Research Article

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Baseline susceptibilities of B- and Q-biotype *Bemisia tabaci* to anthranilic diamides in Arizona

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Abstract

BACKGROUND: Development of pyriproxyfen and neonicotinoid resistance in the B-biotype whitefly and recent introduction of the Q biotype have the potential to threaten current whitefly management programs in Arizona. The possibility of integrating the novel anthranilic diamides chlorantraniliprole and cyantraniliprole into the current program to tackle these threats largely depends on whether these compounds have cross-resistance with pyriproxyfen and neonicotinoids in whiteflies. To address this question, the authors bioassayed a susceptible B-biotype strain, a pyriproxyfen-resistant B-biotype strain, four multiply resistant Q-biotype strains and 16 B-biotype field populations from Arizona with a systemic uptake bioassay developed in the present study.

RESULTS: The magnitude of variations in LC_{50} and LC_{99} among the B-biotype populations or the Q-biotype strains was less than fivefold and tenfold, respectively, for both chlorantraniliprole and cyantraniliprole. The Q-biotype strains were relatively more tolerant than the B-biotype populations. No correlations were observed between the LC_{50} (or LC_{99}) values of the two diamides against the B- and Q-biotype populations tested and their survival rates at a discriminating dose of pyriproxyfen or imidacloprid.

CONCLUSION: These results indicate the absence of cross-resistance between the two anthranilic diamides and the currently used neonicotinoids and pyriproxyfen. Future variation in susceptibility of field populations to chlorantraniliprole and cyantraniliprole could be documented according to the baseline susceptibility range of the populations tested in this study.

Keywords: baseline susceptibility; *Bemisia tabaci*; biotype; chlorantraniliprole; cyantraniliprole; resistance management

1 INTRODUCTION

The sweetpotato whitefly (*Bemisia tabaci*) comprises at least 36 morphologically indistinguishable biotypes.[1] Many of these biotypes, such as the B and Q biotypes, have been recently proposed to represent genetically distinct cryptic species.[1–4] The B biotype, which originated in the Middle East, the Arabian Peninsula or northern Africa,[5] has successfully invaded the world by human-mediated movement of contaminated greenhouse-grown ornamentals since the late 1980s.[1,6] The Q biotype, which originated in the Mediterranean region,[1,2,7] is currently spreading to non-Mediterranean countries via commerce of ornamentals. It has been detected in China,[8] Japan,[9] the United States,[10–13] Mexico[14] and Guatemala.[15]

Invasion of the B biotype devastated crop production in the United States in the late 1980s and early 1990s.[16,17] This is partially because the invading B-biotype populations had *a priori* resistance to broad-spectrum insecticides such as organophosphates, carbamates and pyrethroids available at that time.[17–19] Subsequent strategic and limited uses of two highly effective, selective insect growth regulators (IGRs), pyriproxyfen (a juvenile hormone analog) and buprofezin (a chitin synthesis inhibitor), and several reduced-risk neonicotinoids (imidacloprid, thiamethoxam, dinotefuran and acetamiprid) has since provided effective management of the B biotype.[16,17,19,20–24] Strategic use of these selective insecticides has also dramatically reduced broad-spectrum insecticide use and helped to protect human health, conserve natural enemies and restore farmer's profits.[16,17,25–28]

While the whitefly management program centered on crop- and stage-specific use of the two IGRs and neonicotinoids has been highly effective in the US desert southwest for over a

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decade, and still provides satisfactory management of the B biotype, there are now two potential and serious threats to the continued success of this program. The first challenge is the steady development of resistance of the B biotype to pyriproxyfen and neonicotinoids.[10,29–32] Simulation models based on detailed study of a laboratory-selected strain with over 1000-fold resistance to pyriproxyfen predict that resistance to pyriproxyfen could intensify in a few years.[33,34] Another serious threat to the current whitefly management program is the recent and repeated introduction of the Q biotype.[10,11] Populations of the Q biotype have been associated with severe IGR or neonicotinoid resistance problems in southern Europe[35–38] and Israel.[39–41] To make matters worse, the Q-biotype strains derived from poinsettia collections in Arizona retail nurseries are virtually immune to the IGR pyriproxyfen, highly resistant to the IGR buprofezin and resistant to the neonicotinoid insecticides imidacloprid, acetamiprid and thiamethoxam, as well as pyrethroids synergized with organophosphates (e.g. fenpropathrin + acephate) (Li *et al*., unpublished data).[10,11] Novel selective, reduced-risk chemistries with no cross-resistance to the current insecticides are needed to cope with these two threats.

The most exciting class of novel insecticides developed recently is the diamides, which are divided into phthalic and anthranilic diamides.[42] Cyantraniliprole (Cyazypyr™) and chlorantraniliprole (Rynaxypyr™, Coragen®) are the first two active ingredients from the anthranilic diamide class. The diamide insecticides have a novel mode of action that acts exclusively on the ryanodine receptor in insects, a biochemical site that has not been exploited by any other synthetic insecticide.[42–47] They have extremely low mammalian toxicity because of their specificity to insect ryanodine receptors over mammalian counterparts.[42,43,45,46,48] Field trials have reported that both cyantraniliprole and chlorantraniliprole have efficacy against whiteflies.[49,50]

In the present study, a laboratory bioassay method was first developed for testing the toxicity of cyantraniliprole and chlorantraniliprole to *B. tabaci*. The susceptibilities of geographically discrete field populations of B-biotype whiteflies from Arizona and four laboratory strains of Q-biotype whiteflies to the two anthranilic diamides were then determined. These two anthranilic diamides may be important candidates for integration into the existing whitefly management program to help prevent further increase in pyriproxyfen and neonicotinoid resistance in the B-biotype populations and control the Q biotype when and if it establishes in open-field crops.

2 MATERIALS AND METHODS

2.1 Insecticide

The formulated insecticides in this study were: Coragen® 20SC (chlorantraniliprole; DuPont Crop Protection, Wilmington, DE) and Cyazypyr™ 20SC (cyantraniliprole; DuPont Crop Protection, Wilmington, DE).

2.2 Host plants

The *Bt*-transgenic DP164B2RF (Delta and Pine Land Co., Scott, MS) and the non-transgenic DP491 and DP565 cotton varieties were regularly grown for bioassays or rearing whitefly strains. To grow insect-free cotton plants for rearing whitefly strains, seeds of the *Bt*-transgenic DP164B2RF were planted in 3 L pots containing Redi Earth Peat-Lite Soil mixture (Scott-Sierra Horticulture Products Company, Marysville, OH) and grown in an isolated outdoor greenhouse using seasonal light conditions. Temperatures inside the greenhouse generally fell within the range 25–38 °C. Plants were watered daily and fertilized biweekly (N : P : K = 15 : 30:15; American Plant Food Co., Creve Coeur, MO). Approximately 5 weeks after planting, cotton plants at the 5–6-true-leaf stage were taken to the laboratory for rearing various whitefly populations. Insect-free cotton seedlings for bioassays were obtained from seeds of the three varieties planted in a 40 × 70 × 10 cm plastic tray containing the soil described above and kept in the same isolated greenhouse. After 2 weeks of daily watering and biweekly fertilizing, most plants were at the first true leaf stage with a stem height of ca 12–16 cm and a leaf size of ca 2 cm in diameter (Fig. 1A). These one-true-leaf seedlings were taken to the laboratory and used for bioassays.

2.3 Test insects

2.3.1 *Resistant and susceptible laboratory strains of B. tabaci*

Six laboratory whitefly strains, the B-biotype susceptible Yuma04-S and pyriproxyfen-resistant QC02-R, and the multiply resistant Q biotypes P′06, P′08-52, P′08-53 and P′08-58 (Table 1), were tested with cyantraniliprole and chlorantraniliprole. The Yuma04-S strain is susceptible to pyriproxyfen, neonicotinoids, spiromesifen and buprofezin. It has been reared in the laboratory on cotton seedlings (26 ± 2 °C, 16 : 8 h light : dark) without insecticide exposure since it was collected from a cotton field in Yuma, Arizona, in 2004.

The pyriproxyfen-resistant QC02-R was derived from a cotton collection in Queen Creek, Arizona, in 2002. Two subsequent laboratory selections with pyriproxyfen increased the LC_{50} of this strain to >1000-fold relative to the susceptible strain.[29] This strain has been maintained in the laboratory on cotton seedlings (26 ± 2 °C, 16 : 8 h light : dark) with continuous pyriproxyfen selection pressure (two sprays per generation at 0.1 μg mL^{-1}) since 2002.

The Q-biotype strains P′06, P′08-52, P′08-53 and P′08-58 were collected from different poinsettia collections in December 2006 (P′06) or December 2008 (P′08-52, P′08-53 and P′08-58) from several retail stores in Tucson, Arizona. Like Poinsettia 04,[10,11] the four Q-biotype strains are highly resistant to pyriproxyfen, buprofezin, neonicotinoids and the mixture of fenpropathrin plus acephate (Li *et al*., unpublished data). The P′06 strain has been divided into four substrains and reared on poinsettia, cotton, melon or cowpea (26 ± 2 °C, 60% RH, 16 : 8 h light : dark) without insecticide exposure in isolation from strains of the B biotype. Approximately equal numbers of whitefly adults were taken from the four substrains and used in this study to evaluate the susceptibility of P′06 to cyantraniliprole and chlorantraniliprole.

2.3.2 *Field populations*

A total of 16 field populations, nine collected in 2008 (six from cotton, three from melon) and seven collected in 2009 (two from cotton, five from melon), were assayed to measure the baseline variation in the susceptibility to cyantraniliprole and chlorantraniliprole in field populations of whitefly (Table 1). Each of the 16 populations was established by collecting approximately 5000 whitefly adults using a battery-powered vacuum (Model 4071D; Makita Corp., Anjo, Aichi, Japan) and then directly releasing whiteflies into a cage with fresh, insect-free cotton plants. All populations were maintained on cotton plants with no insecticide selection under the controlled environmental conditions of 26 ± 2 °C, 60% RH and a 16 : 8 h light : dark photoperiod until they were bioassayed with chlorantraniliprole and/or cyantraniliprole in the F0, F1, F2, F3 or F4 generation (Table 1). The 16 field

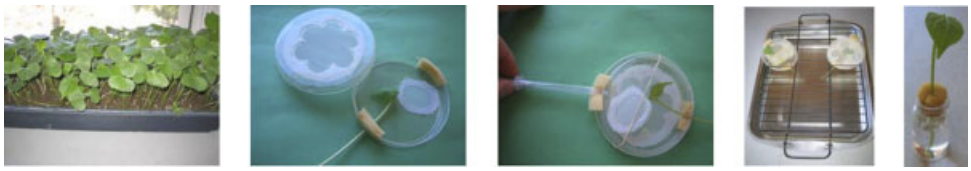

Figure 1. One-true-leaf cotton seedling systemic uptake bioassay for testing susceptibility of cyantraniliprole and chlorantraniliprole against whiteflies. A. Cotton seedlings that reach the first-true-leaf stage are used for bioassays. B. A cotton seedling is cut above the root line. The seedling is then inserted into a modified petri dish with the true leaf inside for egg laying and the stem outside for absorbing water from the pad (see D). C. Ten pairs of whiteflies are transferred into the modified petri dish (egg-laying unit). D. Egg-laying units are lined on a water-containing pad with the stems of the seedlings immersed into water to keep them alive. E. After 24 h of egg laying, adults are removed and the eggs on each seedling are counted and recorded. Each seedling is put into a glass vial containing 20 mL of water (control) and desired concentrations of cyantraniliprole or chlorantraniliprole.

Table 1. Source description of laboratory and field populations tested

Population name	Collection site (county)	Collection date	Number of whiteflies collected	Original host plant	Generation bioassayed	Biotype
08-10 melon	Pinal	23/6/08	5000 adults	Melon	F1	B
08-13 melon	La Paz	7/7/08	5000 adults	Melon	F2	B
08-14 melon	La Paz	7/7/08	5000 adults	Melon	F4	B
08-15 cotton	Yuma	20/7/08	5000 adults	Cotton	F0	B
08-20 cotton	Pinal	27/7/08	5000 adults	Cotton	F2	B
08-25 cotton	Maricopa	11/8/08	5000 adults	Cotton	F3	B
08-31 cotton	Pinal	2/9/08	5000 adults	Cotton	F0	B
08-33 cotton	Maricopa	19/9/08	5000 adults	Cotton	F4	B
08-37 cotton	La Paz	3/10/08	5000 adults	Cotton	F3	B
09-22 cotton	Harquahala	17/8/09	5000 adults	Cotton	F0	B
09-16 melon	Maricopa Ag. Ctr	27/7/09	5000 adults	Melon	F1	B
09-13 melon	Litchfield	6/7/09	5000 adults	Melon	F0	B
09-17 cotton	Tacna	10/8/09	5000 adults	Cotton	F0	B
09-03 melon	Tacna	9/6/09	5000 adults	Melon	F2	B
09-04 melon	S. Gila Valley	9/6/09	5000 adults	Melon	F0	B
09-09b melon	Harquhala	5/10/09	5000 adults	Melon	F2	B
Yuma04-S	Yuma	3/8/04	5000 adults	Cotton	F74	B
QC02-R	Maricopa	16/10/02	5000 adults	Cotton	F105	B
P′06	Pima	4/12/06	Roughly 100 egg/nymphs	Poinsettia	F 27	Q
P′08-53	Pima	16/12/08	Roughly 50 egg/nymphs	Poinsettia	F17	Q
P′08-52	Pima	15/12/08	Roughly 50 egg/nymphs	Poinsettia	F16	Q
P′08-58	Pima	24/12/08	Roughly 50 egg/nymphs	Poinsettia	F16	Q

populations were biotyped by the *VspI*-based *mtCOI* gene PCR-RFLP technique,[12] and all were B-biotype whiteflies (data not shown).

2.4 Bioassay

Based on preliminary experiments indicating that the two diamides have little (chlorantraniliprole) or very low (cyantraniliprole) contact toxicities against *B. tabaci*, a one-true-leaf cotton seedling systemic uptake bioassay was developed for testing the susceptibility of whitefly nymphs to cyantraniliprole and chlorantraniliprole. As illustrated in Fig. 1, cotton seedlings (DP164B2RF, or otherwise indicated) at the one-true-leaf stage (Fig. 1A) were individually cut above the root line from the large tray, leaving 11.43 cm long (or otherwise indicated) stems for uniform insecticide uptake among seedlings. Each seedling was then enclosed in a modified polystyrene petri dish, with its stem passing through a hole (Fig. 1B). Ten pairs of male and female adult whiteflies were aspirated from their rearing cage and then released into the seedling-containing petri dish (oviposition unit) (Fig. 1C). The oviposition units were suspended over a screened moist pad, with the stems of the seedlings dipped into water to keep the seedlings alive (Fig. 1D). After 24 h, the adults were removed and the total number of eggs on each seedling was counted and recorded. Seedlings with >20 eggs were inserted into a 20 mL glass scintillation vial containing water (control) or the desired concentration of Coragen® (chlorantraniliprole, 20% SC formulation) or Cyazypyr™ (cyantraniliprole, 20% SC formulation) in water (Fig. 1E). A total of 4–6 cotton seedlings with >20 eggs were tested for each concentration and control (i.e. 4–6 replicates). The seedlings were held at $26 \pm 1\,^{\circ}C$ and a photoperiod of 16 : 8 h light : dark for the duration of the assay. Mortality was determined on day 14 (or otherwise indicated) after insertion of cotton seedlings into glass vials by counting live nymphs and subtracting that number from the initial number of eggs on each seedling. Dead nymphs were visibly dried/desiccated and essentially popped off the leaf like a dried flake when prodded with a needle. By contrast, live nymphs were visibly not dried and would not pop off the leaf so easily.

Resistance levels of all the field and laboratory populations listed in Table 1 to the current insecticides pyriproxyfen and imidacloprid

(as a representative of neonicotinoids) were determined by discriminating dose bioassays described elsewhere.[10,11]

2.5 Optimization of bioassay

Three optimization experiments were conducted to determine the best observation time, variety and stem height of cotton seedlings. For the cotton variety experiment, three cotton varieties (DP164B2RF, DP491 and DP565) were tested for their effects on the toxicity of chlorantraniliprole against the susceptible Yuma04-S strain. There were three seedling stem height treatments (7.62, 11.43 and 15.24 cm) for the stem height experiment. For determination of the best observation time, the mortality of whiteflies was observed and recorded at five different time points (7, 9, 14, 16 and 18 days after insertion of cotton seedlings into insecticide solutions) using the death criteria described above.

2.6 Statistical analysis

A standard probit analysis was conducted to determine the dose–response line, LC_{50} and LC_{99} values of each strain/population, using Priprobit v.1.63. Statistical differences between LC_{50} or LC_{99} values were determined using the presence or absence of overlap in the 95% fiducial limits (FL). Differences in mortality among different optimization treatments were evaluated by two-way analysis of variance (ANOVA) and Tukey's honestly significant difference test (HSD) with the significance level set at $P < 0.05$. Mortality percentage values of different optimization treatments were arcsine transformed before analysis.

3 RESULTS

3.1 Bioassay optimization

3.1.1 Observation time

In order to determine the best observation time, the toxicological time course of chlorantraniliprole against the susceptible Yuma04-S strain was studied. The mortalities of the control and each concentration (0.1, 0.32, 1 and 3.2 μg mL^{-1}) were determined on days 7, 9, 14, 16 and 18. Two-way ANOVA indicated that significant differences in mortality existed among different concentrations and different observation times (Table 2A). There were also significant interactions between concentration and observation time. Multiple comparison tests showed that the mortality of each concentration increased sharply from day 9 to day 14, while the control mortality remained unchanged (Table 2B). Further increase in mortality after day 14 was not significant (Table 2B). This suggests that day 14 is the optimal observation time for measuring mortality of *Bemisia* nymphs.

3.1.2 Effect of cotton variety

To determine whether the cotton variety had a significant impact on the results of the one-true-leaf systemic bioassay, chlorantraniliprole-induced whitefly mortalities on three different cotton varieties were compared. For all three chlorantraniliprole dosages there were no significant differences in mortality among the three cotton varieties (Table 3). This suggests that the cotton variety is not an important factor for measuring whitefly mortality with the one-true-leaf systemic bioassay.

3.1.3 Effect of the stem height of cotton seedlings

To test whether the stem height of the cotton seedling affected the result of the one-true-leaf systemic bioassay, chlorantraniliprole-induced whitefly mortalities on cotton seedlings with a stem height of 7.62, 11.43 or 15.24 cm were compared. At lower dosages (0.05 and 0.1 μg mL^{-1}) there were no significant differences among the three stem heights (Table 4). At the two higher dosages (0.2 and 0.4 μg mL^{-1}), seedlings with a stem height of 7.62 cm yielded significantly higher mortalities than seedlings with a stem height of 11.43 and 15.24 cm. Thus, seedlings with a fixed stem height should be used. Considering that seedlings with 7.62 cm long stems were less easy to refill with water (control) or insecticide solutions when necessary, and seedlings with 15.24 cm stems took up more growth chamber space, it was recommended that seedlings with a fixed stem height of 11.43 cm be used.

3.2 Baseline susceptibilities of B- and Q-biotype laboratory strains to chlorantraniliprole and cyantraniliprole

Among the six laboratory strains bioassayed for susceptibility to the two anthranilic diamides, the susceptible B-type strain Yuma04-S and the pyriproxyfen-resistant B-biotype strain QC02-R were similarly susceptible to chlorantraniliprole at both the LC_{50} and LC_{99} levels (Table 5). The three multiply resistant Q-biotype strains P′06, P′08-52 and P′08-58 had significantly greater LC_{50} values and were at least 4.79- and 9.79-fold less susceptible to chlorantraniliprole than the two B-biotype laboratory strains at the LC_{50} and LC_{99} levels respectively (Table 5). By contrast, the LC_{50} and LC_{99} values of the four Q-biotype strains P′06, P′08-52, P′08-53 and P′08-58 to cyantraniliprole were not or only marginally (P′08-53 only) different from those of the two B-biotype laboratory strains, based on the presence of overlap in the 95% fiducial limits (Table 6). Interestingly, the pyriproxyfen-resistant B-biotype strain QC02-R was the most susceptible strain at both the LC_{50} and LC_{99} levels, whereas P′08-53 and P′08-52 were the most tolerant strains at the LC_{50} and LC_{99} levels respectively. The LC_{50} values of cyantraniliprole against the six laboratory strains increased in the order: QC02-R (0.019 μg mL^{-1}) < P′06 (0.027 μg mL^{-1}) < Yuma04-S (0.065 μg mL^{-1}) < P′08-52 (0.083 μg mL^{-1}) < P′08-58 (0.106 μg mL^{-1}) < P′08-53 (0.191 μg mL^{-1}).

Further comparison of the LC_{50} and LC_{99} values of the two anthranilic diamides against the six laboratory strains (Tables 5 and 6) showed that cyantraniliprole was more potent against whiteflies than chlorantraniliprole. For example, the LC_{50} values of cyantraniliprole were 1.76-fold (against Yuma04-S), 9.37-fold (against QC02-R strain) and 30.49-fold (against P′06 strain) lower than those of chlorantraniliprole (Tables 5 and 6). Likewise, the LC_{99} values of cyantraniliprole against the QC02-R and P′06 strains were 4.77- and 18.63-fold lower than those of chlorantraniliprole against the two strains respectively.

3.3 Baseline susceptibilities of B-biotype field populations to chlorantraniliprole and cyantraniliprole

Ten B-biotype field populations collected from Arizona cotton or melon fields in 2008 or 2009 (Table 1) were bioassayed for susceptibility to chlorantraniliprole. Their LC_{50} varied 3.09-fold, ranging from 0.143 μg mL^{-1} (08–10 melon) to 0.443 μg mL^{-1} (08–13 melon) (Table 5). Their LC_{99} differed 4.57-fold, ranging from 0.829 μg mL^{-1} (08–15 cotton) to 3.786 μg mL^{-1} (08–13 melon). Their LC_{50} and LC_{99} were 0.80 (08–10 melon) to 2.47 (08–13 melon) and 1.18 (08–15 cotton) to 5.40 (08–13 melon) times those of the pyriproxyfen-susceptible B-type strain Yuma04-S respectively. Overall, based on LC_{50} and LC_{99} values, all B-biotype field populations and laboratory strains were more susceptible to chlorantraniliprole than the three multiply resistant Q-biotype strains (Table 5).

Table 2. Toxic time course of chlorantraniliprole against the susceptible whitefly

A. Two-way ANOVA analysis results

Source of variation	df	SS	MS	*F*	*P*
Concentration	4	81 117.261	20 279.315	110.65282	<0.0001
Observation time	4	11 184.165	2796.0412	15.256425	<0.0001
Interaction	16	6634.1225	414.63266	2.2624173	0.01439
Error	50	9163.4876	183.26975		
Total	74	108 099.04	1460.7978		

B. Tukey–Kramer HSD comparison results

	Average mortality ± SD at different concentrations				
Observation on different days	0.0 μg mL^{-1} (control)	0.1 μg mL^{-1}	0.32 μg mL^{-1}	1 μg mL^{-1}	3.2 μg mL^{-1}
Day 7	5.88 ± 10.19 a	14.56 ± 6.64 a	27.34 ± 16.35 a	61.97 ± 17.21 a	86.80 ± 6.21 a
Day 9	6.62 ± 9.61 a	21.11 ± 5.91 a	44.11 ± 27.34 a	93.32 ± 5.81 b	100.0 ± 0.0 b
Day 14	6.62 ± 9.61 a	58.62 ± 0.47 b	72.14 ± 32.47 a	100.0 ± 0.0 b	100.0 ± 0.0 b
Day 16	7.36 ± 9.18 a	63.97 ± 4.88 b	75.18 ± 31.22 a	100.0 ± 0.0 b	100.0 ± 0.0 b
Day 18	7.36 ± 9.18 a	68.56 ± 3.73 b	80.32 ± 24.39 a	100.0 ± 0.0 b	100.0 ± 0.0 b

Mortality percentage values were arcsine transformed before analysis; the untransformed average mortality of six replicates is presented. Within each column, means followed by the same letter are not significantly different ($P < 0.05$; Tukey's HSD).

Table 3. Effects of cotton seed variety on the toxicity of chlorantraniliprole against the susceptible whitefly

A. Two-way ANOVA analysis results

Source of variation	df	SS	MS	*F*	*P*
Concentration	3	30 631.744	10 210.581	42.460427	<0.0001
Seed variety	2	64.128898	32.064449	5.8683296	0.13333914
Interaction	6	428.64761	71.441268	0.29708658	0.93227
Error	24	5771.3493	240.47289		
Total	35	36 895.87	1054.1677		

B. Tukey–Kramer HSD comparison results

	Average mortality ± SD at different concentrations			
Seed variety	0.0 μg mL^{-1} (control)	0.1 μg mL^{-1}	0.2 μg mL^{-1}	0.4 μg mL^{-1}
Delta pine 164 B2RF (Bollgard 2, *Bt* cotton)	10.57 ± 1.98 a	17.84 ± 11.85 a	59.18 ± 21.00 a	80.32 ± 10.95 a
Delta pine 491(non-*Bt* cotton)	3.55 ± 0.46 a	17.45 ± 7.59 a	47.53 ± 23.12 a	87.28 ± 6.52 a
Delta pine 565 (non-*Bt* cotton)	7.11 ± 4.52 a	22.12 ± 24.29 a	51.43 ± 22.57 a	76.91 ± 20.64 a

Mortality percentage values were arcsine transformed before analysis; the untransformed average mortality of six replicates is presented. Within each column, means followed by the same letter are not significantly different ($P < 0.05$; Tukey's HSD).

Among the seven B-biotype field populations bioassayed for susceptibility to cyantraniliprole, 09–22 cotton was the most susceptible population at both the LC_{50} and LC_{99} levels (Table 6). Relative to this population, the tolerance ratios of the remaining six field populations to cyantraniliprole ranged from 0.94-fold (09–16 melon) to 2.63-fold (08–37 cotton) at the LC_{50} level and from 0.89-fold (08–37 cotton) to 4.38-fold (09-09b melon) at the LC_{99} level. The tolerance ratios of the pyriproxyfen-resistant B-biotype strain QC02-R and the multiply resistant Q-biotype strain P′06, but not the pyriproxyfen-susceptible B-biotype strain Yuma04-S and the other three multiply resistant Q-biotype strains (P′08-52, P′08-53 and P′08-58), fell within the range of the seven field populations, i.e. less than threefold at both the LC_{50} and LC_{99} levels (Table 6).

The 09–13 melon population was the only field population that was bioassayed with both cyantraniliprole and chlorantraniliprole (Tables 5 and 6). As was the case against the laboratory strains, cyantraniliprole was significantly more potent than chlorantraniliprole against this field population. The LC_{50} of cyantraniliprole was 12.52 times lower than that of chlorantraniliprole against the 09–13 melon population, whereas its LC_{99} was 10.11 times lower than that of chlorantraniliprole (Tables 5 and 6).

Table 4. Effects of the stem height of cotton seedlings on the toxicity of chlorantraniliprole against the susceptible whitefly

A. Two-way ANOVA analysis results

Source of variation	Df	SS	MS	*F*	*P*
Concentration	4	41 573.638	10 393.409	149.73764	<0.0001
Stem height	2	814.65093	407.32546	5.8683296	0.00706
Interaction	8	3717.6708	464.70885	6.6950509	<0.0001
Error	30	2082.3241	69.410802		
Total	44	48 188.284	1095.1883		

B. Tukey–Kramer HSD comparison results

	Average mortality ± SD at different concentrations				
Stem height (inch)	0.0 μg mL^{-1} (control)	0.05 μg mL^{-1}	0.1 μg mL^{-1}	0.2 μg mL^{-1}	0.4 μg mL^{-1}
3	12.48 ± 2.73 a	17.11 ± 2.21 a	19.72 ± 17.97 a	86.09 ± 1.47 a	100.0 ± 0.00 a
4.5	10.67 ± 5.97 a	18.28 ± 4.54 a	20.37 ± 3.20 a	45.36 ± 7.00 b	83.37 ± 0.81 b
6	11.96 ± 12.12 a	19.90 ± 2.04 a	37.90 ± 0.35 a	41.32 ± 19.65 b	91.01 ± 7.08 ab

Mortality percentage values were arcsine transformed before analysis; the untransformed average mortality of six replicates is presented. Within each column, means followed by the same letter are not significantly different ($P < 0.05$; Tukey's HSD).

Table 5. Baseline susceptibilities of laboratory and field populations to chlorantraniliprole

Population name	LD-P line	LC50[a] (μg mL^{-1}) (95% FL)	LC99[a] (μg mL^{-1}) (95% FL)	TR[b] at LC_{50}	TR[b] at LC_{99}	% Surviving a discriminating dose of Pyr[c]	% Surviving a discriminating dose of Imi[d]
08-10 melon	$Y = 1.428 + 1.691X$	0.143 (0.097–0.188) a	3.400 (1.852–9.872) a	0.80	4.85	44.0	23.8
08-15 cotton	$Y = 2.596 + 3.309X$	0.164 (0.131–0.196) a	0.829 (0.608–1.363) a	0.92	1.18	44.3	11.6
08-31 cotton	$Y = 1.582 + 2.028X$	0.166 (0.128–0.204) a	2.329 (1.545–4.350) a	0.93	3.32	11.5	4.4
09-17 cotton	$Y = 2.123 + 2.823X$	0.177 (0.145–0.210) a	1.181 (0.830–2.032) a	0.99	1.68	52.2	31.9
Yuma04-S	$Y = 2.931 + 3.929X$	0.179 (0.157–0.201) a	0.702 (0.546–1.042) a	1.0	1.0	5.78	6.2
QC02-R	$Y = 2.716 + 3.836X$	0.196 (0.152–0.249) a	0.792 (0.506–2.227) a	1.09	1.13	99.1	0.72
09-3 melon	$Y = 1.443 + 2.223X$	0.224 (0.169–0.284) a	2.498 (1.507–5.911) a	1.25	3.56	58.5	38.5
09-13 melon	$Y = 1.250 + 2.081X$	0.251 (0.215–0.289) a	3.293 (2.276–5.476) ab	1.40	4.69	47.6	18.1
08-25 cotton	$Y = 1.633 + 3.054X$	0.292 (0.230–0.351) ab	1.686 (1.253–2.656) a	1.63	2.40	42.3	2.8
08-20 cotton	$Y = 1.772 + 4.655X$	0.416 (0.340–0.479) bc	1.316 (1.020–2.125) a	2.32	1.88	36.5	0.0
08-14 melon	$Y = 0.970 + 2.712X$	0.439 (0.355–0.525) bc	3.163 (2.161–5.980) a	2.45	4.51	18.0	16.4
08-13 melon	$Y = 0.883 + 2.496X$	0.443 (0.325–0.552) bc	3.786 (2.776–10.419) ab	2.47	5.40	32.4	12.8
P′06 Q	$Y = 0.169 + 2.578X$	0.860 (0.676–1.026) d	6.870 (4.870–11.889) ab	4.79	9.79	99.8	73.1
P′08-52Q[e]	–	>1.0 f	–	>5.59	–	96.6	48.5
P′08-58 Q[e]	–	>1.0 f	–	>5.59	–	85.3	85.7

[a] LC_{50} and LC_{90} values sharing the same letters are not significantly different based upon the presence of overlap in the 95% fiducial limits (FL).
[b] TR: tolerance ratio = the LC_{50} or LC_{99} of a given population (strain)/the LC_{50} or LC_{99} of the most susceptible population (or strain).
[c] The discriminating dose of Pyr (= pyriproxyfen) is 0.1 μg mL^{-1}, which kills 99.9% of susceptible whiteflies.
[d] The discriminating dose of Imi (= imidacloprid, a representative of neonicotinoids) is 10 μg mL^{-1}, which kills 99.9% of susceptible whiteflies.
[e] The corrected mortalities of the highest concentration (1.0 μg mL^{-1}) of chlorantraniliprole used were 10.65% for P′08-58Q and 24.33% for P′08-52Q.

3.4 Absence of cross-resistance between the two anthranilic diamides and the current insecticides

Except for the Yuma04-S, all the field and laboratory strains tested had low to high levels of resistance to pyriproxyfen, as evidenced by a significant percentage of each strain or population surviving a discriminating dose of pyriproxyfen (0.1 μg mL^{-1}) (Tables 5 and 6), which should kill 99.9% of the susceptible populations.[10] However, the survival percentages of these populations at 0.1 μg mL^{-1} of pyriproxyfen did not correlate with LC_{50} and LC_{99} values of the two diamides against these populations (Tables 5 and 6). For example, QC02-R was the most pyriproxyfen-resistant B-biotype strain, but its LC_{50} and LC_{99} values to the two diamides were no different or even less than (P′08-58) those of the other populations that were susceptible (Yuma04-S) or less resistant to pyriproxyfen (Tables 5 and 6). Likewise, about ten B-biotype field populations (e.g. 08–10 melon, 08–14 melon, 09-3 melon, 09–13 melon and 09–17 cotton in Table 5 and 08–37 cotton, 09-04 melon, 09–13 melon and 09–16 melon in Table 6) and the four Q-biotype strains had low to high levels of resistance to imidacloprid (a representative of neonicotinoids), but their survival rate at a discriminating dose of imidacloprid (10 μg mL^{-1}) did not correlate with their LC_{50} and LC_{99} values to the two diamides (Tables 5 and 6). These results

Table 6. Baseline susceptibilities of laboratory and field populations to Cyantraniliprole

Population name	LD-P line	LC50[a] (95% FL)	LC99[a] (95% FL)	TR[b] at LC_{50}	TR[b] at LC_{99}	% Surviving a discriminating dose of Pyr[c]	% Surviving a discriminating dose of Imi[d]
09-16 melon	$Y = 3.284 + 1.800X$	0.015 (0.011–0.019) a	0.294 (0.192–0.543) a	0.94	2.41	55.5	19.2
09-22 cotton	$Y = 4.721 + 2.624X$	0.016 (0.012–0.020) a	0.122 (0.079–0.267) a	1	1	77.5	3.7
09-13 melon	$Y = 3.348 + 1.933X$	0.019 (0.013–0.025) a	0.296 (0.183–0.636) a	1.19	2.43	47.6	18.1
QC02-R	$Y = 4.647 + 2.688X$	0.019 (0.011–0.026) a	0.137 (0.084–0.377) a	1.19	1.12	99.1	0.72
09-04 melon	$Y = 3.426 + 2.025X$	0.020 (0.015–0.026) a	0.287 (0.187–0.542) a	1.25	2.21	24.1	20.5
08-33 cotton	$Y = 4.254 + 2.670X$	0.026 (0.015–0.036) a	0.190 (0.108–0.706) a	1.63	1.56	42.4	12.1
P'06-Q	$Y = 3.281 + 2.093X$	0.027 (0.017–0.038) a	0.350 (0.217–0.794) a	1.69	2.87	99.8	73.1
09-09b melon	$Y = 2.847 + 1.913X$	0.033 (0.016–0.051) a	0.534 (0.284–1.791) a	2.06	4.38	36.9	0.5
08-37 cotton	$Y = 7.756 + 5.626X$	0.042 (0.031–0.056) ab	0.108 (0.074–0.316) a	2.63	0.89	31.1	16.3
Yuma04-S	$Y = 2.139 + 1.803X$	0.065 (0.011–0.117) a	1.27 (0.558–22.507) ab	4.06	10.41	5.78	6.2
P'08-52 Q	$Y = 2.076 + 1.919X$	0.083 (0.049–0.118) ab	1.350 (0.735–4.218) ab	5.19	11.07	96.6	48.5
P'08-58 Q	$Y = 5.819 + 5.962X$	0.106 (0.089–0.123) ab	0.260 (0.191–0.595) a	6.63	2.13	85.3	85.7
P'08-53 Q	$Y = 2.911 + 4.050X$	0.191 (0.121–0.243) b	0.717 (0.491–1.941) ab	11.94	5.88	93.7	87.4

[a] LC_{50} and LC_{90} values sharing the same letters are not significantly different based upon the presence of overlap in the 95% fiducial limits (FL).
[b] TR: tolerance ratio = the LC_{50} or LC_{99} of a given population (strain)/the LC_{50} or LC_{99} of the most susceptible population (or strain).
[c] The discriminating dose of Pyr (= pyriproxyfen) is 0.1 μg mL^{-1}, which kills 99.9% of susceptible whiteflies.
[d] The discriminating dose of Imi (= imidacloprid, a representative of neonicotinoids) is 10 μg mL^{-1}, which kills 99.9% of susceptible whiteflies.

indicate the absence of cross-resistance between the two diamides and pyriproxyfen or neonicotinoids.

4 DISCUSSION

The possibility of effectively integrating the anthranilic diamides chlorantraniliprole and cyantraniliprole into the Arizona Cross-Commodity Whitefly Management Program to tackle the threats of pyriproxyfen and neonicotinoid resistance in the B-biotype *B. tabaci* and the invasion and potential establishment of the multiply resistant Q-biotype *B. tabaci* may largely depend on whether they have cross-resistance with pyriproxyfen and neonicotinoids. In order to address this question, a cotton seedling bioassay was developed for testing the systemic toxicities of the two new insecticides against *B. tabaci* eggs and nymphs. Six laboratory strains and 16 field populations collected from Arizona cotton or melon fields in 2008 or 2009 were then bioassayed to estimate their dose–response lines (i.e. LC_{50} and LC_{99}) using this one-true-leaf cotton seedling systemic uptake bioassay.

Several lines of evidence obtained from this study demonstrate the absence of cross-resistance between the two novel anthranilic diamides and the currently used pyriproxyfen and neonicotinoids. Firstly, the baseline susceptibility to chlorantraniliprole of the pyriproxyfen-resistant laboratory B-biotype QC02-R was similar to that of the pyriproxyfen- and neonicotinoid-susceptible Yuma04-S strain (Table 5). Secondly, the field populations that are moderately resistant to pyriproxyfen (all the field populations) and neonicotinoids (e.g. 08–10 cotton, 09-3 melon and 09–17 cotton) have baseline susceptibilities to chlorantraniliprole that are similar to that of the susceptible laboratory strain Yuma04-S (Table 5). Thirdly, the response to chlorantraniliprole of the multiply resistant Q-biotype strain P'06 was similar to that of field-collected populations, such as 08–13 melon and 08–14 melon, and was only 3.79- and 8.79-fold less susceptible to chlorantraniliprole than the susceptible B-biotype strain Yuma04-S at the LC_{50} and LC_{99} levels respectively (Table 5). In the case of cyantraniliprole, the susceptible laboratory strain Yuma04-S was actually less susceptible (based on LC_{99} values) than ten of the other twelve populations (the exceptions are the two Q-biotype strains P'08-52 and P'08-53) tested (Table 6), even though all the ten populations were resistant to pyriproxyfen and seven of the ten populations were resistant to imidacloprid (Table 6). Cyantraniliprole was also more potent than chlorantraniliprole, particularly against the multiply resistant Q-biotype strains. Taken together, these results indicate that the two novel anthranilic diamides, especially cyantraniliprole, are suitable candidates for incorporation into the current whitefly management program to control both the resistant B- and Q-biotype whiteflies. The present bioassay results are also consistent with field efficacy trials, which showed that cyantranilprole is highly active against both adult and immature whitefly.[49,50]

Although interpopulation variation in susceptibility to chlorantraniliprole and cyantraniliprole was observed among the B-biotype field populations tested, the magnitude of the differences was small, less than fivefold for both insecticides. These results suggest that the observed susceptibility differences reflect natural variation in chlorantraniliprole and cyantraniliprole susceptibility among the B-biotype whitefly field populations rather than variation caused by prior exposure to selection pressure. Overall, the Q-biotype strains were relatively more tolerant than the B-biotype population, particularly to chlorantraniliprole. However, the magnitude of the interpopulation variation in susceptibility to the two diamides was also less than fivefold among the four Q-biotype strains. Therefore, both B- and Q-biotype whiteflies are considered to be susceptible to chlorantraniliprole and cyantraniliprole across the state of Arizona. Future shifts in susceptibility of the B- and Q-biotype whitefly populations to chlorantraniliprole and cyantraniliprole can now be evaluated on the basis of comparisons with the baseline susceptibility data presented in this study. In addition, a couple of candidate diagnostic concentrations, such as 1 and 10 times the average LC_{99} of the field populations, can be determined on the basis of these baseline susceptibility data and used for monitoring shifts in susceptibility when the two anthranilic diamides are incorporated into whitefly management programs.

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