Modified Maximum Dose Bioassay for Assessing Insecticide Response in Field Populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae)[1]

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Abstract The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) MEAM1 (Hemiptera: Aleyrodidae), continues to be a major pest of vegetable cultivation in Georgia, USA. Field-by-field surveying is an effective approach to determining the susceptibility status of a *B. tabaci* population to an insecticide. During 2020–2022, a modified maximum dose bioassay method was tested to characterize the insecticide response of *B. tabaci* field populations to several commonly used insecticides for whitefly management in Tift Co., GA, and the surrounding areas. A rapid bioassay was used for these evaluations that allowed for field assessments before spray applications to reduce the adult life stage of this species. The results of the evaluations were produced within 24-h following a 24-h root drench period. Our survey suggests that the neonicotinoids dinotefuran and flupyradifurone were the most effective insecticides from the Insecticide Resistance Action Committee (IRAC) group 4A. Cyantraniliprole was also effective, with 88 and 86% adult mortality following exposure to the high (maximum) and low doses, respectively. Conversely, the levels of control using another diamide, cyclaniliprole, were notably lower. Adding a low dose to the high dose provided an early indication of inefficient control with a product potentially indicating an increase in resistance. Specifically, a significant difference between the high and low doses suggests that the dose–response curve had shifted toward resistance development in each *B. tabaci* field population. The proposed bioassay method is meant for systemic insecticides that offer quick responses on adults. The use of this efficient method will improve evaluations prioritizing insecticides for use or rotation in an insecticide resistance management program.

Key Words whitefly, insecticides, neonicotinoids, diamides, maximum dose bioassay

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) MEAM1 (Hemiptera: Aleyrodidae), is a global pest of economically important crops (De Barro et al. 2011). This pest has an extensive host range that includes weeds (Abd-Rabou and Simmons 2010, Barman et al. 2022, De Barro 2011, Kavalappara et al. 2022, Simmons et al. 2008) that aid in its multivoltine existence and distribution in Georgia farmscapes (Gautam et al. 2020, McKenzie et al. 2020). Specifically, *B. tabaci* is a major pest of the

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cotton–vegetable belt in Georgia, USA, and causes economic losses averaging >US $160 million/yr (Li et al. 2021). Direct and indirect crop injuries are rampant in *B. tabaci*-infested crop systems, highlighting a need for an effective management program to suppress *B. tabaci* populations (Brown and Bird 1992, Carriere et al. 2014, Ghosh et al. 2019, Jones 2003, Li et al. 2021, Polston and Capobianco 2013, Shi et al. 2018). Such a management program proved to be challenging given the rapid development of resistance in *B. tabaci* to insecticides, spurring a need for constant insecticide resistance monitoring (De Marchi et al. 2021, Gravalos et al. 2015, Horowitz et al. 2020, Mohammed et al. 2020, Sparks et al. 2020, Wang et al. 2020a, Zheng et al. 2021).

Insecticides are the primary option for *B. tabaci* management in most agricultural production systems, and the options may range from contact to systemic chemistries (De Marchi et al. 2021, Horowitz et al. 2011, Li et al. 2021). Options such as neonicotinoids have been crucial to this heavy reliance due to their soil stability and effective systemic nature, providing some residual control against *B. tabaci* (Horowitz et al. 2020, Perring et al. 2018). Many chemistries of the group, including imidacloprid, thiamethoxam, flupyradifurone, and dinotefuran, are frequently used in management efforts against *B. tabaci*. Diamides such as cyantraniliprole and cyclaniliprole are relatively new chemistries for whitefly management (Lahm et al. 2005, Nauen and Steinbach 2016, Tsukamoto et al. 2021) and offer systemic control of *B. tabaci* with fewer reported control failures than neonicotinoids due to their modes of action (Lahm et al. 2005). Consistent *B. tabaci* management is also seen with other anthranilic diamides with low levels of resistance to their chemistries against *B. tabaci* (Basit 2019, Guo et al. 2020, Horowitz et al. 2020). Additional groups of insecticides, such as insect growth regulators (pyriproxyfen and buprofezin) and ketoenols (spiromesifen and spirotetramat), as well as organic options such as oils, soaps, and detergents (Li et al. 2021), have also been used in these efforts against *B. tabaci*.

Monitoring *B. tabaci* populations is critical to managing insecticide resistance outbreaks and mapping distribution (Caballero et al. 2013a; Castle and Prabhaker 2013; Gauthier et al. 2014; Horowitz et al. 2020; Perier 2023; Perier et al. 2022; Prabhaker et al. 1997; Wang et al. 2018, 2020a, 2020b; Yao et al. 2017; Yukselbaba and Ali 2022). Identified suspected resistant populations can then be subjected to a serial dilution of the active ingredients of common insecticides to produce a dose–response curve and subsequent lethal concentrations (LCs), such as LC_{50}s and LC_{90}s, commonly used for insecticide efficacy characterization. One method that has proved to be an excellent decision tool in resistance monitoring is the maximum dose bioassay. It provides quick and valuable insight into the status of insecticide resistance in whitefly field populations (Cremonez et al. 2023a; De Marchi et al. 2021; Perier et al. 2023a, 2023b). The updated methodology presented herein aims to produce a quick bioassay with increased accuracy by using portable collection tubes for field-by-field evaluation. This study used a modification of the maximum dose bioassay as a high–low dose experiment design. The so-called "high" dose was defined as the maximum labeled rate dosage recommended for managing *B. tabaci*, whereas the "low" dose was set at one tenth of the high dose. A clear understanding of field resistance levels should be the primary driver of pest management and insecticide rotations. Thus, a low dose can facilitate this need. Because the high label rate for an insecticide usually falls between the LC_{70} and LC_{90}, this lower dose would be more

Table 1. Surveyed *Bemisia tabaci* populations from Tift Co., GA, USA, and surrounding areas subjected to the modified maximum dose bioassay.

Population	County	ID	GPS Coordinates (DD)*	*N***	Host
Ponder	Worth	PD-1	31.508053, −83.656430	26	Cantaloupe
LAB-1†	—	LAB-1	31.473839, −83.529010	312	Mix‡
Tifton	Tift	TF-1	31.485385, −83.521330	52	Cucumber
Hort Hill	Tift	HH-1	31.470659, −83.530806	104	Squash
Lewis-Taylor Farms	Tift	L-T1	31.438643, −83.596851	105	Broccoli
Chula	Tift	CH-1	31.524030, −83.528066	52	Zucchini
Gibbs	Tift	GB-1	31.432238, –83.584430	26	Cotton
Lang-Rigdon	Tift	L-R1	31.515645, –83.547805	288	Sweet potato

* Coordinates are in decimal degrees form (DD).
** Total number of experimental units, not counting controls; each unit holds 50 adult whiteflies.
† Laboratory colony.
‡ Maintained with host rotations of cotton and squash.

sensitive and closer to the LC_{50} and potentially highlight the loss of efficacy at an earlier stage than the high "maximum" dose. This approach is meant to complement the other classical bioassays, with its incorporation assisting in distinguishing complex populations based on insecticide response. Therefore, this study aimed to evaluate a rapid bioassay and the addition of a low dose to assess the insecticide response of field *B. tabaci* populations. The rapid nature of this bioassay will provide the option of insecticide evaluation before field spray applications. In turn, it will assist in resistance monitoring by aiding in insecticide selection and rotations.

Materials and Methods

Field surveys were conducted in seven locations around Tift Co., GA, and the surrounding area from June to August 2020–2022, given the region's prominent vegetable and cotton production. Selected sites were obtained with the help of the University of Georgia Extension agents and were scouted at least 24-h before sampling to ensure the presence of sufficient whitefly (*B. tabaci* MEAM1) numbers for bioassays. Sampling was conducted three times per site to collect dose–response data on *B. tabaci* adults through laboratory bioassays. Sampled sites varied in production size, but mostly represented vegetable crops (see Table 1 for site information).

Untreated cotton seedlings (*Gossypium hirsutum* L., Stoneville® ST 4946-GLB2) were used as the standard host for all bioassays conducted, following other reports (Caballero et al. 2013b, Perier et al. 2023a, Schuster et al. 2010, Sparks et al. 2020). The cotton plants selected for this methodology were grown under 30 ± 2°C, 60 ± 5% relative humidity, and a photoperiod of 14:10 (L:D) h. The selection process involved washing and clipping the roots to a length of 5 cm. These prepared cotton

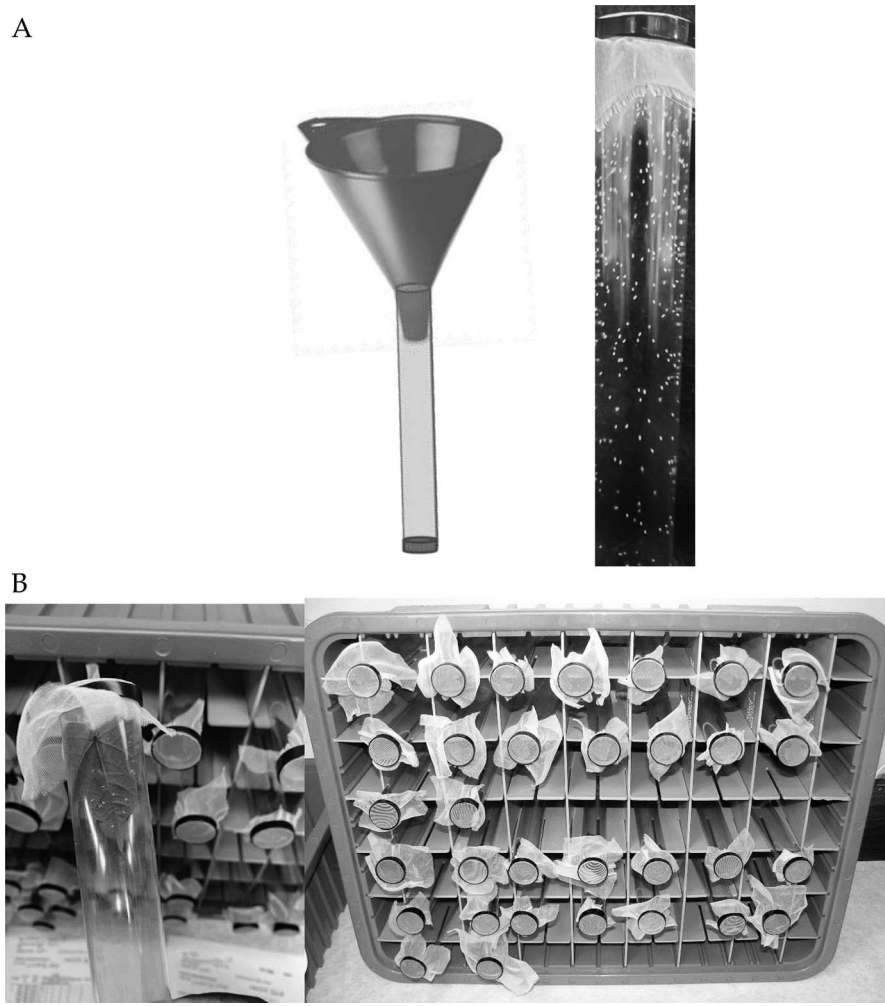

Fig. 1. (A) Funnel apparatus for field collections of *Bemisia tabaci*. (B) Benchtop setup for testing adult mortality.

plants were then inserted into the respective treatments as part of the experimental procedure following the steps outlined by Perier et al. (2023a).

A colony of *B. tabaci* cryptic species MEAM1 (LAB-1) maintained in a pesticide-free environment for at least 4 yr on rotations of squash (*Cucurbita pepo* L. subsp. *pepo* var. Golden Summer Crookneck) and cotton was used as the colony comparison for insecticide evaluations. The relative susceptibility of the colony (LAB-1) served as a reference for the expected susceptibility of the field populations to the tested insecticide (Caballero et al. 2013b).

Adult whiteflies were collected from all populations by using a funnel apparatus (Fig. 1) and stored in screened transparent plastic tubes (diameter: 2.86 cm; length:

Table 2. Evaluated insecticides against *Bemisia tabaci* populations from Tift Co., GA, USA, and surrounding areas.

Active Ingredient (a.i.)	IRAC Group	Commercial Trade Name	Rate (maximum/ha)*	Amount/L**	a.i./L†
Dinotefuran	4A	Venom 70SG	280.2 g	0.30 g	125.67 mg
Cyantraniliprole	28	Exirel 0.83SC	986.5 ml	1.06 ml	104.8 mg
Flupyradifurone	4D	Sivanto Prime 1.67SL	876.9 ml	0.94 ml	188 mg
Thiamethoxam	4A	Actara 25WDG	385.3 g	42 g	0.13 g
Imidacloprid	4A	Admire Pro 4.6F	160.8 ml	0.18 ml	98 mg
Cyclaniliprole	28	Harvanta	1.20 L	1.28 ml	64.34 mg
Water	—	Control (check)	—	—	—

* Maximum label rate of a product per hectare.
** Amount of formulated product per liter per hectare.
† Amount of active ingridient (a.i.) per liter per hectare.

20.3 cm, ClearTec® Packaging, Park Hill, MO) for the bioassay. Thirty-six tubes were collected per site per sample, with each containing at least 50 whiteflies. During transport to the laboratory, the samples were briefly insulated and cooled with ice packs to mitigate mortality due to transportation from the field; the travel time averaged 30 min from each collection site. As such, whiteflies, on average, were bioassayed within 1.5-h after field collection, including a 1-h acclimation period. Adult age and sex were not determined for this study, nor were they included as factors in any analysis.

Bioassays were conducted at the Coastal Plain Experiment Station in Tifton, GA. Ambient conditions for the bioassays were 27 ± 2°C, 50% relative humidity, and a photoperiod of 24:0 (L:D) h for both the treatment period and the bioassay. Several populations of *B. tabaci* (Table 1) were subjected to a high dose of six insecticides: imidacloprid, dinotefuran, thiamethoxam, flupyradifurone (Insecticide Resistance Action Committee [IRAC] group 4), and cyantraniliprole and cyclaniliprole (IRAC group 28), in a dose–response bioassay. An additional "low" dose of each insecticide, one tenth of the high dose, was also tested (Table 2).

Stock solutions made up to 500 ml for each insecticide were used following previously published protocols (Perier et al. 2023a) and only modified for the single additional dose. This "low" dose was created by diluting 50 ml of the insecticide stock with 450 ml of water. The selected cotton plants were 3 weeks old (with at least one terminal true leaf with a 4-cm width) at the time of use. Similarly to the bioassay steps outlined by Perier et al. (2023a), only the terminal true leaf was used in this study after a 24-h root drench of the plant in the treatments and check (untreated control) at experimental conditions. Treated leaves were used to bioassay adult whiteflies and were placed inside the collection tubes toward a benchtop light. Treatments and check concentrations were replicated four times per bioassay, with mortality recorded immediately after leaf insertion into the tubes and again after 24-h.

Mortality data were corrected using Abbott's formula (Abbott 1925) for consistency with the checks and treatments across all locations. An additional threshold of 40% was established for mortality in the check. This threshold was used to

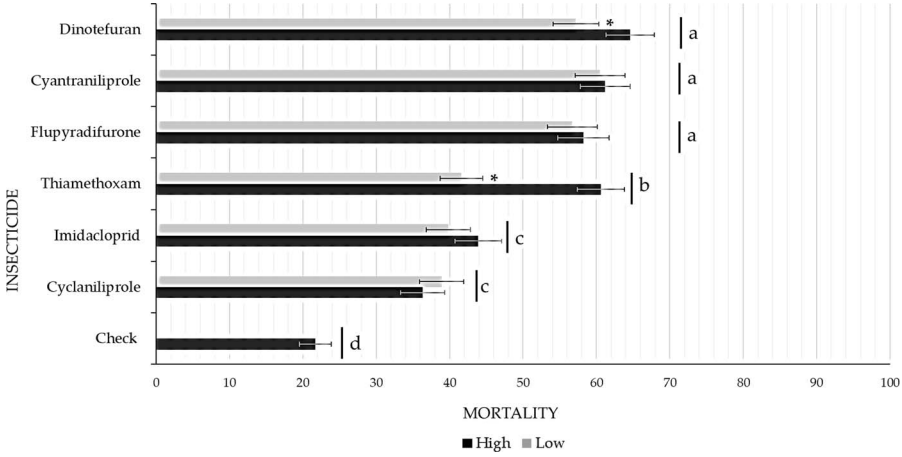

Fig. 2. Mortality of *Bemisia tabaci* across all populations in response to exposure to six insecticide treatments at high and low doses. An asterisk (*) over a bar indicates a significant difference ($P < 0.05$) between high and low doses. The same letter behind the line indicates no significant difference in insecticide efficacy ($P < 0.05$).

remove experimental sets with mortality in the check above the threshold. The final dataset subjected to analysis was percentage data and therefore had a binomial distribution. To ensure normality, the data were transformed (log transformed) and Gaussian error distribution was confirmed using residual and normality plots (Fernandez 1992) in SAS Enterprise Guide v. 8.3 (SAS Institute Inc., Cary, NC). Proc GLM was then used to determine the chemical, dosage, and population differences of the transformed data, whereas pairwise associations and differences were categorized using the LSMEANS statement and LINES option ($P = 0.05$). The separated responses were then ranked according to their effectiveness at managing *B. tabaci*. Overall comparisons involved analysis of either the pooled populations or pooled doses.

Results and Discussion

The differences in *B. tabaci* mortality were a result of treatment ($F_{5,1200} = 31.79$; $P < 0.0001$), dose ($F_{2,1200} = 71.14$; $P < 0.0001$), and population ($F_{7,1200} = 102.44$, $P < 0.0001$). Significant interactions were only seen in parings including dose, mainly dose and chemical ($F_{5,1200} = 4.61$; $P < 0.001$) and dose and population ($F_{14,1200} = 3.76$; $P < 0.0001$). When all three interacted, there was no significant impact on mortality, nor was there an interaction between chemicals and population. When mortality was pooled for each population, all treatments were significantly separated from the check, untreated control (Fig. 2). Similarly, treatments were separated regardless of dose (high and low). They were then ranked according to their efficacy (Fig. 2). At this level, dinotefuran, cyantraniliprole, and flupyradifurone caused the highest mortality on *B. tabaci*, followed by thiamethoxam, with imidacloprid

and cyclaniliprole offering the least contribution in the Tift Co. and surrounding area (Fig. 2).

Regarding insecticide dose, only two insecticides, dinotefuran and thiamethoxam, showed dose-related different responses (high or low dose). For the other insecticides, the doses were comparable at $P < 0.05$ (Fig. 2). However, clearer separation could be seen for each chemical when the population was considered an effect at $P < 0.05$ (Fig. 3). Separation was observed for several insecticides at different locations for a specific dose (Fig. 3), while at the same time there was a lack of separation among the doses for the other chemicals when population was not considered (Fig. 2), illustrating the need for site specific data. Certain populations exhibited higher mortality at the lower dose than at the high doses (e.g., TF-1, Figs. 3E). Interestingly, there were cases where mortality between the doses was unchanged and had no separation (e.g., CH-1, Figs. 3C); as such, more evaluations would be required to determine the insecticide response of these locations to these insecticides (LC_{50} bioassays). Or, perhaps, the lack of dose separation in these populations aids in characterizing the overall efficacy of these insecticides. Nevertheless, the trends identified in the lower dose, that is, good or poor efficacy, were also evident in the high dose, as seen with the Ponder population and many of the insecticides (Fig. 3).

The 24-h maximum dose bioassay used in this study revealed higher mortality rates in several populations of *B. tabaci* when exposed to the high dose of the tested insecticides. Overall, cyantraniliprole was highly effective in the Georgia *B. tabaci* populations, resulting in an average of 88.7% mortality across all field populations at the high dose. Notably, at the low dose, the average mortality was 86% for the same insecticide, indicating substantial control. Therefore, using the proposed low-dose estimates the same level of adequate control of *B. tabaci* for cyantraniliprole. In the LT, Gibbs, and Lang-Rigdon populations, a higher mortality rate was obtained at the low dose of cyantraniliprole, whereas in Chula and Ponder, susceptibility to the insecticide was already evident at the low dose, highlighting the potential benefit of dose characterization in individual populations. The general efficacy of dinotefuran and flupyradifurone, compared with the other two neonicotinoid insecticides tested at the high dose, was consistent with previous reports from Florida, USA (De Marchi et al. 2021, Smith et al. 2016). In these Georgia *B. tabaci* populations, dinotefuran exhibited higher efficacy, resulting in high mortality at the high dose. The efficacy of imidacloprid and thiamethoxam in these same populations was poor, warranting further resistance management. Similarly, less effective control was obtained with cyclaniliprole, as the results revealed low *B. tabaci* mortality following exposure. In imidacloprid, poor efficacy was evident at the low dose except for the Tifton and Ponder populations. This was expected, given the potential for fast upregulation of metabolic mechanisms in response to imidacloprid exposure (Karunker et al. 2008, Perier 2023). However, the shifts in mortality observed in these populations due to exposure to the low dose of imidacloprid hint toward the existence of sublethal effects. Some biological processes, such as fecundity and other behavioral changes, could be disrupted (He et al. 2011, Nauen et al. 1998, Sohrabi et al. 2011, Wang et al. 2016). However, sublethal effects were beyond the scope of this study, but could provide insight for improved resistance management as seen with other neonicotinoids (Wang et al. 2016).

Bemisia tabaci mortality was surprisingly different in response to the two diamides tested in this study. Although cyantraniliprole exposure resulted in higher *B. tabaci*

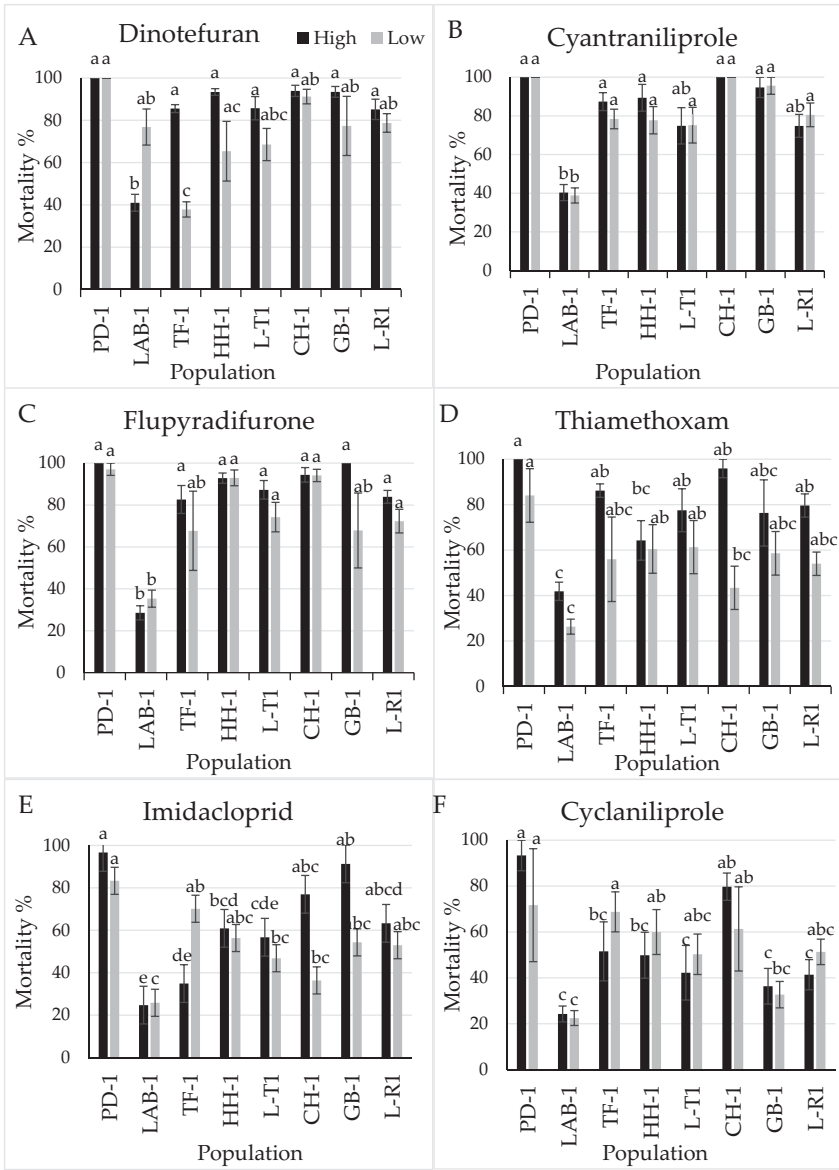

Fig. 3. Mortality of *Bemisia tabaci* across eight populations in response to exposure to six insecticide treatments at the high and low doses: dinotefuran (A), cyantraniliprole (B), flupyradifurone (C), thiamethoxam (D), imidacloprid (E), and cyclaniliprole (F). *x*-axis (populations): PD-1 = Ponder, LAB-1 = laboratory colony, TF-1 = Tifton, HH-1 = HortHill, L-T1 = Lewis-Taylor farms, CH-1 = Chula, GB-1 = Gibbs, L-R1 = Lang-Rigdon. For bars of the same color, the same letter over the bar indicates no significant difference ($P < 0.05$).

mortality, exposure to cyclaniliprole (another relatively new anthranilic diamide with a novel mode of action; Tsukamoto et al. 2021) yielded an insufficient *B. tabaci* control in this study. In other locations, cyclaniliprole continues to offer control with low levels of resistance (Gill and Chong 2021, Guo et al. 2020). As such, further evaluation is needed to confirm whether cyclaniliprole has poor uptake in plants from root drench applications, as translocation throughout the plant would be limited. This is notable because differences in response for nymphs were also seen when sprays and drenches were previously compared (Gill and Chong 2021). This could explain the poor control observed in this study from cyclaniliprole, given the root drench insecticide treatment method. Nevertheless, cyclaniliprole separated from the check and offered some levels of *B. tabaci* control.

Chula and Ponder, on average, were the most susceptible populations tested against the insecticides. By contrast, the laboratory colony LAB-GA was the most resistant to these insecticides, possibly because the original colony was created from resistant populations at a time when heavy *B. tabaci* infestation plagued Tift Co. and its neighboring areas. With the lack of interfering field environmental factors, such as genetic diversity from migrating populations, in a controlled ecosystem, such as colony rearing conditions, an established case of resistance could be sustained by haplodiploidy (Denholm et al. 1998). Therefore, certain resistance traits may persist without contention in a colony, allowing more susceptible populations to exist in field populations. However, the identification of these populations would require more than just a "maximum dose" testing and warrant future efforts.

In this study, we propose a rapid bioassay that produces insecticide response data within a 48-h time frame (the approach involves 24-h systemic treatment of the plants followed by a 24-h experimental period). Although insecticide activity can persist past this period, other factors may interfere with the recorded results. In this study, leaf desiccation and whitefly feeding behavioral changes were considered when standardizing to the 24-h experiment period once the leaf was detached. Mainly, these factors could contribute to mortality with a longer experiment duration. Not to mention the potential degradation of the applied insecticide. Because *B. tabaci* is a systemic feeder, a systemic approach was the goal of this study. However, this methodology targets insecticides that offer quick responses on adults as well as nymphs, not necessarily growth regulators, which require longer experimental times. In addition, the tested insecticide should have some systemic nature that allows for translocation from the roots. There is a possibility of differences between this systemic approach and other insecticide evaluation methodologies that tend to offer complete contact coverage of the treatment plant or direct application to the insect (Yu 2014). However, earlier comparisons found no such differences, with mortality only being due to the treatments, regardless of the methodology (Sparks et al. 2020). Plant uptake and retention of the chemicals being tested can also be estimated for greater accuracy, thereby correlating response to the treatment concentrations applied (Perier et al. 2023a). Therefore, if the molecular chemistry of the insecticide allows, it can be tested with this methodology and more accurately. The low dose is proposed as a companion to the high dose and is meant to provide more information on complex populations that may benefit from more long-term control options using sublethal approaches. The application of the low dose identified many areas of concern during this study. Initially, there was a need for field characterization, as insecticide response was impacted by the geographical

location of the population. Moreover, the application rate of these insecticides at the high dose control was sufficient for most. However, the low dose identified populations that could be actively selected for resistant populations due to reduced mortality at the higher dose. Also, the low dose provides a clearer starting point for future LC_{50} evaluations that would be based on the high dose and require multiple adjustments of serial doses.

Including a low dose alongside a high dose (=maximum labeled rate) bioassay is a crucial enhancement to the original maximum dose bioassay proposed by De Marchi et al. (2021). Incorporating the low dose creates a more comprehensive understanding of the dose–response relationship, because the high dose alone provides only a single data point on the hypothetical dose–response curve of a given population to a specific compound. This addition improves the bioassay and aligns with the need for faster assay development, because it requires only two different dosages instead of a larger set of concentrations (Perier et al. 2023b, Yu 2014). This reduction in doses saves time, reduces the sample size, and minimizes the material required for the laboratory bioassay test. Therefore, the modified high–low dose bioassay proposed in this study provides a quick, easy to setup tool for integration into insecticide resistance management strategies. Further studies regarding model validity for precision and accuracy comparing the laboratory bioassays with real-world in-field responses are needed (Cremonez et al. 2023a, 2023b).

This study evaluated commonly used insecticides for *B. tabaci* management. Greater control was found with the novel neonicotinoids dinotefuran and flupyradifurone than with imidacloprid and thiamethoxam. As such, these two novel neonicotinoids should be considered as alternatives. With the diamides, only cyantraniliprole offered substantial control. The use of the low dose in this study identified field trends that were different from those displayed at the high dose. As such, the impact of population should always be considered during these evaluations. The addition of the low dose provided more insight into the insecticide response of each population and could be used for evaluations prioritizing insecticides for use or rotation.

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