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RESEARCH

New Insecticides for Management of Tomato Yellow Leaf Curl, a Virus Vectored by the Silverleaf Whitefly, *Bemisia tabaci*

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ABSTRACT. Greenhouse studies using a randomized complete block design were carried out to evaluate the effect of six insecticides on transmission of Tomato yellow leaf curl virus (TYLCV) by the silverleaf whitefly, *Bemisia tabaci* biotype B Gennadius (Hemiptera: Aleyrodidae) to tomato, *Lycopersicon esculentum* (Miller) (Solanales: Solanaceae), seedlings that were inoculated with whiteflies from a TYLCV colony in cages 3, 7, or 14 d after treatment with insecticide. The purpose was to reveal differences in residual efficacy of four materials that are nearing registration for use on tomato—cyazypyr, flupyradifurone, pyrafluquinazon, and sulfoxaflor—and to compare them with two established insecticides, pymetrozine and a zeta-cypermethrin/bifenthrin combination. Differences in efficacy were expected because these six materials represent five distinct modes of action and both contact and systemic materials. Percentage of tomato seedlings expressing virus symptoms tended to be lowest in seedlings treated with flupyradifurone. The zeta-cypermethrin/bifenthrin insecticide demonstrated comparable efficacy to flupyradifurone in some trials at 3 and 7 d after treatment inoculations, but not the 14 d after treatment inoculation. Pyrafluquinazon was not statistically different from cyazypyr or sulfoxaflor in percentage of plants with virus symptoms in any trial. Percentage virus in the cyazypyr and sulfoxaflor treatments was not statistically different in the 3 and 7 d after treatment inoculations. Among seedlings treated with insecticide, percentage with virus symptoms tended to be highest in the seedlings treated with pymetrozine.

Key Words: begomovirus, *Bemisia tabaci*, Geminiviridae, *tomato yellow leaf curl virus*, whitefly insecticidal control

Tomato yellow leaf curl virus (TYLCV) is one of the most economically important diseases of tomato globally (Moriones and Navas-Castillo 2010). TYLCV is a begomovirus in the Geminiviridae. TYLCV was first detected in Florida, one of the foremost producers of fresh market tomatoes in the United States (USDA NASS 2011), in 1997 (Polston et al. 1997). TYLCV can cause loss of entire fields (Mosler et al. 2009). It is vectored persistently by the silverleaf whitefly, *Bemisia tabaci* biotype B (Gennadius) (Hemiptera: Aleyrodidae). Mehta et al. (1994) demonstrated that feeding by only one infected whitefly can lead to transmission of TYLCV; however, transmission efficiency is increased at densities of five infected adults per plant. Whiteflies can inoculate a tomato plant with TYLCV after 15 min of feeding. There is a latent period of about 24 h between acquiring the virus and the ability to transmit it (Mehta et al. 1994).

Management of TYLCV includes reducing viral inoculum by destroying crop residues, using reflective mulches to repel the vector in early stages of crop growth, planting TYLCV-resistant varieties when appropriate, and treating plants with a combination of at-plant, drip injected, and foliar insecticides (Schuster et al. 2008b). Protection of the crop from TYLCV during the first 5 or 6 wk after transplanting is crucial to reduce yield losses (Sakia and Muniyappa 1989). The development of insecticide resistance and the loss of insecticide registrations have contributed to the need for new insecticides to manage *B. tabaci* and TYLCV in Florida and other tomato growing regions. Intensive use of neonicotinoid insecticides to manage *B. tabaci* has led to the development of tolerance to certain neonicotinoids among some populations of whitefly in Florida (Schuster et al. 2008b, 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, Ma et al. 2010). The registration of endosulfan, a cyclodiene insecticide used to manage whitefly, is being withdrawn for use on Florida tomato in 2014 (Environmental Protection Agency 2010).

Insecticides that will be registered for use in the United States by 2014 and that have demonstrated efficacy in suppressing *B. tabaci* include cyazypyr (also known as cyantraniliprole) (DuPont Corp.; www.dupont.com), flupyradifurone (Bayer Crop Science; www.cropscience.bayer.com), pyrifluquinazon (Nichino; www.nichino.net), and sulfoxaflor (Dow AgroScience; www.dowagro.com). Research indicates that cyzaypyr, flupyradifurone, and pyrifluquinazon may reduce the transmission of certain viruses (Schuster et al. 2008a, Jacobson and Kennedy 2011, Palumbo 2012). Pymetrozine (Fulfill, Syngenta Corp.; www.syngenta-us.com) and a pyrethroid combination, zeta-cypermethrin and bifenthrin (Hero, FMC Corp.; www.fmc.com) are registered insecticides that were included as treatments in the evaluations. Pymetrozine was included as a treatment because it has been implicated in the reduction of transmission of TYLCV (Polston and Sherwood 2003) and like zeta-cypermethrin/bifenthrin, it is commonly used by Florida tomato growers to manage *B. tabaci* (*Vegetable Production Handbook for Florida* 2013).

Cyazypyr is in the anthranilic diamide group of insecticides and kills by disrupting calcium metabolism via the ryanodine receptors. Flupyradifurone and sulfoxaflor are neonicotinoid insecticides, which bind agonistically to nicotinic acetylcholine receptors in the central nervous system. The mode of action of pyrifluquinazon is unknown. Pymetrozine is in the pyridine-azomethine group of insecticides. It paralyzes cibarium muscles and prevents certain Hemiptera from feeding. Zeta-cypermethrin and bifenthrin are sodium channel modulators possessing quick "knock down" properties. Cyazypyr, flupyradifurone, pymetrozine, and sulfoxaflor are xylem mobile and both root and foliarly systemic (Bextine et al. 2004, Foster et al. 2011). Pyrifluquinazon is translaminar and zeta-cypermthrin + bifenthrin works primarily through contact.

Table 1. Type III tests of fixed effects evaluating the effect of insecticide, DAT with insecticide that seedlings were exposed to whiteflies from the TYLCV colony, and their interaction on % of plants with virus symptoms

			Trial 1		Trial 2		Trial 3	
Effect	DF	Error DF	*F* value	Pr > *F*	*F* value	Pr > *F*	*F* value	Pr > *F*
Insecticide	6	819	192.76	<0.0001	2,881.36	<0.0001	3,385.53	<0.0001
DAT	2	819	91.25	<0.0001	119.7	<0.0001	133.23	<0.0001
Insecticide × DAT	12	819	11.26	<0.0001	122.75	<0.0001	98.06	<0.0001

Greenhouse studies were carried out to evaluate the effect of these six materials on transmission of TYLCV by *B. tabaci* on tomato seedlings when seedlings were confined with whiteflies reared on TYLCV-infected tomatoes 3, 7, or 14 d after a single application of each material. These intervals were chosen to evaluate residual activity based on short, intermediate, and long intervals from the perspective of field applications of insecticides to tomato. Because of the importance of protecting the tomato crop from virus in the first weeks after transplanting, seedlings were tested at a stage representative of early establishment in the field. Information was collected on residual efficacy of these materials to better understand the best placement of materials with different modes of action in a whitefly or TYLCV management program. This information, when combined with field evaluations of these products, will contribute to the development of whitefly management guidelines for tomato growers.

Materials and Methods

Three factorial experiments, with insecticide treatments as the first factor and whitefly introductions to the plants at different periods after treatment as the second factor, were carried out using a randomized complete block design with each treatment combination replicated four times. Trial 1 was initiated on 3 October 2011; trial 2 on 8 December 2011; and trial 3 on 20 January 2012. "Florida 47" tomato plants were grown in a greenhouse from seed, and thinned to one plant per cell, in 128, 1½ in^2, cell Speedling trays (Speedling Inc., Ruskin, FL) cut into sections of four rows of eight cells each. Insecticide treatments were applied when seedlings had four true leaves, 4–5 wk after planting. The maximum labeled rate per acre per application was used for registered products, and the manufacturer's suggested per acre rate was used for products nearing registration. The amounts per acre for each material were converted to a per plant rate based on 3,630 plants/acre (8,970 plants/ha) and a treatment volume of 50 gallons/acre (468 liter/ha). The resulting concentrations of the active ingredients were: 0.320 g/liter cyazypyr (DuPont DPX-HGW86 10 SE); 0.437 g/liter flupyradifurone (Bayer Sivanto 200SL); 0.206 g/liter pymetrozine (Syngenta Fulfill Insecticide); 0.108 g/liter pyrifluquinazon (Nichino NNI-0101 20SC); 0.210 g/liter sulfoxaflor (Dow AgroSciences GF-2032 240SC); 0.059 g/liter zeta-cypermethrin + 0.178 g/liter bifenthrin (FMC Hero Insecticide). Each insecticide preparation was mixed in 5 gallons of water (18.927 liter) and applied to thoroughly wet 360 tomato seedlings (12 trays of 32 plants each), without excessive run-off. Applications were made with a hand-held CO_2-powered sprayer, pressurized to 60 psi and outfitted with a single nozzle with a D-5 disk and No. 45 core (Spraying Systems Co., Glendale Heights, IL). In addition to the six-insecticide treatments, an untreated control was included in the evaluations. Plants in the untreated control were exposed to viruliferous whitefly 3, 7, or 14 d after other treatments received insecticide, but received no insecticide applications.

After insecticides were applied, each Speedling tray was placed inside a cage made of nylon organza cloth with a Velcro closure sewn into the top and supported by a 33 × 43 × 23 cm PVC-pipe frame. Each caged Speedling tray was placed on a food service tray and then all were arranged in a randomized complete block design. Treated seedlings were maintained in a greenhouse at an average of 27°C and 65% relative humidity and natural light. Based on previous experience, transmission studies are more effective in when the plant and vector are maintained initially in natural light.

In total, 100 whitefly adults were introduced into each of the seedling cages at 3, 7, or 14 d after treatment with the insecticides. These 100 whiteflies per cage produce a ratio of three adults per plant to ensure exposure of each plant to the virus without providing excessive pressure. Adult whiteflies were collected from a colony maintained on TYLCV-infected tomato plants at the University of Florida Gulf Coast Research and Education Center since 2005.

In total, 10 seedlings were removed from each tray 3 wk after whiteflies were introduced and maintained in a larger cage for an additional 3 wk to allow symptoms of TYLCV to develop under controlled conditions. The seedlings remaining in the trays were used to evaluate treatment effects on egg densities in a separate trial (Smith and Giurcanu 2013). The 10 seedlings were potted in a 3.79 l pot with Fafard 3B potting mix (Conrad Fafard, Inc., Agawam, MA) and placed in a whitefly free 61 × 61 × 61 cm Lumite screen cage (BioQuip Products, Rancho Dominguez, CA) in a growth room, where they were maintained on a 13:11-h light: dark cycle at 23–27°C and ∼30% relative humidity. Plants were watered and fertilized as needed with 20-20-20 water soluble plant food with micronutrients (J. R. Peters Inc., Allentown, PA). Six weeks after exposure to whiteflies, the plants were examined and the number of plants with unambiguous symptoms of TYLCV—stunted, curled upper leaves with bright yellow edges, and interveinal areas—were recorded as infected.

Statistical Analysis. A Generalized Linear Model (GLM) was fit for the binary outcome TYLCV and classification variables insecticide treatment and waiting period before inoculation with whiteflies from the TYLCV colony (days after treatment [DAT]). Tukey–Kramer multiple pairwise comparisons were carried out and the corresponding letter groupings assigned in order to group the proportions in homogeneous groups of significantly greater proportions. The statistical analysis was performed in SAS/GLM using SAS/STAT and SAS/IML software, Version 9.3 of the SAS System for Windows (SAS 2011).

Results

Percent Virus among Treatments. When data from the three trials were pooled and analyzed as one dataset, the influence of "Trial" as a factor on percentage TYLCV was highly significant ($F_{2,\ 2,457} = 374.60$; $P < 0.01$). For this reason, the results of each trial were discussed separately. The effect of insecticide, DAT with insecticide, and the interaction of the two were highly significant for each trial (Table 1).

The same treatment or DAT combinations produced different percentages of plants with TYLCV symptoms in the three trials, and statistical differences among treatments were not always the same in the three trials. However, a relatively consistent relationship among treatment, DAT, and virus incidence was apparent across trials (Table 2). Virus incidence in the untreated control ranged from a low of 70% at 7 and 14 DAT in the second trial to a high of 100% in each DAT in the third trial. This indicates that the amount of viral inoculum present in the batches of 100 whitefly adults used to infect plants varied from trial to trial, and presumably from cage to cage within a trial. Possible reasons for this are discussed below.

Table 2. Percentage of plants (±SEM) with TYLCV symptoms when exposed to whiteflies from a TYLCV colony 3, 7, or 14 DAT with insecticides

Insecticide treatment	DAT 3	DAT 7	DAT 14
Trial 1			
Untreated	95 ± 0.03 A	75 ± 0.06 A	95 ± 0.03 A
Cyazypyr	15 ± 0.05 C	20 ± 0.06 B	42 ± 0.07 C
Flupyradifurone	0 ± – D	0 ± – D	2 ± 0.02 D
Pymetrozine	63 ± 0.07 B	72 ± 0.07 A	85 ± 0.05 AB
Pyrifluquinazon	13 ± 0.05 C	33 ± 0.07 B	70 ± 0.07 ABC
Sulfoxaflor	25 ± 0.06 C	25 ± 0.06 B	77 ± 0.06 AB
Zeta-cypermethrin/bifenthrin	15 ± 0.05 C	10 ± 0.04 C	60 ± 0.07 BC
*F*6,819	542	823	7.51
P	<0.0001	<0.001	<0.0001
Trial 2			
Untreated	90 ± 0.04 A	70 ± 0.07 A	70 ± 0.07 A
Cyazypyr	18 ± 0.06 B	28 ± 0.07 B	33 ± 0.07 BC
Flupyradifurone	0 ± – C	0 ± – C	0 ± – D
Pymetrozine	13 ± 0.05 B	18 ± 0.06 B	55 ± 0.07 AB
Pyrifluquinazon	20 ± 0.06 B	18 ± 0.06 B	13 ± 0.05 C
Sulfoxaflor	15 ± 0.05 B	35 ± 0.07 B	32 ± 0.07 BC
Zeta-cypermethrin/bifenthrin	0 ± − C	0 ± − C	23 ± 0.06 BC
*F*6,819	843	1,170	1,220
P	<0.0001	<0.001	<0.0001
Trial 3			
Untreated	100 ± − A	100 ± − A	100 ± – A
Cyazypyr	13 ± 0.05 B	10 ± 0.04 C	30 ± 0.07 BC
Flupyradifurone	0 ± – C	0 ± – D	5 ± 0.002 C
Pymetrozine	30 ± 0.07 B	63 ± 0.07 B	40 ± 0.07 B
Pyrifluquinazon	8 ± 0.04 B	10 ± 0.04 C	5 ± 0.03 C
Sulfoxaflor	10 ± 0.04 B	13 ± 0.05 C	23 ± 0.06 BC
Zeta-cypermethrin/bifenthrin	0 ± – C	5 ± 0.03 C	20 ± 0.06 BC
*F*6,819	3,678	2,753	742
P	<0.0001	<0.0001	<0.0001

For each trial, means within a column not followed by the same letter are significantly different by the Tukey–Kramer method.

Table 3. *F*-tests indicating differences within each insecticide treatment of percentage plants with TYLCV symptoms when exposed to whiteflies from a TYLCV colony 3, 7, or 14 DAT with the insecticide

Insecticide treatment		Trial 1	Trial 2	Trial 3
Untreated	$F_{2,819}$	*3.15*	2.59	0.00
	Pr > *F*	<0.05	>0.05	>0.05
Cyazypyr	$F_{2,819}$	4.04	1.16	2.95
	Pr > *F*	<0.05	>0.05	>0.05
Flupyradifurone	$F_{2,819}$	78.46	0.00	122.19
	Pr > *F*	<0.01	>0.05	<0.01
Pymetrozine	$F_{2,819}$	4.62	9.09	4.21
	Pr > *F*	<0.05	<0.01	<0.05
Pyrifluquinazon	$F_{2,819}$	11.62	0.40	0.34
	Pr > *F*	<0.01	>0.05	>0.05
Sulfoxaflor	$F_{2,819}$	12.23	2.19	1.28
	Pr > *F*	<0.01	>0.05	>0.05
Zeta-cypermethrin/bifenthrin	$F_{2,819}$	12.30	808.04	578.24
	Pr > *F*	<0.01	<0.01	<0.01

ns (not significant). $P > 0.05$, *$0.05 > P > 0.01$, **$P < 0.01$.

Overall, virus incidence was highest in the untreated control and lowest in the flupyradifurone treatment (Table 2). Among the insecticides, virus incidence tended to be higher in the pymetrozine treatment. Percent virus was significantly higher in the untreated control than all other treatments at 3 DAT for each trial and at 7 and 14 DAT in the third trial. Virus incidence in the flupyradifurone treatment was statistically lower than the untreated control and pymetrozine treatment in all trials, and lower than the cyazypyr and sulfoxaflor treatments in all trials with the exception of 14 DAT in the third trial.

Virus incidence was zero in the flupyradifurone treatment at 3 and 7 DAT for each trial and was not >5% at 14 DAT in any trial. Percentage virus in the zeta-cypermethrin + bifenthrin treatment was statistically lower than all other treatments except flupyradifurone at 3 DAT in the second and third trial, and statistically lower than all other treatments except flupyradifurone at 7 DAT in the first and second trial. The percentage of plants with virus symptoms in the zeta-cypermethrin + bifenthrin treatment was not statistically different from pymetrozine at 14 DAT in any trial. This indicates that the residual effect of the zeta-cypermethin/bifenthrin treatment was reduced at 14 d relative to its efficacy 3 and 7 d after application.

Percentage of plants with virus symptoms in the pyrifluquinazon treatment was not statistically different from the cyazypyr and sulfoxaflor treatments in any trial (Table 2). Percentage of plants with virus symptoms was higher in the cyazypyr treatment than sulfoxaflor at 14 DAT in trial 3 but not in any other comparison. Percentage of plants with virus symptoms was always lower in the cyazypyr treatment than in the untreated control. The number of plants with virus symptoms was always lower in the pyrifluquinazon and sulfoxaflor treatments than the untreated control with the exception of the 14 DAT comparison in the first trial, when these two treatments were not statistically different from the untreated control.

Percentage virus symptoms in the pymetrozine treatment were not statistically different from the untreated control at 7 DAT in the first trial and at 14 DAT in the first and second trials. Percent virus was statistically higher in the pymetrozine treatment than all other insecticide treatments at 3 and 7 DAT in trial 1 and 7 DAT in trial 3.

Percent Virus within the Same Treatment at 3, 7, and 14 DAT. Zeta-cypermethrin/bifenthrin was the only treatment in which percent virus symptoms was statistically higher at 14 DAT than 3 DAT in each trial (Table 3; see Table 2 for virus percentages). In the pymetrozine treatment, percent virus was higher in the 14 DAT than 3 DAT in the first and second trial, and higher at 7 DAT than 3 DAT in the third trial. Percent virus in the flupyradifurone treatment was statistically higher at 14 DAT than 3 and 7 DAT in the first and third trial; however, percent virus at 3 and 7 DAT was zero in this treatment in each experiment. Percent virus at 14 DAT was not higher than 5% in the flupyradifurone treatment in any trial. Percent virus in the cyazypyr, pyrifluquinazone, and sulfoxaflor treatments was higher at 14 DAT than 3 and 7 DAT in trial 1, but not in subsequent trials. In the first trial, percentage virus was statistically lower at 7 DAT than 3 and 14 DAT in the untreated control, but not in other trials.

Discussion

With few exceptions, the percentage of tomato seedlings with TYLCV symptoms was higher in untreated plants than in plants treated with insecticide. The percentage of plants with TYLCV symptoms in the pymetrozine treatment was not statistically different from the untreated control in three of the nine inoculations. Percentage TYLCV symptoms was always lowest in the flupyradifurone treatment. In the first trial, plants inoculated at 14 d demonstrated a significantly greater percentage of TYLCV symptoms than those inoculated at 3 d in each insecticide treatment; however, only zeta-cypermethrin + bifenthrin consistently had higher percentages of TYLCV symptoms in the 14 d after treatment inoculation than the 3 d after treatment inoculation in the three trials. This indicates a greater loss of residual efficacy for the zeta-cypermethrin/bifenthrin treatment at 14 d after treatment than for the other materials evaluated. Reduction in efficacy 2 wk after application is consistent with a contact material such as zeta-cypermethrin + bifenthrin. The percentage of plants with TYLCV symptoms in the zeta-cypermethrin + bifenthrin treatment at 3 and 7 d after treatment inoculations was never >10% and was 0% in three comparisons. The fact that the pyrethroid combination was comparable to flupyradifurone, a highly effective neonicotinoid, points to the ongoing usefulness of pyrethroids for managing whiteflies in areas where resistance to pyrethroids has not developed.

The range of percentage virus observed in plants receiving the same treatment in the three repetitions of the experiment may be due to variability in the amount of viral inoculum present in the 100 whiteflies introduced into each cage. Whitefly adults were aspirated from TYLCV-infected tomato plants without controlling for age or sex with the assumption that 100 whiteflies raised on TYLCV-infected tomato should be enough to transmit the virus to 100% of the 30 seedlings in each cage. However, gender and age affect the ability of whiteflies to transmit TYLCV, and a preponderant number of males or older females could result in reduced transmission of the virus. Czosnek et al. (2001) found that most 1- to 2-wk-old female *B. tabaci* that had fed for 48 h on TYLCV-infected tomato could transmit the virus, but that number dropped to 60% in 3-wk-old females. Only 20% of 1- to 2-wk-old males could transmit the virus after a 48-h acquisition period. These variables may explain why virus transmission was <100% in some of the control cages. It is also possible that although a large number of putatively viruliferous whiteflies were confined with tomato seedlings in a small cage, these whiteflies may have aggregated on certain seedlings and not fed on other seedlings. The amount of viral inoculum will be variable in both laboratory and field populations of viruliferous *B. tabaci*. Despite this variability, consistent patterns were revealed in the percentages of plants expressing symptoms of TYLCV under different insecticide and virus exposure combinations.

The results of this set of trials indicate that each material tested can contribute to the suppression of transmission of TYLCV. Flupyradifurone stands out for the level and duration of TYLCV suppression. However, it is promising that materials with distinct modes of action are effective in suppressing the virus, because growers must employ a variety of toxicological pathways to offset the development of insecticide resistance to any one group. Evaluations of these and other materials as at-plant and foliar spray programs for managing *B. tabaci* and TYLCV are ongoing, and will contribute to the development of guidelines for tomato growers in Florida and elsewhere to manage this key pest.

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