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Bioassay to Evaluate Resistance Levels in Tarnished Plant Bug (Hemiptera: Miridae) Populations in Alabama to Common Cotton Insecticides

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ABSTRACT

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), has emerged as the major insect pest of cotton in the mid-southern United States following the eradication of the boll weevil and the introduction of genetically modified Bt cotton for caterpillar pests. The objective of this study is to evaluate tarnished plant bug resistance to the five most common insecticides used for control across six distinct growing regions. Glass-vial bioassays were used to evaluate resistance of field populations in a laboratory setting. Elevated levels of resistance of tarnished plant bug to bifenthrin and, to a lesser degree, imidacloprid have been reported in various regions of Alabama when compared to a susceptible lab population. Bifenthrin resistance was found to be high (12 – 13.4-fold resistance levels) in three of the six counties tested in 2023. Populations showed the lowest resistance levels to dicrotophos in 2023 and 2024.There is a limited number of chemical classes available for tarnished plant bug control , therefore further resistance monitoring is necessary to inform management strategies and to slow the development of resistance. This will contribute to the overall goal of establishing and maintaining the most cost efficient and efficacious control programs for tarnished plant bug in Alabama

Keywords: Alabama, bioassay, insecticide, Lygus, resistance, Tarnished Plant Bug

INTRODUCTION

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is consistently the most damaging single pest of cotton in the mid-southern United States (Musser et al. 2009). Tarnished plant bug (TPB) is a highly polyphagous insect with over 700 plant hosts (Esquivel & Mowery 2007). In the 2022 growing season in Alabama, TPB caused $16.6 M in damage, over $6 M more than the next most damaging pest. Of the 426,458 acres of cotton planted in Alabama, 100% of the acreage was infested and 95% of the acres were treated an average of 2.4 times for TPB (Cook et al. 2022). This pest causes direct damage and yield loss in cotton through feeding and subsequent abscission of pinhead squares (flower buds) as well as feeding on larger squares and small bolls (Cleveland & Smith 1968). TPB feed by piercing the developing buds and injecting saliva that breaks down plant tissue so it can be ingested (Layton 2000). Yield losses can be directly attributed to the loss of first position pinhead squares and the introduction of pathogens into fruiting structures. These pathogens cause further damage to seed and lint in bolls that are not abscised (George et al. 2021).

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Furthermore, TPB feeding on medium or larger squares results in flowers that are damaged; the resulting injury, which is referred to as "dirty blooms," manifests in the reproductive parts of the cotton flower and results in misshapen bolls due to incomplete fertilization. Such misshapen bolls are described as "hawk-billed" (Layton 2000).

Prior to the introduction of *Bt* (*Bacillus thuringiensis*) transgenic cotton and the completion of boll weevil eradication in Alabama, TPB were not major pests of cotton (George et al. 2021). With the decrease in broad-spectrum insecticide sprays associated with boll weevil eradication and the genetic control of Lepidopteran pests with *Bt cotton* , TPB emerged as an opportunistic economic pest (Musser et al. 2007). Resistance of TPB populations to insecticides such as bifenthrin and acephate has been well documented over the last few decades in the mid-southern U.S. following these changes (Snodgrass 1996; Hollingsworth et al. 1997; Snodgrass & Scott 2000; Snodgrass & Scott 2003; Snodgrass et al. 2009; Parys et al. 2018; Dorman et al. 2020; Catchot et al. 2022).

Snodgrass (1996) first identified pyrethroid resistance in TPB and later found that resistance to the organophosphate acephate could confer cross-resistance to additional organophosphates (Snodgrass et al. 2009). Non-chemical strategies such as varietal selection, planting date, and field border management provide limited control but chemical management is still needed for adequate control of TPB (Adams et al. 2013). Given the growing problem with insecticide resistance and cross-resistance in TPB, resistance monitoring is an important tool in TPB pest management. This information helps to highlight the importance of rotating insecticide chemistries and modes of action both during the season and from year to year to reduce the selective pressure applied to these insect pest populations. The objective of this study is to evaluate TPB resistance to the five most common insecticides used for control across six distinct cotton producing regions in Alabama. We hypothesize that geographically distinct populations will have region-specific resistance with changes in resistance from region to region.

METHODS AND MATERIALS

TPB populations were collected in six distinct cotton-producing regions of Alabama: southwest (Monroe County), southeast (Henry County), central west (Dallas County), central east (Macon County), northwest (Limestone County), and northeast (Cherokee County) (Fig. 1). These populations were obtained from uncultivated hosts daisy fleabane (*Erigeron annuus*) and crimson clover (*Trifolium incarnatum*) between May-June 2023 and May-June 2024 . These collections were made prior to the growing season to get a baseline resistance in the population prior to the introduction of insecticides. Insects were collected using a sweep net (38 X 38 cm) and aspirator (Carolina Biologicals; Burlington, NC). Each collection was placed in a plastic container (Plastic Screw-Top Canister (1 Gallon), Mainstays; Bentonville, AR) with shredded copy paper as a substrate to increase the surface area available inside the container. TPB populations remained in the lab for 24 hours after collection to acclimate the insects to the laboratory environment following field collection and remove any dead or moribund insects. No less than 100 individuals were tested for each location (N = 3517).

A laboratory colony of adult TPB was obtained from Mississippi State University (Mississippi State, MS, USA) for use as a susceptible baseline for calculating resistance ratios. This colony has been maintained for >10 years following details outlined by (Cohen 2000; Musser et al. 2012). The insects were reared under controlled conditions (27°C and 16:8 (L:D) h).

Bioassays were performed using the glass vial method described by Snodgrass et al. (1996, 2009) and can indicate technical resistance in a laboratory setting. Scintillation vials (20-mL, VWR Scientific; Radnor, PA) were submerged in a 10% Clorox solution for at least two days prior to testing, triple rinsed with tap water and heated until dry on a hot dog roller (Great Northern Commercial 1650-Watts 30-Hot Dog 11-Roller Grilling Machine; Lorain, OH).

The technical grade insecticides bifenthrin, acephate and dicrotophos (VWR Scientific; Radnor, PA) were prepared using a coated vial technique as described by Snodgrass (1996).

Technical grade thiamethoxam and imidacloprid were prepared using a floral foam method as described by Teague and Tugwell (1996). These methods were chosen according to the mode of action of the respective insecticides. Bifenthrin, acephate and dicrotophos are contact insecticides, whereas thiamethoxam and imidacloprid are most effective when ingested (Snodgrass *et al.* 2008). Each insecticide was prepared using a serial dilution of a stock solution of insecticide/ acetone in concentrations of 0.1, 0.316, 1.0, 3.16, 10.0, and 31.6 ug/vial for the coated vial assays. Coated vials of pure acetone were used as a negative control. The same concentrations of a 10 % honey water and insecticide solution were used for floral foam treated vials. A 10 % honey water solution was used as a negative control in these assays.

Vial preparation was conducted on the day of testing For the coated vials, 0.25 mL of solution was pipetted into each vial and the vials were rolled until dry under a fume hood on the unheated hot dog roller. The drying process allowed the acetone to evaporate, and the insecticide was left as a residue on the inner surface of the vial (Snodgrass 1996). When dry, a 1.3 cm piece of green bean was placed inside each vial as a food source. Green beans (*Phaseolus vulgaris*), were soaked in a 10 % Clorox solution for five minutes for surface sterilization, then rinsed with tap water for five minutes and allowed to dry prior to being cut into pieces and placed in vials. Two adult TPB were used per vial, with a minimum of nine replicates for each concentration per insecticide per location. Each vial was closed with a cotton ball to prevent insect escape. Vials were kept at room temperature (approximately 20°C) and mortality assessed and recorded after 24 hours. For each floral foam vial, a disk of wettable floral foam (Oasis Floral Products; Kent, OH) measuring approximately 12 mm X 12 mm was obtained using a coring device (Freeshu; Leizhou, Guangdong China). One disk was placed in each vial; 0.5 mL of solution was pipetted onto each floral foam disk. One tarnished plant bug was added per vial and each vial was closed with a cotton ball; a minimum of 18 replicates for each concentration per insecticide per location were used. Adult TPB were considered dead if they could not right themselves from a supine position in five seconds or did not move when gently prodded.

Data were analyzed using Polo probit software (LeOra Software LLC, Berkshire, UK). Resistance ratios (RR50) were calculated by dividing the LC50 of the field populations to the LC50 of the laboratory colony. For this study, populations with a resistance ratio less than three were considered as having low resistance levels, between three and 10 medium resistance levels, and more than 10 high resistance levels (Dorman et al. 2020).

RESULTS

Resistance levels to acephate was low (0.00–2.6-fold) in all regions sampled for 2023 and 2024 (Table 1). Resistance levels to bifenthrin was high in three of the six regions tested in 2023 (Table 2): Monroe Co.(12.0-fold), Macon Co. (13.4-fold), and Limestone Co. (12.8-fold). Only the Cherokee Co. population showed resistance to dicrotophos in either year (Table 3), with a resistance ratio of 1.9-fold. All regions had at least medium resistance to imidacloprid (3.2–5.9-fold) except Cherokee Co., which showed high resistance (15.45-fold) in 2023 (Table 4). In 2024 all regions showed low to medium resistance levels to imidacloprid (1.5–4.9-fold) (Table 4). All regions tested had low to medium resistance levels to thiamethoxam (1.7–4.9-fold) except for Dallas and Cherokee counties in 2024 (Table 5). No region had high resistance levels to more than one insecticide tested. Populations had the most resistance to bifenthrin across the state of Alabama.

DISCUSSION

These data add to TPB resistance data that has been made available over the last several decades. There has not been a recent comprehensive evaluation of resistance in Alabama TPB populations, however Dorman et al. (2020) collected and tested Alabama populations in the northeast, northwest, and central east for resistance to acephate, bifenthrin, and thiamethoxam in 2018 and 2019. There were similarities when compared to our results with acephate, but the two studies differed significantly concerning bifenthrin.

Dorman et al. (2020) reported low to medium resistance to bifenthrin in the central east (0.4–3.8) whereas our results found a RR50 of 13.4 (high) in 2023 (Table 2). High chi-squared values (ten or more) suggest that there is a large amount of variability in the data that could be rectified by a larger data set. However, the variability in these data is common for laboratory bioassays, which emphasizes the need to make thorough field observations for the best control recommendations. A lack of overlapping confidence intervals between the "high" resistance level populations and the susceptible strain is a good indication of true differences.

Tarnished plant bug age has been documented as a significant factor when assessing mortality. Adults over 10 days old are reported to have significantly higher mortality than those 10 days or younger (Snodgrass 1996). While laboratory colony ages could be controlled, field populations were of unknown and likely variable age ranges. Therefore, the potential age differences may have skewed the results of field collected bioassays in either direction. If many older individuals were collected, that population would be significantly more susceptible to a given insecticide and the opposite may be true for younger individuals.

The prevalence of resistance to bifenthrin and, to a lesser extent, imidacloprid could be correlated with the intensity of their respective usage. Since its registration in cotton in 1978, bifenthrin has been used extensively in TPB and other cotton insect management programs.

Resistance to bifenthrin and subsequent cross-resistance to other pyrethroids has been documented in the Mississippi Delta since the mid-1990's. Resistance to most classes of available insecticides has also been reported in the region (George et al. 2021). Furthermore, that resistance has been shown to increase in populations over the growing season (Dorman et al. 2020). These data suggest that continued monitoring of TPB insecticide resistance is important to determine recommended control measures as different regions of a state, as demonstrated here and in other trials, can have varying levels of resistance to respective insecticides. The disparate nature of these growing regions can help to decrease the rate of resistance formation due to a relative lack of gene flow when compared to large, dense regions of production. To inform best practices moving forward, understanding insecticide use rates for the different growing regions of Alabama would be beneficial. With these data, localized integrated resistance management plans could be implemented in these disparate areas.

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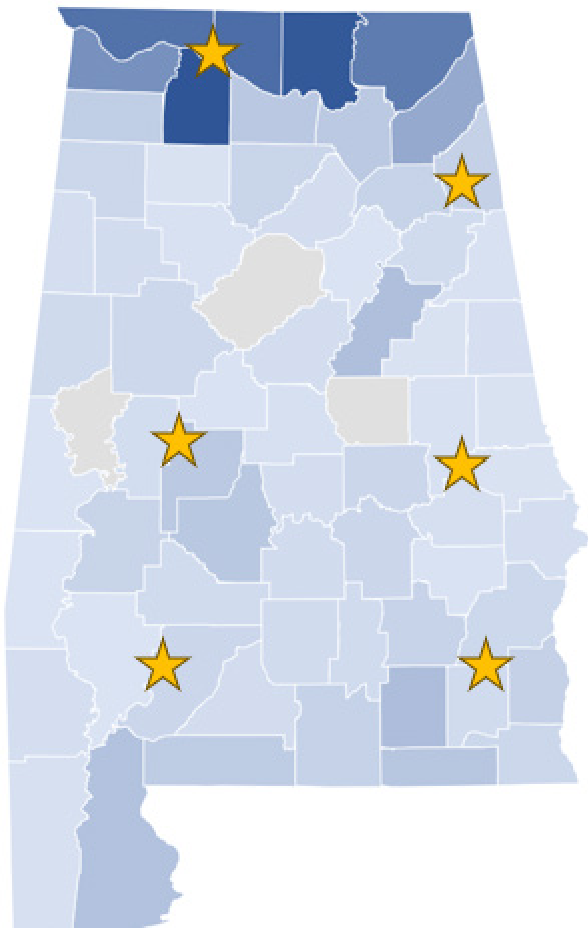

Figure 1. Map of Alabama. The darker regions indicate higher cotton acreage. The stars represent the six areas sampled for tarnished plant bug populations.

Table 1. Log-probit bioassays on adult tarnished plant bug to technical grade acephate. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance

Location	*LC 50*[a]	X^2	*N=*[b]	*CI*	*RR 50*[c]
2023 Monroe Co., AL	0.818	26.83	112	-*	-
2023 Henry Co., AL	8.670	0.468	126	6.379 - 11.952	2.108
2023 Dallas Co., AL	10.70	3.888	126	-*	2.602
2023 Macon Co., AL	7.893	0.468	126	-*	1.920
2023 Limestone Co., AL	4.825	4.259	116	2.405 – 10.360	1.173
2023 Cherokee Co., AL	9.976	2.486	128	3.680 - 20.310	2.426
2024 Monroe Co., AL	1.012	3.36	126	0.472 - 1.929	-
2024 Henry Co., AL	1.470	0.95	126	0.950 - 2.250	-
2024 Dallas Co., AL	8.153	3.75	126	5.021 - 14.727	1.983
2024 Macon Co., AL	1.240	6.39	126	0.345 - 3.914	-
2024 Limestone Co., AL	5.071	2.37	126	1.110 - 8.293	1.233
2024 Cherokee Co., AL	7.41	1.083	126	4.723 - 12.504	1.802
2023 Laboratory	4.112	19.655	140	-*	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR50 calculated using the LC50 values of a susceptible laboratory colony from Mississippi State University
*Confidence interval could not be obtained due to increased variability in the data set

Table 2. Log-probit bioassays on adult tarnished plant bug to technical grade bifenthrin. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*LC 50*[a]	X^2	*N=*[b]	*CI*	*RR 50*[c]
2023 Monroe Co., AL	2.648	4.109	112	1.089 - 6.446	11.982
2023 Henry Co., AL	1.740	43.558	126	-*	7.873
2023 Dallas Co., AL	0.450	5.689	126	-*	2.036
2023 Macon Co., AL	2.962	9.502	126	0.577 - 9.874	13.403
2023 Limestone Co., AL	2.821	5.764	126	0.629 – 17.993	12.765
2023 Cherokee Co., AL	0.128	4.640	126	0.001 - 0.764	-
2024 Monroe Co., AL	0.897	3.34	126	0.462 - 1.492	4.059
2024 Henry Co., AL	0.226	5.68	126	0.020 - 0.871	1.023
2024 Dallas Co., AL	0.008	1.217	126	0.000 - 0.058	-
2024 Macon Co., AL	0.808	5.98	126	0.181 - 2.692	3.656
2024 Limestone Co., AL	0.639	3.62	126	0.265 - 1.315	2.891
2024 Cherokee Co., AL	0.600	2.59	126	0.172 - 1.084	2.715
2023 Laboratory	0.221	7.833	140	0.053 - 0.498	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR50 calculated using the LC50 values of a susceptible laboratory colony from Mississippi State University
*Confidence interval could not be obtained due to increased variability in the data set

Table 3. Log-probit bioassays on adult tarnished plant bug to technical grade dicrotophos. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance

Location	*LC 50*[a]	X^2	*N=*[b]	*CI*	*RR 50*[c]
2023 Monroe Co., AL	0.629	20.737	112	-*	-
2023 Henry Co., AL	1.508	0.792	126	0.847 - 2.170	-
2023 Dallas Co., AL	0.450	5.689	126	-*	-
2023 Macon Co., AL	0.807	0.943	126	0.577 - 1.130	-
2023 Limestone Co., AL	1.18	0.965	126	0.848 – 1.670	-
2023 Cherokee Co., AL	3.294	3.383	126	2.197 - 4.986	1.902
2024 Monroe Co., AL	0.427	6.57	126	0.065 - 1.222	-
2024 Henry Co., AL	0.411	12.95	126	0.040 - 1.724	-
2024 Dallas Co., AL	0.154	13.17	126	0.006 - 0.735	-
2024 Macon Co., AL	0.429	1.93	126	0.216 - 0.650	-
2024 Limestone Co., AL	0.471	1.59	126	0.202 - 0.743	-
2024 Cherokee Co., AL	0.575	7.66	126	0.074 - 1.871	-
Laboratory	1.732	1.532	140	1.235 - 2.452	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR50 calculated using the LC50 values of a susceptible laboratory colony from Mississippi State University
*Confidence interval could not be obtained due to increased variability in the data set

Table 4. Log-probit bioassays on adult tarnished plant bug to technical grade imidacloprid. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*LC 50*[a]	X^2	*N=*[b]	*CI*	*RR 50*[c]
2023 Monroe Co., AL	2.371	1.718	126	0.434 - 5.570	5.854
2023 Henry Co., AL	2.135	3.72	126	1.119 - 3.870	5.272
2023 Dallas Co., AL	2.030	1.49	126	0.604 - 4.200	5.012
2023 Macon Co., AL	1.290	6.50	126	0.153 - 3.652	3.185
2023 Limestone Co., AL	1.280	6.70	126	0.297 – 3.790	3.160
2023 Cherokee Co., AL	6.258	3.596	126	1.848 - 52.675	15.452
2024 Monroe Co., AL	2.015	2.37	126	0.750 - 5.875	4.975
2024 Henry Co., AL	0.627	5.09	126	0.090 - 2.468	1.548
2024 Dallas Co., AL	0.908	3.809	126	0.464 - 1.524	2.242
2024 Macon Co., AL	0.975	4.14	126	0.179 - 3.998	2.407
2024 Limestone Co., AL	0.701	7.36	126	0.055 - 3.853	1.730
2024 Cherokee Co., AL	1.332	0.74	126	0.574 - 2.838	3.289
Laboratory	0.405	4.944	126	0.076 - 1.206	-

[a] Units represented as ug/vial^{-1}

[b] Total number of tarnished plant bug adults

[c] RR50 calculated using the LC50 values of a susceptible laboratory colony from Mississippi State University

Table 5. Log-probit bioassays on adult tarnished plant bug to technical grade thiamethoxam. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*LC 50*[a]	X^2	*N=*[b]	*CI*	*RR 50*[c]
2023 Monroe Co., AL	1.218	7.894	126	0.213 - 4.622	3.904
2023 Henry Co., AL	0.803	3.55	126	0.334 - 1.638	2.574
2023 Dallas Co., AL	0.544	2.56	126	0.163 - 0.970	1.744
2023 Macon Co., AL	0.812	0.869	126	0.264 - 1.551	2.603
2023 Limestone Co., AL	1.298	2.95	126	0.565 - 2.586	4.160
2023 Cherokee Co., AL	1.525	1.837	126	0.163 - 4.396	4.888
2024 Monroe Co., AL	0.723	8.23	126	0.087 - 2.563	2.317
2024 Henry Co., AL	0.945	4.23	126	0.289 - 2.350	3.029
2024 Dallas Co., AL	0.191	0.43	126	0.069 - 0.399	-
2024 Macon Co., AL	0.996	6.26	126	0.191 - 3.312	3.192
2024 Limestone Co., AL	0.730	2.74	126	0.250 - 1.782	2.330
2024 Cherokee Co., AL	0.171	1.34	126	0.058 - 0.371	-
Laboratory	0.312	4.52	126	0.982 - 1.666	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR50 calculated using the LC50 values of a susceptible laboratory colony from Mississippi State University