Uptake and Retention of Imidacloprid and Cyantraniliprole in Cotton for the Control of *Bemisia tabaci* (Hemiptera: Aleyrodidae)[1]

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J. Entomol. Sci. 58(4): 434–446 (October 2023)
DOI: 10.18474/JES22-77

Abstract Plant tissue bioassays are a standard approach for bioassaying insects such as the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), an insect that specializes in systemic feeding on the phloem in leaves by using a piercing-sucking mouthpart apparatus. Systemic insecticides remain the most effective approach to whitefly management; however, little work has been done to quantify the amount of insecticide active ingredient that a species is exposed to when feeding. This study was conducted to estimate the imidacloprid and cyantraniliprole concentrations present in cotton (*Gossypium hirsutum* L.) leaves 24 h after a root drench for systemic toxicological bioassays. Insecticide active-ingredient quantification involved liquid chromatography–tandem mass spectrometry. Comparable concentration responses also were conducted to indicate the mortality of the sweetpotato whitefly at the tested concentrations. The results indicated significant active-ingredient retention with higher concentrations of insecticide treatments, which corresponded with higher sweetpotato whitefly mortality. Specifically, for imidacloprid and cyantraniliprole, the average slopes and intercepts of the log parts per billion of leaf tissue concentration to milligrams of active ingredient per liter of treatment solution were $y = 4.08\ x + 0.83$ and $y = 6.22\ x + 0.47$, respectively. These formulae estimate leaf tissue concentrations that can be linked to insect insecticide exposure in the leaves, with 50–73% of the overall variability explained. Significant correlations also were observed between the root drench concentrations, leaf tissue concentrations, and sweetpotato whitefly mortality.

Key Words residues, insecticides, cotton, whitefly, leaf tissue

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a significant pest of crop production globally. Whiteflies are polyphagous and feed on plant sap by penetrating the vascular bundle with a mouthpart apparatus adapted for piercing and sucking (Perier et al. 2022, Rosemarie et al. 1995). Probing as a result of feeding, which has been confirmed using electrical penetration graph techniques (Tjallingii 1985), appears to be the primary route for insecticide exposure. Historically, systemic insecticides have been the primary strategy

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for whitefly control to mitigate crop damage and, most importantly, virus transmission. However, reduced efficacy is a growing issue associated with preventative applications of systemic insecticides that increase resistance selection pressures (Perier et al. 2022).

Cotton is a major crop in many countries, including the United States, especially within the subtropical region known as the Cotton Belt (Khan et al. 2020). Insecticides, systemic or otherwise, are frequently used to protect plant production from whiteflies and other insect pests (Anees and Shad 2020). The homogeneous distribution of insecticides throughout a plant is essential for effective chemical control (de Boer and Satchivi 2014), regardless of the application method (Pes et al. 2020). Therefore, the need to quantify insecticide efficacy for prolonged pest control increased literature reports detailing various bioassay methods for testing (Adamczyk et al. 1999, Jansson et al. 1997, Kanga et al. 2021, Liu and Stansly 1995, Portilla 2020, Snodgrass 1996, Sparks et al. 2020; https://irac-online.org/methods/; accessed 1 December 2022). However, reports correlating the insecticide concentration applied to the plants with the insecticide concentration retained by the treated plant are lacking.

Two commonly used insecticide formulations for whitefly control are Admire® Pro 4F (active ingredient [a.i.] imidacloprid, Bayer Crop Science, Research Triangle Park, NC) and Exirel® 0.83SC (active ingredient cyantraniliprole, FMC, Philadelphia, PA), belonging to the Insecticide Resistance Action Committee (IRAC) group 4a (neonicotinoids) and group 28 (diamides), respectively. These chemicals have insecticidal effects on piercing-sucking insects (Horowitz et al. 2011, Perier et al. 2022, Sparks et al. 2020), even as a systemic application (Sparks et al. 2020). However, whether systemic (piercing-sucking) feeding is the primary route of insecticide exposure for the sweetpotato whitefly is not fully known or lacks quantifiable reports, mainly because insecticide efficacy tests encompass many application methods, such as contact, dipping, injection, or topical (Yu 2014), to name a few. Insecticides, once applied, are typically used in concentration–response tests within the first 24 h, partly to minimize degradation or metabolic decomposition of the applied active ingredient in or on the plant and partly to maximize testing efficiency (Sparks et al. 2020). By contrast, residue studies often evaluate the persistence of an active ingredient in plant tissue for periods after application, regardless of the metabolic alteration, because they focus on the resilience of the chemical (Gunther and Blinn 1956). Herein, we propose evaluating both efficacy and residue concentrations of imidacloprid and cyantraniliprole to improve insecticide efficacy evaluations.

It is essential to quantify the concentration of an insecticide's active ingredient when considering insecticide regulation policies and crop safety (Gunther and Blinn 1956). However, the same can be said for efficacy trials to evaluate the insecticide residues to which an insect pest might be exposed when feeding on plant parts, such as the leaves, $>$24 h after insecticide application. Including insecticide leaf tissue data (residue) improves the accuracy of insecticide concentration–response evaluations because the plant insecticide residue data can be adjusted to incorporate insecticide metabolites that form in the plant. It is therefore possible to study plant residues by allowing the plant to process the insecticide but halting uptake at a specified time interval, such as 24 h, to limit residues for a

specific application method. There is a need for standardizing the methodology of insecticide efficacy trials that serve as the basis for toxicological bioassays meant to explain concentration response to given treatment concentrations. In this study, we aimed to correlate the imidacloprid and cyantraniliprole concentrations in cotton leaf tissue with a serial dilution of treatment concentrations used to study insecticide response in bioassay trials by using a modified insecticide bioassay recommended by Sparks et al. (2020). We also used the presence of an imidacloprid metabolite, "olefin," to confirm the retention of concentrations of imidacloprid. Our hypothesis was that increasing insecticide concentration in a root drench would result in a proportional increase in the concentration of the active ingredient retained in the leaf tissue, with a proportional increase in whitefly mortality.

Materials and Methods

The study was conducted in a laboratory at the Coastal Plains Research Station, University of Georgia, in Tifton. *Bemisia tabaci* used in the experiment were collected from ongoing colonies housed at the Coastal Plains Research Station. Whiteflies were maintained in a rearing room for 4 yr at 25 ± 3°C and a photoperiod of 14 L:10 D h. They were reared on rotations of cotton and squash (*Cucurbita pepo* L. subsp. *pepo* var. Golden Summer Crookneck) seedlings in trays. Three-week-old cotton plants were used for all insecticide and control treatments. Cotton plants were grown using untreated seeds (variety ST 4946GLB2) in PRO-MIX® soil medium (with Osmocote® blend fertilizer added, N–P–K = 19–5–8) in a growth chamber at 30 ± 2°C with a relative humidity (RH) of 60% and a photoperiod of 14 L:10 D h. Cotton plants were selected for use if they had at least two true leaves, with at least one of the terminal leaves having a width of 4 cm. For all bioassay experiments, the soil was washed from the roots of all plants, and the roots were clipped to 5 cm in length. Plants were then immediately placed in treatment or control solutions.

The insecticide formulations used were Admire Pro 4F (label rate 67.43 ml/0.41 ha, imidacloprid at 2.09 kg a.i./3.79 L, Bayer Crop Science) and Exirel 0.83SC (label rate 399.24 ml/0.41 ha, cyantraniliprole at 0.38 kg a.i./3.79 L, FMC). All insecticide containers (250-ml research samples) of the formulated product were well agitated before use. Stock solutions of 1,000 ml were prepared using the recommended rates for each insecticide formulation (Exirel, 1.06 ml L^{-1} of product; Admire Pro, 0.18 ml L^{-1} of product). A dilution series of each insecticide was also prepared in 500 ml of tap water. Higher treatment concentrations were prepared directly in 500 ml of tap water and required no stock solution. All solutions (control included) were prepared in labeled 0.946-L plastic cups. The amount of product required to prepare the stock was calculated using the following formula for 378.5 L of water: $x = a/b$, where x is amount of product required to make the stock solution, a is the recommended insecticide label rate converted to milliliters, and b is the volume of solvent (378.5 L) converted to milliliters.

Leaf tissue insecticide concentration analysis. Nine treatment solutions corresponded to the following assigned multipliers: 0.001, 0.01, 0.1, 1.0, 10, 50, 100, 1,000, and 10,000 (Table 1). By serially diluting the 100-multiplier solution, 0.001, 0.01, 0.1, 1.0, and 10 treatment solutions were created. Solution 1,000 was prepared as a 1/10 dilution of the 10,000-multiplier solution, whereas solution 50 was

Table 1. Tabular assignment of the multiplier value to each respective insecticide milligrams of active ingredient per liter for analysis consistency and associated bioassay mortality of whitefly adults.

	Treatment (mg a.i./L*)		Mean (±SE) mortality of *B. tabaci*	
Multiplier	Imidacloprid**	Cyantraniliprole**	Imidacloprid**	Cyantraniliprole**
0	0	0	9.3 (±8.06)	28.65 (±7.87)
0.001	0.00098	0.001048	2.21 (±2.21)	8.9 (±1.88)
0.01	0.0098	0.01048	5.6 (±2.72)	36.41 (±9.05)
0.1	0.098	0.1048	25.73 (±12.21)	27.2 (±4.74)
1	0.98	1.048	14.41 (±4.02)	47.13 (±15.56)
10	9.80	10.48	36.03 (±6.04)	67.35 (±2.59)
50	49.00	52.40	31.49 (±3.19)	51.79 (±12.69)
100	98.00	104.80	56.97 (±4.66)	76.3 (±7.78)
1,000	98,000	104,800	—***	—
10,000	980,000	1,048,000	84.35 (±3.58)	73.58 (±4.56)

* mg a.i. L^{-1}, milligrams of active ingredient per liter.
** Insecticide formulation: imidacloprid, Admire Pro and cyantraniliprole, Exirel.
*** Dashes indicate no mean mortality for *B. tabaci* calculated.

a 1/4 dilution of the stock solution. Finally, treatment solution 10,000 was prepared directly to 500 ml by adding a high product concentration for each insecticide formulation (Exirel, 53 ml of product; Admire Pro, 9.8 ml of product). A control (0 ml) of tap water also was used.

Before liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, the leaf samples were prepared as follows. The experimental conditions were maintained at an ambient temperature of 27 ± 2°C with a 50% RH and continuous lighting on a laboratory countertop. Each 0.946-L plastic cup had one of the nine treatment solutions or a control. In each cup, four plants (roots washed and trimmed), representing four replicates per treatment, were added and left to undergo a root drench for 24 h. Following the treatment period, a large terminal true leaf (at least 4 cm in width) from each plant was excised and placed individually in a manila seed envelope labeled according to insecticide, treatment concentration, and replication number. Each envelope was processed individually. The selected leaves were allowed to air dry in the partially opened envelope at room temperature for 1 wk in darkness to improve the accuracy of the leaf tissue insecticide concentration data collection. Air drying was essential to remove the moisture and increase the nanogram-level insecticide detection from the leaves' true mass.

In total, 160 2-ml screw cap centrifuge tubes containing two ceramic beads were prepared beforehand for weighing the leaves and were labeled similarly to the manila seed envelopes. Dried leaf mass was measured in the pretared tube with beads by using a NewClassic MF analytical scale (model MS104S/03, Mettler Toledo, Columbus, OH). Acetonitrile (1.5 ml) was added to the tubes, and samples

were allowed to sit for 5 min after which the leaf samples were pulverized using a VWR® bead mill homogenizer (VWR International, Radnor, PA) at a speed setting of 4 (4,000 rpm) with time intervals of four cycles of 30 s with a delay of 10 s. Samples were pulverized until powdered in acetonitrile. After processing, the samples were wrapped in aluminum foil to reduce light exposure and stored in a −40°C freezer until the LC-MS/MS analysis.

Sample extracts were shipped in coolers with ice packs to Villanova University (Villanova, PA) for insecticide residue analysis on the leaf tissue samples. Sample extracts were centrifuged at 8,500 rpm for 5 min. Following centrifugation, approximately 200 μl of supernatant acetonitrile was pipetted into an autosampler vial containing a 400-μl vial insert for LC-MS/MS analysis. A Shimadzu Prominence high-performance liquid chromatography (HPLC) system consisting of binary Shimadzu LC-20AD pumps, a SIL-20A autosampler, and a CTO-20A column oven (Shimadzu, Colombia, MD) was used under Analyst software (SCIEX, Framingham, MA) control. A Phenomenex Gemini-NX column (C18, 250 × 4.6 mm, 5-μm particle) fitted with a 2-mm guard column and heated to 50°C was used for separation. The HPLC mobile phase for both imidacloprid and cyantraniliprole consisted of 10 mM ammonium formate in water (aqueous) and acetonitrile (organic). For imidacloprid and metabolites analysis, a gradient program of 75/25 (aqueous/organic, 1.0-min hold) ramped to 5/95 (over 10 min) with a 3-min hold at 5/95 at a total column flow of 1.0 ml/min afforded baseline-resolved LC separation of imidacloprid-olefin (hereafter referred to as olefin) and imidacloprid. For cyantraniliprole analysis, a gradient program of 70/30 (aqueous/organic, 1.0-min hold), ramped to 5/95 (over 7 min) with a 3-min hold at 5/95 at a total column flow of 1.0 ml/min was used. Mass spectrometric detection was performed with a SCIEX 3200 QTRAP triple quadrupole mass spectrometer operated in positive electrospray ionization (ESI) mode. Multiple reaction monitoring (MRM) transitions for imidacloprid (256/211, declustering potential [DP] = 28 V, collision energy [CE] = 23 V), imidacloprid olefin (254/209, DP = 26 V, CE = 22 V), and cyantraniliprole (475/286, DP = 20 V, CE = 24 V) were optimized by infusion of pure standards. Optimized ESI source parameters were as follows: curtain gas, 172.4 kPa; ESI nebulizer gas, 413.7 kPa; auxiliary gas, 413.7 kPa; ESI probe temperature, 550°C, and ion spray voltage, 5,500 V. The dwell time for each MRM transition was 1 s. Calibration standards were prepared in the 20–2,000-ppb range for imidacloprid and olefin and in the 3–4,000-ppb range for cyantraniliprole. The leaf residue concentration limit of detection (LOD) is calculated at a signal-to-noise ratio of 3 based on 10-μl replicate injections of the low-concentration standard. The imidacloprid, olefin, and cyantraniliprole LOD was 0.93, 1.12, and 0.22, respectively.

***Bemisia tabaci* adult bioassay.** Three-week-old cotton plants were systemically treated for 24 h before being used in the bioassay. Systemic treatment methods were similar to the methods described above; however, treatment concentrations differed slightly with the removal of the 1,000 multiplier to accommodate the available specimens in the testing population. Nevertheless, several concentrations (cyantraniliprole: 0.001048, 0.01048, 0.1048, 1.048, 10.48, 52.40, 104.80, and 1,048,000 mg a.i. L^{-1}; imidacloprid: 0.00098, 0.0098, 0.098, 0.98, 9.8, 49, 98, and 980,000 mg a.i. L^{-1}) and a check (control, distilled water) were prepared and used as treatments and controls for the bioassay. Adults were collected in clear plastic tubes (diameter, 2.86 cm; length, 20.3 cm; ClearTec® Packaging, Park Hill, MO) screened at both ends with

nylon chiffon. Thirty-six tubes containing 30 adults each were collected and placed in a blue bin on a countertop to acclimate to experimental conditions of 27 ± 2°C, 50% RH, and continuous lighting for 1 h. Four replicates were prepared per treatment and control solutions. An excised terminal true leaf (at least 4 cm in width) for either a control or treatment was added to each tube containing whiteflies after the acclimation period. An initial mortality count was conducted after resealing the tube. The bioassay continued for 24 h under experimental conditions. After 24 h, a mortality count was recorded for each tube.

Statistical analyses. Generated data were analyzed using PROC GLM, PROC PROBIT, PROC REG, and PROC CORR in SAS® Enterprise Guide, Version 8.3 (SAS Institute Inc., Cary, NC). Adult bioassay data were analyzed using PROC PROBIT to determine the concentration responsible for 50% mortality of the population (LC_{50}). Mortality was recorded as percent mortality and corrected using Abbot's formula before being log transformed (Abbott 1925).

For the leaf tissue concentration analyses, the initial analysis was conducted using PROC GLM. Analysis variables included the treatment (milligrams of active ingredient of each insecticide in solution, multiplier) and the response (residue concentration, parts per billion in the leaf). Residual plots were used to confirm the need for log transformation of the dataset (Fernandez 1992). Using PROC REG, regression lines and equations were created using log multiplier (log milligram of active ingredient per liter) and leaf tissue concentrations (log parts per billion) to produce graphs correlating treatment and response. The regression equations estimate the quantity of treatment concentration retained in a leaf following insecticidal treatments. Data were animated using PROC SGPLOT in SAS Enterprise Guide with treatment concentrations (milligrams of active ingredient per liter = log multiplier) on the *x*-axis and quantified insecticide leaf tissue concentrations (log parts per billion) on the *y*-axis. Finally, insecticide concentrations in the serial dilution root drench and leaf residue analysis were correlated with whitefly mortality by using PROC CORR.

Results and Discussion

Leaf residue analysis. Using LC-MS/MS, we confirmed the translocation of the imidacloprid and cyantraniliprole treatments from the roots (via drench application) to the leaves. As expected, the cyantraniliprole, imidacloprid, and olefin concentrations in the leaf tissue increased significantly with increasing insecticide treatment concentrations applied to the roots (Fig. 1) (imidacloprid: $F_{1,78} = 115.40$, $P < 0.0001$; cyantraniliprole: $F_{1,78} = 63.69$, $P < 0.0001$) (Table 2). Moreover, each chemical had a different leaf tissue concentration of active ingredient retained relative to the treatment concentration. This retention was likely due to the different chemical characteristics of each insecticide. Based on these results, we suggest that standardizing methodologies is necessary for accurate estimates of insecticide residue concentrations in the leaf tissue and, eventually, insect mortality response. Bioassays can be adjusted to fit a specific insecticide. However, predicting the potential response to a chemical following contact or systemic insecticide application could also depend on the bioassay method. As such, a "multiplier" (Table 1) was assigned to each milligram of active ingredient per liter for analysis consistency across both insecticides based on the dilution ratios of each respective label rate.

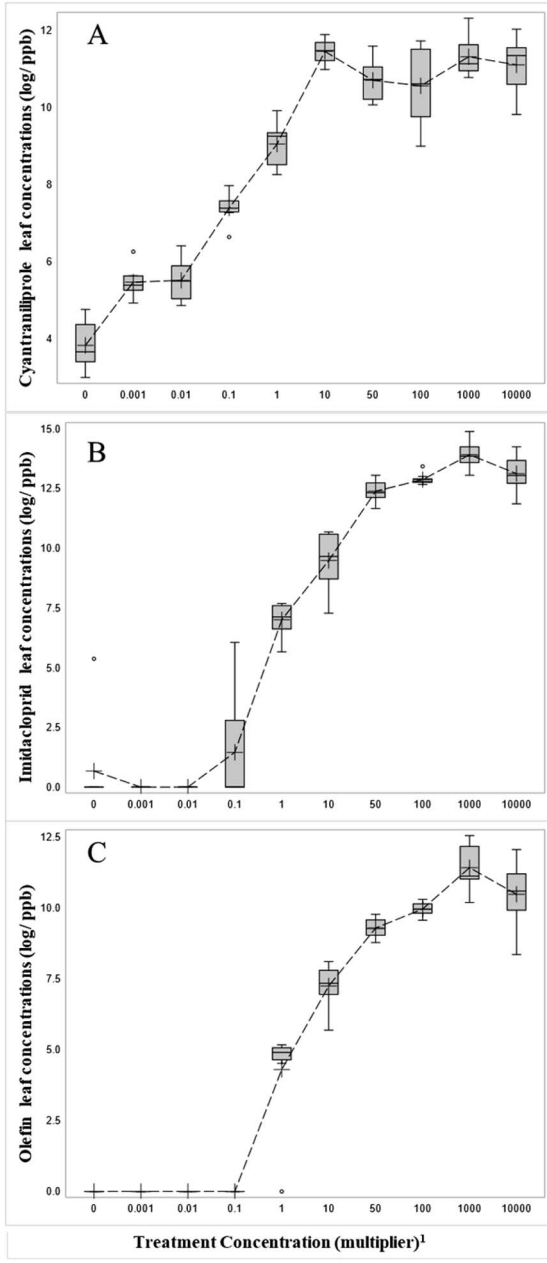

Fig. 1. Leaf tissue treatment concentrations based on dilutions of cyantraniliprole and imidacloprid. (A) Cyantraniliprole. (B) imidacloprid. (C) Olefin, an imidacloprid metabolite (imidacloprid-olefin). (multiplier) = insecticide treatment concentration of 0, 0.001048, 0.01048, 0.1048,

However, discussed inferences are described using the milligrams of active ingredient per liter for the relative insecticide because they refer to the treatment concentrations of each insecticide in solution.

Specifically, the cyantraniliprole concentrations tapered off in cotton leaves at the treatment concentration of 10.48 mg a.i. L^{-1} (Table 1, multiplier = 10; Fig. 1A). However, leaf tissue concentrations were confirmed as low as 0.001048 mg a.i. L^{-1} of the applied cyantraniliprole, which preceded a steep linear increase until the plateau, as depicted in Fig. 1A. This plateau might have been the limit of cyantraniliprole retention for a cotton plant at this plant age (3 wk). Interestingly, the reduced risk of phytotoxicity with diamides such as cyantraniliprole is not without limitation, because our data showed that a high-rate systemic application of this insecticide formulation above this plateau residue concentration would fail to be translocated.

By contrast, imidacloprid residue concentrations were only confirmed in treatment concentrations above 0.0098 mg a.i. L^{-1} (Table 1, multiplier = 0.01; Fig. 1B). The absence of an apparent plateau meant that imidacloprid uptake and retention would persist to higher concentration retention once applied. However, additional higher concentrations would likely result in increased phytotoxic impacts, as observed in other plants following an imidacloprid application (Ebel et al. 2000, Gorman et al. 2007).

In addition, this experiment identified a toxicologically relevant metabolite of imidacloprid called olefin. Generally, imidacloprid and olefin have a linear relationship (Sur and Stork 2003) as imidacloprid metabolizes into olefin in plants following application. The long persistence of olefin in plant tissue allows for continuous residue measurement in other plant systems (Benton et al. 2015). Therefore, the similarities in residue concentrations between Fig. 1B and C were expected and indicate the potential use of olefin to identify imidacloprid residues in cotton (Table 2).

Therefore, when evaluating both insecticides for future use, the above-mentioned results highlighted the ability to use higher treatment concentrations of imidacloprid in cotton test plants, but with consideration for potential phytotoxicity effects on concentration–response curves. In addition, the highest cyantraniliprole treatment concentrations may not be achievable in cotton test plants due to the translocation of smaller amounts at the highest rates. Even so, a significant regression was achieved for each insecticide, and the slope + intercept (Table 2) could be used to determine the active-ingredient retention in future insecticide efficacy trials. For example, using the average insecticide residue concentration equation for imidacloprid, $y = 4.08\,x + 0.83$ (Table 2), let x represent the milligrams of active ingredient per liter of the insecticide treatment concentration being applied (0.009 ml L^{-1} of product = 9.8 mg a.i. L^{-1} of imidacloprid). The expected residue concentration in

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1.048, 10.48, 52.40, 104.80, 104,800, and 1,048,000 mg a.i. L^{-1} for cyantraniliprole (A) and 0.00098, 0.0098, 0.098, 0.98, 9.80, 49.00, 98.00, 98,000, and 980,000 mg a.i. L^{-1} for imidacloprid (B) and olefin (C). Mid-bar shows the median insecticide residue concentration at each treatment concentration; + shows the mean leaf residue concentration location at each treatment concentration; and boxes portray the first to third percentiles of the data, with whiskers indicating the lower and upper values of the data. Small circles represent outliers.

Table 2. Tabulated unilateral runs of the leaf residue analysis, showing average regression equations for leaf tissue insecticide concentration estimation.

Insecticide	Run	*n*	df	*P*	*F*	R^2 (%)	Slope + intercept*
Imidacloprid**							
	1	36	1	<0.001	22.17	39.46	$y = 5.17x + 0.60$
	2	80	1	<0.001	115.40	59.67	$y = 3.63x + 0.94$
	Avg.++	116	1	<0.001	130.12	53.30	$y = 4.08x + 0.83$
Cyantraniliprole**							
	1	40	1	<0.001	37.04	49.36	$y = 4.56x + 0.58$
	2	80	1	<0.001	63.69	44.95	$y = 7.18x + 0.39$
	Avg.++	120	1	<0.001	92.45	43.93	$y = 6.22x + 0.47$
Olefin+							
	1	36	1	<0.001	17.47	33.94	$y = 2.40x + 0.46$
	2	80	1	<0.001	149.31	65.69	$y = 2.30x + 0.80$
	Avg. ++	116	1	<0.001	135.09	54.23	$y = 2.33x + 0.69$

* Leaf tissue treatment concentration estimation from generated regression lines.
** Insecticide formulation: imidacloprid, Admire Pro and cyantraniliprole, Exirel.
+ Olefin is an imidacloprid metabolite (imidacloprid-olefin).
++ Average regression of both unilateral runs, resulting equation estimates leaf tissue concentration from drench application of insecticides.

the leaf would be calculated using "log ($y = 40.81$)." Therefore, 1.61 ppb imidacloprid would be retained in the leaf tissue. This value falls between 1 and 10 on the *x*-axis (multiplier) of Fig. 1B and corresponds to 0.98–9.8 mg a.i. L^{-1} in Table 2. Confirming active-ingredient retention (insecticide exposure) stands to increase the accuracy of toxicological bioassays.

***Bemisia tabaci* adult bioassay.** The natural response of *B. tabaci* to cyantraniliprole and imidacloprid was evaluated to determine the LC_{50} of both insecticides (Fig. 2). In the tested *B. tabaci* population, cyantraniliprole was more effective at inducing adult mortality (LC_{50} = 48.17 [2.58–839.52] mg a.i. L^{-1}) than imidacloprid (LC_{50} = 493.70 [145.13–1,559] mg a.i. L^{-1}). The concentration–response slope indicates the tolerance level toward both insecticides for the species. Another critical component of insecticide bioassays, the slope value, is often compared with the tested population's susceptibility variation (Yu 2014). The steepness of the imidacloprid slope may indicate a more homogenous response in contrast with the slope of cyantraniliprole (Fig. 2).

Higher tolerance to imidacloprid could result from increased exposure to the insecticide in its various agricultural formulations (Horowitz et al. 2011). Many uses exist in which milligrams of active ingredient has not yet been translated into direct exposure, as reported herein. No such reports on cyantraniliprole have been documented. Bioassays serve to indicate the development of resistance to a given

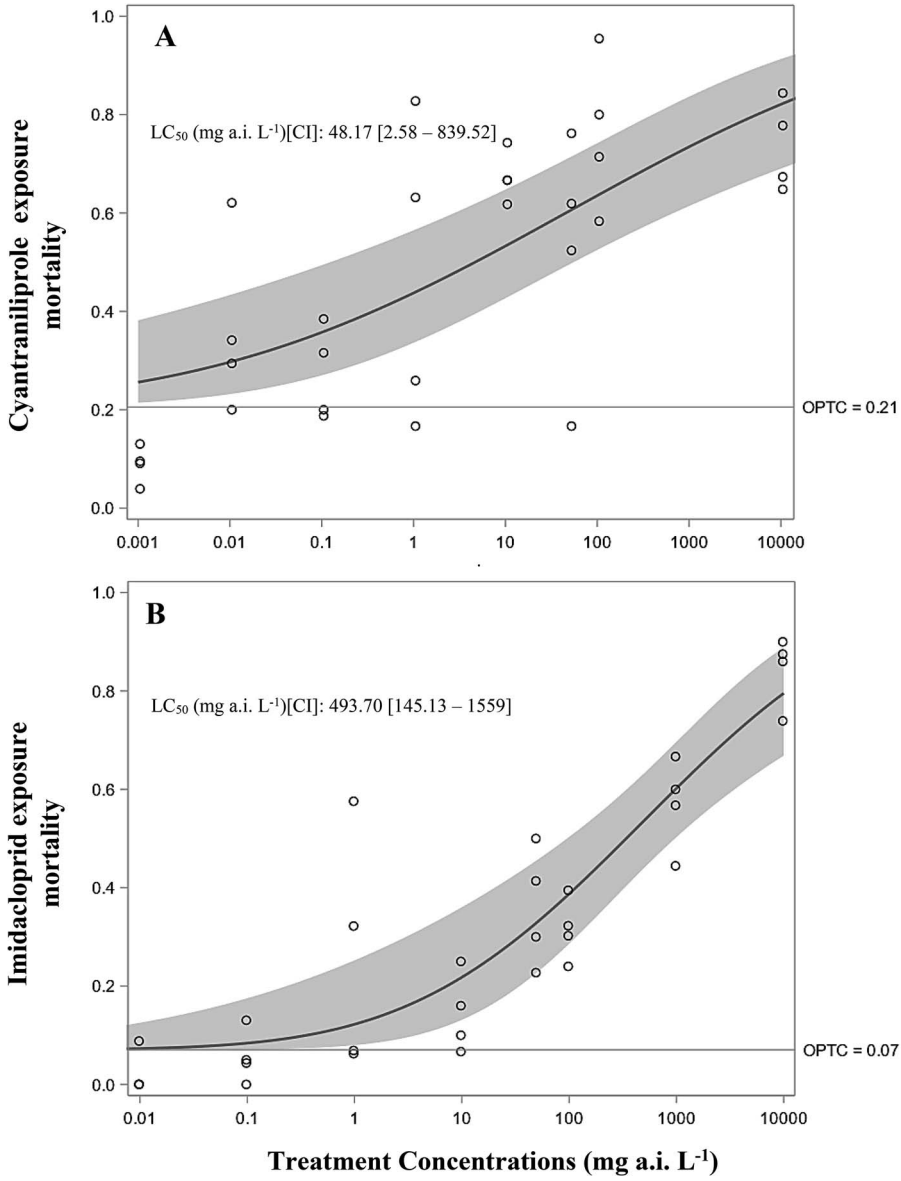

Fig. 2. Adult *Bemesia tabaci* concentration–response curves following exposure to cyantraniliprole and imidacloprid in a 24-h leaf drench bioassay. (A) Cyantraniliprole. (B) Imidacloprid. CI, fiducial limits, represented by shading on both graphs.

insecticide chemistry. Our approach incorporates the biological response of the treated plant in regulating *B. tabaci* insecticide exposure and potential insecticide efficacy.

Regarding concentration response, higher insecticide concentrations were needed for greater *B. tabaci* mortality. However, LC-MS/MS reveals that, at least for cyantraniliprole, the plant begins halting the chemical uptake after an applied rate of 10.48 mg a.i. L^{-1}. Therefore, although the median lethal concentration (LC_{50}) for cyantraniliprole was 48.17 mg a.i. L^{-1} (at least four times higher), the insecticidal activity of the active ingredient in the plant might not be the same. Similar to imidacloprid, cyantraniliprole is metabolized into a form that has increased toxicity toward the pest insects. Some metabolites have been identified in other plant systems, but the lack of reference standards is an ongoing challenge for proper identification (Huynh et al. 2021). If similar to olefin, a cyantraniliprole metabolite in cotton would increase detection accuracy. Imidacloprid uptake in cotton plants was linear and increased with increasing insecticide concentrations. The high LC_{50} required for adult mortality could result from increased exposure due to the lack of plant regulation.

There was a positive and significant correlation between the root drench concentration and leaf tissue concentration for both imidacloprid and cyantraniliprole ($n = 36$, $R^2 = 0.72$, $P < 0.0001$ and $n = 36$, $R^2 = 0.64$, $P < 0.0001$, respectively). Similarly, positive and significant relationships between the root drench concentration and sweetpotato whitefly mortality occurred with both insecticides ($n = 36$, $R^2 = 0.63$, $P < 0.0001$ and $n = 36$, $R^2 = 0.52$, $P = 0.0012$, respectively). Finally, the leaf residue and sweetpotato whitefly mortality for imidacloprid and cyantraniliprole also indicated positive and significant relationships ($n = 36$, $R^2 = 0.74$, $P < 0.0001$ and $n = 36$, $R^2 = 0.66$, $P = 0.0012$, respectively). Stronger correlations were seen in the results from exposure to imidacloprid. As mentioned earlier, uptake of the chemical appeared linear and could explain this strong relationship between root treatment and plant leaf tissue concentration. Nevertheless, a portion of the root treatment can remain in the solution due to the 24-h limit, resulting in a lower Pearson correlation coefficient for the relationship. Higher imidacloprid concentrations were required to induce whitefly mortality in the plant tissue concentration study, indicating resistance to the insecticide. Uptake and retention of the chemical also were lower for smaller treatments, which could also reduce the strength of the correlation. For cyantraniliprole, correlations were moderate, with the weakest relationship being that between sweetpotato whitefly mortality and root drench concentration. Our results indicated that cyantraniliprole uptake is heavily regulated by cotton. Knowing this, plus the moderate strength of the other relationships, the regulation of the chemical in the plant may influence the insecticide's effectiveness.

In summary, we presented the leaf issue concentration outcomes of imidacloprid and cyantraniliprole subjected to two separate trials to produce slope equations for the estimation of leaf tissue insecticide retention (cyantraniliprole, $y = 6.22\,x + 0.47$; imidacloprid, $y = 4.08\,x + 0.83$; Table 2). The model was significant in both trials, with at least 50.42–73.28% variability being explained. We also found significant correlations between the root drench concentrations, leaf tissue residue concentrations, and sweetpotato whitefly mortality. The correlations also indicated that there are links between leaf tissue concentrations and *B. tabaci* mortality that are not necessarily impacted by the amount of milligrams of active ingredient used as a treatment. Nevertheless, there was an apparent proportional

increase in leaf tissue concentration from root drench concentrations that resulted in mortality. Therefore, insecticide efficacy is subjected to the plant's metabolism.

Acknowledgments

This work was partly funded by a USDA-Agricultural Research Service (ARS) project (6080-22000-027-18-S) entitled "Managing Whiteflies and Whitefly-Transmitted Viruses in Vegetable Crops in the Southeastern U.S." Studies that contributed to our understanding of this subject matter were conducted using the Georgia Agricultural Experiment Stations laboratory facilities. We greatly appreciate the assistance of internal reviewers at the University of Georgia Tifton Campus and the USDA-ARS. In addition, we acknowledge the University of Georgia Libraries and the Web of Science database. This article reports the results of the research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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