TOXICITY OF SELECTED INSECTICIDES AND INSECTICIDE MIXTURES ADULT BROWN STINK BUGS (HETEROPTERA: PENTATOMIDAE)

Juan D. López, Jr.
M. A. Latheef
USDA-ARS, APMRU
College Station, TX
Bill Ree
Texas AgriLife Extension
Bryan, TX

Abstract

Glass vial bioassays were conducted to evaluate the toxicity of selected insecticides and insecticide mixtures to brown stink bug (BSB), *Euschistus servus* (Say) collected from black light traps, cotton plants and weeds in farming areas in the Brazos Valley of Texas. The organophosphate dicrotophos was 5- and 18-fold more toxic than acephate and chlorpyrifos, respectively. The order of toxicity to BSB for synthetic pyrethroids was: bifenthrin = zeta-cypermethrin = gamma-cyhalothrin > lambda cyhalothrin > cypermethrin. Mixtures of technical insecticides prepared in the same proportions as commercial formulations were significantly more toxic than their commercial formulations to BSB. The lack of potentiation of the mixtures may be due to absence of additivity or synergism in the composition of active and inert ingredients used in the formulations or the decreased concentrations of each component in the formulated mixtures. Tolerance to lambda-cyhalothrin and cypermethrin by BSB increased 2- and 3-fold from 2005 through 2009, and 2006 through 2007, respectively,. Increased tolerance to synthetic pyrethroids in field-collected BSB may have contributed to increased captures of BSB in black light traps in 2007 and greater and later occurrence on cotton and weeds in the Brazos Valley during 2009.

Introduction

The most important stink bug pests of cotton in the United States are the southern green stink bug (SGSB), *Nezara viridula* (L.), green stink bug (GSB), *Acrosternum hilare* (Say), and brown stink bug (BSB), *Euschistus servus* (Say) (Roach 1988). These pests have long been occasional and secondary pests of cotton with little economic importance in the United States (Swan and Papp 1972; McPherson and McPherson 2000). However, several workers have reported that the eradication of the boll weevil and the adoption of Bollgard® cotton has caused reduction in pesticide applications in the Southeast and other areas of the Cotton Belt and that this decline in insecticide use has probably allowed other insects such as stink bugs to assume greater importance as economically important pests of cotton (Turnipseed *et al*. 1995; Greene *et al*. 1999; Roof and Arnette 2000; Emfinger *et al*. 2001; Karner and Goodson 2002). Stink bugs cause hard-lock and boll rot from feeding on the bolls that is associated with pathogens (Medrano *et al*. 2009) and which contributes to loss in lint and seed cotton yield (Emfinger *et al*. 2004, Willrich *et al*. 2004a, 2004b, 2004c). Insecticides still remain the only tool to control stink bugs (Emfinger *et al*. 2001, Willrich *et al*. 2003). It is, therefore, important to evaluate stink bugs' susceptibility to insecticides year over year, and to assess the efficacy of newly released formulations against adult species of the insect. We selected BSB for this study because it is recognized as being more difficult to control with insecticides, is becoming more prevalent in the Brazos Valley, and unlike SGSB, has not been evaluated extensively for susceptibility to insecticides in the study area.

The objectives of this study were to evaluate *E. servus* adult susceptibility to selected technical insecticides, assess temporal variation in toxicity and determine efficacy of mixtures of such technical insecticides vis-à-vis their commercial formulations.

Materials and Methods

Insects

Brown stink bug adults were captured in blacklight (BL) traps operated adjacent to cotton fields in Burleson CO., near College Station, TX. Each trap was equipped with a 40-W fluorescent lamps (40 BL) mounted vertically between four baffles and above a funnel (30 inches diameter) (Hollingsworth and Hartstack 1972). The original collection containers were replaced with an inverted capture canister from a Texas cone trap (Hartstack *et al.* 1979) to keep the insects alive. Pieces from paper bags were crinkled and placed inside the containers to allow the adults to separate from the other insects captured by climbing on the paper. Stink bugs were collected from BL traps each day and brought to the laboratory and kept in a walk-in chamber maintained at 55° F. Stink bugs were sorted from the rest of the captured insects and placed inside a cage containing green beans, *Phaseolus vulgaris* (L.), fresh sweet corn, *Zea mays* (L.), and 10% sucrose solution (wt:vol) in a 4-oz Glad® plastic cup with a lid through which two cotton wicks each 6 inches long were inserted. Adult BSB were also collected from late cotton by placing an insect net with the opening close to the terminals and hitting them with a stick to to catch the adults. Adults were also collected from weeds on turn rows close to cotton either in the same way as from cotton or manually. Collected adults were placed in ziplock bags and taken to the laboratory for caging as previously described. They were tested within 2-3 days of capture.

Insecticides

Technical insecticides were purchased from ChemService, West Chester, PA., and were stored in a freezer. The purity of the technical insecticides averaged 98%. Stock cultures of various insecticides were prepared by weighing the chemical in a Sartorius® Balance (Model No. LA120S) accurate to 0.0001 g. Stock solutions of technical grade insecticides were mixed with acetone (assay 99.5%). Formulated insecticides, Cobalt™ (clorpyrifos -30%, gamma-cyhalothrin – 0.54%), Hero™ (zeta-cypermethrin – 3.75%, bifentrhin – 11.25%) and Endigo ™ (lambda-cyhalothrin – 9.48%, thiamethoxam – 12.6%) were mixed with 95% ethyl alcohol to enhance solubility. Various concentrations of the insecticides were then prepared using the serial dilution procedure. All insecticide solutions were kept in a freezer for 30 days, and discarded and thereafter, fresh solutions were prepared.

AVT

Adult vial test (AVT) procedures were similar to those described earlier (Plapp *et al*. 1987; Snodgrass 1996; Emfinger *et al*. 2001; Willrich *et al*. 2003). One-half ml of each concentration was pipetted into a 20-ml scintillation vial. Vials were placed over a hot dog roller (Star Grill-Max®) using the lowest heat until the acetone was evaporated leaving behind a thin film of insecticide residues inside the vials.

Regardless of sex, stink bugs were placed inside each insecticide-treated vial at two adults per vial, and the mouth of the vial was closed with a boll of cotton. An untreated control was maintained for all tests. Vials were kept in an environmental room maintained at 80° F, RH >60% and a photoperiod of 14:10 h L:D. Mortality was determined after 24 h. Stink bugs were considered dead when they could not right themselves after placing them upside down on a paper towel. Lethal concentration (LC) values for the mortality data were computed using Proc Probit procedure (SAS 2002, version 9.2). LC values were significantly different if 95% confidence limits (CL) did not overlap.

Results and Discussion

The dosage mortality equations provided good fit for all technical insecticides for 24 hour responses (Table 1). The data are presented by insecticide classes based on LC_{50}s (Lower-Upper Confidence Limits). LC_{50} values of selected organophosphates and pyrethroids for BSB are shown in Table 1. Among the organophosphates tested, dicrotophos was the most toxic insecticide with a LC_{50} value of 0.30 (0.24-0.37: 95% CLs) µg /vial at 24 h. Dicrotophos was 5- and 18-fold more toxic than acephate and chlorpyrifos, respectively, to brown stink bug. Willrich et al. (2003) reported that acephate was more toxic to BSB in Louisiana than dicrotophos. This suggests that AVT bioassay for different insecticides is required on a regional basis because of differences in environment, insecticide usage patterns, and cropping patterns. Bifenthrin, zeta-cypermethrin and γ-cyhalothrin were similar in toxicity to BSB with LC_{50} values being 0.27 (0.18-0.51) µg/vial, 0.33 (0.27-0.43) µg/vial, and 0.35 (0.30-0.42) µg/vial, respectively. Cypermethrin was the least toxic pyrethroid with LC_{50} value of 1.35 (1.01-1.90) µg./vial and was significantly different from λ-cyhalothrin with LC_{50} value of 0.78 (0.64-0.94) µg/vial.

The contact toxicity of mixtures of technical insecticides and their commercial formulations is presented in Table 2. Cobalt technical was more toxic to BSB with the LC_{50} being 75.56 (55.92-97.33) µg/vial than its commercial formulation with an LC_{50} of 75.56 (55.92-97.33) µg A.I./vial. Similarly, Hero technical with an LC_{50} of 0.02 µg /vial (0.002-0.03) was more toxic to BSB than its commercial formulation with an LC50 of 5.14 A.I.µg/vial (4.66-6.21). Also, Endigo technical was more toxic to BSB with the LC_{50} being 0.18 (0.14-0.22) µg./vial than its commercial formulation with its LC_{50} being 32.00 (25.79-43.74) µg A.I./vial. These data suggest that the lack of potentiation of the insecticide mixtures in commercial formulations may be due to absence of additivity or synergism in the composition of active and inert ingredients or the lower concentrations of each component in the mixture formulations.

Table 1. Lethal concentration (LC) (µg/vial) data (24 h) using adult vial bioassay for contact toxicity of technical insecticides to *Euschistus servus* collected in black light traps, cotton plants and weed species.

Insecticide	N	Slope ± SE	LC_{50}	95% CL	χ^2	$P > \chi^2$
Organophosphates						
Acephate	102	2.74 ± 0.47	1.38c	1.01-1.81	3.47	0.32
Dicrotophos	59	4.83 ± 1.33	0.30b	0.24-0.37	0.02	0.90
Chlopyrifos	180	3.58 ± 0.66	5.00a	4.27-5.67	1.53	0.46
Pyrethroids						
Bifenthrin	59	2.06 ± 0.46	0.27c	0.18-0.51	3.48	0.18
Cypermethrin	129	1.93 ± 0.34	1.35a	1.01-1.90	2.36	0.50
Zeta-cypermethrin	106	5.81 ± 1.10	0.33c	0.27-0.43	2.75	0.25
λ-cyhalothrin	318	1.83 ± 0.24	0.78b	0.64-0.94	5.61	0.13
γ-cyhalothrin	379	1.87 ± 0.25	0.35c	0.30-0.42	2.16	0.54

LC_{50} values were computed using Proc Probit procedures of SAS (2002). Means within each insecticide group followed by the same lower case letter are not significantly different (P=5%).

Table 2. Lethal concentration (LC) (µg/vial or µg A.I./vial) data (24 h) for contact toxicity of technical insecticide mixtures and EC formulations to *Euschistus servus* captured in black light traps using adult vial bioassay.

Insecticide Mixtures	N	Slope	LC_{50}	95% CL	χ^2	$P > \chi^2$
Cobalt Technical	185	5.95 ± 0.84	1.30b	1.18-1.44	0.38	0.94
Cobalt EC	70	3.11 ± 0.82	75.56a	55.92-97.33	0.32	0.57
Hero Technical	90	1.93 ± 0.63	0.02b	0.002-0.03	1.43	0.49
Hero EC	71	8.26 ± 2.19	5.14a	4.66-6.21	0.28	0.59
Endigo Technical	101	3.55 ± 0.60	0.18b	0.14-0.22	1.91	0.59
Endigo EC	80	3.53 ± 0.69	32.00a	25.79-43.74	0.15	0.70

LC_{50} values were computed using Proc Probit procedures of SAS (2002). Means within each column within each insecticide category followed by the same lower case letter are not significantly different (P=5%).

Figure 1 shows that BSB developed 3-fold tolerance to cypermethrin from 2005 to 2009 in the Brazos Valley of Texas. The LC_{50} for cypermethrin in 2005 was 0.49 (0.29-0.93) µg A.I./vial and increased to 1.35 (1.01-1.90) µg A.I./vial in 2009. Similarly, LC_{50} for λ-cyhalothrin was twice as high in 2007 compared to 2006. Furthermore, there was evidence of increased numbers of BSBs captured in BL traps in 2007 (Fig. 3), and much higher numbers of BSB were observed/collected from cotton plants and weeds in the study area late in the season in 2009.

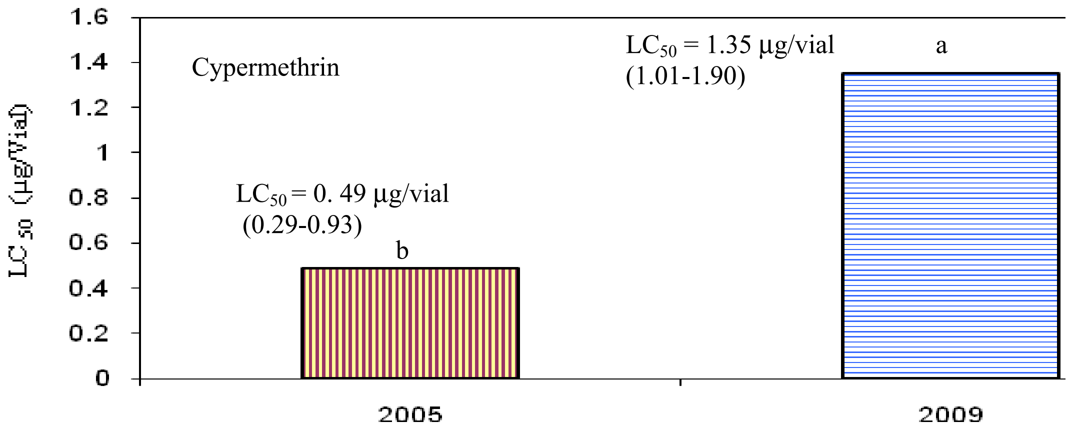

Figure 1. Contact toxicity of cypermethrin to brown stink bug during 2005 and 2009. Means followed by the same lower case letter are not significantly different (*P*=5%)

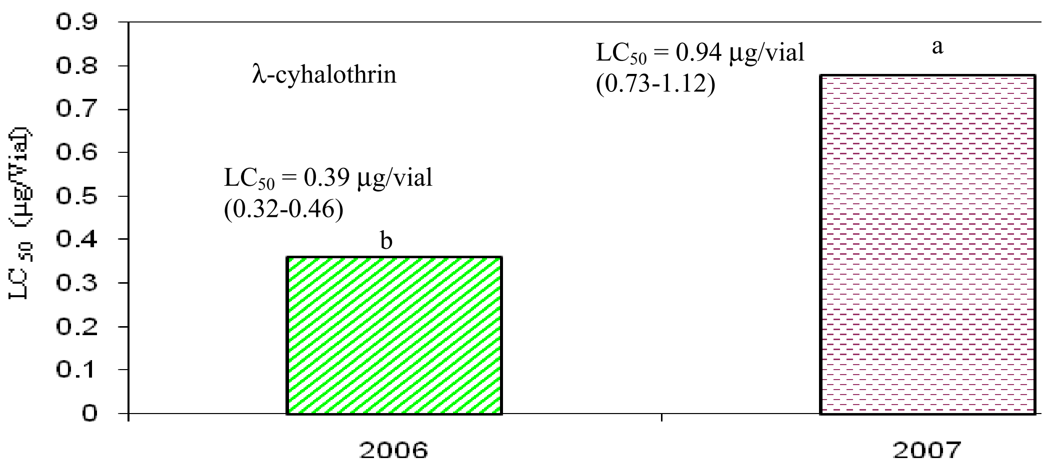

Figure 2. Contact toxicity of λ-cyhalothrin to brown stink bug during 2006 and 2007. Means followed by the same lower case letter are not significantly different (*P*=5%).

Figure 3. Relative abundance of brown stink bugs captured in BL traps in the Brazos Valley during August from 2003 through 2009.

Acknowledgements

We thank Curtis Hubbard, Chris Parker, Parker Knutson, and Lori Nemec for technical help in this study.

Disclaimer

Mention of a commercial or proprietary product does not constitute an endorsement for its use by the U. S. Department of Agriculture.

References

Emfinger, K., B.R. Leonard, J. Gore, and D. Cook. 2001. Insecticide toxicity to southern green, *Nezara viridula* (L.), and brown, *Euschistus servus* (Say), stink bugs. Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis, TN, pp. 1159-1161.

Emfinger, K.D., B.R.Leonard, M.M. Willrich, J.D. Siebert, J.H. Fife, J.S. Russell, J. Gore, and J.J. Adamczyk. 2004. Defining boll and yield tolerance to late-season cotton insect pests in Louisiana. Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis, TN, pp. 278-281.

Greene, J.K., S.G. Turnipseed, M.J. Sullivan, and G.A. Herzog. 1999. Boll damage by the southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 92:941-944.

Hartstack, A.W., J.A. Witz, and D.R. Buck. 1979. Moth traps for the tobacco budworm. J. Econ. Entomol. 72:519-522.

Hollingsworth, J.P., and A.W. Hartstack, Jr. 1972. Effects of components on insect light trap performance. Trans. ASAE. 15:924-927.

Karner, M., and J. Goodson. 2002. Stink bugs – an emerging pest in Oklahoma cotton fields. Proc. Beltwide Cotton Prod. Res. Conf, National Cotton Council of America, Memphis, TN, pp. 3.

McPherson, J.E., and R.M. McPherson. 2000. Stink bugs of economic importance in America north of Mexico. Chapter 4, CRC Press, Boca Raton, FL, pp. 77-99.

Medrano, E.G., J. Esquivel, A. Bell, J. Greene, P. Robets, J. Bacheler, J. Marois, D. Wright, R. Nichols, and J. Lopez. 2009. Potential for *Nezara viridula* (Hemiptera: Pentatomidae) to transmit bacterial and fungal pathogens into cotton bolls. Curr. Microbiol. 59:405-412.

Plapp F.W., G.M. McWhorter, and W.H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm in Texas. Proc. Beltwide Cotton Prod. Res. Conf., pp. National Cotton Council of America, Memphis, TN, pp. 324-326.

Roach S. H. 1988. Stink bugs in cotton and estimation of damage caused by their fruiting structures. Proc. Beltwide Cotton Prod. Res. Conf, National Cotton Council of America, Memphis, TN, pp. 292-294.

Roof, M. E., and F. Arnette. 2000. Monitoring stink bugs in cotton in South Carolina. Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis, TN, pp. 1122-1123.

SAS 2002. Version 9.2, SAS Institute, Cary, NC.

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

Swan, L.A., and C.S. Papp. 1972. The common insects of North America. Harper & Row Publ. New York, NY, 750 pp.

Turnipseed, S.G., M.J. Sullivan, J.E. Mann, and M.E. Roof. 1995. Secondary pests in transgenic Bt cotton in South Carolina. *In* Proc. Beltwide Cotton Conf., National Cotton Council. pp. 768-769.

Willrich, M.M., B.R. Leonard, and D.R. Cook. 2003. Laboratory and field evaluations of insecticide toxicity to stink bugs (Heteroptera: Pentatomidae). J. Cotton Sci. 7:156-163.

Willrich, M.M., B.R. Leonard, and G.B. Padgett. 2004a. Influence of southern green stink bug, *Nezara viridula* L. on late-season yield losses in cotton, *Gossypium hirsutum* L. Environ. Entomol. 33:1095-1101.

Willrich, M.M., B.R. Leonard, and J. Temple. 2004b. Injury to preflowering and flowering cotton by brown stink bug and southern green stink bug. J. Econ. Entomol. 97:924-933.

Willrich, M. M., B. R. Leonard, R. H. Gable, and L. R. Lamotte. 2004c. Boll injury and yield losses in cotton associated with brown stink bug (Heteroptera: Pentatomidae) during flowering. J. Econ. Entomol. 97:1928-1934.