GLASS-VIAL INSECTICIDE ASSAYS AND DETOXIFICATION SYSTEM COMPARISONS OF MISSISSIPPI POPULATIONS OF *LYGUS LINEOLARIS*

Daniel E. Fleming
Natraj Krishnan
Fred R. Musser
Mississippi State University
Mississippi State, MS

Abstract

Lygus lineolaris is a major pest of cotton in Mississippi but is much more damaging to cotton in the Delta region of the state compared to the damage in the Hills region of the state. The reason for this difference is not understood but has been thought to be a result of higher insecticide resistance in the Delta region. Research was conducted in 2014 to compare insecticide LC_{50}s, mortality, and glutathione S-transferase activity in Mississippi populations of *L. lineolaris*. Results of this research indicated no regional differences, however the county of collection significantly affected mortality and glutathione S-transferase activity. The data do not explain the higher level of economic damage caused by the Delta populations of *L. lineolaris* but indicate that variation in insecticide tolerance and detoxification enzymes occurs in Mississippi populations of *L. lineolaris*.

Introduction

Lygus lineolaris (Palisot de Beauvois) (Hemiptera: Miridae) has become a major pest of cotton (*Gossypium hirsutum* L.) in Mississippi. Since 1979 it has frequently been considered one of the three most costly insect pests of Mississippi cotton (Williams 1997), and since 2003, it has been estimated that *L. lineolaris* was the target of 4.1 insecticide applications with an average yearly cost of $110 per hectare (Williams 1979-2012). The estimated losses and costs of control from *L. lineolaris* damage are not uniform across the state. Since 1986 the cotton insect losses of Mississippi have been separated into two regions, the Delta and the Hills (Figure 1). The Delta is the region of western Mississippi that is the flood plain of the Mississippi and Yazoo rivers and includes the area west of the Loess Bluffs (Figure 1). The Hills region is the remainder of the state. The Delta region is intensively farmed and has less non-cultivated areas than the Hills region which typically has smaller fields with adjacent non-cultivated areas. The number of insecticide applications targeting *L. lineolaris* in the Delta region has been estimated to be 3.1 fold greater than in the Hills region, and the costs of control plus the value of the cotton lost per hectare to *L. lineolaris* has been estimated to be approximately 3.7 fold greater in the Delta than in the Hills (Williams 1979-2012).

The reason for the increased pest pressure in the Delta region is largely unknown. Higher rates of insecticide resistance in the Delta are often considered a cause for the dissimilarity between the regions. Insecticide resistance in *L. lineolaris* in the United States, especially in the Mississippi Delta region, has been confirmed for several classes of insecticides, including: pyrethroids, organophosphates, organochlorines, carbamates, and cyclodienes (Cleveland and Furr 1980, Cleveland 1985, Dennehy and Russell 1996, Hollingsworth et al. 1997, Holloway et al. 1998, Pankey et al. 1996, Snodgrass and Scott 1988, Snodgrass 1994, Snodgrass and Elzen 1995, Snodgrass 1996a,b, Snodgrass and Scott 1999, 2000, 2002, Snodgrass and Scott 2003, Snodgrass et al. 2009). Higher levels of insecticide resistance have been reported from *L. lineolaris* populations in regions of more frequent insecticide use, such as the Mississippi Delta (Cleveland and Furr 1980, Cleveland 1985, Hollingsworth et al. 1997, Snodgrass 1996b,), and within these regions variation in insecticide resistance exists (Snodgrass and Scott 1988, Snodgrass 1994, Snodgrass 1996a, Snodgrass and Scott 2000, 2002, Snodgrass and Scott 2003, Snodgrass et al. 2009). Concurrent studies of insecticide resistance focusing on Mississippi Delta and Hills populations of *L. lineolaris* are not known to have been published.

If insecticide resistance is a factor in differences in *L. lineolaris* injury to cotton, then levels of insecticide detoxification enzymes may also be dissimilar. Three enzymes are commonly associated with metabolic insecticide detoxification: cytochrome P-450 (Feyereisen et al. 1995, Feyereisen 1999, Kasai and Scott 2000, Puinean et al. 2010, Tomita et al. 1995), esterases (Campbell et al. 1998, Karunaratne and Hemingway 2001, Liu and Han 2003, Raghavendra et al. 1998, Smyth et al. 2000, Valles 1998), and glutathione S-transferase (Armstrong 1997, Fournier et al. 1987, Oppenoorth et al. 1972). These enzymes have been studied in *L. lineolaris* and vary in relation to insecticide resistance. Gene expression, mRNA levels, and activity of these enzymes have all been shown to be higher in resistant *L. lineolaris* strains and with exposure to insecticides (Zhu and Snodgrass 2003, Zhu et al. 2004, Zhu et al. 2007, Zhu et al. 2011, Zhu et al. 2012, Zhu and Luttrell 2012, 2014).

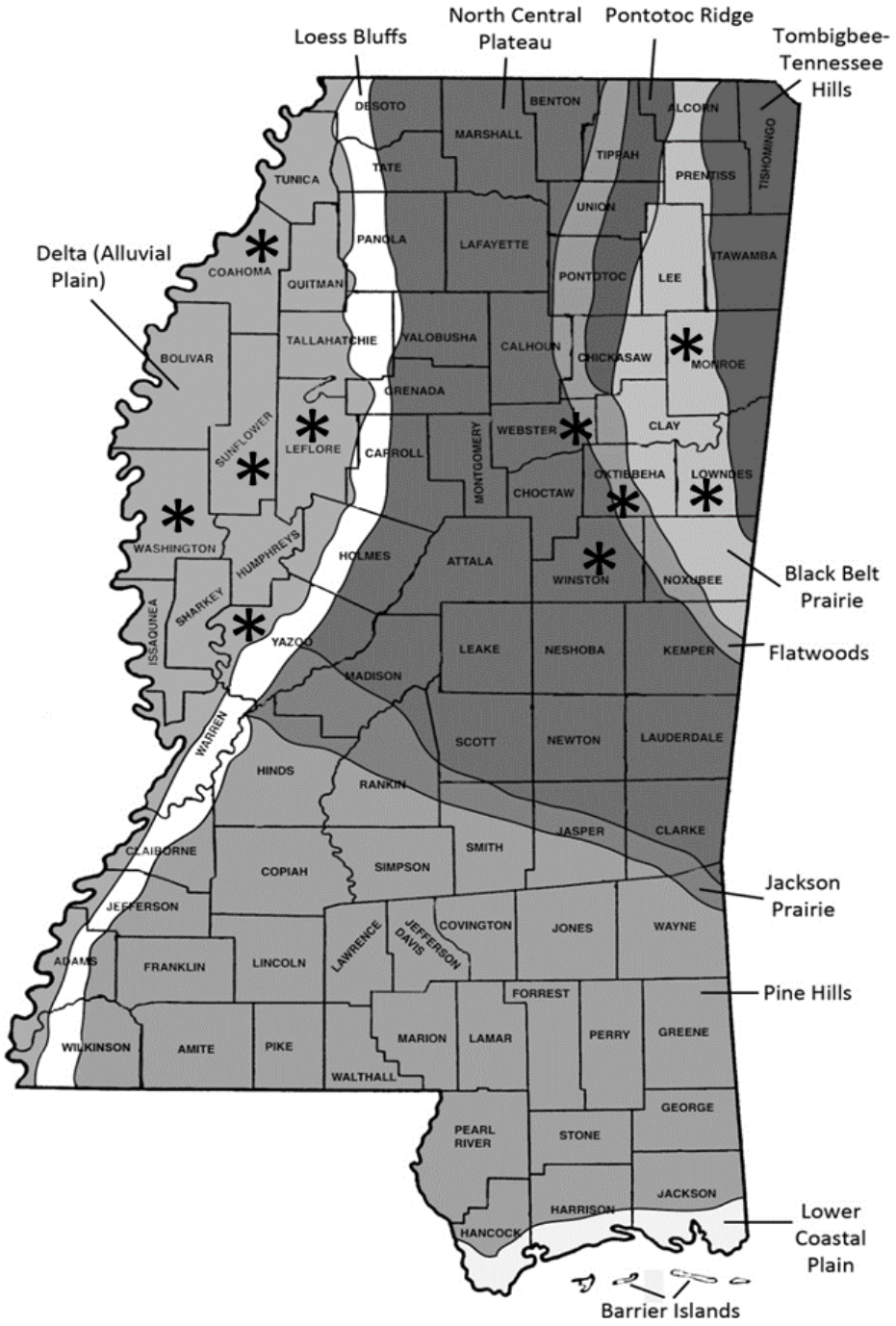

Figure 1. Geographic regions of Mississippi. The delta (alluvial plain) is the Delta region referred to in this paper, while the Hills refers to the rest of the state. The primary cotton growing areas of the Hills region are the areas east of the loess bluffs and north of the pine hills.
* indicates counties of collection sites.

The purpose of this study is to compare insecticide tolerance in Delta and Hills populations of *L. lineolaris*. Experiments were conducted to evaluate insecticide tolerance and glutathione S-transferase (GST) activity in

Mississippi populations of *L. lineolaris*. Regional differences in insecticide tolerance and glutathione S-transferase activity may explain why *L. lineolaris* is a more serious pest of cotton in the Delta than in the Hills.

Materials and Methods

Insect rearing

Colonies of *L. lineolaris* were collected weekly from wild host plants at five locations (one location per week per region) (Figure 1 (*)) in both the Delta and Hills regions during the late spring/ early summer of 2014. Insects were collected with sweep nets, placed into plastic containers, and aspirated into cricket cages for transport back to the Mississippi State University Insect Rearing Center. Insects were then emptied into 8.3 liter plastic containers filled with shredded paper. A fine mesh screen was placed on the containers and held down with the self-sealing lids of these containers which are modified with the center of the lid removed. Screen covers and shredded paper were replaced weekly to reduce contamination from fecal material. Insect colonies were kept in a rearing chamber maintained at 26.7 ± 2° C at a relative humidity of 65 ± 5% with a 16:8 light:dark cycle. The insects were fed an oligidic artificial diet, similar to that of Cohen (2000), presented in a 5 x 5 cm Parafilm (Bemis Company Inc., Neenah, WI, www.parafilm.com) packet placed on top of the screens. Oviposition packets consisting of a 4% carrageenan solution presented in a 5 x 5 cm Parafilm were also placed on top of the screens. Oviposition packets containing eggs were transferred three times weekly into 8.3 liter plastic containers similar to those housing the parent colonies. These containers remained in the same rearing chamber as the parent containers and nymphs were reared on artificial diet to fourth instar.

Insecticide bioassays

A glass-vial bioassay was used to determine LC_{50}s and mortality for acephate, permethrin, and imidacloprid. Technical grade acephate and permethrin (Chem Service, West Chester, PA, http://www.chemservice.com) were dissolved and diluted in acetone to make the dose solutions for the experiment. 20 ml scintillation vials were treated with a dose of acephate (1.875, 3.75, 7.5, 15, or 30 µg/vial) and permethrin (1.25, 2.5, 5, 10, or 20 µg/vial) by pipetting 500µl of solution into the vials and rolling the vials on a repurposed Star® flat roller grill (heating element removed) until the acetone had evaporated. Flats of vials were stored on a shelf and covered by aluminum foil until use. When less than half of the insects died at the highest tested dose, the assay was conducted again with higher doses included (60 and 120 µg acephate/vial or 40 and 80 µg permethrin/vial). Cut pieces of green beans (0.2 g) were placed in the vials to serve as a food source for the insects. Two 4th instar nymphs were placed in each vial by gently scooping them from the sides of the rearing container into the vial. A cotton ball was placed in the mouth of the vial to prevent the nymphs from escaping. Vials were placed into the same rearing chamber as the colonies and mortality was recorded after 24 hours. Technical grade imidacloprid (Chem Service, West Chester, PA, http://www.chemservice.com) was dissolved in acetone and diluted in honey water to make the doses (0.022, 0.067, 0.2, 0.6, 1.8 µg/cap) for the experiment. 300 µl of solution was placed into caps of 1.5 ml SealRite® microcentrifuge tubes (USA Scientific, Inc., www.usascientific.com) that had been removed from the tube. Caps were then covered in Parafilm by pressing the Parafilm over the lip of the cap with a lid from an ultra-fine tip Sharpie® marker. Filled caps were placed lip side up in 20 ml scintillation vials. Two fourth instar nymphs were transferred into each vial by gently scooping the nymphs into the vials from the sides of the container. A cotton ball was placed in the top of the vial to prevent the nymphs from escaping. Vials were placed into the same rearing chamber as the colonies and mortality was recorded after 72 hours. Each insecticide treatment was replicated three times with ten nymphs per replicate and all replicates had an insecticide free treatment as a check.

Glutathione S-transferase activity

Insects from the same field colonies were used to test for GST activity. Three replicates of twelve insects each were taken from each colony and replicates were divided into four subsamples of three insects each. The method of enzyme isolation and measurement was similar to that of Habig et al. 1974 with some modifications. Subsamples were homogenized in 500µl of a 100 mM potassium-phosphate homogenizing buffer (Fisher Scientific, www.fishersci.com) (approximately 2:1 dibasic:monobasic to obtain a pH 7.0) containing 2 mM EDTA disodium salt (Fisher Scientific, www.fishersci.com) and 0.1% Triton X-100 (Sigma-Aldrich, www.sigmaaldrich.com). The homogenate was centrifuged at 3000 g for 5 minutes and supernatant removed and re-centrifuged at 10000 g for 15 minutes. Supernatant was then diluted 10:1 in a 100 mM potassium-phosphate assay buffer (approximately 2:1 dibasic:monobasic to obtain a pH 7.0) containing 0.1 % Triton X-100. 20 µl of diluted sample supernatant, 150 µl of assay buffer, and 20 µl of L-glutathione reduced (GSH) (Sigma-Aldrich, www.sigmaaldrich.com) were pipetted, in triplicate, into wells of a 96-well clear, flat-bottom plate (Fisher Scientific, www.fishersci.com). Six wells on each

plate were used for 170 µl of assay buffer and 20 µl of GSH that served as a negative control. 10 µl 1-chloro-2,4-dinitrobenzene (CDNB) substrate (Sigma-Aldrich, www.sigmaaldrich.com) was added to all wells. Plates were placed in a BioTek Synergy H1 Multi-mode plate reader (www.biotek.com), shaken for two seconds orbitally, and absorption was read every minute for ten minutes at 340 nm. Net change in absorbance was calculated by subtracting the absorbance per minute of the negative control from the absorbance per minute of the samples then dividing by the extinction coefficient of 0.00503 (Chemical 2014) and multiplying by the dilution factor of 10 to give nmol of enzyme per minute per ml. The protein of each sample was estimated using the BCA method (Smith et al. 1985). Glutathione S-transferase activity was expressed as nmol per minute per mg of protein by dividing the net change in absorbance by the estimate of protein of each sample.

Statistics

Analyses of insecticide assays were conducted in SAS 9.3 using the Proc Probit procedure to determine LC_{50}s and the Proc Mixed procedure to compare LC_{50}s between regions. Insecticide rates common to all colonies for each insecticide (acephate= 30 µg/vial; permethrin= 20 µg/vial; imidacloprid= 0.6 µg/cap) were used to further examine differences between region and colonies using the Proc Mixed procedure. Region and county were independently analyzed as main effects and replicates were analyzed within colony. Analysis of GST activity was performed with the Proc Glimmix procedure of SAS 9.3. Region and county were independently analyzed as main effects and subsample within replicate, replicate within county, and subsample by replicate within county were treated as random factors.

Results

Insecticide assays

LC_{50}s ranged from 9.96 to 27.35 µg/vial for acephate, 5.84 to 43.69 µg/vial for permethrin and 0.04 to 0.24 µg/cap for imidacloprid (Table 1). Region did not have a significant effect on LC_{50} levels for acephate (df= 1,8; F= 0.77; p= 0.41), permethrin (df= 1, 8; F= 1.39; p= 0.27), or imidacloprid (df= 1,8; F= 0.84; p= 0.39). The Washington County colony was 2.7 fold more tolerant of acephate than the numerically most susceptible colony from Webster County. The Washington County colony was also 7.5 fold more tolerant to permethrin than the numerically most susceptible colony from Webster County. The Yazoo County colony was 6 fold more tolerant of imidacloprid than the numerically most susceptible colony from Oktibbeha County. Table 1 shows the LC_{50}s and statistical output for each county.

As expected given that LC_{50}s did not vary by region, region was not a significant factor of mortality at a common concentration of acephate (df= 1, 28; F= 0.11; p= 0.74), permethrin (df= 1, 28; F= 3.66; p=0.07), or imidacloprid (df= 1, 28; F= 3.32; p= 0.08). However, mortality did vary among counties for acephate (df= 9, 20; F=5.03; p<0.01), permethrin (df= 9, 20; F= 5.48; p<0.01), and imidacloprid (df= 9, 20; F=2.78; p= 0.03). Mortality for acephate ranged from 50 to 93 % at 30 µg/vial, mortality for permethrin ranged from 27 to 87 % at 20 µg/vail, and mortality for imidacloprid ranged from 80 to 97 % at 0.6 µg/vial. Figure 2 shows the mortality of each insecticide by county.

Glutathione S-transferase activity

Region did not have a significant effect on GST activity (df= 1, 25.79; F= 2.32; p=0.14), however county significantly affected GST activity levels (df= 9, 17.63; F=3.43; p= 0.01). GST activity ranged from 2.05 (Lowndes County) to 3.42 (Washington County) nmol/min/mg protein, a 1.7 fold difference. Figure 3 shows GST activity by county. Differences from the lowest to highest GST activity are not as pronounced as differences in mortality however the Washington County colony had the highest GST activity, which is in agreement with the LC_{50} and mortality data.

Discussion

The results of this study indicated that insecticide tolerance and GST activity are not different between the Delta and Hills regions overall, based on the five colonies tested from each region. However, region was nearly significant in regards to mortality for permethrin (p=0.07) and imidacloprid (p=0.08) suggesting higher tolerance for these insecticides in the Delta. The primary cause of this higher tolerance to permethrin primarily came from the Washington County colony and higher tolerance to imidacloprid primarily came from the Yazoo County colony. How common these more resistant populations are throughout Mississippi remains unknown. GST activity also suggested a trend (p=0.14) for higher GST activity in the Delta region but this is again possibly due to the higher GST activity in the Washington County colony alone. Additional research is being conducted to evaluate cytochrome p-450 and esterase activity levels.

Table 1. Statistics and LC_{50} estimates of *L. lineolaris* colonies from Mississippi.

			Goodness of Fit (Pearson)		Chi-Square	LC50 (µg insecticide)		
Insecticide	**Region**	**County**	**value**	**prob>Chi**	**(Wald)**	**rate**	**lower**	**upper**
Acephate	**Delta**	**Coahoma**	10.93	0.62	47.53	11.57	9.36	14.04
		Leflore	14.20	0.36	51.75	11.81	9.32	14.84
		Sunflower	5.81	0.95	43.89	14.36	10.91	18.70
		Washington	16.82	0.21	51.15	27.35	21.88	33.85
		Yazoo	11.58	0.89	29.07	11.00	8.25	14.13
	Hills	**Lowndes**	10.20	0.68	35.38	11.28	8.37	16.20
		Monroe	12.91	0.45	49.49	14.80	11.62	18.90
		Oktibbeha	14.55	0.34	38.28	12.20	9.93	14.96
		Webster	14.45	0.34	55.01	9.96	8.04	12.51
		Winston	8.28	0.83	36.85	13.79	10.67	18.77
Imidacloprid	**Delta**	**Coahoma**	12.50	0.49	31.61	0.10	0.06	0.17
		Leflore	10.67	0.64	29.37	0.08	0.04	0.12
		Sunflower	8.70	0.80	27.41	0.14	0.08	0.22
		Washington	6.94	0.91	37.50	0.11	0.07	0.16
		Yazoo	5.63	0.96	13.84	0.24	0.10	0.44
	Hills	**Lowndes**	4.37	0.99	24.34	0.11	0.05	0.18
		Monroe	11.24	0.59	37.22	0.12	0.07	0.19
		Oktibbeha	10.57	0.65	29.41	0.04	0.02	0.06
		Webster	12.11	0.52	15.19	0.10	0.04	0.21
		Winston	3.17	1.00	25.19	0.15	0.09	0.23
Permethrin	**Delta**	**Coahoma**	16.90	0.20	36.00	13.19	9.80	18.63
		Leflore	13.61	0.40	34.57	11.28	8.22	15.76
		Sunflower	13.21	0.43	43.32	11.32	9.08	13.92
		Washington	12.37	0.50	25.77	43.69	35.05	72.89
		Yazoo	6.19	0.94	49.74	11.69	9.91	14.98
	Hills	**Lowndes**	17.35	0.18	45.47	9.34	7.36	12.44
		Monroe	12.86	0.46	45.39	8.58	6.53	11.10
		Oktibbeha	8.06	0.84	22.71	13.02	9.46	21.40
		Webster	15.31	0.29	54.02	5.84	4.63	7.40
		Winston	14.39	0.35	36.86	15.49	11.99	20.61

The lack of significant regional differences does not help explain the reason for the increased number of insecticide applications required in Delta cotton and the higher amount of feeding losses to Delta cotton. These data, as well as the data of (Adams et al. 2014) indicate that there is variation in the biology and physiology of *L. lineolaris* populations in Mississippi. The reasons for these variations are unknown but may include host plant species abundance, insecticide selection pressure, obstacles to movement, or other factors. A better understanding of these factors may reveal the cause of the higher economic costs to cotton from *L. lineolaris* in the Delta region.

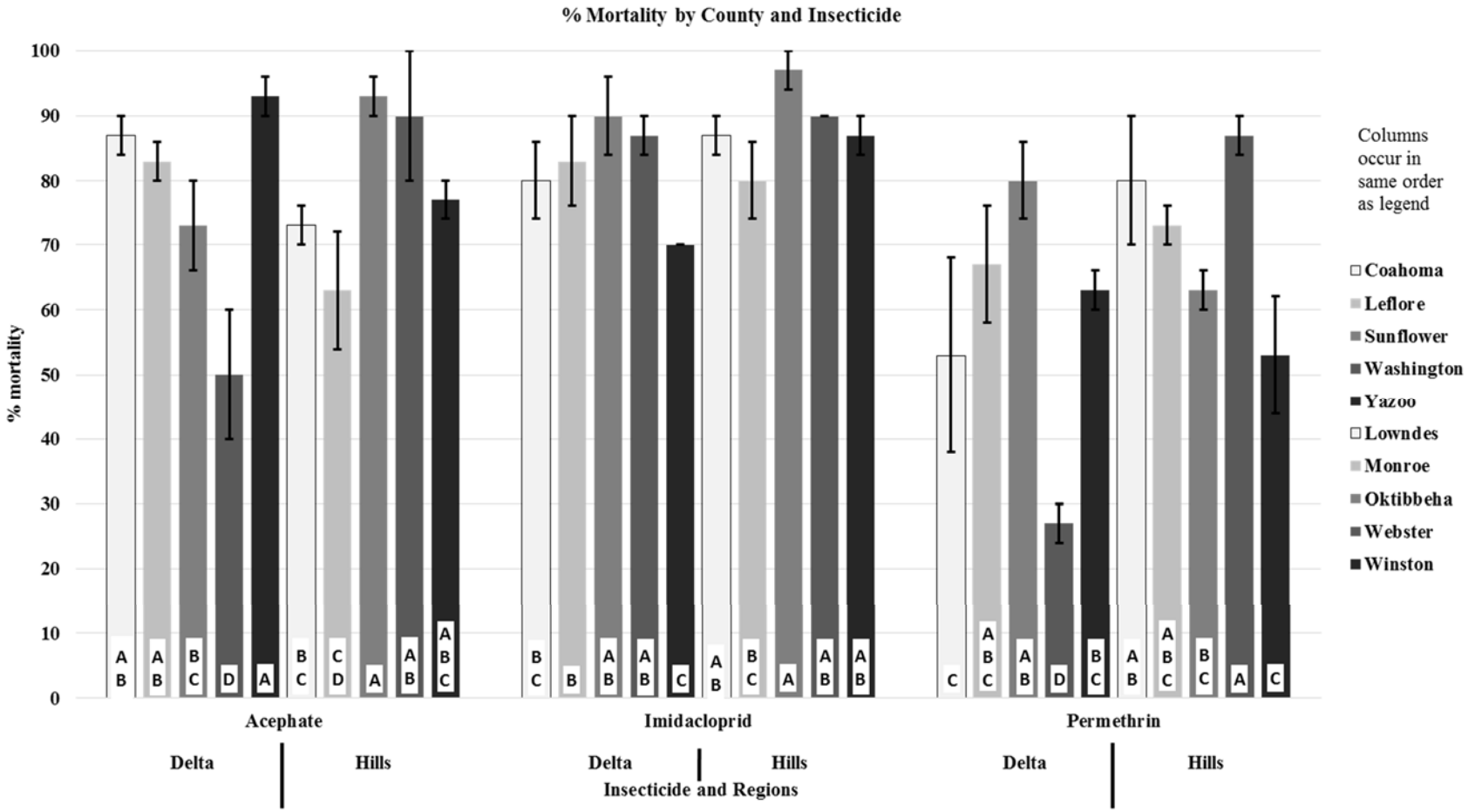

Figure 2. Mean mortality of *L. lineolaris* colonies exposed to three insecticides.

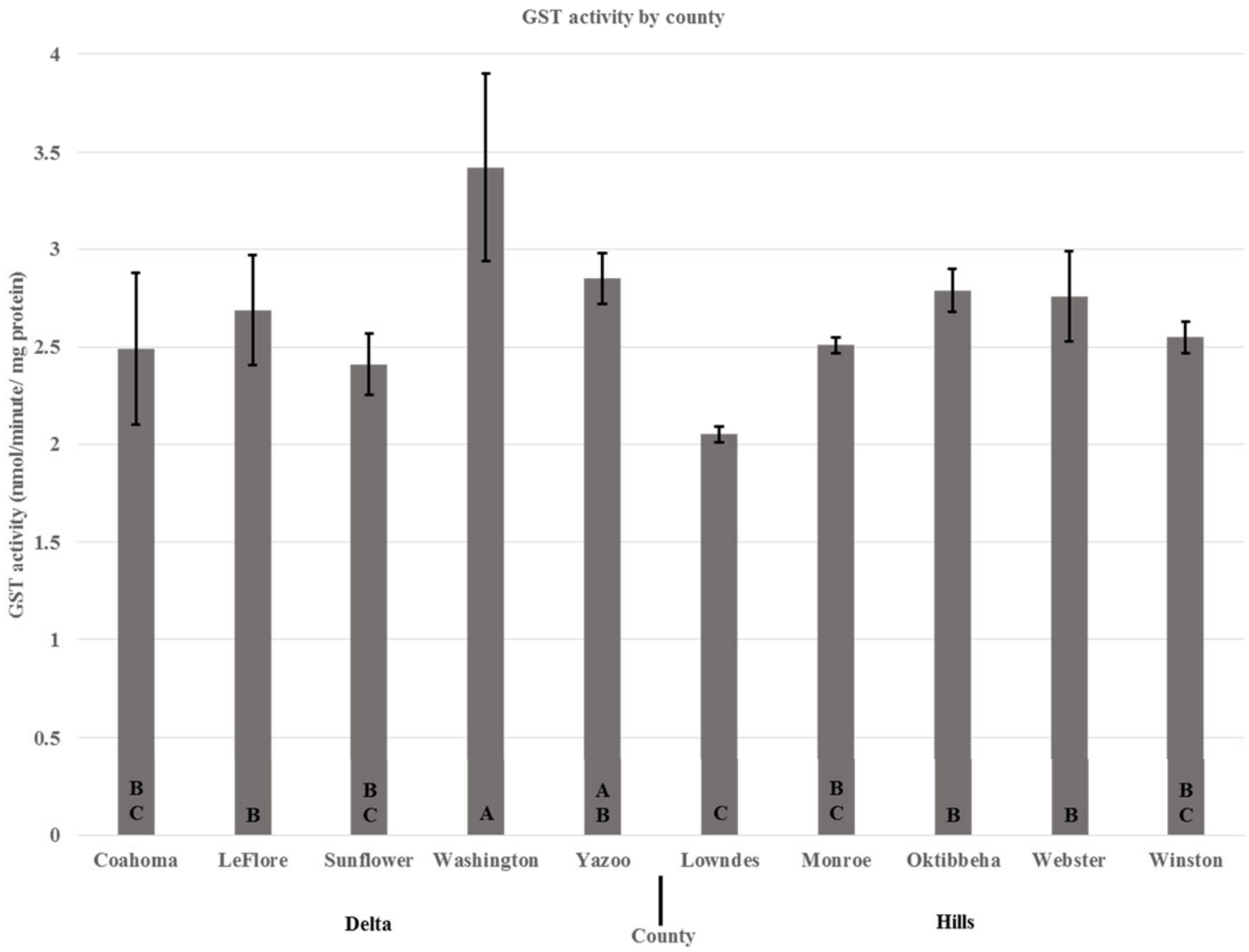

Figure 3. Mean glutathione S-transferase activity (nmol/minute/mg protein) in Mississippi *L. lineolaris* colonies.

References

Adams, B., J. Gore, A. Catchot, F. Musser, and D. Dodds. 2014. A biological comparison of tarnished plant bug (Hemiptera: Miridae) populations from Mississippi's major agricultural regions. Environ. Entomol. 43: 782-786.

Armstrong, R. N. 1997. Structure, catalytic mechanism, and evolution of the glutathione transferases. Chemical Research in Toxicology 10: 2-18.

Campbell, P. M., R. D. Newcomb, R. J. Russell, and J. G. Oakeshott. 1998. Two different amino acid substitutions in the ali-esterase, E3, conver alternative types of organophosphorus insecticide-resistance in the sheep blowfly, *Lucilia cuprina*. Insect Biochemistry and Molecular Biology 28: 139-150.

Cayman Chemical. 2014. Glutathione S-transferase assay kit. (https://www.caymanchem.com/pdfs/703302.pdf).

Cleveland, T. C., and R. E. Furr. 1980. Toxicity of methyl parathion applied topically to tarnished plant bugs. J. Georgia Entomol. Soc. 15: 304-307.

Cleveland, T. C. 1985. Toxicity of several insecticides applied topically to tarnished plant bugs. J. Entomol. Sci. 2: 95-97.

Dennehy, T. J., and J. S. Russell. 1996. Susceptibility of *Lygus* bug populations in Arizona to acephate (Orthene®) and Bifenthrin (Capture®) with related contrasts of other insecticides, pp. 771-777. *In*, Proceedings of the Beltwide Cotton Conference.

Feyereisen, R., J. F. Andersen, F. A. Carino, M. B. Cohen, and J. F. Koener. 1995. Cytochrome P450 in the house fly: structure, catalytic activity and regulation of expression of CYP6A1 in an insecticide-resistant strain. Pesticide Science 43: 233-239.

Feyereisen, R. 1999. Cytochrome P450-mediated metabolic resistance to insecticides in the house fly, *Musca domestica*. Nippon Nogeikagaku Kaishi 73: 1043-1051.

Fournier, D., A. Cuany, M. Pralavorio, J. M. Bride, and J. B. Berge. 1987. Analysis of methidathion resistance mechanisms in Phytoseilus persimilis. Pesticide Biochemistry and Physiology 28: 271-278.

Habig, W. H., M. J. Pabst, and W. B. Jakoby. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. The Journal of Experimental Biological Chemistry 249: 7130-7139.

Hollingsworth, R. G., D. C. Steinkraus, and N. P. Tugwell. 1997. Responses of Arkansas populations of tarnished plant bugs (Heteroptera: Miridae) to insecticides, and tolerance differences between nymphs and adults. J. Econ. Entomol. 90: 21-26.

Holloway, J. W., B. R. Leonard, J. A. Ottea, J. H. Pankey, J. B. Graves, and G. Snodgrass. 1998. Insecticide resistance and synergism of pyrethroid toxicity in the tarnished plant bug, *Lygus lineolaris*, pp. 947-948. *In*, Proceedings of the Beltwide Cotton Conference, San Diego, CA.

Karunaratne, S. H. P. P., and J. Hemingway. 2001. Malathion resistance and prevalence of the malathion carboxylesterase mechanism in populations of mosquito vectors of disease in Sri Lanka. Bulletin of the World Health Organization 79: 1060-1064.

Kasai, S., and J. G. Scott. 2000. Overexpression of cytochrome P450 CYP6D1 is associated with monooxygenase-mediated pyrethroid resistance in house flies from Georgia. Pesticide Biochemistry and Physiology 68: 34-41.

Liu, Z.-W., and Z.-J. Han. 2003. The roles of carboxylesterase and AChE insensitivity in malathion resistance development in brown planthopper. Acta Entomologica Sinica 46: 250-253.

Oppenoorth, F. J., V. Rupes, S. el Bashir, N. W. H. Houx, and S. Voerman. 1972. Glutathione-dependent degradation of parathion and its significance for resistance in the housefly. Pesticide Biochemistry and Physiology 2: 262-269.

Pankey, J. H., B. R. Leonard, J. B. Graves, and E. Burris. 1996. Toxicity of acephate, cypermethrin, and oxamyl to tarnished plant bugs in vial bioassays and cage studies in cotton, pp. 882-887. *In*, Proceedings of the Beltwide Cotton Conferences, Nashville, TN.

Puinean, A. M., I. Denholm, N. S. Millar, R. Nauen, and M. S. Williamson. 2010. Characterisation of imidacloprid resistance mechanisms in the brown planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae). Pesticide Biochemistry and Physiology 97: 129-132.

Raghavendra, K., S. K. Subbarao, M. K. K. Pillai, and V. P. Sharma. 1998. Biochemical mechanisms of malathion resistance in Indian *Anopheles culicifacies* (Diptera: Culicidae) sibling species A, B, and C: microplate assays and synergistic studies. Ann. Entomol. Soc. Am. 91: 834-839.

Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150: 76-85.

Smyth, K. A., T. M. Boyce, R. J. Russell, and J. G. Oakeshott. 2000. MCE activities and malathion resistances in field populations of the Australian sheep blowfly (*Lucilia cuprina*). Heredity (Edinb) 84: 63-72.

Snodgrass, G. L., and W. P. Scott. 1988. Tolerance of the tarnished plant bug to dimethoate and acephate in different areas of the Mississippi Delta, pp. 294-295. *In*, Proceedings of the 1988 Beltwide Cotton Production Research Conferences, New Orleans, LA.

Snodgrass, G. L. 1994. Pyrethroid resistance in a field population of the tarnished plant bug in cotton in the Mississippi delta, pp. 1186-1187. *In*, Proceedings of the 1994 Beltwide Cotton Conference, San Diego, CA.

Snodgrass, G. L., and G. W. Elzen. 1995. Insecticide resistance in a tarnished plant bug population in cotton in the Mississippi Delta. Southwest. Entomol. 20: 317-323.

Snodgrass, G. L. 1996a. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89: 783-790.

Snodgrass, G. L. 1996b. Glass-vial bioassay to estimate insecticide resistance in adult tarnished plant bugs (Heteroptera: Miridae). J. Econ. Entomol. 89: 1053-1059.

Snodgrass, G. L., and W. P. Scott. 1999. A discriminating-dose bioassay for detecting pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations. Southwest. Entomol. 24: 301-308.

Snodgrass, G. L., and W. P. Scott. 2000. Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations during a three-year period in the delta area of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 93: 441-446.

Snodgrass, G. L., and W. P. Scott. 2002. Tolerance to acephate in tarnished plant bug (Heteroptera: Miridae) populations in the Mississippi River Delta. Southwest. Entomol. 27: 191-199.

Snodgrass, G. L., and W. P. Scott. 2003. Effect of ULV malathion use in boll weevil (Coleoptera: Curculionidae) eradication on resistance in the tarnished plant bug (Heteroptera: Miridae). J. Econ. Entomol. 96: 902-908.

Snodgrass, G. L., J. Gore, C. A. Abel, and R. Jackson. 2009. Acephate resistance in populations of the tarnished plant bug (Heteroptera: Miridae) from the Mississippi River delta. J. Econ. Entomol. 102: 699-707.

Tomita, T., N. Liu, F. F. Smith, P. Sridhar, and J. G. Scott. 1995. Molecular mechanisms involved in increased expression of a cytochrome P450 responsible for pyrethroid resistance in the housefly, *Musca domestica*. Insect Mol Biol 4: 135-140.

Valles, S. M. 1998. Toxicological and biochemical studies with field populations of the German cockroach, *Blatella germanica*. Pesticide Biochemistry and Physiology 62: 190-200.

Williams, M. R. 1979-2012. Cotton crop loss data. (http://www.biochemistry.msstate.edu/resources/cottoncrop.asp).

Zhu, Y. C., and G. L. Snodgrass. 2003. Cytochrome P450CYP6X1 cDNAs and mRNA expression levels in three strains of the tarnished plant bug Lygus lineolaris (Heteroptera : Miridae) having different susceptibilities to pyrethroid insecticide. Insect Mol Biol 12: 39-49.

Zhu, Y. C., G. L. Snodgrass, and M. S. Chen. 2004. Enhanced esterase gene expression and activity in a malathion-resistant strain of the tarnished plant bug, Lygus lineolaris. Insect Biochemistry and Molecular Biology 34: 1175-1186.

Zhu, Y. C., G. L. Snodgrass, and M. S. Chen. 2007. Comparative study on glutathione S-transferase activity, cDNA, and gene expression between malathion susceptible and resistant strains of the tarnished plant bug, *Lygus lineolaris*. Pesticide Biochemistry and Physiology 87: 62-72.

Zhu, Y. C., S. West, G. Snodgrass, and R. Luttrell. 2011. Variability of resistance-related enzyme activities in field populations of the tarnished plant bug, *Lygus lineolaris*. Pesticide Biochemistry and Physiology 99: 265-273.

Zhu, Y. C., Z. Guo, Y. He, and R. Luttrell. 2012. Microarray analysis of gene regulations and potential association with acephate-resistance and fitness cost in *Lygus lineolaris*. PLoS ONE 7: 1-17.

Zhu, Y. C., and R. Luttrell. 2012. Variation of acephate susceptibility and correlation with esterase and glutathione S-transferase activities in field populations of the tarnished plant bug, *Lygus lineolaris*. Pesticide Biochemistry and Physiology 103: 202-209.

Zhu, Y. C., and R. Luttrell. 2014. Altered gene regulation and potential association with metabolic resistance development to imidacloprid in the tarnished plant bug, *Lygus lineolaris*. Pest Management Science in press: in press