Effectiveness of Cyantraniliprole for Managing *Bemisia tabaci* (Hemiptera: Aleyrodidae) and Interfering with Transmission of Tomato Yellow Leaf Curl Virus on Tomato

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ABSTRACT Cyantraniliprole is the second xylem-systemic active ingredient in the new anthranilic diamide class. Greenhouse (2006), growth chamber (2007), and field studies (2009–2010) were conducted to determine the efficacy of cyantraniliprole for managing *Bemisia tabaci* (Gennadius) biotype B and in interfering with transmission of tomato yellow leaf curl virus (TYLCV) by this whitefly. Cyantraniliprole applied as soil treatments (200 SC) or foliar sprays (100 OD) provided excellent adult whitefly control, TYLCV suppression, and reduced oviposition and nymph survival, comparable to current standards. The positive results observed in these greenhouse experiments with a high level of insect pressure (10× the field threshold of one adult per plant) and disease pressure (five adults per plant, with a high level of confidence that TYLCV virulent adults were used), indicate a great potential for cyantraniliprole to be used in a whitefly management program. Field evaluations of soil drench treatments confirmed the suppression of TYLCV transmission demonstrated in the greenhouse studies. Field studies in 2009 and 2010 showed that cyantraniliprole (200 SC) provided TYLCV suppression for 2 wk after a drench application, when using a susceptible (2009) or imidacloprid-tolerant (2010) whitefly population. Cyantraniliprole was demonstrated to be a promising tool for management of TYLCV in tomato production, which is very difficult and expensive, and which has limited options. The integration of cyantraniliprole into a resistance management program will help to ensure the continued sustainability of this and current insecticides used for the management of insect vectors, including whiteflies and the TYLCV they spreads.

KEY WORDS Cyantraniliprole, Cyazypyr, anthranilic diamides, sweetpotato whitefly, *Bemisia tabaci*

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) biotype B, is a major pest of some of the most important food and fiber crops in the world, transmitting 10% of all known plant viruses (Fauquet et al. 2005, 2008; Tay et al. 2012). *B. tabaci* biotype B was introduced into Florida in 1987, followed a decade later by the introduction of the tomato yellow leaf curl virus (TYLCV), the most devastating virus (genus Begomovirus, family Gemiviridae) for tomato crops (Hamon and Salguero 1987, Price 1987, Schuster et al. 1990, Simone et al. 1990, Kring et al. 1991, Polston et al. 1999, Polston and Lapidot 2007). Since then, TYLCV, vectored exclusively by this whitefly species, annually persists as the key phytosanitary constraint among tomato producers. Fresh market tomatoes are one of the most produced vegetables in Florida, having generated US$435 and US$268 million in 2011 and 2012, respectively (U.S. Department of Agriculture–National Agricultural Statistics Service [USDA-NASS] 2013). Tomato crop sanitation relies heavily on insecticides to control the vector and suppress TYLCV incidence and severity, which has resulted in the development of a broad spectrum of resistance by the whitefly vector to most insecticide chemistries in some US states and other countries (Insecticide Resistance Action Committee [IRAC] MoA group 1A, 1B, 2A, 3A, 4A, 4C, 7C, 9B, 16; Prabhaker et al. 1985, 1992; Dittrich et al. 1990; Cahill et al. 1996a,b,c; Wolfenbarger et al. 1998; Palumbo et al. 2001; Toscano et al. 2001; Li et al. 2003; Riley and Tan 2004; Horowitz et al. 2007; Ahmad et al. 2010; Gorman et al. 2010; Schuster et al. 2010; Caballero et al. 2013a; Longhurst et al. 2013; Nauen et al. 2013a,b; IRAC 2014).

Cyantraniliprole (Cyazypyr, DuPont Crop Protection, Wilmington, DE) is the second active ingredient in the new anthranilic diamide class of chemistry (IRAC MoA group 28). The chemistry acts as a modulator of the ryanodine receptors, which are ubiquitous calcium channel regulators (Lahm et al. 2012, Jeanguenat 2013, IRAC 2014). Insecticides in this chemistry class have a novel mode of action, selectively activating

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and binding to the ryanodine receptors in insect striate muscle cells, provoking calcium release from intracellular stores located in the sarcoplasmic reticulum and inducing gradual muscle contraction, thus causing impaired regulation and feeding, paralysis, and, consequently, death (Lahm et al. 2005, 2007, 2009, 2012; Cordova et al. 2006, 2007; Legocki et al. 2008; Tang and Tao 2008; Wilks et al. 2008; Jeanguenat 2013). Cyantraniliprole, a xylem systemic insecticide with translaminar activity and having root systemicity and foliar penetration, is the first compound in the diamide class that has demonstrated cross-spectrum activity on chewing and sucking arthropod pests (Lahm et al. 2012, Tiwari and Stelinski 2013, Barry et al. 2014). It has high potency on key whitefly species, including *Bemisia tabaci* (Castle et al. 2009; Palumbo 2009, 2010; Stansly et al. 2010; Cameron et al. 2011).

The unique mode of action of cyantraniliprole on insect muscles induces rapid feeding cessation, resulting in inhibition of feeding damage, reproduction, and mobility, and as a consequence, reduction of virus transmission, which has been demonstrated on thrips, aphids, and whiteflies, vectors of Tospovirus, Potyvirus, and Begomovirus (TYLCV), respectively (Jacobson and Kennedy 2011, 2013a,b; Cameron et al. 2013, 2014). Overall, rapid impaired feeding, wide chewing–sucking pest spectrum, and safety to beneficial arthropods (Dinter et al. 2012, Misra 2012, Amarasekare and Shearer 2013, Funderburk et al. 2013, Tiwari and Stelinski 2013) make this anthranilic diamide insecticide a promising tool in integrated pest management programs for *B. tabaci*. Cyantraniliprole received regulatory approval by the U.S. Environmental Protection Agency (US EPA) on January 24, 2014 and by the State of Florida on February 5, 2014 and is labeled for use on tomatoes for whitefly control (Florida Department of Agriculture and Costumer Services [FDACS] 2014, US EPA 2014). Preliminary studies have shown cyantraniliprole to be effective on immature and adult whitefly and TYLCV suppression (Portillo et al. 2009, Schuster et al. 2009, D.J.S. personal communication). In addition, reduction in thrips-transmitted tomato spotted wilt virus and impact on feeding behavior of thrips and aphids has been reported (Jacobson and Kennedy 2011, 2013a,b). Therefore, the objective of the present study was to conduct greenhouse and field studies to determine the efficacy of cyantraniliprole (Cyazypyr 200 SC and 100 OD) for managing *B. tabaci* and for interfering with transmission of the TYLCV by the whitefly.

Materials and Methods

Greenhouse Trials. *Plants.* Tomato (*Solanum lycopersicum* L., 'Florida 47') seedlings were grown in Styrofoam transplant trays containing 32 cells, with one tomato seedling in each cell. Tomato seedlings were transplanted when they reached the two true-leaf growth stage.

Whiteflies. Adults used to infest the tomato plants in all studies were reared in the laboratory on tomato plants, 'Lanai', at the University of Florida/IFAS, Gulf Coast Research & Education Center. Nonvirulent adults were obtained directly from a virus-free colony maintained on tomato plants for >25 yr. TYLCV virulent adults were obtained from a colony that had been reared continuously on TYLCV-infected tomato plants in a temperature-controlled room for >10 yr.

Soil Soak or Drench. In 2006, treatments were applied as soil soaks by immersing a transplant tray for 30 min into food serving trays (68 cm in length by 45 cm in width) containing aqueous treatment solutions. Testing determined that under these conditions, each tray of 32 cells absorbed 280 ml of aqueous solution. In 2007, treatments were applied as soil drenches by pipetting 3.5 ml of solution into each plant cell. Testing determined that this volume was sufficient to thoroughly wet the soil in each cell without leaching out the bottom. Treatments consisted of three rates of cyantraniliprole 200 μg (AI)/ml (Cyazypyr 200 SC; DuPont Crop Protection, Wilmington, DE) tested at 1, 2.5, and 5 mg (AI) per transplant in 2006 and 0.7, 2.5, and 5 mg (AI) per transplant in 2007. Imidacloprid (Admire 240 μg (AI)/ml or Admire Pro 550 μg (AI)/ml; Bayer Crop Science, Kansas, MO) was used as a standard treatment at the label rate for this type of application: 0.7 mg (AI) per plant in both experiments. In total, four trays (replicates) were used per each of the five treatments, including an untreated control.

Foliar Spray. Treatments were applied for 10 s per tray using a CO_2 backpack sprayer with one hollow cone nozzle (D-5 disk and no. 45 core) at 60 psi that delivered 214 ml per tray, an equivalent of 560 liter/ha. Care was taken so that both abaxial and adaxial leaf surfaces were covered, directing the nozzle first over the tops of the seedlings to cover the upper leaf surfaces and then directed upward from the soil surface to cover the lower leaf surfaces. Treatments consisted of three or two rates of cyantraniliprole (Cyazypyr 100 OD) tested at 50, 75, and 100 g (AI)/ha in 2006, and at 75 and 112 g (AI)/ha alone or 75 g (AI)/ha + 0.5% v:v methylated seed oil (MSO) in 2007. Pymetrozine, 50% (AI) wt:wt (Fulfill 50 WDG; Syngenta Crop Protection Inc., Greensboro, NC) at 100 g (AI)/ha was used as a standard treatment in both years. In total, four trays (replicates) were used for each of the five treatments, including an untreated control.

Clip Cage Evaluations. Seven days after treatment (soil soak or drench) or 2 h after the foliar spray, four plants were removed from each tray per treatment and transplanted into 15.25 cm (6″) diameter pots and placed into a room maintained at 22.2 to 25.6°C and photoperiod of 12:12 (L:D) h. A single clip cage was added to a leaflet on each potted plant, four clip cages per tray (replication), four replications total ($n = 16$ clip cages per treatment). Each clip cage was infested with 10 nonvirulent whitefly adults for a total 160 adults per treatment. The number of dead adults was counted daily for the next 3 d, after which the remaining live adults were counted and removed. The number of eggs laid (10 d after treatment [DAT] for soil soak or drench, 3 DAT for foliar spray, and 3 d postinfestation for both) and the number of live and dead nymphs (17 DAT for

soil soak or drench, 10 DAT for foliar spray, and 10 d postinfestation for both) were also recorded in 2007. A nymph was considered dead if it looked desiccated, flat, brownish in color, and the margins were curling or unattached.

Large Cage Evaluations. The remaining 28 plants in each of the four trays per treatment above were left in the trays, placing one tray into each cage (44 cm in length by 23 cm in width by 32 cm in height) constructed with polyvinyl chloride pipe frames and covered with organdy cloth bags with a lengthwise slit at the top sealed with Velcro (Velcro USA Inc. Troy, MI). TYLCV-infected adult whiteflies were immediately released into each cage at a rate of five adults per plant. The cages were randomized on benches in a greenhouse (2006) or in the above temperature-controlled room (2007) with four replications. Three days later (10 DAT for soil soak or drench and 3 DAT for foliar spray), the number of surviving adults on all plants were counted and removed, and the number of eggs laid on the middle 10 plants of each tray were also counted. The trays were then returned to the cages. Seven days later (17 DAT for soil soak or drench, 10 DAT for foliar spray, and 10 d postinfestation for both), the number of live and dead nymphs were counted for the middle 10 plants in each tray. At that time, all of the trays were drenched with imidacloprid at 0.7 mg (AI) per plant, as described above, to kill whitefly adults and nymphs, and the plants were fertilized. Three weeks later, the plants were visually inspected for obvious symptoms of TYLCV infection. Plant tissue was collected from selected plants with typical TYLCV symptoms and from all plants with weak or questionable symptoms to confirm the presence of TYLCV using polymerase chain reaction (PCR). Reactions used the sense primer C473 (Ghanim et al. 1998) and antisense primer PTY1v2406 (Nakhla et al. 1993), which amplify a 859-bp DNA product specific to TYLCV.

Field Trials. *Plants.* Seeds of the ground growing type tomato variety, UF hybrid 8107, were seeded and grown for 6 wk in Styrofoam transplant trays containing 128 cells, with one seed in each cell. Seedlings were field-transplanted when reaching the two true-leaf growth stage on 28–29 September in 2009 and 24–25 March in 2010. Beds were irrigated with a single drip tube per row with emitters spaced every 30 cm at a rate of 1 liter/h (10 psi).

Whiteflies. Viruliferous adults from the TYLCV-infected colony were used in 2009; this is the same colony that was used for the greenhouse studies. The colony used in 2010 was collected the previous fall from a commercial tomato field, and had been maintained on TYLCV-infected tomato plants treated with the LC_{50} (0.347 µg [AI]/ml) of imidacloprid for whitefly adults (based on the susceptible laboratory colony in 2010; Caballero et al. 2013a). The RR_{50} compared with the susceptible laboratory colony was ~10-fold. Both colonies were maintained in the above temperature-controlled rooms at the University of Florida/IFAS, Gulf Coast Research & Education Center.

Treatments. Each plot consisted of a single, bed centered, 12.2 m (40 feet) long row, with 20 plants each spaced 0.6 m (2 feet) apart with an inter-bed distance of 1.5 m (5 feet). After transplanting, soil drench treatments were applied by pouring 100 ml of insecticide solution around each plant. Cyantraniliprole SC 200 µg (AI)/ml was applied at 20 mg (AI) per plant in 2009 and at 20, 10, and 5 mg (AI) per plant in 2010. Imidacloprid 550 µg (AI)/ml was included as a standard treatment in both years at 30 mg (AI) per plant. Immediately after drenching, each plot was covered with UV-resistant, point-bonded polypropylene seed bed insect-proof covers supported with wire hoops spaced at 1.22 m (4 feet) to avoid collapse over the plants (Grower's Solution, LLC, code AG09, Cookeville, TN). Whitefly infestation dates were at 3, 7, 14, and 28 d after drenching in 2009 and 7, 14, and 21 d after drenching in 2010. At every infestation date, 10 mixed age and gender TYLCV viruliferous whitefly adults collected in individual aspirator tubes were released at the base of each plant by making small holes on the side of cover cages. Three weeks after each infestation date, all 20 plants per replicate were uncovered and visually inspected, recording incidence and severity of TYLCV infection (Seem 1984), based on a grading scale from 0 to 5 modified from Polston and Sherwood (2003).

0 = healthy, no symptoms.
1 = very mild, partial chlorosis in some areas of developing leaflet.
2 = severe chlorosis but no curling and normal plant size and on occasion chlorosis in developed leaflets.
3 = chlorosis, initial curling and slight plant reduction in size.
4 = at least two leaflets deformed and reduced size, chlorosis, some stunted.
5 = completely stunted, deformed and reduced leaf and plant size, severe chlorosis.

An index of symptom severity or mean symptom severity was calculated by multiplying the number of plants in each rating category by the number of plants within each symptom rating (0 to 5), summing across all the rates and dividing by the total number of plants showing symptoms (Polston and Sherwood 2003).

Statistical Analyses. *Greenhouse Trials.* Treatments were arranged in a randomized complete block design with four replications. Data were subjected to analyses of variance (ANOVAs), and means were compared using the least significant different (LSD, $P \leq 0.05$; PROC GLM, SAS Institute Inc. 2008, Cary, NC).

Field Trials. Treatments were evaluated in a split-plot design with four replications, having infestation dates as the main plot, and insecticides and untreated controls as subplots, both designated at random. Treatment effects were analyzed separately within each infestation date, TYLCV incidence and severity data were subjected to ANOVAs and means were compared using the LSD ($P \leq 0.05$) in the event of a significant

Table 1. Impact of cyantraniliprole 200 SC or imidacloprid 550 SC in soil soak (2006) and drench (2007) experiments on adult and nymph *B. tabaci* mortality through time (clip cage experiments) exposed for 3 d to tomato plants treated 7 d earlier

		Clip Cage Evaluation								
		% Adult mortality[b] (DAE[c])						No. of immature		
		2006			2007			2007		
Treatment[a]	mg (AI) per plant	1	2	3	1	2	3	Eggs	Live nymphs	Dead nymphs
Cyantraniliprole 200 SC	0.7	___[d]	___	___	59.3bc	73.8b	100b	3b	<1b	3b
	1.0	11.4a	61.2bc	94.9bc	___	___	___	___	___	___
	2.5	6.0a	44.5b	78.4b	56.0bc	71.2b	100b	3b	<1b	3b
	5.0	3.7a	49.6bc	89.4bc	62.4c	77.5b	100b	2b	<1b	3b
Imidacloprid 550 SC	0.7	46.6b	67.8c	96.1c	49.1b	69.1b	96.0b	4b	1b	3b
Control	0.0	0.6a	1.8a	7.9a	8.2a	16a	17.1a	45a	32a	8a
LSD		5.82	11.29	8.05	14.08	12.63	5.94	6	8	5
$F_{4,72}$		83.13	41.69	170.42	19.85	32.81	303.07	73.75	28.42	1.78
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1422

[a] Tomato plants were soaked/drenched 7 d prior to exposing adult whiteflies in clip cages.
[b] Mortality percentage are based on 160 adults in four replicates per treatment.
[c] Days after exposure (DAE).
[d] Not tested. Means within a column followed by the same letter are not significantly different ($P \geq 0.05$; LSD; PROC GLM, SAS Institute Inc. 2008).

Table 2. Impact of cyantraniliprole 100 OD or pymetrozine 50 WDG in foliar spray experiments on adult and nymph *B. tabaci* mortality through time (clip cage experiments) exposed to foliar residues on tomato leaves treated 2 h earlier

		Clip cage evaluation								
		% Adult Mortality (DAE)						No. of Immature		
		2006			2007			2007		
Treatment[a]	g (AI) per ha	1	2	3	1	2	3	Eggs	Live nymphs	Dead nymphs
Cyantraniliprole 100 OD	50	26.4bc	55.8b	91.1b	___	___	___	___	___	___
	75	33.8bc	76.6c	95.7b	27.7b	55.5b	92.0b	3b	<1b	3b
	75+ MSO	___	___	___	45.8c	65.9b	99.4b	2b	<1b	2a
	100	40.5c	82.5c	98.2b	___	___	___	___	___	___
	112	___	___	___	15.4a	37.2c	92.6b	4b	<1b	3b
Pymetrozine 50 WDG	100	28.8bc	53.4c	87.4b	26.2b	43.6c	84.4b	5b	1b	5c
Control	0.0	7.7a	11.9a	15.0a	8.0a	12.5a	18.1a	30a	18a	<1a
LSD		9.98	9.77	5.57	13.08	11.8	7.96	6	5	2
$F_{4,72}$		11.98	64.05	316.99	9.55	23.55	140.76	34.3	22.75	6.52
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002

[a] Tomato plants were sprayed and allowed to dry for 2 h prior to exposing adult whiteflies in clip cages. Means within a column followed by the same letter are not significantly different ($P \geq 0.05$; LSD; PROC GLM, SAS Institute Inc. 2008).

F value ($P \leq 0.05$; PROC GLM, SAS Institute Inc. 2008).

Results

Greenhouse Trials. In the clip cage evaluations, cyantraniliprole 200 SC applied via soil soak (2006) appeared to result in slower adult mortality as compared with a soil drench at 1 d after exposure (DAE; 2007); however, this comparison has to be taken with caution as the two methods were not tested in the same experiment and higher mortality was observed in the controls in 2007 (Table 1). All treatments, including imidacloprid, provided statistically similar adult mortality at 2 and 3 DAE. No clear dose response was observed with the soil soak or drench applications of cyantraniliprole. Control survival level achieved ranged from 83 to 92% after 3 d of exposure. Cyantraniliprole 100 OD applied via foliar sprays provided significant adult whitefly mortality in both years as compared with the untreated control, and was comparable with that of pymetrozine (Table 2). All cyantraniliprole rates applied as foliar spray provided similar adult mortality. The use of methylated seed oil in 2007 appeared to speed up control at 1 DAE, but all treatments were similar by 3 DAE. After 3 d of exposure, cyantraniliprole provided from >92 to nearly 100% control. All soil and foliar treatments resulted in fewer eggs and fewer live nymphs compared with the control, except for dead nymphs (Tables 1 and 2).

In the large cage evaluations, cyantraniliprole (200 SC) applied via soil soak or drench at 0.7–1, 2.5, and 5 mg (AI) per plant or as foliar sprays (100 OD) at 50–112 g (AI)/ha provided significant reductions in the number of whitefly adults, eggs, and the subsequent

Table 3. Impact of cyantraniliprole 200 SC or imidacloprid 550 in soil soak (2006) and drench (2007) experiments on TYLCV transmission and adult survival, oviposition, and the resulting nymph survival of *B. tabaci* (large cage experiments with TYLCV viruliferous *B. tabaci* adults) exposed for 3 d to tomato plants treated 7 d earlier

		Large cage evaluation (no. per 10 plants[a])								
		2006				2007				
Treatment	mg (AI) per plant	Adults	Eggs	Nymphs	TYLCV incidence	Adults	Eggs	Live nymphs	Dead nymphs	TYLCV incidence
Cyantraniliprole 200 SC	0.7	0b	4b	<1b	26.7b	1b	2b	6b	1a	25bc
	2.5	0b	3b	0b	16.4c	<1b	3b	1b	0b	8.7c
	5.0	<1b	5b	2b	19.8c	<1b	1b	2b	<1a	11.5c
Imidacloprid 550 SC	0.7	1b	7b	<1b	23.2c	1b	1b	7b	2a	36b
Control	0.0	20a	88a	70a	58.7a	17a	43a	65a	2a	81.7a
LSD		4	35	19	12.7	11	29	32	2	17.76
$F_{4,12}$		41.03	10.73	25.35	18.68	4.62	3.86	6.8	2.15	26.33
P-value		<0.0001	0.0006	<0.0001	<0.0001	0.0173	0.0305	0.0042	0.1367	<0.0001

[a] Average of four replicates. Means within a column followed by the same letter are not significantly different ($P \geq 0.05$; LSD; PROC GLM, SAS Institute Inc. 2008).

Table 4. Impact of cyantraniliprole 100 OD or pymetrozine 50 WDG in foliar spray experiments on TYLCV transmission and adult survival, oviposition, and the resulting nymph survival of *B. tabaci* (large cage experiments with TYLCV viruliferous *B. tabaci* adults) exposed for 3 d to foliar residues on tomato plants treated 2 h earlier

		Large cage evaluation (no. per 10 plants)								
		2006				2007				
Treatment	g (AI) per ha	Adults	Eggs	Nymphs	TYLCV incidence	Adults	Eggs	Live nymphs	Dead nymphs	TYLCV incidence
Cyantraniliprole 100 OD	50	0b	5b	1b	12.6b	___	___	___	___	___
	75	2b	8b	1b	12.5b	2a	5b	9a	5a	24.2b
	75+MSO	___	___	___	___	1a	2b	5a	4a	4c
	100	2b	3b	0b	5.4b	___	___	___	___	___
	112	___	___	___	___	1a	4b	7a	5a	18.7b
Pymetrozine 50 WDG	100	1b	2b	0b	0.9b	3a	4b	8a	1a	17b
Control	0.0	17a	110a	132a	77.7a	10a	67a	19a	0a	69.7a
LSD		7	40	35	12.91	6	62	16	9	13.56
$F_{4,12}$		10.5	13.39	26.77	57.03	3.24	2.03	1.16	0.53	32.69
P-value		0.0007	0.0002	<0.0001	<0.0001	0.0507	0.1536	0.3774	0.7166	<0.0001

Means within a column followed by the same letter are not significantly different ($P \geq 0.05$; LSD; PROC GLM, SAS Institute Inc. 2008).

nymphs compared with the control in 2006 and 2007. Reductions were comparable to that of the soil soak or drench standard imidacloprid at 0.7 mg (AI) per plant and the foliar standard pymetrozine at 100 g (AI)/ha (Tables 3 and 4).

Soil or foliar applications of cyantraniliprole significantly reduced the incidence of TYLCV infection as compared with the untreated control (Tables 3 and 4). Cyantraniliprole as a soil soak or drench at all rates tested resulted in similar to or better TYLCV protection than imidacloprid (Table 3). Treatments were generally more effective in reducing TYLCV infection when applied foliarly than in the soil. Cyantraniliprole at 50–112 g (AI)/ha showed a flat dose response, being comparable with pymetrozine at 100 g (AI)/ha (Table 4). The use of MSO in 2007 significantly improved whitefly adult control and reduced TYLCV incidence compared with cyantraniliprole alone at the same rate or any of the other treatments. Visual observations of TYLCV correlated with specific PCR amplification product in 100% of plants with typical symptoms and in >85% of plants showing weak or questionable TYLCV symptoms.

Field Trials. Interactions between treatments and infestation dates were significant in 2009 ($F = 20.12$; df = 9; $P < 0.0001$; and $F = 17.79$; df = 9; $P < 0.0001$) for both incidence and severity, respectively. Therefore, treatment effects (subplots) were analyzed separately within the infestation date (main plots). Both cyantraniliprole 200 SC and imidacloprid resulted in a significant reduction in the percentage of infected plants with TYLCV infection, with a 15–24% incidence, as compared with plants in the untreated, infested control plots, which had ~90% incidence when infested 3 and 7 DAT (Table 5). In addition, the severity or symptom intensity was also significantly different for both insecticides compared with the untreated, infested control; however, there was no significant difference between the two insecticides at 3 and 7 d after drenching. By 14 DAT, cyantraniliprole still resulted in a significant reduction in TYLCV symptomatic plants, with ~25% incidence, while plants treated with imidacloprid had ~48% infection, TYLCV incidence in plots treated with both insecticides was significantly lower compared with the untreated, infested control, which had

Table 5. TYLCV incidence (percentage) and severity index (intensity) by DAT and cumulative average when viruliferous *B. tabaci* adults were released at 3, 7, 14, and 28 d after soil drench application of cyantraniliprole 200 SC or imidacloprid 550 SC

		TYLCV incidence and severity (DAT[a])									
		3		7		14		28		Average across all rates	
Treatment	mg (AI) per plant	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Cyantraniliprole 200 SC	20	15.00b	0.4250b	24.11b	0.6729b	24.64c	0.6357c	79.45a	1.0455a	35.56c	0.6905c
Imidacloprid 550 SC	30	21.25b	0.6375b	23.75b	0.6750b	47.87b	1.3158b	80.79a	1.0917a	43.17b	0.9270b
Infested control	0.0	90.00a	2.5875a	89.79a	2.0648a	77.50a	1.9750a	88.75a	1.0813a	86.48a	1.9261a
Noninfested control	0.0	0.00c	0.0000c	0.00c	0.0000c	0.00d	0.0000d	0.00b	0.0000b	0.00d	0.0000d
LSD		9.68	0.2900	10.63	0.3067	12.16	0.3438	10.15	0.1520	5.27	0.1399
F		131.98	121.24	101.30	61.56	58.03	48.25	132.09	97.34	81.91	70.52
df (treat, error)		(3, 313)	(3, 313)	(3, 310)	(3, 310)	(3, 308)	(3, 308)	(3, 309)	(3, 309)	(3, 1240)	(3, 1240)
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Field trial, fall 2009.

[a] Days after treatment (DAT). Means within a column followed by the same letter are not significantly different ($P \geq 0.05$; LSD; PROC GLM, SAS Institute Inc. 2008).

Table 6. TYLCV incidence (percentage) and severity index (intensity) by DAT and cumulative average when viruliferous *B. tabaci* adults were released at 7, 14, and 21 d after soil drench application of cyantraniliprole 200 SC or imidacloprid 550 SC)

		TYLCV incidence and severity (DAT)							
		7		14		21		Average across all dates	
Treatment	mg (AI) per plant	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Cyantraniliprole 200 SC	20	26.25c	0.9625c	80.00b	2.2125b	92.50b	1.8500c	66.25d	1.6750d
	10	33.75c	1.0875c	91.25a	2.5125b	97.50ab	1.9625c	74.17c	1.8542d
	5	32.50c	1.0750c	91.25a	2.9250a	98.75a	2.2000b	74.17c	2.0667c
Imidacloprid 550 SC	30	57.50b	2.2250b	95.00a	3.0625a	97.50ab	2.5125a	83.33b	2.6000b
Infested control	0.0	92.50a	3.6500a	96.25a	3.1875a	97.50ab	2.6125a	95.42a	3.1500a
Noninfested control	0.0	0.00d	0.0000d	0.00c	0.0000c	0.00c	0.0000d	0.00e	0.0000e
LSD		12.41	0.4804	8.11	0.3264	5.02	0.2276	5.17	0.2074
F		49.64	53.79	165.03	102.93	478.81	136.47	122.64	59.13
df (treat, error)		(5, 471)	(5, 471)	(5, 471)	(5, 471)	(5, 471)	(5, 471)	(5, 1404)	(5, 1404)
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Field trial, spring 2010.

Means within a column followed by the same letter are not significantly different ($P \geq 0.05$; LSD; PROC GLM, SAS Institute Inc. 2008).

~78% TYLCV incidence. Cyantraniliprole showed a significantly lower severity index damage compared with the standard imidacloprid. The disease severity in both insecticides differed from that of the untreated, infested control at 14 d after drenching. Neither insecticide reduced the percentage of symptomatic plants or the intensity of symptoms when plants were infested 28 DAT. The overall average incidence and severity of cyantraniliprole was significantly lower compared with imidacloprid and the untreated infested control (Table 5).

In 2010, using the imidacloprid-tolerant whitefly colony, interactions between treatments and infestation dates were also significant, as in 2009 ($F = 27.25$; df = 10, $P < 0.0001$ and $F = 14.85$; df = 10, $P < 0.0001$), for both incidence and severity, respectively. Therefore, treatment effects (subplots) were analyzed separately within infestation date (main plots). The 20, 10, and 5 mg (AI) per plant rates of cyantraniliprole significantly reduced TYLCV incidence when infested 7 DAT compared with the standard and the untreated, infested control, with ~30% plants with symptoms of TYLCV infection compared with ~58% in the standard imidacloprid, and ~93% in the untreated, infested control plots (Table 6). The TYLCV symptom intensity was also significantly lower with the three rates of cyantraniliprole than the severity observed with imidacloprid and the untreated infested control. Results at 14 d after the drench application indicate that only cyantraniliprole at the 20 mg (AI) per plant rate significantly reduced TYLCV incidence as compared with imidacloprid and the untreated, infested control, but TYLCV incidence levels were high, with 80% in the cyantraniliprole 20 mg (AI) per plant plots and ~95% in the imidacloprid and untreated, infested control plots. TYLCV severity index at 14 DAT was significantly lower in plots treated with cyantraniliprole at 10 and 20 mg (AI) per plant than imidacloprid and the untreated, infested control. When plots were infested 21 d after drenching, neither cyantraniliprole nor imidacloprid, or untreated, infested control reduced the percentage of TYLCV symptomatic plants. However, the three rates of cyantraniliprole resulted in significantly lower TYLCV symptom intensity than imidacloprid and the untreated, infested control. When comparing the average incidence and severity over the three evaluation dates,

plots treated with the three rates of cyantraniliprole resulted in significantly lower TYLCV than imidacloprid and the untreated, infested control (Table 6). None of the plants in the untreated, noninfested control plots showed TYLCV symptoms, indicating that plants had no virus in the nursery greenhouse and that the cages protected from accidently getting whiteflies into the tunnels in the field during both years of the study.

Discussion

TYLCV is a monopartite Begomovirus transmitted in a persistent, circulative way and can be acquired during an access period as short as 10 min by adults, which is followed by a latent period of 4 to 21 h, and it can then be transmitted in as little as 10 min. The virus lives in the insect vector most of its life span (Duffus 1996, Polston et al. 1999). Whiteflies acquire the virus when feeding, which then goes to the stomach and passes from the midgut to the hemolymph. The virus then moves to the salivary glands, where it replicates and is retransmitted to other healthy plants during the process of feeding. Whiteflies have piercing–sucking mouthparts and feed on phloem sap of plants, where the TYLCV reside, but it has also been demonstrated that during the probing process, they also feed on water in the xylem and on other cells and intracellular spaces for an indeterminate time, so it is likely that whiteflies are affected by the insecticide early in the feeding process (Polston and Sherwood 2003, Cameron et al. 2013, Barry et al. 2014, Civolani et al. 2014). Feed in the xylem also helps the whitely to regulate osmotic potential (Pompon et al. 2011). Thus, it is very important to know the virus–vector relationship when selecting an insecticide to interfere with virus transmission. Cyantraniliprole and the standard insecticides imidacloprid and pymetrozine used for comparison in these studies are similar in that all are xylem systemic and have translaminar activity. Pymetrozine is also phloem systemic. All three have a different mode of action and appear to act quickly enough on adult whiteflies to reduce TYLCV transmission (Naun et al. 1998, Polston and Sherwood 2003, Cameron et al 2013).

Cyantraniliprole applied as foliar sprays or soil treatments provided adult whitefly control and TYLCV suppression, and reduced oviposition and nymph survival, as good as or better than the tested standards, although mortality was higher in controls in the latter method. When cyantraniliprole SC and imidacloprid were applied as soil soaks (Table 1), imidacloprid induced higher mortality at 1 DAT, probably because imidacloprid (IRAC MoA group 4A) is a fast-acting nicotinic acetylcholine receptor agonist that kills rapidly and demonstrates antifeedant properties (Naun et al. 1998, IRAC 2014). Cyantraniliprole (IRAC MoA group 28) induces rapid feeding cessation caused by the release of calcium from the striate muscle cells, thus provoking paralysis and death (Lahm et al. 2012, Jeanguenat 2013, IRAC 2014); however, insect mortality may be slower. At 2 and 3 DAT, mortality was similar with both insecticides. Applying cyantraniliprole as a soil drench resulted in greater mortality at 1 DAT (Table 1), thus suggesting better uptake of the insecticide. Foliar applications of cyantraniliprole OD and pymetrozine in 2006 and 2007 resulted in statistically similar levels of mortality (Table 2). Both insecticides have some foliar contact activity, with translaminar movement. Pymetrozine (IRAC MoA group 9B) is a rapid feeding inhibitor that causes immediate and irreversible blocking of stylet penetration, resulting in starvation and death (Kayser et al. 1994, Polston and Sherwood 2003, IRAC 2014). Combining cyantraniliprole with MSO in 2007 significantly improved whitefly adult control and reduced TYLCV incidence compared with cyantraniliprole alone at the same rate and compared with any of the other treatments. However, the application of MSO alone or combined with pymetrozine were not included in this study. It is well-known that some penetrating adjuvants can help certain insecticides improve translaminar activity and thus result in better control of sucking pests. It is also known that oils can have a direct impact on mortality of certain sucking pests, including whiteflies. The former is pointed out by Lahm et al. (2012, p. 1422), when using foliar formulations of cyantraniliprole. Therefore, the interpretation and comparisons between treatments have to take this into account. The data in this study showed low adult mortality on days 1 and 2, yet with low TYLCV incidence following application of cyantraniliprole (both with the systemic soil and foliar applications), and data from an electrical penetration graph study of the probing behavior of adult *B. tabaci* biotype Q (Hemiptera: Aleyrodidae) following exposure to cyantraniliprole indicates that feeding cessation is a key factor in the reduction of virus transmission with cyantraniliprole (Civolani et al. 2014). Feeding cessation has also been shown to be a key factor for imidacloprid and pymetrozine. Insect mortality is also a factor for all these insecticides, especially under field conditions, where secondary spread of the virus can be reduced by killing and reducing pest populations.

Incidence (% infected plants) and severity (intensity of the infection) are indexes to evaluate the impact of TYLCV transmission by *B. tabaci* in tomato plants (Seem 1984). The degree of infection expressed in treatments can be compared with noninfested–nontreated controls (0% infection) and with infested–nontreated control (high infection). Lower incidence and severity are both indicators of better feeding suppression and better interference with TYLCV transmission, and therefore better indicators of plant vigor, which should be reflected in a better yield. The positive results observed in these greenhouse experiments with a high level of insect pressure (10× the field threshold of one adult per plant) and disease pressure (five adults per plant, with a high level of confidence that TYLCV virulent adults were used) indicate a great potential for cyantraniliprole as a component of a whitefly management program. Field evaluations of soil drench treatments confirmed the suppression of TYLCV transmission in the greenhouse studies, but the residual impact on suppression was not consistent. Field studies

conducted in 2009 and 2010 demonstrated suppression of TYLCV after 2 wk of drench application, even when an imidacloprid-tolerant colony was used in 2010. Whitefly incidence and severity was higher in 2010, particularly in plots infested 14 DAT. Weather conditions were very different in the two studies, fall 2009 and spring 2010, impacting plant growth and possibly the ability of the vector to transmit the virus and perhaps the incubation period. As explained above, a soil application by itself would not be recommended.

Management of TYLCV is very difficult and expensive and has limited options, often requiring significant changes in production and management practices and yield expectations (Hilje et al. 2001, Yang et al. 2004). Results from this study indicate that cyantraniliprole, applied as a drench at transplanting (SC formulation) or foliarly (OD formulation), will provide tomato growers in Florida with an excellent alternative to manage TYLCV and its vector, *B. tabaci*. Baseline studies conducted from 2008 to 2010 in Arizona and Florida confirmed total susceptibility and absence of cross-resistance to other insecticides of field-collected populations of immature and adult whiteflies to cyantraniliprole (Li et al. 2012, Caballero et al. 2013b). Prevention or delay of resistance is based on chemistry rotations, minimizing the continuous use of the same modes of action and target sites. A susceptibility monitoring program is an element of a resistance program (Castle et al. 2009). The use of cyantraniliprole in a TYLCV management program that address rotations with other available options, including insect resistance management considerations for the use of other group 28 insecticides, will be critical to preserve the efficacy of this new tool (Smith 2013). Sustainable use of cyantraniliprole following the recent registration at the beginning of 2014 will be fundamental to the successful management of insect vectors, including *B. tabaci* and the TYLCV disease it spreads.

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