

# Patterns of Tarnished Plant Bug (Hemiptera: Miridae) Resistance to Pyrethroid Insecticides in the Lower Mississippi Delta for 2008–2015: Linkage to Pyrethroid Use and Cotton Insect Management

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## Abstract

Populations of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), from the Lower Mississippi Delta regions of Arkansas, Louisiana, and Mississippi were evaluated from 2008 through 2015 for susceptibility to pyrethroid insecticides using a diagnostic-dose assay with permethrin. Resulting data add to the compilation of pyrethroid susceptibility data carefully tracked in this pest since 1994 and provide continuing evidence of high frequencies of pyrethroid resistance in field populations of the tarnished plant bug. Resistance levels are variable, and some populations remain susceptible suggesting practical value in the continued use of the diagnostic-dose assays prior to pyrethroid treatments. Recent studies with dose–response models suggest that levels of pyrethroid resistance in some populations may still be evolving, with some populations requiring higher doses to reach levels of control comparable to those observed 10 yr ago. Concerns for frequent use of multiple classes of insecticides and possible selection for tarnished plant bugs with metabolic resistance mechanisms capable of detoxifying available insecticide chemistries warrant continued efforts to manage resistance in this important crop pest. Associations among measured pyrethroid resistance levels, published data on annual use of pyrethroid insecticides, and annual estimates of cotton insect losses and control costs were explored and summarized for the 8 yr of this investigation. Mortality of tarnished plant bugs at the diagnostic-dose of permethrin was negatively correlated with kilograms of pyrethroids applied per acre of harvested cropland.

**Key words:** *Lygus lineolaris*, pyrethroid, insecticide resistance

The Mississippi Cooperative Extension Service recently recommended specific tank-mixtures of pyrethroids in combination with other insecticides for controlling tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) (Catchot 2017). This represents a major change in strategic use of pyrethroids, and an example of the evolving interrelationships between resistance management practices for this highly polyphagous pest of cotton and those for the heliothine pest complex [bollworm (*Helicoverpa zea* Boddie) (Lepidoptera: Noctuidae) and tobacco budworm (*Heliothis virescens* F.) (Lepidoptera: Noctuidae)], a group of species previously dependent upon chemical control (Luttrell 1994) but now largely controlled via widespread planting of Bt cottons (Luttrell and Jackson 2012, Luttrell et al. 2015). The Mississippi Cooperative Extension Service also no longer recommends pyrethroid insecticides for heliothine control (Catchot 2017), marking the end of 37 yrs of pyrethroids specifically applied for this purpose. A window-strategy for use of pyrethroids on cotton only after first-bloom is still recommended as it has been in the Mississippi recommendations since

1993 (Head 1993). This window-use strategy was developed initially in an effort to delay pyrethroid resistance evolution in the tobacco budworm (Anonymous 1986). Pyrethroid-resistant populations of tobacco budworm were first detected in the Mississippi Delta in 1986 (Luttrell et al. 1987).

Pyrethroids were first recommended for use on cotton in Mississippi for control of bollworm and tobacco budworm in 1979 following a conditional registration (MCES 1979). Elliott (1989) provides a summary of pyrethroids and his vision for the importance of these insecticides in modern agriculture including use in the animal sector for control of ectoparasites as well as the health and household insecticide market. Palmquist et al. (2012) provides an updated evaluation of pyrethroid insecticides including use patterns and ecotoxicity, and they describe how the pyrethroids as a group replaced many organophosphate insecticides due to better selectivity on target pests and less persistence than organochlorine insecticides. For all of these reasons, pyrethroids were preferred insecticides for controlling many pests of cotton, not just heliothines. Snodgrass

(2014) and [Luttrell and King \(2014\)](#) provide overviews of the sometimes controversial use of pyrethroids for early-season pests of cotton.

[Snodgrass \(1994\)](#) first reported pyrethroid resistance in tarnished plant bugs collected from cotton near Schlater, MS during July and August of 1993, 14 yr after the first use of pyrethroids on cotton in Mississippi and 7 yr after the first documentation of pyrethroid resistance in tobacco budworm ([Luttrell et al. 1987](#)). [Snodgrass \(1996b\)](#) further evaluated the problem, reported expanding examples of pyrethroid and organophosphate resistance in tarnished plant bug populations, and provided rationale for expanded monitoring of pyrethroid resistance given the impact of widespread pyrethroid use on multiple pests and efforts to preserve their critical role in cotton production. This monitoring effort spanned 25 yr of published scientific works including [Snodgrass \(1994\)](#), [Snodgrass and Elzen \(1995\)](#), [Snodgrass \(1996a,b\)](#), [Snodgrass and Scott \(2000\)](#), [Snodgrass et al. \(2008\)](#), [Snodgrass et al. \(2009\)](#), and [Parys et al. \(2017\)](#). [Snodgrass and Scott \(1999\)](#) developed a discriminating-dose assay that could detect pyrethroid-resistant tarnished plant bug populations and predict possible control problems before treatments were initiated. This assay was based on a 3-h observation of field-collected bugs exposed to glass-vials treated with 15 µg of permethrin. [Snodgrass et al. \(2008\)](#) reported results of studies conducted with both the diagnostic-dose assay and traditional dose-response studies that rely on development of probit mortality regression lines. They concluded that either a  $LC_{50}$  of 24 µg of permethrin per vial at 24 h or 60% or less mortality at the diagnostic-dose (15 µg permethrin per vial at 3 h) could be used to predict field control problems with tarnished plant bugs exposed to pyrethroid insecticides.

Reported here are additional studies conducted from 2008 through 2015 to further study the evolution of pyrethroid resistance in populations of the tarnished plant bugs from the Lower Mississippi Delta. Studies in 2014 and 2015 also included dose-response regressions for comparison to those published by [Snodgrass et al. \(2008\)](#). Temporal and spatial patterns of plant bug mortality at the diagnostic-dose were compared to reported use rates of pyrethroids for individual counties/parishes within the study area and estimates of cotton insect losses and control costs annually accumulated by the National Cotton Council to explore linkages between tarnished plant bug resistance and cotton insect management practices.

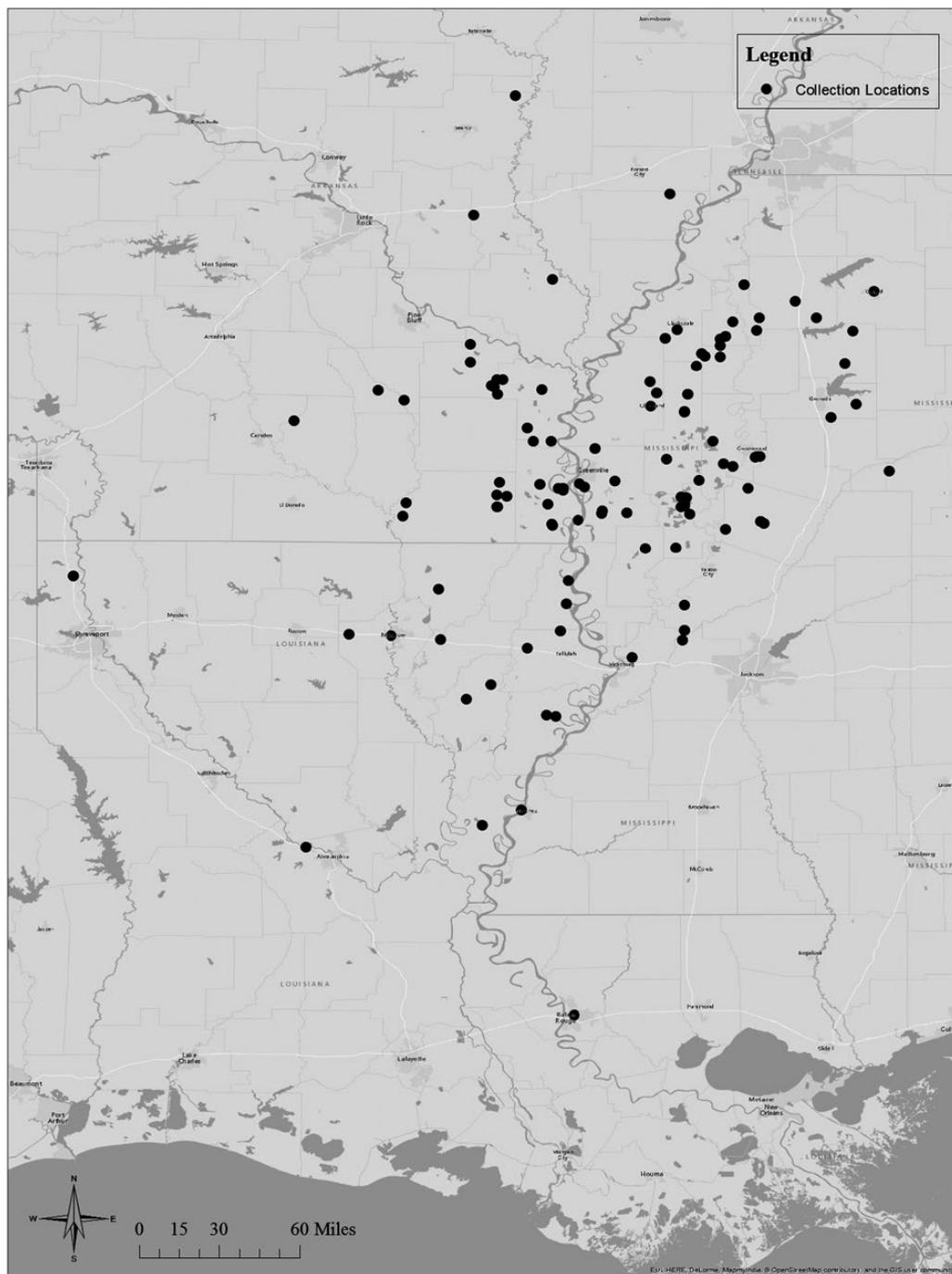
## Material and Methods

Methods and procedures for collecting information on the susceptibility of tarnished plant bugs to pyrethroid insecticides were those described in depth by [Snodgrass \(1996a,b\)](#), [Snodgrass and Scott \(1999, 2000\)](#), and [Snodgrass et al. \(2008, 2009\)](#) for 2008 through 2013. Slightly different procedures were used in 2014 and 2015, and specific modifications are described below. Tarnished plant bug populations across the Lower Mississippi Delta region of Arkansas, Louisiana, and Mississippi ([Fig. 1](#)) were collected from multiple locations each year of the 8-yr study and exposed to a diagnostic-dose assay using glass-vials treated with permethrin (15 µg per vial). A handheld GPS unit was used to record collection locations (Garmin Monterra, Olathe, KS) in 2014 and 2015. Earlier collections, those from 2008 through 2013, were associated with specific latitudes and longitudes by identifying previous collection sites from written records and locating the site on a digital Google Earth map. Insects were collected by sweeping known hosts [primarily the seasonal sequence of broadleaf weeds described by [Snodgrass et al. \(1984\)](#)], usually from road ditches and field borders described in the previous studies by [Snodgrass and colleagues](#), and transporting

the collected insects to the USDA ARS Southern Insect Management Research Unit laboratory in Stoneville, MS. Insects were kept for 24 h in 3.8-liter cardboard containers with green bean pods (*Phaseolus vulgaris* L.) as food prior to the assays on the following day. The diagnostic-dose assay was administered in 20-ml glass scintillation vials as described by [Snodgrass and Scott \(1999\)](#). Technical grade permethrin purchased from Chem Service (West Chester, PA) was dissolved in acetone and pipetted in 0.5-ml aliquots into each vial (15 µg per vial). The vials were rolled on a hot dog roller without heat under a fume hood until the permethrin residue was dry. All assays from 2008 through 2013 included 50 adult tarnished plant bugs from the previous day field collection. Two bugs were added to each vial and mortality was determined after 3 h of exposure. Each test vial contained a piece of green bean pod (~3-cm long) for food.

In 2014 and 2015, studies were focused more on linking the 2008–2013 data, and the previous collective work of the [Snodgrass](#) laboratory on pyrethroid resistance, to future resistance studies focused more on field ecology and evolving population genetics. Creating an experimental bridge to the previous work was essential, and thus repeating the procedures of [Snodgrass et al. \(2008 2009\)](#) as much as possible was a high priority. However, we were also interested in re-evaluating dose-response models with multiple doses in 2014 and 2015 as the more than two-decade long exposure to pyrethroids may have selected for additional resistance mechanisms ([Zhu and Snodgrass 2003](#), [Zhu and Luttrell 2012](#)) and altered some of the previous tight-relationships between diagnostic-dose procedures and full dose-response models described by [Snodgrass and Scott \(2000\)](#) and [Snodgrass et al. \(2008\)](#). Thus, studies in 2014 and 2015 used a range of test concentrations (0-, 0.5-, 1.5-, 5-, 15-, and 50-µg permethrin per vial). They also included a laboratory reference colony as an additional experimental control. Observations of surviving insects were made at 3 and 24 h, thereby allowing data from the 15 µg per vial dose to be compared to that of the 2008–2015 studies. Sample size was reduced in individual assays in 2014 and 2015 as other studies were being simultaneously conducted with other insecticides, and insects were needed for multiple test concentrations in the permethrin studies. A minimum of 10 individuals were tested at each concentration (two per vial), and the test was repeated with additional replicates (up to a maximum of three) with the same collection of insects. As a result of these procedures, the number of insects examined at the diagnostic dose of 15 µg per vial would have ranged from 10 to 30 for a given collection. Dose-mortality regressions were conducted on the 24-h observations using probit analysis (PROC PROBIT, SAS 9.4, SAS Institute Incorporated, 2013). Data were discarded for individual tests that failed chi-square tests for significance of slope or chi-square goodness of fit ( $P = 0.05$ ).

A USDA ARS laboratory colony was added to the monitoring assays as an additional experimental control (generally weekly) in 2014 and 2015. Procedures were the same as those for the field collections. The laboratory-reared insects were from a colony originally established in 1998 at the USDA-ARS Biological Control Rearing and Research Unit in Starkville, MS. Insects were originally collected from weedy host plants in Chickasaw Co., MS ([Cohen 2000](#)). The colony was moved to the USDA-ARS National Biological Control Laboratory in 2006 and to the USDA-ARS Southern Insect Management Research Unit in 2010 ([Portilla et al. 2011](#)). The colony is routinely reared under controlled abiotic conditions in environmental chambers (constant 27°C, 60% relative humidity (RH), and a photoperiod of 16:8 [L:D] h) following details outlined in [Portilla et al. \(2011\)](#) to provide large numbers of even-aged insects. Assays with the USDA colony were conducted exactly as those with the field colonies, but the insects were mixed sex adults (50F: 50M) of a



**Fig. 1.** Sites of tarnished plant bug collections in the Arkansas, Louisiana, and Mississippi Delta.

uniform age (1- to 2-d-old adults). [Snodgrass \(1996a\)](#) described the effects of age of tarnished plant bugs on susceptibility to insecticides.

Survival rates observed at the diagnostic-dose were studied for influences of time of insect collection (year and month within year) and geographic location of collection (latitude, longitude, and geographic groupings of the data). Collection areas were grouped for analysis into regions, which included Arkansas (almost all samples were from the Southeast Delta Region of Arkansas), Crossett (a location in Arkansas used as a historical benchmark of susceptibility), East Mississippi Delta (Mississippi sample locations along the geographic boundary of the Delta and Hill regions), Louisiana (almost all samples were from the Northeast Louisiana Delta region), the USDA ARS Laboratory colony reared on meridic diet (only included

as a control comparison), North Mississippi Delta (samples from Mississippi Delta Counties north of US Highway 82), and South Mississippi Delta (samples from Mississippi Delta Counties south of US Highway 82). Relationships to latitude and longitude were studied via linear models using Multivariate Procedures in JMP, Version 11.1.

Additional exploratory research was conducted by examining possible relationships between survival of plant bugs at the diagnostic-dose, estimated pyrethroid-insecticide use by collecting county-level information from the USGS National Water Quality Assessment Program's Pesticide National Synthesis Project ([Stone 2013](#), [Thelin and Stone 2013](#), [Baker and Stone 2015](#), [USGS 2017](#)), and annual estimates of cotton insect loss and control for the

different regions (Williams 2017). The full USGS pesticide dataset was subset by FIPS code to only include states in the Midsouth using the “EPest-high” estimates of insecticide application for individual counties. Insecticides were categorized by their mode of action and pooled by state and county. The data presented here include all pyrethroids applied to harvested cropland. The grouping of regions differed for insect loss and control cost estimates and included Arkansas (the entire state), Southeast Arkansas, Louisiana (the entire state), Mississippi (the entire state), and the Mississippi Delta. Data were also pooled across the South Delta Region (combination of estimates from Louisiana, Mississippi Delta and Southeast Arkansas) and across all three states (Arkansas, Louisiana, Mississippi) for some observations. Analyses using overlapping geographic areas were completed using repeated measures analyses of variance (ANOVA) to account for non-independence in JMP. Linear relationships between observed survival at the diagnostic dose and county-level estimates of kg of pyrethroids used per acre of harvested cropland were developed and studied. Estimated use of pyrethroid insecticides was also studied by ANOVA to detect any differences in use patterns across the temporal and spatial scales of the study. Similarly, temporal and spatial patterns of tarnished plant bug mortality at the diagnostic-dose and recorded annual use of pyrethroid insecticides for each county within the study area were compared to the National Cotton Council’s annual cotton insect loss estimates for Arkansas, Louisiana, and Mississippi for the years 2008 through 2015 (Williams 2017).

## Results

Geographic locations of the field sample sites are shown in Fig. 1. Descriptive statistics associated with the samples over years and groupings of geographic locations within the Delta are summarized in Tables 1 and 2, respectively. Table 2 also includes comparative information on diagnostic-dose mortality of insects from the Crossett, AR, control site and studies conducted against the USDA ARS diet-reared colony in 2014 and 2015. When tarnished plant bug mortality at the diagnostic dose was studied by ANOVA for effect of region (Arkansas, Louisiana, North Mississippi Delta, and South Mississippi Delta), there was no effect on resulting mortality ( $df = 3$ ,  $F = 1.2785$ ,  $P = 0.2825$ ). There were also no significant influences of latitude ( $n = 234$ ,  $r^2 = 0.0065$ ,  $F = 1.5360$ ,  $P = 0.2165$ ) or longitude ( $n = 234$ ,  $r^2 = 0.0025$ ,  $F = 0.5907$ ,  $P = 0.4429$ ) of collection site on resulting mortality at the diagnostic-dose across the study. Data for the East Mississippi Delta reported in Table 2 were grouped into

the North and South Mississippi Delta for this analysis. There was a significant effect ( $df = 7$ ,  $F = 4.2677$ ,  $P = 0.0002$ ) of year (Fig. 2) with average mortality at the diagnostic-dose in 2011 (77.7%) and 2008 (70.8%) exceeding that of 2015 (58.9%), 2014 (57.5%), 2010 (53.9%), 2009 (53.6%), and 2013 (49.5%). The average mortality in 2012 (63.8%) was intermediate and not different from any other year. The data were also studied for effects of months within years. Month of collection had a significant effect on mortality ( $df = 8$ ,  $F = 5.009$ ,  $P < 0.0001$ ). Average mortality in April (82.6%), October (80.0%), and May (78.4%) was greater than that of July (50.2%) and September (50.2%). Average mortality in June (67.0%) and August (67.9%) was intermediate between the two groups. During some years, samples were also collected in November (20% mortality) and December (45% mortality), but by this time of the season, tarnished plant bugs are generally entering diapause (Snodgrass 2003) and accumulating fat reserves that may impact response to the insecticides. Based on the Snodgrass et al. (2008) suggestion that field populations with less than 60% mortality at the diagnostic-dose would cause field control problems, we estimate that the number of populations difficult to control would have ranged from 4% in 2011 to 62% in 2013 (Table 1).

Mortality of tarnished plant bugs from the Crossett, AR, locations averaged 86.7%, a response similar to that previously reported by Snodgrass et al. (2008). Average responses for all geographic regions within the Delta and the USDA ARS laboratory colony fed solely on a meridic diet ranged from 48.8% (Arkansas) to 67% (Louisiana) and were less than that of the average for the Crossett control location (Table 2). Mortality of the USDA ARS laboratory colony also exhibited significant variable response across the different tests (range of 20–90% mortality, 95% CI of 46.7–65.2% mortality). Average dose–response regressions obtained via probit analysis of the USDA ARS laboratory colony, and the field colonies collected in 2014 through 2015, indicated no difference in average responses at the 3-h observation time based on overlap of 95% confidence limits. At 24 h, the USDA laboratory colony had significantly higher mortality at the 50  $\mu\text{g}$  per vial dose than the average of responses for the field colonies. There were no differences at lower doses, and average slopes of the regression lines were similar (Table 3). Mortality at the standard 3-h observation of insects exposed to 15  $\mu\text{g}$  permethrin per vial, at the 3-h observation of insects exposed to 50  $\mu\text{g}$  permethrin per vial, and the calculated  $LC_{50}$  across all doses at 3 h were compared via simple pairwise correlation to the measured 24 h  $LC_{50}$  (Table 3). Across all colonies ( $n = 59$ ), mortality at the 50  $\mu\text{g}$  dose at 3 h was more closely correlated to 24-h  $LC_{50}$  ( $r = -0.5286$ ,

**Table 1.** Summary statistics for diagnostic-dose response of tarnished plant bugs across all samples for each year 2008 through 2015

Year	Number of samples tested	Mean $\pm$ SEM % mortality <sup>a</sup>	Range in % mortality observed	95% CI	Number of samples with % mortality < 60	% Populations considered resistant <sup>b</sup>	Number of samples with % mortality > 90	% Populations considered susceptible
2008	64	71 $\pm$ 3.11	6–98	64.72–76.91	16	25.0	12	18.8
2009	76	54 $\pm$ 3.84	2–98	46.05–61.11	40	52.6	14	18.4
2010	19	54 $\pm$ 7.25	0–94	39.68–68.11	9	47.4	2	10.5
2011	23	78 $\pm$ 2.15	53–97	73.32–81.75	1	4.3	2	8.7
2012	42	21 $\pm$ 3.66	4–96	14.27–28.62	18	42.9	4	9.5
2013	29	49 $\pm$ 5.17	14–98	39.35–59.61	18	62.1	4	13.8
2014	52	58 $\pm$ 3.95	0–100	49.76–65.24	24	44.4	3	5.6
2015	14	59 $\pm$ 7.37	0–100	44.50–73.39	8	42.1	4	21.1

<sup>a</sup>Diagnostic response is mortality of adult tarnished plant bugs after three hours of exposure to glass vials treated with 15  $\mu\text{g}$  permethrin per vial.

<sup>b</sup>Resistance is based on the recommendation of Snodgrass et al. (2008) that mortality of 60% or less at the diagnostic dose would indicate field control problems.

<sup>c</sup>Susceptibility is based on the  $LC_{90}$  diagnostic dose of 15  $\mu\text{g}$  per vial after 3 h of exposure (Snodgrass and Scott 1999)

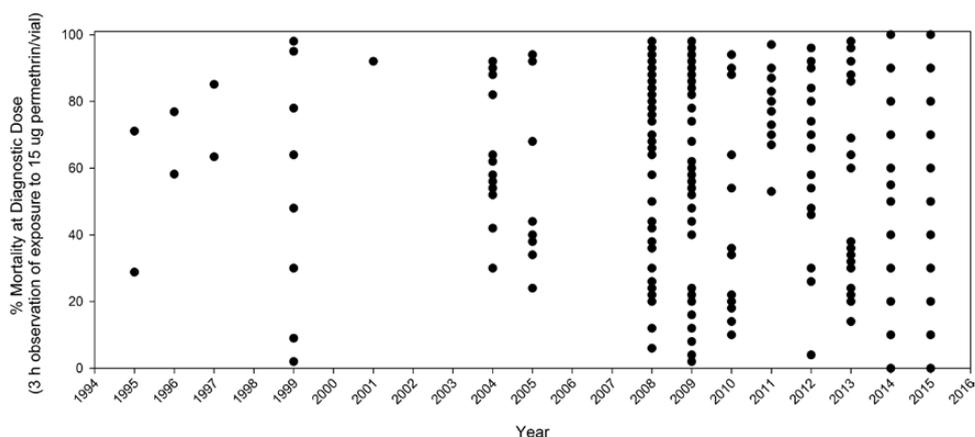
**Table 2.** Summary statistics for diagnostic dose response of tarnished plant bugs across all samples for each sample area 2008 through 2015

Area	Number of samples tested	Mean $\pm$ SEM % mortality <sup>a</sup>	Range in % mortality observed	95% CI	Number of samples with % mortality < 60	% Populations Considered Resistant <sup>b</sup>	Number of Samples with % Mortality > 90	% Populations Considered Susceptible <sup>c</sup>
Arkansas	45	49 $\pm$ 4.38	4–94	40.68–57.87	28	61.4	4	8.9
Crossett, Arkansas	3	87 $\pm$ 3.33	80–90	80.13–93.20	0	0.0	0	0.0
East Mississippi Delta	20	66 $\pm$ 7.76	4–98	51.00–81.42	9	42.1	7	35.0
Louisiana	52	67 $\pm$ 3.82	2–100	59.51–74.47	15	28.8	8	15.4
USDA Lab Diet Reared	22	56 $\pm$ 4.73	20–90	46.67–65.20	10	50.0	0	0.0
North Mississippi Delta	85	62 $\pm$ 3.16	0–100	55.34–67.74	32	84.3	14	16.5
South Mississippi Delta	99	61 $\pm$ 3.06	8–100	54.65–66.66	40	40.0	12	12.1

<sup>a</sup>Diagnostic response is mortality of adult tarnished plant bugs after three h of exposure to glass vials treated with 15  $\mu$ g permethrin per vial.

<sup>b</sup>Resistance is based on the recommendation of Snodgrass et al. (2008) that mortality of 60% or less at the diagnostic dose would indicate field control problems.

<sup>c</sup>Susceptibility is based on the LC<sub>90</sub> diagnostic dose of 15  $\mu$ g per vial after 3 h of exposure (Snodgrass and Scott 1999).



**Fig. 2.** Individual responses of tarnished plant bug populations in the Mississippi Delta to a diagnostic dose of permethrin in a glass vial assay, 1994 through 2015. Data for 1994 through 2005 were from Snodgrass (1994, 1996a), Snodgrass and Elzen (1995), Snodgrass et al. (1999, 2008, 2009), and Snodgrass and Scott (2000).

$P < 0.0001$ ) than the standard 3-h observation at 15  $\mu$ g per vial ( $r = -0.2555$ ,  $P = 0.05510$ ) (Table 4). A similar trend was observed with pairwise comparisons across the field colonies only ( $n = 43$ ), but not with comparisons across the different tests with the USDA ARS laboratory colony where the relationship between observations at 3 h with the 15  $\mu$ g per vial dose and the 24-h LC<sub>50</sub> was highly significant ( $r = -0.8818$ ,  $P < 0.0001$ ) and similar to that previously reported by Snodgrass et al. (2008). The relationship between 3-h observation of mortality at the 50  $\mu$ g per vial dose and the 24-h LC<sub>50</sub> was not significant ( $P = 0.3653$ ), as almost all insects were dead at that concentration.

Across the entire study period of 2008 through 2015, average annual use of pyrethroids was greater in the Mississippi Delta (0.0145-kg pyrethroid/acre of harvested cropland) than it was in Louisiana (0.0073-kg pyrethroid/acre of harvested cropland). Pyrethroid use in Southeast Arkansas was more variable across years and was intermediate between that of the Mississippi Delta Region and Louisiana (0.011 kg/acre of harvested cropland). Across the entire region, pyrethroid use when studied by ANOVA, varied significantly by year ( $df = 7$ ,  $F = 5.9358$ ,  $P < 0.0001$ ) with amounts applied during 2010 (0.0163 kg/harvested acre), 2013 (0.0163 kg/harvested acre), 2012 (0.0151 kg/harvested acre), and 2009 (0.0151 kg/harvested acre) exceeding that applied in 2008 (0.0008 kg/harvested acre). Those for 2011 (0.0120 kg/harvested acre), 2014 (0.0115 kg/harvested acre), and 2015 (0.0100 kg/harvested acre) were intermediate in amounts applied. There was no significant relationship between year and

diagnostic-dose mortality when studied by linear regression using the entire dataset ( $n = 233$ ,  $F = 0.0831$ ,  $P = 0.7734$ ; Table 5). There was a significant negative linear relationship ( $n = 233$ ,  $r^2 = 0.0214$ , intercept  $\pm$  SE =  $70.888 \pm 3.682$ , slope =  $-55.677 \pm 1.768$ ,  $F = 5.1008$ ,  $P = 0.0248$ ) between kg pyrethroid applied/acre of harvested cropland across the study and resulting tarnished plant bug mortality at the diagnostic dose (Fig. 3, Table 5), though the low  $R^2$  indicates that the data are highly variable.

Since the empirical estimates of tarnished plant bug mortality at the diagnostic-dose developed by Snodgrass et al. (2008) were primarily from field samples taken in the South Delta (delta regions of Louisiana, Mississippi, and Southeast Arkansas), mean kg of pyrethroids per acre of harvested cropland and mean mortality of tarnished plant bugs at the diagnostic-dose were averaged for each year across the South Delta Region, and compared to averaged estimates of insect loss and control costs adjusted for the South Delta region. Adjustments were relative to the annual cotton acreages for each of the individual regions comprising the collective total (Table 6). When these data were studied by ANOVA, there were no significant differences across years in amount of pyrethroid applied per harvested crop acre, mean mortality of tarnished plant bugs at the diagnostic dose, mean number of insecticide applications for plant bugs, mean acres of cotton (although they were highly variable and there was a consistent decrease), mean estimated % crop loss to insects, mean number of foliar applications for all pests, mean cost of all foliar insecticides per acre, or mean cost of individual insecticide

**Table 3.** Summary statistics for dose-response regression of laboratory and field populations of tarnished plant bug exposed to permethrin in glass vial assays in 2014 and 2015 experiments

	No. of regressions	Mean $\pm$ SEM	Minimum value observed <sup>a</sup>	Maximum value observed <sup>a</sup>	95% CI
USDA Lab Diet-Fed Colony					
24-h Slope	16	2.4 $\pm$ 0.15	1.5	3.5	2.14–2.74
24-h LC <sub>50</sub>	16	7.6 $\pm$ 1.21	1.5	18.2	5.26–9.99
24-h LC <sub>90</sub>	15	22 $\pm$ 3.91	0.5	44.0	14.32–29.66
24-h % Mort (0 $\mu$ g per vial)	16	0	0	0	0
24-h % Mort (0.5 $\mu$ g per vial)	16	6.6 $\pm$ 2.69	0	40	1.29–11.83
24-h % Mort (1.5 $\mu$ g per vial)	16	18.9 $\pm$ 6.27	0	70	6.62–31.2
24-h % Mort (5 $\mu$ g per vial)	16	39.1 $\pm$ 6.55	10	100	26.22–51.9
24-h % Mort (15 $\mu$ g per vial)	16	71.6 $\pm$ 5.99	20	100	59.83–83.29
24-h % Mort (50 $\mu$ g per vial)	16	99.2 $\pm$ 0.44	95	100	98.36–100.08
3-h Slope	15	2.29 $\pm$ 0.94	0	4.2	1.81–2.77
3-h LC <sub>50</sub>	15	9.07 $\pm$ 5.09	1.3	18.2	6.39–11.74
3-h LC <sub>90</sub>	15	33.81 $\pm$ 22.31	5.3	29.95	22.12–45.49
3-h % Mort (0 $\mu$ g per vial)	15	0	0	0	0
3-h % Mort (0.5 $\mu$ g per vial)	15	4 $\pm$ 2.14	0	30	0–8.19
3-h % Mort (1.5 $\mu$ g per vial)	15	16.3 $\pm$ 6.77	0	80	3.07–29.6
3-h % Mort (5 $\mu$ g per vial)	15	41.8 $\pm$ 8.11	0	90	25.94–57.73
3-h % Mort (15 $\mu$ g per vial)	15	68 $\pm$ 5.78	20	100	56.68–79.32
3-h % Mort (50 $\mu$ g per vial)	15	97 $\pm$ 1.53	80	100	94.01–99.99
Field Colonies					
24-h Slope	43	2.1 $\pm$ 0.19	0.5	5.4	1.75–2.49
24-h LC <sub>50</sub>	43	6.1 $\pm$ 1.56	0.3	55.5	3.02–9.12
24-h LC <sub>90</sub>	42	164.4 $\pm$ 101.95	0.0	4277.0	0–364.27
24-h % Mort (0 $\mu$ g per vial)	43	1.1 $\pm$ 0.57	0	20	0–2.2
24-h % Mort (0.5 $\mu$ g per vial)	43	13.4 $\pm$ 2.57	0	60	8.35–18.43
24-h % Mort (1.5 $\mu$ g per vial)	43	32.9 $\pm$ 2.98	0	80	27.06–38.75
24-h % Mort (5 $\mu$ g per vial)	43	64.4 $\pm$ 3.80	0	100	56.9–71.81
24-h % Mort (15 $\mu$ g per vial)	43	80 $\pm$ 3.79	10	100	72.56–87.44
24-h % Mort (50 $\mu$ g per vial)	43	93.7 $\pm$ 1.67	60	100	90.46–96.98
3-h Slope	42	3.47 $\pm$ 4.48	0.1	19.1	2.12–4.82
3-h LC <sub>50</sub>	42	15.92 $\pm$ 24.92	0.92	145.81	7.99–23.84
3-h LC <sub>90</sub>	42	771.92 $\pm$ 4255.19	2.94	26303	0–2124.88
3-h % Mort (0 $\mu$ g per vial)	42	0.2 $\pm$ 0.24	0	10	0–0.7
3-h % Mort (0.5 $\mu$ g per vial)	42	2.4 $\pm$ 1.48	0	60	0–5.28
3-h % Mort (1.5 $\mu$ g per vial)	42	14.3 $\pm$ 2.46	0	70	9.46–19.11
3-h % Mort (5 $\mu$ g per vial)	42	38.9 $\pm$ 4.19	0	100	30.72–47.13
3-h % Mort (15 $\mu$ g per vial)	42	66.4 $\pm$ 4.87	0	100	56.89–75.96
3-h % Mort (50 $\mu$ g per vial)	42	84.5 $\pm$ 3.39	10	100	77.87–91.18

<sup>a</sup>LC<sub>50</sub> and LC<sub>90</sub> values are expressed as microgram of permethrin per vial.

**Table 4.** Correlation coefficients between observations of mortality at 3-h test concentrations of 15- and 50- $\mu$ g permethrin per vial, estimated LC<sub>50</sub> values at 3- and 24-h measured LC<sub>50</sub>s in 2014 through 2015 glass vial assays with an USDA Laboratory colony and field colonies from the Mississippi Delta

	Correlation coefficient ( <i>r</i> )	Significance probability
All 2014–2015 Dose–response regressions ( <i>n</i> = 59)		
3-h Mort 15 $\mu$ g    24-h LC <sub>50</sub>	–0.2555	0.0551
3-h Mort 50 $\mu$ g    24-h LC <sub>50</sub>	–0.5286	<0.0001*
3-h LC <sub>50</sub> 24-h LC <sub>50</sub>	–0.0663	0.624
2014–2015 Dose–response regressions for field colonies ( <i>n</i> = 43)		
3-h Mort 15 $\mu$ g    24-h LC <sub>50</sub>	–0.2829	0.0695
3-h Mort 50 $\mu$ g    24-h LC <sub>50</sub>	–0.5355	0.0003*
3-h LC <sub>50</sub> 24-h LC <sub>50</sub>	–0.0615	0.6989
2014–2015 Dose–response regressions for USDA Lab ( <i>n</i> = 16)		
3-h Mort 15 $\mu$ g    24-h LC <sub>50</sub>	–0.8818	<0.0001*
3-h Mort 50 $\mu$ g    24-h LC <sub>50</sub>	–0.2518	0.3653
3-h LC <sub>50</sub> 24-h LC <sub>50</sub>	0.6036	0.0172*

\*Significant correlation at *P*=0.05.

applications. Significant differences across years were detected in the mean number of insecticide applications for bollworms and the mean yield in kilograms of lint per acre. More insecticide applications for bollworm were made in 2010 (1.95/acre) than in 2008 (0.64/acre), 2012 (0.55/acre), and 2013 (0.53/acre). Yield was highest in 2013 (545 kg lint/acre), which was significantly greater than that of 2008 (346 kg lint/acre) and 2009 (371 kg lint/acre). When these South Delta metrics were studied by pairwise simple correlation using Multivariate Procedures in JMP Version 11.1, significant or near-significant correlations were detected between acres of cotton and kg of pyrethroid/acre of harvested cropland (*n* = 32, *r* = 0.3549, *P* = 0.0463), number of applications for plant bugs and year (*n* = 32, *r* = 0.3252, *P* = 0.0694), and acres of cotton and year (*n* = 32, *r* = –0.3553, *P* = 0.0460). All other pairwise comparisons were not significant (*P* = 0.05).

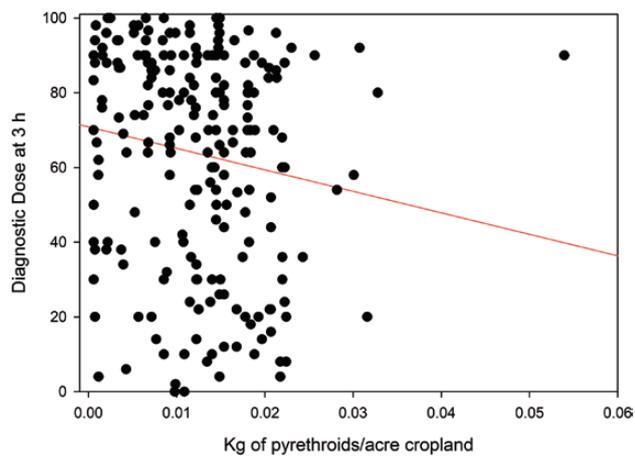
## Discussion

Results of this study confirm the continued presence of pyrethroid resistance in tarnished plant bug populations in the Lower Mississippi Delta. Levels (or frequencies) of resistance appear to be as great as or

**Table 5.** Regression equations describing diagnostic dose mortality (DD 3 h) as a function of pyrethroid use (kg pyrethroid/acre) and diagnostic dose mortality and pyrethroid use as functions of assay sample year, month, latitude and longitude

Dependent variable	Independent variable	<i>n</i>	<i>r</i> <sup>2</sup>	Intercept ± SE	Slope ± SE	F Ratio	Prob > F
DD 3 h	kg pyrethroid/acre	233	0.0214	70.89 ± 3.61	-55.68 ± 1.77	5.1008	0.0248*
DD 3 h	sample year	233	0.0003	7.13 ± 18.79	-0.002 ± 0.009	0.0831	0.7734
DD 3 h	sample month	233	0.0988	104.08 ± 8.212	-5.75 ± 1.14	25.3121	<0.0001*
DD 3 h	sample latitude	233	0.0066	164.91 ± 81.67	-3.02 ± 2.44	1.536	0.2165
DD 3 h	sample longitude	233	0.0025	248.31 ± 240.19	-2.03 ± 2.64	0.5907	0.4429
kg pyrethroid/acre	sample year	233	0.0058	-23.00 ± 21.45	0.01 ± 0.011	1.3561	0.2454
kg pyrethroid/acre	sample month	233	0.0132	0.01 ± 0.002	0.001 ± 0.0003	3.1053	0.0794
kg pyrethroid/acre	sample latitude	233	0.0311	-0.04 ± 0.02	0.002 ± 0.0001	7.4453	0.0068*
kg pyrethroid/acre	sample longitude	233	0.0152	0.13 ± 0.06	-0.001 ± 0.0002	3.6406	0.0576

\*Statistically significant regression model ( $P = 0.05$ ).



**Fig. 3.** Relationship between tarnished plant bug mortality at the diagnostic dose (DD of 15 µg of permethrin per vial for 3 h) and kg pyrethroid applied per acre of cropland across the 2008–2015 study period. (Regression  $Y = 70.89 - 575.68X$ .)

greater than those previously reported. Snodgrass et al. (2008) tested 20 field populations (excluding the Crossett control location) and found that 45% of the populations had mortalities less than 60% at the 15 µg per vial diagnostic dose of permethrin. They also found 20% of the populations to be susceptible with mortalities greater than 90% at the diagnostic dose. Over the 8 yr of this study, a total of 301 field populations were collected and assayed at the diagnostic-dose. Of these, 41% were classified as resistant (<60% mortality), and 14% were classified as susceptible (>90% mortality) using Snodgrass' criteria. Those populations between 60 and 90% mortality were not classified as either resistant or susceptible. While differences among years were evident (Table 1: range of 4 to 62% of populations resistant), the percent of tarnished plant bug populations considered to be resistant (42.1%) and susceptible (21.1%) in 2015 were almost identical to those reported by Snodgrass et al (2008) (45 and 20%, respectively). Differences in average mortality at the diagnostic dose were not significant in analyses, but the percentage of populations considered to be resistant suggested that sample sites in Arkansas (primarily Southeast Arkansas) and the North Mississippi Delta had higher frequencies of resistant populations (61 and 84%, respectively) than Louisiana (28%), East Mississippi Delta areas bordering the Hills (42%), and South Mississippi Delta (40%). The percent of populations considered to be susceptible was numerically greatest for the East Mississippi Delta (35%). Populations susceptible in other regions were less than the 20% of populations reported to be susceptible (>90% mortality at diagnostic-dose) by Snodgrass et al. (2008).

The Crossett control location still appears to be susceptible to permethrin (87% mortality at the diagnostic-dose). Snodgrass et al. (2008) reported mortality of 92% for the Crossett colony exposed to the diagnostic-dose. The USDA ARS laboratory colony fed solely on mericid diet is not susceptible and exhibits variability from test to test (Tables 2 and 3) comparable to the variability in response among field populations. This laboratory colony is known to be more tolerant than many field colonies to several classes of insecticide chemistries (Parys et al. 2015, Parys et al. 2017). The physiological basis for this variability and general tolerance to permethrin is deserving of further investigation, especially since the current diagnostic-dose assay utilizes insects directly from the field with no knowledge of insect age or previous food source. Nutrition and host plant effect insect susceptibility to a number of different insecticides (Gordon 1961, Wood et al. 1981, Yu 1982, Liang et al. 2007, Jensen et al. 2016). An alternative approach would be to rear tarnished plant bugs on green beans or other plant materials similar to the procedures used by Snodgrass and Scott (1999) in developing the glass-vial assay for tarnished plant bugs and perhaps compare field control of cotton infested with the USDA Laboratory colony to that of feral field populations. Our recent field collections of insects from the Crossett area were all made in May and early-June. Perera et al. (2015) studied the population genetics of 15 tarnished plant bug populations in the Mississippi Delta by using 13 microsatellite markers and found a general trend for increased gene flow between populations later in the season. They also postulated that selection by insecticide sprays in cotton could have played a role in the temporal variation observed in genetic structure. Perhaps, populations of tarnished plant bug from Crossett location later in the year would express more resistance.

Comparison of the diagnostic-dose response at 3 h with 24 h dose-response regressions yielded different results from that reported by Snodgrass et al. (2008). Mortality of bugs after 3 h of exposure to the 15 µg per vial dose of permethrin was highly correlated with the  $LC_{50}$  at 24 h across 16 experiments with the USDA Laboratory colony as reported for the field populations in Snodgrass et al. (2008). However, with the 43 field populations studied in 2014 and 2015 (Table 3), mortality of bugs exposed to a 50 µg per vial dose was more strongly correlated to 24-h  $LC_{50}$ s than that for the 15 µg per vial dose. This may suggest continued selection for additional resistance mechanisms. Zhu and Snodgrass (2003) measured elevated cytochrome P450 levels in pyrethroid resistant strains of the tarnished plant bug. Zhu and Luttrell (2015) and Fleming et al. (2016) reported elevated levels of esterases and glutathione-S transferase in Mississippi populations of the tarnished plant bug. Additional research is needed to determine the impact of selection on tarnished plant bugs by one class of insecticide on resistance of tarnished plant

**Table 6.** Mean (SEM) pyrethroid use, tarnished plant bug mortality at a diagnostic dose, and estimates of insecticide use and cotton acreage for the South Delta Region of the Midlands

Year	Mean ± SEM kg pyrethroid/ acre cropland	Mean ± SEM % tarnished plant bug mortality at diagnostic dose	Mean ± SEM acres of cotton
2008	0.008 ± 0.0017	66.3 ± 6.9	411758 ± 114465.4
2009	0.013 ± 0.0022	55.9 ± 10.1	318819 ± 100748.4
2010	0.021 ± 0.0081	53.5 ± 3.1	375333 ± 84994.8
2011	0.011 ± 0.0019	76.6 ± 1.4	451667 ± 96709.8
2012	0.012 ± 0.0036	58.4 ± 7.1	384167 ± 105122.3
2013	0.011 ± 0.0042	48.7 ± 3.4	220333 ± 54913.1
2014	0.006 ± 0.004	71.2 ± 14.6	260936 ± 47290.4
2015	0.009 ± 0.0015	76.6 ± 0.9	185000 ± 22546.2
ANOVA	df = 7; F = 1.3105; P = 0.3073	df = 7; F = 1.8434; P = 0.1515	df = 7; F = 1.2973; P = 0.3129
Year	Mean ± SEM no. insecticide applications for bollworm	Mean ± SEM no. all foliar insecticide applications	Mean ± SEM no. insecticide applications for plant bugs
2008	0.64 ± 0.054 b	5.3 ± 1.2	3.18 ± 0.907
2009	1.14 ± 0.308 ab	7 ± 1.1	4.02 ± 1.081
2010	1.95 ± 0.479 a	7.2 ± 1.3	3.84 ± 1.027
2011	1.42 ± 0.385 ab	8.6 ± 0.4	4.97 ± 1.021
2012	0.55 ± 0.161 b	11 ± 1	4.85 ± 0.676
2013	0.53 ± 0.07 b	10.8 ± 0.8	4.85 ± 0.93
2014	0.77 ± 0.035 ab	7 ± 2.4	4.84 ± 0.463
2015	0.93 ± 0.066 ab	7.6 ± 1.7	5.25 ± 0.627
ANOVA	df = 7; F = 3.7809; P = 0.0130	df = 7; F = 1.1801; P = 0.3620	df = 7; F = 0.6712; P = 0.6937

\*Means within a column followed by a common letter do not differ significantly, Tukey's HSD test ( $P = 0.05$ ).

bugs to other classes of insecticide, especially since current chemical controls are failing and growers are applying increased numbers of applications for control of these bugs on cotton (Gore et al. 2014, Fleming et al. 2016). The presence of multiple resistance mechanisms would not be surprising given the nearly 40 yr of exposure of tarnished plant bugs to pyrethroids and the recognition that control problems were associated with multiple classes of insecticides when pyrethroid resistance was first detected (Snodgrass 1996b, Scott and Snodgrass 2000). In a review of insecticide trials for tarnished plant bugs in 1999, Reed et al. (1999) reported that pyrethroids provided 94% control of tarnished plant bugs in 1982, 73% in 1986, and about 56% in the late 1990s. They also reported a sharp decline in efficacy of organophosphates for control of tarnished plant bug.

The exploration of possible associations among measured pyrethroid susceptibility in tarnished plant bug populations, recorded county/parish level use of pyrethroids and annual estimates of cotton insect loss and control costs provided a few statistically significant relationships. Mortality at the diagnostic dose was negatively correlated with kilogram of pyrethroid applied per acre of harvested cropland and month of sample indicating that resistance levels increase with increased use of pyrethroids and expanded exposure over the growing season. This is logical and a likely influence given the dynamics of cotton acreage and the sequence of multiple insecticide treatments used in cotton. Pyrethroid use was positively correlated with latitude indicating that more northern sample sites (i.e., North Delta) were associated with more use of pyrethroids. This again could be indirectly associated with concentrations of cotton acreage within the landscape. Linear relationships between measured plant bug mortality and cotton insect loss estimates were not observed. There was a statistically significant positive relationship between year and number of applications per acre for plant bugs and a statistically significant negative relationship between year and acres of cotton. The recorded use of pyrethroids per acre of cropland was also positively related to the acres of cotton grown in a

county/parish. There were no major differences among the states in estimated numbers of insecticide applications made for plant bugs or all insects.

Collectively, this study confirms the previous trends documented by Snodgrass et al. (2008) and suggests that tarnished plant bug resistance to pyrethroids is still present. Given the extensive use of insecticides to control tarnished plant bug in the Delta region and the risk of selection for detoxifying mechanisms that may render multiple classes of insecticide ineffective, efforts should be intensified to develop management systems less dependent on insecticides. The development of the diagnostic-dose assay by Snodgrass and Scott (1999) provided a tool to determine if a plant bug population was susceptible or resistant prior to treatment. Our work indicates that this diagnostic tool is still relevant and needed for monitoring. These tools can provide important information reasonably quickly regarding the value of applying pyrethroid treatments for tarnished plant bug in cotton.

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