3, 7 and 14 DAT in 2 trials. Nymph densities at 3, 7 and 14 DAT were intermediary in one trial each.

Pymetrozine. Egg densities were significantly higher in the pymetrozine treatment than other insecticide treatments at 3 DAT for 2 trials, but not significantly different from cyazypyr, pyrifluquinazon, or sulfoxaflor in trial 2. Egg densities were significantly higher at 7 DAT for pymetrozine than other insecticide treatments in 2 trials, but not different from the cyazypyr treatment in trial 2. Egg densities were significantly higher in the pymetrozine treatment at 14 DAT than other insecticide treatments in 2 trials, but not statistically different from zeta-cypermethrin/bifenthrin, pyrifluquinazon, or cyazypyr at 14 DAT in Trial 1.

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Nymph densities were significantly higher at 3 and 7 DAT than other insecticide treatments in trials 1 and 3, but only significantly higher than the zeta-cypermethrin treatment at 3 and 7 DAT in trial 2. Nymph densities were consistently higher in the pymetrozine treatments at 14 DAT than cyazypyr, flupyradifurone, and sulfoxaflor.

DISCUSSION

With few exceptions, egg and nymph densities were significantly lower on tomato seedlings treated with insecticides than the untreated control, whether whitefly adults were introduced 3, 7 or 14 days after the insecticide treatment. Egg and nymph densities on treated plants tended to be very low, and in many instances densities in different treatments were not statistically different. Under the conditions of this experiment, the 4 materials that are nearing registration demonstrated a high degree of efficacy with regard to reducing egg density even when whiteflies were introduced into cages 14 days after the material had been applied.

The combination of a quick knock down pyrethroid (zeta-cypermethrin) with a long residual pyrethroid (bifenthrin) produced among the lowest egg and nymph densities at 3 and 7 DAT. The egg density results were comparable to those produced by the 4 products nearing registration, all but one of which is systemic (pyrifluquinazon). The zeta-cypermethrin/bifenthrin treatment but did not demonstrate the same residual activity at 14 DAT as products nearing registration. The higher densities of eggs and nymphs in the pymetrozine treatment are consistent with the label of the product, which indicates suppression only of whiteflies. Low egg and nymph densities across treatments and whitefly exposure dates confirm that each of the materials nearing registration can contribute to suppression of *B. tabaci*. However no treatment resulted in complete suppression of oviposition, even under the controlled conditions of a greenhouse study.

With the exception of pyrifluquinazon, which primarily affects first instar crawlers of *B. tabaci*, the active ingredient in each insecticide treatment has general nymphicidal activity. Overall, treatment effects on nymph densities were similar to treatment effects on egg densities. This indicates that nymph densities were largely a reflection of egg densities, and that treatments did not reduce egg viability under the conditions of this trial.

The whitefly colony used in this trial has been maintained in culture for several years, but was augmented with whitefly adults collected from the Balm, Florida area 2-3 months before the initiation of the trial. While the responses of the whiteflies in this trial may have been representative of whitefly populations in west central Florida, whitefly populations from different areas that have been subjected to distinct insecticidal pressure may demonstrate a different pattern of response. From the perspective of insecticide resistance management, it is promising for growers of crops attacked by *B. tabaci* that novel materials with distinct modes of action are nearing commercial availability. Information from this and other trials carried out in the greenhouse and field will be used to provide guidelines to growers regarding effective rotations of insecticides with distinct modes of action for management of *B. tabaci*. While the focus of this study was foliar application of materials, systemic materials included in this trial have the advantage that they can be applied as at-planting drenches and drip-injected insecticides.

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