Susceptibility of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Adult Populations to Imidacloprid in Georgia, USA[1]

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Abstract Imidacloprid has been one of the most widely used insecticides for managing the sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in the United States since the U.S. Environmental Protection Agency first registered it in 1994. A major whitefly control failure occurred in the state of Georgia in 2017 when *B. tabaci*–induced economic losses in horticultural and field crops surpassed approximately US$160 million. Vegetable growers have historically used imidacloprid for whitefly management, which likely led to the insecticide control failures of whiteflies in spring vegetables. Despite this, only a single site documentation of imidacloprid resistance in adults from 2007 in Georgia exists, making the current status unknown. Thus, it is likely that Georgia has an ongoing risk of imidacloprid-resistant *B. tabaci* infestations. No multicounty, extensive survey for imidacloprid dose response in whitefly exists for the state of Georgia. Therefore, an adult mortality bioassay of a range of imidacloprid concentrations was used to evaluate *B. tabaci* populations from several counties in South Georgia, where most of these economic losses occurred. This included a maximum dose concentration representing the current highest labeled rate. Dose response to the insecticide was not uniform across locations, with whiteflies in several areas displaying unexpected susceptibility to imidacloprid. Median lethal concentrations (LC_{50}s) ranging from 0.02 to 196.05 mg of active ingredient per liter in Georgia whitefly populations were substantially lower than the reference Florida whitefly population. This baseline information for the state is critical to future evaluations of this insecticide in resistance management programs.

Key Words survey, insecticide resistance, whiteflies, neonicotinoid, bioassay

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is prolific, polyphagous, and adept at crop invasion and infestation (De Barro 2011). The species distribution can be associated with a variety of cropping systems that includes vegetables, row crops, herbs, and ornamental crops, while also extending to many weed hosts that promote its multivoltine persistence (Barman et al. 2022, De Barro 2011, De Marchi et al. 2021, Simmons et al. 2008) within the tropical and subtropical regions of the world (Costa et al. 1993, Gangwar and Gangwar 2018). Economic losses resulting from *B. tabaci* are both direct or indirect, with damages

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caused by plant feeding (direct) inducing the loss of photosynthetic ability through honeydew–facilitated sooty mold growth (Gangwar and Gangwar 2018). Similarly, its ability to transmit plant viruses (indirect) (Caballero et al. 2015, Dutta et al. 2018, Jones 2003, Kavalappara et al. 2022) and its resilience against insecticides regardless of the mode of action (Horowitz et al. 2011, 2020) have caused substantial negative economic impacts. Globally, concerns for control failures due to insecticide resistance in *B. tabaci* have increased in the last decade (Dangelo et al. 2018). This is mainly due to current management programs for *B. tabaci*, which involve the frequent use of insecticides and thereby select for resistant populations (Basit 2019, LaTora et al. 2022, Perier et al. 2022).

Bemisia tabaci has historically been identified as an insect pest of economic importance (Mound and Halsey 1978), with reputation surging in the United States due to the severely damaging B cryptic species (strain) displacing the A cryptic species (strain) in the late 1980s to early 1990s (Brown et al. 1995). Within Georgia, commodity groups, including vegetables, row crops, and forage crops, which combined account for approximately 29% of the total Farm Gate value in 2021 (Stubbs 2020), are frequently subjected to *B. tabaci* infestations. Since 2017, annual economic losses due to *B. tabaci* outbreaks in these Georgia crops have been estimated at US$161 million (Li et al. 2021). The increased economic losses due to whiteflies in Georgia can be partly attributed to reduced insecticide efficacy. Two *B. tabaci* cryptic species have been identified in Georgia whitefly populations, Middle East–Asia Minor 1 (MEAM1, formerly biotype B) and Mediterranean (formerly biotype Q). MEAM1 is the nearly exclusive cryptic species reported from field populations, especially in the southern half of Georgia (McKenzie et al. 2020). The MEAM1 population has displayed resistance to a wide range of insecticides, including imidacloprid (Horowitz et al. 2020, Perier et al. 2022).

Imidacloprid, a neonicotinoid, is a chloronicotinyl insecticide with a high systemic ability that is targeted toward piercing-sucking insects such as whiteflies. However, the efficacy of this insecticide for whitefly control is threatened by *B. tabaci* populations exhibiting resistance (Mullins 1993, Prabhaker et al. 1997). In Georgia, imidacloprid resistance was documented in Tift Co. in 2007 (Schuster et al. 2008). Notably, in some plant species, a heavy infestation of whiteflies results in the silvering of the leaves due to feeding, especially in cucurbits. Therefore, the persistence of the silverleaf symptoms throughout the growing season, regardless of consistently higher rates of imidacloprid applied to manage whiteflies, can be an indicator of a resistant population, as observed in squash production in that county. Imidacloprid resistance appears to be primarily metabolic, associated with an overexpression of detoxification enzymes known as cytochrome P450s (Karunker et al. 2008, Nauen et al. 2013, 2015, Perier et al. 2022). These reports list P450s associated with imidacloprid resistance that are suitable for monitoring and evaluating at-risk whitefly populations going forward. Nevertheless, field surveys are still required to quantify the biological response to imidacloprid to characterize the population phenotypes. In this study, we report the results of a survey of several farms throughout South Georgia concerning an assessment of the current imidacloprid response of *B. tabaci* field populations. The objective was to determine the current efficacy level of imidacloprid in South Georgia and its potential as a control option for whiteflies. As

Table 1. Surveyed whitefly populations from South Georgia counties and Marion County, Florida, subjected to imidacloprid bioassay.

Population Name	State	County	*N**	Host
Colq–BDO	Georgia	Colquitt	27	Cabbage
Gra–C	Georgia	Grady	12	Zucchini
Mit–C	Georgia	Mitchell	20	Pumpkin
Prc–B	Georgia	Pierce	4	Cotton
Sum–P	Georgia	Sumter	10	Pumpkin
Tom–P	Georgia	Thomas	4	Squash
Tif–TT	Georgia	Tift	26	Tomato, broccoli
Tmb–R	Georgia	Toombs	24	Pumpkin
Wil–O	Georgia	Wilcox	4	Cotton
Wot–PS	Georgia	Worth	16	Cotton, zucchini
Mar–C	Florida	Marion	6	Soybean
LAB–col**	—	—	19	Mix†

* Total number of experimental units, excluding control; each unit holds 30 adult whiteflies.
** Laboratory colony.
† Maintained with rotations of cotton and squash.

such, we hypothesized that there is no difference in dose response when individual populations of whiteflies across South Georgia are subjected to a bioassay of imidacloprid concentrations. With this information, it would be possible to discern a current baseline of response to the insecticide for future field efficacy evaluations, especially as neonicotinoid insecticides remain a key chemical control option for mitigating economic losses because of *B. tabaci* infestation.

Methods and Materials

A laboratory colony of MEAM1 *B. tabaci* (Table 1, Lab–col) maintained on rotations of untreated squash (*Cucurbita pepo* L. subsp. *pepo* var. Golden summer crookneck) and cotton (*Gossypium hirsutum* L., Stoneville® ST 4946GLB2) was established from a pre-existing laboratory colony maintained for at least 4 yr without exposure to imidacloprid. Imidacloprid susceptibility in both the laboratory and field populations was determined by comparing the relative susceptibility of these populations to the insecticide to the most susceptible population, usually the laboratory colony (Caballero et al. 2013b). For the susceptible check population, the most susceptible field population from the survey, Grd–CO (Table1), was used and kept in colony for the duration of this study and continues to be maintained for future studies as an imidacloprid susceptible population.

Adult whiteflies (all MEAM1; Perier 2023) from 11 sites throughout South Georgia were collected using a yellow funnel, and transparent plastic tubes (diameter, 2.86 cm; length, 20.3 cm; ClearTec® Packaging, Park Hill, MO) screened at both ends with nylon chiffon for bioassaying. A Florida (Marion Co.) population also was included to reference the imidacloprid response of *B. tabaci* in the neighboring state. Thirty-six tubes were collected per site, each containing at least 30 whiteflies. Tifton, GA (Tift Co.) was set as the starting location for collection due to the historically heavy infestation of *B. tabaci*. Other 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 sufficient whitefly numbers for bioassays. During transport, the tubes were kept insulated with icepacks to lower travel temperatures and promote whitefly survival. Mortality during travel using this method was rare and ensured sufficient numbers even after long travel distances (~4 h). On average, whiteflies were bioassayed within 4.5 h after field collection. Whitefly age and sex were unknown due to the method of collection. However, mortality due to age was controlled by allowing at least 1 h for acclimation of the collected whiteflies to the experimental conditions. Dead whiteflies after the acclimation period were then removed before testing. Collections were conducted from early May to late October 2021 and from early June to mid-November 2022, aligning with heavy infestation months for whiteflies in Georgia. Sampled sites (Table 1) varied from research stations to commercial farms and included crops such as cotton, legumes, brassicas, and cucurbits. Each site was sampled at least three times to collect dose–response data on the species through laboratory bioassays.

Experimental conditions for the study were ambient at a temperature of 27 ± 2°C, relative humidity of 50%, and a photoperiod of 24:0 (L:D) h for both the treatment period and the bioassays. Adult whiteflies were subjected to a dose–response bioassay of several concentrations (0.00098, 0.0098, 0.098, 0.98, 9.8, 49, 98, and 980,000 mg active ingredient [a.i.] L^{-1}) of imidacloprid (brand name, Admire® Pro 4F; label rate, 67.43 ml/acre IRAC group: 4A, Bayer Crop Science, Research Triangle Park, NC) and a check (untreated control, distilled water), labeled accordingly as treatment solutions.

This study used 3-wk-old cotton (*G. hirsutum* var. ST4946GLB2, untreated) plants as the standard host for all bioassays. Cotton plants were grown using PRO–MIX® soil medium (with Osmocote® blend fertilizer added, NPK= 19–5–8) with germination and plant development limited to growth chambers (30 ± 2°C with a relative humidity of 60% and a photoperiod of 14:10 [L:D] h) to ensure non-infested plants. When selected (terminal true leaf of at least 4 cm in width), the root systems were washed and clipped (5 cm long) before being immediately placed in treatment solutions (Perier 2023). Plants were left in treatment solutions for 24 h to undergo systemic (root drench) treatment at experimental conditions. Treated or check leaves were inserted into the transparent collection tubes holding the whiteflies after the acclimation period. Treatment and check concentrations were replicated four times per bioassay, with whitefly mortality recorded at leaf insertion into collection tubes and again after 24 h.

Corrected mortality, using Abbott's formula (Abbott 1925), was log transformed. Gaussian error distribution was confirmed using residual and normality plots (Fernandez 1992) in SAS® Enterprise Guide v. 8.3 (SAS Institute Inc., Cary, NC). Data were subjected to PROC PROBIT and PROC REG analyses to estimate the

Table 2. Imidacloprid survey summary and insecticide response status of sampled whitefly populations throughout South Georgia and Marion County, Florida, during 2021–2022.

Population Name	*N*	% Maximum Dose Mortality*	Maximum Dose Status**	(LC_{90}/Maximum Dose) Ratio†	LC_{90} Ratio Status†
Colq–BDO	27	71.01	R	235,603.48	R
Gra–C	12	96.08	S	0.38	S
Mit–C	20	87.23	R	22,504.21	R
Prc–B	4	66.83	R	197.90	R
Sum–P	10	88.26	R	47.84	R
Tom–P	4	83.56	R	12,757.55	R
Tif–TT	26	82.79	R	104.14	R
Tmb–R	24	87.57	R	315.41	R
Wil–O	4	97.08	S	0.51	S
Wot–PS	16	73.05	R	5,129.49	R
Mar–C	6	54.30	R	92,899.72	R
LAB–col	19	45.52	R	224,822.80	R

* Mean percent mortality following label rate (98 mg a.i. L^{-1}) imidacloprid exposure ($F_{3,11} = 9.09$; $R^2 =$ 39.3%; $P \leq 0.0001$).

** Status: S, susceptible (mortality 96–100%); R, resistant (mortality <90%) (Pusawang et al. 2022).

† Ratio relating to response for *B. tabaci* at the LC_{90}. Ratio status: R, Resistant (>1); S, Susceptible (<1).

dose–response median lethal concentration (LC_{50}) values and respective fiducial limits, slope, and SE of regression lines and χ^2 values for the tested populations. Resistance ratios for 50% mortality were also calculated as a division of field LC_{50}s by the susceptible population LC_{50}. A representative susceptibility status for each population was created to represent adult whitefly response due to exposure to either the maximum dose or the LC_{90} of imidacloprid, which represents a critical dose capable of managing 90% of the population (Table 2). For the maximum dose status, mortality was grouped as either S, susceptible (adult mortality ranging from 96 to 100%) or R, resistant (adult mortality <90%), similar to grouping conditions used by Pusawang et al. (2022) in other species. Referring to the LC_{90} ratio status of *B. tabaci* to imidacloprid, an LC_{90} ratio was calculated as the LC_{90} divided by the maximum dose, respectively, for each population. LC_{90} ratio status was then grouped as either R, Resistant (>1) or S, Susceptible (<1).

Results

Eleven *B. tabaci* field populations were evaluated for imidacloprid efficacy (Table 1). Adult mortality at the recommended label rate (98 mg a.i. L^{-1}) averaged 83.35% in Georgia and only 54.3% in Marion Co., FL ($F_{2,171} = 31.97$; $R^2 = 28.29\%$;

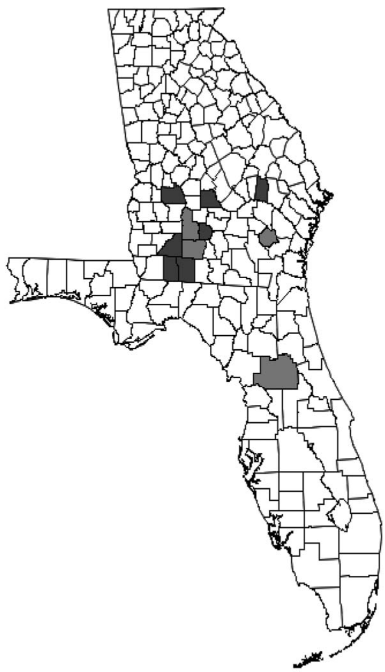

Fig. 1. Imidacloprid control at the maximum dose for *B. tabaci* populations in Georgia counties and Marion County, FL, excluding untreated control, 2021–2022.

$P < 0.0001$). Moreover, only two populations from the 12 *B. tabaci* populations (laboratory colony included) tested were considered susceptible (mortality >96%) to the label rate of imidacloprid ($F_{3,11} = 9.09$; $R^2 = 39.3\%$; $P < 0.0001$). The remaining nine field populations were resistant, as expressed by the lower mortality observed at the maximum dose (Table 2). The susceptibility statuses observed at the maximum dose were consistent with those obtained using the LC_{90} ratios (Table 2), which meant that response to imidacloprid was consistent at both evaluation concentrations. Nevertheless, 7 locations had sufficient management of whitefly populations with >80% adult mortality following imidacloprid bioassays (susceptible populations included), whereas the remaining four whitefly populations from other locations had approximately 40–80% adult mortality (Fig. 1).

The tested laboratory colony was less susceptible to imidacloprid than the sampled field populations. As such, a susceptible field population (Gra–C, Grady Co., GA) was used to compute the resistant ratios reported in Table 3. Except for Pierce and Marion counties, LC_{50} values reported were relatively low, with the susceptible Gra–C population having the lowest LC_{50} (0.02, fiducial limit = 0.0013–0.0404). Regarding state response, the Florida representative population, Marion Co., was at least 18-fold more resistant than the highest reported Georgia whitefly population (Pierce Co.).

Table 3. Imidacloprid response of field-collected whiteflies compared with reference resistant and susceptible populations of *B. tabaci* from this survey.

Population Name	LC_{50} (95% FL) (mg a.i. L^{-1})	Slope ($\pm$SE)	Probit Fit Line χ^2	Probit Fit Line df	Probit Fit Line *P*	RR_{max}*	RR_{min}**
Colq–BDO	42.36 (0.0011–4255)	0.22 ($\pm$0.07)	9.97	1	0.0016	0.01	2,118
Gra–C	0.02 (0.0013–0.0404)	0.36 ($\pm$0.04)	74.62	1	<0.0001	0.00001	—
Mit–C	41.54 (—)	0.27 ($\pm$0.15)	3.45	1	0.0632	0.01	2,077
Prc–B	196.05 (—)	0.64 ($\pm$0.25)	6.63	1	0.0100	0.05	9,802.5
Sum–P	16.86 (0.0001–212)	0.52 ($\pm$0.17)	9.38	1	0.0022	0.004	843
Tom–P	0.03 (—)	0.17 ($\pm$0.10)	2.92	1	0.0875	0.000001	1.5
Tif–TT	21.44 (0.006–225)	0.48 ($\pm$0.14)	11.89	1	0.0006	0.005	1072
Tmb–R	18.56 (—)	0.40 ($\pm$0.14)	8.35	1	0.0039	0.004	928
Wil–O	4.06 (—)	1.12 ($\pm$0.46)	6.60	1	0.0102	0.001	203
Wot–PS	2.13 (0.005–38.8)	0.24 ($\pm$0.05)	16.75	1	<0.0001	0.001	106.5
Mar–C	3,643 (—)	0.38 ($\pm$0.15)	6.75	1	0.0094	0.87	182,150
LAB–col	4,181 (—)	0.34 ($\pm$0.20)	3.08	1	0.0793	—	209,050

* Resistant ratio, calculated as (LC_{50} of field population)/(LC_{50} of Lab–SC–maximum concentration for LC_{50} or resistant).

** Resistant ratio, calculated as (LC_{50} of field population)/(LC_{50} of Gra–C–minimum concentration for LC_{50} or susceptible). Values in parentheses indicate 95% fiducial limit (FL).

Discussion

Imidacloprid is a widely used insecticide that has been in circulation globally for nearly 3 decades (De Marchi et al. 2021, Horowitz et al. 2011, Mullins 1993). Given the novelty of its mode of action following its registration by the U.S. Environmental Protection Agency, its repetitive use on many agronomic crops is unsurprising. Even now, imidacloprid is still being used for seed treatments of crops due to its systemic nature (Horowitz et al. 2011). The overuse of imidacloprid likely gave rise to the current global resistant status to the insecticide in species such as *B. tabaci* (De Barro et al. 2011, Castle and Prabhaker 2013, Ahmad and Khan 2017, Basit 2019). Not only is *B. tabaci* prolific but also its adaptive survivability allows it to rapidly select for resistant populations (Perier et al. 2022). As such, control failures with the insecticide in the species are frequent (Cremonez et al. 2023, Dangelo et al. 2018, Horowitz et al. 2020, Prabhaker et al. 1997).

In neighboring states, such as Florida, a susceptibility to imidacloprid profile for *B. tabaci* already exists and has since been used to monitor changes in response in Georgia populations (De Marchi et al. 2021, Caballero et al. 2013a, Smith and Nagle 2014, Smith et al. 2016). However, no such profile has been established for Georgia. Instead, comparisons are often made using the data available from Florida or Florida *B. tabaci* populations (McKenzie et al. 2020, Sparks et al. 2020). Similar to these reports, our study added a Florida *B. tabaci* population to reference the difference in susceptibility between the two states. The results highlighted a significant difference between the highest reported LC_{50} in Georgia and that of Marion Co. in Florida. This difference in imidacloprid response between the Florida and Georgia *B. tabaci* populations highlighted the need for the creation of a Georgia imidacloprid profile. Future evaluations of the insecticide would benefit from comparisons with a Georgia baseline for greater accuracy on the region's *B. tabaci* resistance development. Nevertheless, the extensive work on insecticide resistance management (IRM) programs in Florida would still be beneficial to IRM efforts in Georgia, after some tailoring, given the pockets of susceptibility to imidacloprid in the state.

In this study, a systemic insecticide treatment procedure was used during efficacy and dose–response evaluations due to the potential of the methodology to mimic residual treatment from seed treatments. Similarly, reports have compared the efficacy of imidacloprid from seed treatments and root drenches, but stated that higher amounts of imidacloprid were retained in plant tissue from seed treatments due to contact with larger amounts of the insecticide (Tang et al. 2020). Regardless, a recent report has indicated that imidacloprid uptake and retention are linear and can be correlated with a root drench from a series of concentrations (Perier et al. 2023). In this instance, the exact exposure amount was quantifiable and applied using the same methodology as this study. As such, the rapid survey of imidacloprid presented herein would accurately depict field response. This is mainly because mortality can be determined based on the applied rate of imidacloprid, with some potential environmental and plant metabolism loss accounted for. These possible avenues for losing some amounts of insecticide are not considered in contact applications. If considering the photodegradation of the insecticide, contact applications may only be viable for 43 min (Wamhoff and Schneider 1999) before environmental loss. By contrast, a systemic approach allows for the metabolic alteration of imidacloprid to a new chemical (such as olefin) that can still be detected in the plant (Perier et al. 2023) and, in some cases, may be more toxic to insect pests than the originally applied insecticide.

Our survey confirmed susceptibility to imidacloprid in two *B. tabaci* populations in two separate locations in Georgia. However, average whitefly mortality for Georgia indicates a high resistance level in field populations to imidacloprid. Several locations still had relatively high control and could trend toward future susceptibility with proper management. For the few susceptible locations, imidacloprid might be the least expensive management option for *B. tabaci* populations, but annual monitoring with at least the maximum dose bioassay is recommended. Still, all of these areas would benefit from an annually managed resistance management program to prevent selection for resistance. Although it is unknown what led to these instances of imidacloprid susceptibility, the seasonal rotation of imidacloprid applications could have helped to maintain susceptibility. Historically, insecticide rotations can maintain susceptibility and reduce resistance to an insecticide, but require annual evaluation to be sure that this standard practice is working. In the meantime, finding new insecticides for improving efficacy against whiteflies in farmscapes is needed.

In conclusion, the use of imidacloprid in the state of Georgia requires extensive resistance management, as whiteflies in eight counties were found to be resistant to the insecticide. Populations in the two remaining Georgia counties evaluated were susceptible, with high mortality following imidacloprid treatments. Overall, MEAM1 *B. tabaci* populations in Georgia are resistant to imidacloprid, but less so than the evaluated Florida population. Comparisons of efficacy would be helpful if made using susceptible reference material from Georgia populations going forward for better accuracy.

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