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ARTHROPOD MANAGEMENT

Insecticide Resistance Monitoring of Tarnished Plant Bug (Hemiptera: Miridae) Populations in the Mid-Southern United States

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ABSTRACT

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is the target for multiple insecticide applications in cotton in the mid-southern U.S. Resistance to several insecticide classes has been documented, so monitoring of resistance levels to insecticides currently used is needed before field control failures occur. Several populations were tested to estimate resistance levels for commonly used insecticides during 2017 to 2019. On average, 25 to 40% of populations were determined to be resistant to thiamethoxam, imidacloprid, and/or sulfoxaflor. There were no differences among years in mean imidacloprid and thiamethoxam LC_{50} values; however, the mean LC_{50} increased from 2017 to 2019 for sulfoxaflor. No differences in resistance were detected between the two primary agricultural regions of the Mid-South (Hills and Mississippi River Delta) for any of the insecticides. For each insecticide, the range between the most and least susceptible populations was greatest in the Hills region during 2018. Susceptibility to thiamethoxam was the most variable followed by imidacloprid and sulfoxaflor. It is important to continue monitoring for resistance because continued selection pressure is likely to lead to widespread reduced efficacy in the future.

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a major pest of cotton in the mid-southern U.S. (Cook and Threet, 2019). Management efforts for this pest is essential in most fields every year due to fruit injury and yield losses. Studies show that planting early, choosing short-season varieties, and keeping the field edges clean reduces crop damage from *L. lineolaris* (Adams et al., 2013), yet management for this pest often requires multiple applications of insecticides (Cook and Threet, 2019). For control of *L. lineolaris*, neonicotinoid (imidacloprid and thiamethoxam), sulfoximine (sulfoxaflor), and/or insect growth regulator (novaluron) insecticides generally are applied first followed by organophosphate insecticides or mixtures including organophosphate insecticides later in the season (Catchot et al., 2019). After years of multiple insecticide applications, the presence of insecticide-resistant populations of *L. lineolaris* is to be expected (Helps et al., 2020; Roush, 1993). Documenting changes in insecticide susceptibility is needed to minimize crop losses due to resistant *L. lineolaris*. Resistance is measured as a change in susceptibility, so it is critical to establish a baseline of susceptibility.

Pyrethroids were readily adopted by cotton producers in the early 1980s because of their high efficacy and broad spectrum of control. After 14 years of pyrethroid use on cotton, *L. lineolaris* populations exhibited resistance in multiple locations in Mississippi (Snodgrass, 1996b). More recent studies have reported resistant populations of *L. lineolaris* to pyrethroids, as well as some carbamates and organophosphates (Hollingsworth et al., 1997; Pankey et al., 1996, 2017; Snodgrass and Scott, 2000; Snodgrass et al., 2009). The addition of neonicotinoid insecticides in the late 1990s helped minimize and delay insecticide resistance, but scattered reports of *Lygus* with resistance to imidacloprid or thiamethoxam are now present (Dorman et al., 2020; Parys et al., 2017; Zhu and Luttrell, 2015). Sulfoxaflor was introduced in 2012, and a baseline for susceptibility of mid-southern U.S. populations of *L. lineolaris* to sulfoxaflor was established during 2015 (Parys et al., 2017).

Here, the authors provide new resistance monitoring results for *L. lineolaris* for 63 populations collected during 2017 to 2019 from two well-

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defined agricultural regions of the mid-southern U.S.: Delta and Hills (Fig. 1). The Delta has a high percentage of cultivated area with large fields in close proximity, whereas the Hills region has a lower percentage of cultivated area, with smaller, more isolated fields (NASS, 2017). Both regions grow cotton and have *L. lineolaris*, but *L. lineolaris* pressure and insecticide application frequency tends to be greater in the Delta region (Cook and Threet, 2019; Fleming et al., 2015). Populations of *L. lineolaris* from both regions were collected during 2017 to 2019 and assayed with thiamethoxam, imidacloprid, and sulfoxaflor to compare the level of susceptibility of these populations with a laboratory colony. These data, combined with previously published data (Parys et al., 2017), will be useful in documenting changes in susceptibility to these insecticides of *L. lineolaris* populations in the future.

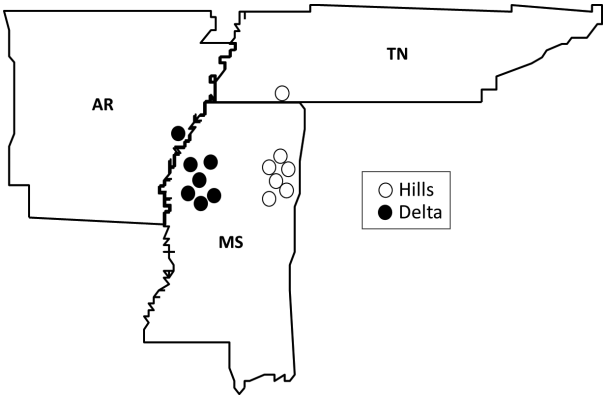

Figure 1. Location of counties and region label (Hills or Delta) where *L. lineolaris* collections were made during 2017-2019. Multiple collections were made within each county except in AR.

MATERIALS AND METHODS

Laboratory Colony. A colony of *L. lineolaris* established in 2005 at Mississippi State University was used in the assays described below. This colony was collected from uncultivated hosts in several agricultural regions of Mississippi and has been periodically supplemented with field-collected *L. lineolaris* since establishment. The colony was reared in 40-cm x 25-cm x 13-cm plastic containers as described by Musser et al. (2012) and maintained at 27 °C, 70% relative humidity with a 14:10 (L:D) cycle. The colony was fed a semi-solid oligidic diet (Cohen, 2000) that also included 33.6 ppm fumagillin (Musser et al., 2012). Diet was presented in Parafilm® (Pechiney Plastic Packaging, Menasha, WI) packets and changed three times per week. Egg packets were made with a 4% carrageenan (Gelcarin GP 812, FMC, Rockland ME) solution in a Parafilm® packet, placed on the top of the rearing containers, and changed three times per week.

Field Collections. Adult *L. lineolaris* populations were collected from uncultivated flowering plants (primarily fleabanes, *Erigeron* spp.) in cotton production regions of Mississippi, Tennessee, and Arkansas using sweep nets May through August 2017 to 2019. Adults were aspirated into containers and fed fresh green beans or host plants to keep the insects alive until the bioassays were conducted. The containers were transported to Mississippi State University within 24 h of collection and bioassays were conducted within 48 h of collection.

Bioassays. All assays were conducted using 20-ml glass scintillation vials. Prior to use in assays, vials were submerged in a bleach-water solution of 250 ml 7.5% ai sodium hypochlorite /20 l water for 2 d, individually triple rinsed with tap water, placed upside down in a vial rack and baked at 150 °C for 3 h. After vials cooled to room temperature, they were rinsed with acetone and placed in a chemical fume hood until dry.

Assay methodology was based on insecticide mode of action. Susceptibility to analytical grade sulfoxaflor (99.5% pure, ChemService, West Chester, PA), primarily a contact insecticide (Parys et al., 2017), was assayed using a coated vial. A sulfoxaflor-acetone solution (250 µl), prepared in five concentrations by serial dilution ranging from 31.6 to 0.1 µg sulfoxaflor/vial or a control of pure acetone, was dispensed into each vial. The vial was then placed immediately on an unheated roller in a chemical fume hood and rolled until dry. Vials were treated within 24 h of beginning the assay. A surface-sterilized piece of fresh green bean was added to each vial as a food source. Two *L. lineolaris* adults were placed in each vial and the vial was capped with a cotton ball. Vials were kept at room temperature and mortality was assessed after 24 h (Parys et al., 2017).

Analytical grade imidacloprid (98.3% pure, ChemService) and thiamethoxam (99.5% pure, ChemService) are most active through ingestion, so these insecticides were tested using floral foam (Snodgrass et al., 2008a). Using a cork borer, a 12-mm diameter plug was removed from a block of "wet style" floral foam (Oasis Floral Products, Kent, OH) and cut into circular disks 12 mm thick.

One floral foam disk was placed inside each vial and 0.5 ml of a 10% honey-water solution containing one of five insecticide concentrations ranging from 31.6 to 0.1 µg/ml of solution or a control of 10% honey-water solution was pipetted carefully into the floral foam so that no droplets were outside the floral foam. A single *L. lineolaris* adult was placed in each vial and the vial was capped with a cotton ball. Vials were kept at room temperature and mortality was assessed after 24 h (Parys et al., 2017). Approximately 30 insects were tested at each concentration for all assays.

Data Analysis. Concentration-mortality data were analyzed using probit analysis (PROC PROBIT, SAS 9.4, SAS Institute; Cary, NC) to estimate an LC_{50} for each assay. Assays with a good fit to the probit model (χ^2 goodness of fit test with $p > 0.10$) were used to evaluate the impact of collection region, year, and source. Responses were subjected to analysis of variance (PROC GLIMMIX, SAS 9.4) to evaluate whether region (Delta or Hills) and year were significant factors for each insecticide. All the field assays were then jointly compared to the laboratory colony assays to analyze the impact of *L. lineolaris* source (field or lab) on the concentration-mortality relationship. For both analyses, the logit link function and the binomial distribution were modeled (Lappi and Luoranen, 2018; Martin et al., 2003) and degrees of freedom were calculated using the Kenward-Roger method; Fisher's Protected LSD test with $\alpha = 0.05$ was used to separate means. Means and standard errors of LC_{50}s were calculated for presentation purposes using PROC MEANS (SAS 9.4); however, the raw data were used to estimate the impact of region, year, and source.

Previous research groups routinely used field populations of *L. lineolaris* from Crossett, AR as the susceptible population (Parys et al., 2017, 2018; Snodgrass, 1996a, 1996b; Snodgrass and Scott, 2000; Snodgrass et al., 2008b, 2009), although Parys et al. (2017) expressed concern about the sustainability of this methodology. The Crossett area has pine and timber production but no row-crop production. Efforts to collect *L. lineolaris* from Crossett, AR for this study were unsuccessful. Furthermore, using a new field collection for a baseline is not ideal because the genetics could change over time and results tend to be more variable than results from a laboratory colony (Parys et al., 2017).

The authors created an unconventional baseline by taking the mean of the five lowest field-population assays for each insecticide as the baseline of a susceptible population. Resistance ratios were calculated by dividing the LC_{50} of each population by the mean LC_{50} of the five lowest field populations for each insecticide.

RESULTS

The proportion of usable assays (those having a good fit to the probit model and a significant response to concentration) to total assays was 53% during 2017, 68% during 2018, and 79% during 2019. Usable data over all three insecticides and three years totaled 63 assays on field populations and seven assays on the laboratory colony. LC_{50} estimates for field populations varied widely for imidacloprid (Table 1), sulfoxaflor (Table 2), and thiamethoxam (Table 3).

The concentration-mortality relationship did not vary for any compound tested with respect to region (imidacloprid $F = 0.05$, df = 1,15.62, $p = 0.82$; sulfoxaflor $F = 0.02$, df = 1,14.74, $p = 0.89$; thiamethoxam $F = 0.11$, df = 1,19.67, $p = 0.75$). For imidacloprid and thiamethoxam, the collection year was also not a factor (Table 4). However, year was a factor for sulfoxaflor assays, with 2018 and 2019 being higher than 2017 (Table 4).

Assays of all field-collected populations were compared to laboratory-colony assays for each insecticide. The dose-mortality relationship for imidacloprid was higher for the laboratory colony than the field collections, but the dose-mortality relationship was not different between laboratory and field collections for sulfoxaflor and thiamethoxam (Table 4). However, because there were only two to three assays conducted with the laboratory colony, statistical power for detecting these differences was limited.

Using the mean of the lowest five field-colony LC_{50} estimates as the baseline for susceptible populations, between 25 and 40% of the field populations tested for each insecticide had resistance ratios greater than 10. Yearly fluctuations were similar, ranging from 11 to 50%, except for sulfoxaflor during 2019, when 75% of the populations had resistance ratios greater than 10 (Table 4). Using this susceptible population baseline, all assays on the laboratory colony with all three insecticides resulted in resistance ratios >10 (Table 4), even though the laboratory population was not exposed to any insecticide in more than 10 years, which is longer than sulfoxaflor has been on the market (EPA, 2019).

Table 1. Individual 24-h floral foam bioassays of imidacloprid on *L. lineolaris*. All counties are in Mississippi. LC_{50} estimates and 95% fiducial limits are expressed in ppm of active ingredient. Green shaded lines indicate the assay was used to create the baseline for susceptibility

Year	Region	County	Goodness of Fit			Probit Slope			LC_{50}	
			X^2	df	P	Mean	SEM	P	Estimate	95% FL
2017	Delta	Humphreys	6.42	4	0.17	0.39	0.11	0.0005	2.63	0.46-9.20
2017	Delta	Sunflower	1.86	4	0.76	0.33	0.07	<.0001	0.99	0.25-2.45
2017	Delta	Tallahatchie	1.84	4	0.76	0.59	0.13	<.0001	0.65	0.13-1.42
2018	Delta	Leflore	3.05	4	0.55	0.22	0.05	<.0001	0.88	0.28-2.92
2018	Delta	Sunflower	6.97	4	0.14	0.56	0.08	<.0001	0.73	0.45-1.14
2018	Delta	Sunflower	4.3	4	0.37	1.45	0.41	0.0004	2.55	1.43-3.49
2018	Delta	Tallahatchie	1.19	4	0.88	0.69	0.13	<.0001	0.08	0.04-0.13
2017	Hills	Clay	3.32	3	0.34	0.52	0.12	<.0001	0.45	0.12-0.88
2017	Hills	Lowndes	2.89	3	0.41	0.48	0.09	<.0001	4.01	2.18-7.05
2017	Hills	Noxubee	1.48	3	0.69	0.42	0.10	<.0001	9.27	4.52-20.92
2017	Hills	Oktibbeha	2.64	3	0.45	0.47	0.10	<.0001	0.49	0.16-0.93
2018	Hills	Clay	3.73	4	0.44	0.38	0.08	<.0001	1.15	0.21-3.02
2018	Hills	Lowndes	4.45	4	0.35	0.46	0.09	<.0001	0.38	0.12-0.75
2018	Hills	Monroe	3.39	4	0.49	0.34	0.06	<.0001	1.22	0.57-2.33
2018	Hills	Noxubee	6.73	4	0.15	0.21	0.06	0.0005	0.03	0.00-0.15
2018	Hills	Oktibbeha	3.36	4	0.50	1.03	0.28	0.0002	0.92	0.38-1.38
2019	Hills	Lowndes	2.01	4	0.73	0.54	0.12	<.0001	0.28	0.07-0.58
2019	Hills	Noxubee	1.57	4	0.81	0.49	0.09	<.0001	0.30	0.09-0.66
2019	Hills	Oktibbeha	2.67	4	0.61	0.84	0.22	0.0001	2.31	1.11-3.56
2019	Hills	Winston	4.32	2	0.12	0.62	0.16	0.0001	0.24	0.06-0.49
2019	Lab		6.75	4	0.15	0.34	0.06	<.0001	3.41	1.54-10.04
2019	Lab		5.85	4	0.21	0.39	0.07	<.0001	3.34	1.69-6.52

Table 2. Individual 24-h rolled vial bioassays of sulfoxaflor on *L. lineolaris*. All counties are in Mississippi except where the state abbreviation is included. LC_{50} estimates and 95% fiducial limits are expressed in ppm of active ingredient. Green shaded lines indicate the assay was used to create the baseline for susceptibility

Year	Region	County	Goodness of Fit			Probit Slope			LC_{50}	
			X^2	df	P	Mean	SEM	P	Estimate	95% FL
2017	Delta	Humphreys	0.64	3	0.89	0.50	0.09	<.0001	5.38	3.01-9.43
2017	Delta	Leflore	5.24	3	0.16	0.27	0.08	0.0009	0.57	0.01-2.75
2017	Delta	Sunflower	2.10	3	0.55	0.83	0.21	<.0001	11.2	5.66-18.9
2017	Delta	Tallahatchie	4.95	3	0.18	0.61	0.13	<.0001	0.86	0.35-1.52
2018	Delta	Leflore	5.67	3	0.13	0.62	0.16	<.0001	3.82	1.40-7.01
2018	Delta	Tallahatchie	0.31	3	0.96	0.31	0.09	0.0003	15.4	6.14-77.8
2018	Delta	Washington	4.80	3	0.19	0.36	0.12	0.0038	17.1	4.21-58.6
2018	Delta	Washington	4.32	3	0.23	0.49	0.08	<.0001	1.08	0.53-1.83
2018	Delta	Washington	0.45	3	0.93	0.47	0.08	<.0001	1.49	0.74-2.53
2019	Delta	Lee, AR	6.09	3	0.11	0.55	0.21	0.0109	21.3	7.07-54.9
2019	Delta	Leflore	3.43	3	0.33	1.04	0.24	<.0001	26.3	18.7-46.4
2017	Hills	Clay	2.50	3	0.48	0.57	0.10	<.0001	1.17	0.60-1.95

Table 2. continued

Year	Region	County	Goodness of Fit			Probit Slope			LC_{50}	
			X^2	df	P	Mean	SEM	P	Estimate	95% FL
2017	Hills	Lowndes	3.50	3	0.32	0.67	0.19	0.0004	0.63	0.06-1.69
2017	Hills	Noxubee	4.47	3	0.22	0.68	0.20	0.0008	1.80	0.28-3.74
2017	Hills	Oktibbeha	1.33	3	0.72	0.70	0.14	<.0001	1.13	0.35-2.20
2018	Hills	Lowndes	4.64	3	0.20	0.46	0.08	<.0001	11.8	5.95-28.3
2018	Hills	Noxubee	1.17	3	0.76	0.58	0.20	0.0029	33.8	17.5-182
2018	Hills	Oktibbeha	4.52	3	0.21	0.47	0.15	0.0022	5.37	0.71-13.1
2019	Hills	Lowndes	4.31	3	0.23	0.45	0.14	0.0013	3.08	0.29-8.96
2019	Hills	Noxubee	3.26	3	0.35	0.29	0.08	0.0002	13.1	3.42-68.5
2019	Lab		3.70	4	0.45	0.42	0.07	<.0001	9.42	4.12-18.9
2019	Lab		3.63	4	0.46	0.69	0.14	<.0001	35.3	21.9-59.1

Table 3. Individual 24-h floral foam bioassays of thiamethoxam on *L. lineolaris*. All counties are in Mississippi except where the state abbreviation is included. LC_{50} estimates and 95% fiducial limits are expressed in ppm of active ingredient. Green shaded lines indicate the assay was used to create the baseline for susceptibility

Year	Region	County	Goodness of Fit			Probit Slope			LC_{50}	
			X^2	df	P	Mean	SEM	P	Estimate	95% FL
2017	Delta	Bolivar	3.16	4	0.53	0.20	0.06	0.0016	6.00	0.29-42.5
2017	Delta	Humphreys	1.52	4	0.82	0.29	0.06	<.0001	13.1	5.60-51.6
2017	Delta	Sunflower	2.66	4	0.62	0.44	0.09	<.0001	0.09	0.01-0.24
2017	Delta	Tallahatchie	1.71	4	0.79	0.52	0.11	<.0001	0.19	0.02-0.62
2018	Delta	Sunflower	1.67	4	0.80	0.46	0.09	<.0001	0.12	0.04-0.27
2018	Delta	Tallahatchie	3.05	4	0.55	0.52	0.13	<.0001	0.23	0.03-0.57
2018	Delta	Washington	7.14	4	0.13	0.47	0.12	0.0001	1.37	0.20-3.00
2018	Delta	Washington	5.23	4	0.26	0.55	0.08	<.0001	0.11	0.06-0.17
2019	Delta	Washington	1.94	4	0.75	0.41	0.08	<.0001	0.18	0.03-0.51
2017	Hills	Clay	0.87	3	0.83	0.33	0.09	0.0002	0.18	0.01-0.53
2017	Hills	Lowndes	5.41	4	0.25	0.46	0.13	0.0003	2.52	0.47-5.38
2017	Hills	Noxubee	3.93	3	0.27	0.50	0.09	<.0001	1.02	0.48-1.74
2017	Hills	Oktibbeha	6.57	4	0.16	0.45	0.10	<.0001	0.07	0.01-0.15
2017	Hills	Gibson, TN	1.31	4	0.86	0.37	0.09	<.0001	0.31	0.01-1.41
2018	Hills	Clay	3.36	4	0.50	0.28	0.06	<.0001	3.92	0.68-14.5
2018	Hills	Lowndes	0.29	4	0.99	0.33	0.06	<.0001	0.65	0.25-1.45
2018	Hills	Noxubee	4.2	4	0.38	0.27	0.11	0.0186	0.004	0.00-0.55
2018	Hills	Oktibbeha	0.26	3	0.97	0.30	0.12	0.0097	0.02	0.00-0.57
2019	Hills	Lowndes	6.66	4	0.15	0.49	0.07	<.0001	0.23	0.11-0.42
2019	Hills	Oktibbeha	0.52	4	0.97	0.47	0.07	<.0001	0.40	0.20-0.69
2019	Hills	Gibson, TN	3	4	0.56	0.51	0.10	<.0001	0.56	0.22-1.04
2019	Hills	Gibson, TN	1.25	4	0.87	0.28	0.06	<.0001	0.13	0.02-0.34
2019	Hills	Winston	0.95	4	0.92	0.37	0.08	<.0001	0.05	0.01-0.17
2019	Lab		5.47	4	0.24	0.26	0.06	<.0001	0.77	0.04-3.84
2020	Lab		5.75	4	0.22	0.46	0.08	<.0001	0.60	0.18-1.35
2020	Lab		3.35	4	0.50	0.42	0.13	0.002	0.57	0.00-2.72

Table 4. Mean LC_{50} estimates for imidacloprid, sulfoxaflor and thiamethoxam by year for *L. lineolaris* collections made from the Midsouthern US during 2017-2019 and all field collections compared to a laboratory colony

Variable	N[z]	LC_{50} (ppm)[y]			Resistance Ratios >10[w]
		Mean (SEM)[x]	Lowest	Highest	
Imidacloprid					
Year: 2017	7	2.64 (1.21) a	0.45	9.27	43%
Year: 2018	9	0.88 (0.25) a	0.03	2.55	11%
Year: 2019	4	0.78 (0.51) a	0.24	2.31	25%
Source: Field	20	1.48 (0.47) B	0.03	9.27	25%
Source: Lab	2	3.38 (0.04) A	3.34	3.41	100%
Sulfoxaflor					
Year: 2017	8	2.84 (1.31) b	0.57	11.2	13%
Year: 2018	8	11.2 (3.89) a	1.08	33.8	50%
Year: 2019	4	16.0 (5.08) a	3.08	26.3	75%
Source: Field	20	8.81 (2.17) A	0.57	33.8	40%
Source: Lab	2	22.4 (13.0) A	9.42	35.3	100%
Thiamethoxam					
Year: 2017	9	2.61 (1.47) a	0.07	13.14	44%
Year: 2018	8	0.80 (0.47) a	0.004	3.92	38%
Year: 2019	6	0.26 (0.08) a	0.05	0.56	17%
Source: Field	23	1.37 (0.62) A	0.004	13.14	35%
Source: Lab	3	0.65 (0.06) A	0.57	0.77	100%

[z] N= number of good fit populations.

[y] LC_{50} values reported in parts per million of active ingredient. LC_{50} estimates followed by the same letter within an insecticide and variable type are not significantly different (Fisher's Protected LSD test with $\alpha = 0.05$).

[x] Statistics for year. imidacloprid ($F = 0.47$; df = 2,15.57; $p = 0.63$), sulfoxaflor ($F = 9.31$; df = 2,15.06; $p < 0.01$), thiamethoxam ($F = 0.12$; df = 2,19.58; $p = 0.88$). Statistics for source: imidacloprid ($F = 6.04$; df = 1,19.56; $p = 0.02$), sulfoxaflor ($F = 3.57$; df = 1,19.1; $p = 0.07$), thiamethoxam ($F = 0.78$; df = 1,24.24; $p = 0.39$).

[w] Percentage of populations tested with resistance ratios > 10. Resistance ratios were calculated by dividing the LC_{50} by the mean LC_{50} of the 5 lowest field populations for the insecticide. The mean LC_{50} of the 5 lowest field populations per chemical: imidacloprid (0.19 ppm), sulfoxaflor (0.85 ppm), thiamethoxam (0.05 ppm).

DISCUSSION

The Mississippi River Delta region is farmed more intensively and annually tends to have higher insect pressure than the Hills region. As a result, more insecticide applications are made on cotton in the Delta region than in the Hills region (Fleming et al., 2015). Snodgrass and colleagues, primarily testing populations from the Delta region, showed resistance of *L. lineolaris* to insecticides in multiple classes: pyrethroids (Snodgrass, 1996b; Snodgrass et al., 2008b, 2009), carbamates and organophosphates (Snodgrass and Elzen, 1995; Snodgrass and Scott, 2002; Snodgrass et al., 2009), and neonicotinoids (Snodgrass et al., 2008a). Calculated resistance ratios reported here commonly exceed 10, but the LC_{50} values are generally within ranges previously reported (Parys et al., 2017). Similarly, Parys et al. (2017) generally found high variability in susceptibility of *L. lineolaris* populations across the Mid-South region. They concluded that most populations were susceptible to neonicotinoids, but several populations exhibited high levels of resistance. For sulfoxaflor, Parys et al. (2017) reported minimum and maximum LC_{50} values of 0.26 and 45.82, respectively; here, the authors found a minimum LC_{50} of 0.57 and a maximum of 33.8. Dorman et al. (2020) reported slightly elevated resistance ratios in North Carolina and Virginia with sulfoxaflor compared to a laboratory colony. However, thiamethoxam resistance ratios calculated by Dorman et al. (2020) were not different from the laboratory colony. Contrary to expectations, resistance was equally frequent in the Delta

and Hills regions for all insecticides. The increasing number of populations with elevated resistance ratios for sulfoxaflor over time is concerning, as 75% of the four populations tested in 2019 exceeded 10-fold resistance ratio. Further monitoring should be conducted to determine if this trend is real or a statistical anomaly.

The authors are not aware of any reports of field control failures with sulfoxaflor, but there have been some anecdotal reports of less than satisfactory field control using neonicotinoids. This is consistent with the observation of a greater range in susceptibility for the neonicotinoids reported here and by Parys et al. (2017) and is likely an indication of ongoing field selection for resistance. It is important to continue monitoring for resistance because continued selection for resistance is likely to lead to widespread reduced efficacy from these compounds in the future. How rapidly this will occur depends on the intensity of selection, the amount of movement between selected and unselected populations, and any fitness costs associated with resistance. Cross resistance among these insecticides is also a possible factor. Although sulfoxaflor is not a neonicotinoid and has activity on some neonicotinoid-resistant insects (Sparks et al., 2013), its mode of action is similar to neonicotinoids, so some resistance mechanisms might impact all these chemistries. Although field populations from the same county and year were assayed for multiple insecticides, these were often collected from different locations on different dates, so these data should not be used to evaluate cross resistance between insecticides.

The laboratory colony used in this manuscript provided consistent data, but it did not appear to represent a baseline for *L. lineolaris* insecticide susceptibility because the LC_{50}s for the laboratory colony were always more than 10-fold higher than the lowest field populations. Because the field populations were tested within 48 h of collection, the health and vigor of the field populations were not comparable to the laboratory colony.

Although laboratory colonies are generally equally or more susceptible to insecticides compared to susceptible field populations (Ali and Luttrell, 2007; Dorman et al., 2020), environmental stress and nutrition can impact insecticide susceptibility (Gordon, 1961; Jensen et al., 2016; Kulma et al., 2013; Wood et al., 1981). For example, a well-fed susceptible laboratory colony can appear more resistant to insecticides than a stressed, but equally susceptible field population. To use a laboratory colony as a baseline for resistance to a stressed field collection, the stressed colony should either be maintained until the stresses are removed, probably by testing the next generation, which increases the effort needed for data collection, or a stress-correction factor should be applied if the stress is consistent among field collections. At present a stress-correction factor is not known, so in this study the average LC_{50} from the lowest five bioassays was used as a baseline. This field-based baseline is not ideal because it requires multiple susceptible populations and will vary depending on how many populations are tested. In contrast, a laboratory-based baseline only requires a single laboratory colony that is easily reared and consistently available with minimal genetic variability.

Despite these limitations, for this study the unconventional baseline method appears to have provided a reasonably consistent baseline estimate over the three insecticides. Comparing the lowest five field-collection assays to the laboratory-colony assays showed the laboratory colony to be 13, 18, and 26 times more resistant for thiamethoxam, imidacloprid, and sulfoxaflor, respectively. One would expect this ratio to be consistent across insecticides if the reduced susceptibility of the laboratory colony is a function of rearing conditions, but variable if the genetics of resistance were important factors. Because the ratio of the laboratory colony LC_{50} to lowest field-population LC_{50} was fairly consistent over all three insecticides, the authors believe this method for establishing a baseline for insecticide susceptibility was reasonable and provided a useful method of evaluating the development of resistance in field populations. The authors do not recommend routinely using such an approach but suggest conducting research to establish an appropriate stress-compensation factor. Based on these data, it appears that this factor would reduce the observed laboratory-colony LC_{50} in the range of 13- to 26-fold to create the baseline. Establishing this baseline with a laboratory colony will reduce effort and variability compared to testing newly collected field populations from non-agricultural areas or testing multiple field populations from agricultural areas. Furthermore, as insecticides continue to be used across the landscape and average LC_{50}s increase, finding truly susceptible field populations to use as a baseline could become difficult.

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