MODIFIED AVT: SUSCEPTIBILITY OF STINK BUGS TO SELECTED INSECTICIDES

M.M. Willrich, K. Emfinger, B.R. Leonard, D.R. Cook and J. Gore
Louisiana State University Agricultural Center
Louisiana Agricultural Experiment Station
Baton Rouge, LA

Abstract

The adult vial test (AVT) was modified to evaluate insecticide susceptibility of two species of stink bugs, brown stink bug, *Euschistus servus* (Say), and southern green stink bug, *Nezara viridula* (L.), encountered in Louisiana. Acephate, dicrotophos, bifenthrin, cypermethrin, and *lambda*-cyhalothrin were tested between species, life stages, and over the duration of the 2001 growing season. There were no differences in responses to an insecticide between early and late-season collections within each species. Brown stink bug adults were most sensitive to acephate, bifenthrin, and cypermethrin as compared to *lambda*-cyhalothrin. In contrast, southern green stink bug adults were highly sensitive to bifenthrin and *lambda*-cyhalothrin. The *lambda*-cyhalothrin LC_{50} for brown stink bug adults was 51 times higher than that of southern green stink bug adults. Southern green stink bug nymphs were significantly less sensitive to *lambda*-cyhalothrin than were adults.

Introduction

During the last five years, abundance and occurrence of stink bugs, including brown stink bug, *Euschistus servus* (Say), and southern green stink bug, *Nezara viridula* (L.), has increased in mid-south and southeastern cotton, *Gossypium hirsutum* L., producing states. In Louisiana, the number of acres infested with stink bug species in 1995 through 2000 has risen from 8,367 to 374,699, respectively (Williams 1996, 2001). During the 2001 growing season in Louisiana, one to two insecticide applications per acre were targeted against stink bugs (R.D. Bagwell, personal communication).

The rise in pest status of this insect complex may be related to a general reduction in the use of broad-spectrum insecticides. Eradication of the boll weevil, *Anthonomous grandis grandis* Boheman, development of selective insecticides that are less toxic to sucking insects and coleopterans, and introduction of genetically engineered *Bacillus thuringiensis* (*Bt*) cotton have contributed to fewer insecticide applications against cotton pests (Greene and Herzog 1999, Leonard et al. 1999, Roberts 1999, Bachelor and Mott 2000, Roof and Arnette 2000).

Initiating control measures against stink bugs in cotton requires more than detecting the pest and determining the infestation level. Proper identification of species and developmental stages is necessary because insecticide susceptibility varies among species and life stages (McPherson et al. 1979). McPherson et al. (1979) demonstrated that *Edessa bifida* (Say) adults had a significantly higher LD_{50} than adults of other stink bug species when exposed to methyl parathion. The LD_{50}'s for southern green stink bug, green stink bug, *Acrosternum hilare* (Say), and brown stink bug fifth instar nymphs also were higher than that for their corresponding adults.

Recent field and laboratory studies indicate brown stink bug is more difficult to control compared to southern green stink bug. *Lambda*-cyhalothrin has not provided satisfactory control of brown stink bug in the field (Fitzpatrick et al. 2001). Additionally, Emfinger et al. (2001) demonstrated pyrethroids (cypermethrin, *lambda*-cyhalothrin, and cyfluthrin), with the exception of bifenthrin, to be significantly more toxic to southern green stink bug compared to brown stink bug in the adult vial test (AVT). Beginning in 2001, insecticide recommendations for cotton and soybean, *Glycine max* (L.), in Louisiana were separated for brown stink bug and southern green/green stink bug (Bagwell et al. 2001, Baldwin et al. 2001).

Due to the differences in insecticide susceptibility among stink bug species, and the increasing importance of these pests in cotton and soybean, monitoring for insecticide resistance and changes in susceptibility are critical. The AVT was initially developed by Plapp et al. (1987) for adult tobacco budworm, *Heliothis virescens* (F.), and has since been modified for adults and/or larvae of several other insect species, including tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Campanhola and Plapp 1989, Mink et al. 1993, Archer et al. 1994, Snodgrass 1996). The AVT is an attractive monitoring tool because it requires inexpensive equipment, offers rapid results, and can be used by growers, crop consultants, and researchers (Plapp et al. 1987, Snodgrass 1996). There is limited data available on stink bug susceptibility to insecticides and the AVT could become important for establishing baseline dose mortality data.

The objectives of these studies were 1) to evaluate the AVT for stink bug adults and nymphs, 2) to compare insecticide susceptibility to acephate, dicrotophos, bifenthrin, cypermethrin, and *lambda*-cyhalothrin between species and life stages, and

3) to determine if susceptibility changes during the growing season (early-season vs late-season) within a species. These data will serve as a reference for future stink bug monitoring and insecticide susceptibility programs in Louisiana.

Materials and Methods

Insects

Brown stink bug adults and southern green stink bug adults and nymphs were obtained early-season (May and June 2001) from mustard, *Brassica* spp., and corn, *Zea mays* L., and late-season (August and September 2001) from soybean in northeast Louisiana. Insects were collected using a standard 38.1 cm diameter sweep net or hand-removed from the plant. Collections were made ca. 24-h prior to insecticide exposure in the AVT. Insects were held in a polypropylene cage (30.0 x 30.0 x 30.0 cm, BugDorm, Megaview Science Education Services CO. Ltd., Taichung, Taiwan) to reduce mortality from physical injury and to eliminate parasitized insects. The stink bugs were fed washed green beans, *Phaseolus vulgaris* (L.), and shelled peanuts, *Arachis hypogae* L.

Adult Vial Test

AVT procedures similar to those described by Plapp et al. (1987) were used to evaluate the activity of dicrotophos, bifenthrin, *lambda*-cyhalothrin, cypermethrin, and acephate against brown stink bug adults and southern green stink bug adults and nymphs (4-5th instars). Stock solutions of acephate (99.6% w/w, Valent USA Corporation, Walnut Creek, CA), dicrotophos (98% w/w, Chem Service, West Chester, PA), bifenthrin (99% w/w, Chem Service, West Chester, PA), cypermethrin (59% w/w *cis*, 39% w/w *trans*, Chem Service, West Chester, PA), and *lambda*-cyhalothrin (98.7% w/w, Syngenta Ag Products, Richmond, CA) were developed by dissolving technical grade samples in acetone. Serial dilutions were made from each stock solution to yield the desired insecticide concentrations. The number and range of concentrations varied for each insecticide [(insecticide, number of concentrations, range of concentrations in µg/vial); (acephate, 7, 0.05-1.0); (dicrotophos, 7, 0.1-5.0); (bifenthrin, 8, 0.05-2.5); (cypermethrin, 8, 0.25-10.0); (*lambda*-cyhalothrin, 8, 0.05-2.5)]. The interior surface of 20 ml glass scintillation vials was coated with insecticide by pipetting 0.5 ml of the appropriate insecticide solution into the vials. These vials were rotated on a modified hot dog roller (heating element disconnected) until all of the acetone had evaporated. Vials were stored in a dark environment until used in bioassays.

Stink bugs were placed into insecticide-treated and non-treated vials (1 adult or nymph/vial). A minimum of 10 insects for each particular species and/or life stage were subjected to a dose for each bioassay. No food was provided for insects during the AVT. Mortality was determined 4-h after exposure. The criterion for mortality was the inability of the insect to assume an upright posture after being dislodged from the vial. Bioassays conducted within a three week period for a particular species and/or life stage were pooled for data analysis. Mortality for treated vials was corrected for natural mortality in the non-treated vials using using Abbott's formula (Abbott 1925). Corrected mortality data was subjected to probit analysis using Polo PC (LeOra Software 1987) and LC_{50} and 95% confidence intervals were estimated. LC_{50} values were considered significant if 95% confidence intervals did not overlap.

Results and Discussion

There was no significant difference in response of brown stink bug adult populations to insecticides between early and late-season collections (Tables 1, 2, 3). Two collections of southern green stink bug adults were exposed to bifenthrin, and there was no significant difference in the response (Table 1). These data suggest stink bug susceptibility did not change between early and late-season collections. Data for early and late-season collections were pooled within species to compare overall toxicity among insecticides.

Lambda-cyhalothrin was significantly more toxic than bifenthrin to southern green stink bug adults (Figure 1). The LC_{50} of *lambda*-cyhalothrin and bifenthrin was 0.05 and 0.24 µg/vial, respectively. Southern green stink bug nymphs (4-5th instar) were significantly less sensitive (8.5 times) to *lambda*-cyhalothrin than adults.

LC_{50}'s of brown stink bug adults were 0.17, 0.39, 0.87, 1.09, and 2.55 µg/vial for acephate, bifenthrin, cypermethrin, dicrotophos, and *lambda*-cyhalothrin, respectively (Figure 2). Brown stink bug adults were significantly more sensitive to acephate than all other insecticides tested. In contrast, *lambda*-cyhalothrin was significantly less toxic to brown stink bug adults relative to all other insecticides, with exception of dicrotophos. There were no differences in responses between cypermethrin and dicrotophos.

Southern green stink bug adults are significantly more sensitive to *lambda*-cyhalothrin than brown stink bugs. The LC_{50} of brown stink bug adults to *lambda*-cyhalothrin was 51 times higher than that of southern green stink bug adults. This is consistent with Emfinger et al. (2001) in which LC_{50}'s for brown stink bugs and southern green stink bugs were 0.47 and 0.11 µg/vial, respectively. When LC_{50}'s for southern green stink bug nymphs and brown stink bug adults were compared, the difference in response to *lambda*-cyhalothrin between species was [still significantly different but] dramatically reduced. The LC_{50} for brown stink bug adults was 6.3 times higher than that of southern green stink bug nymphs. Bifenthrin toxicity between both species of adults was similar.

Acknowledgments

The authors wish to thank the numerous student workers at the Macon Ridge Research Station for their assistance with these studies. Also, the authors wish to thank the LSU Ag Center, Cotton Incorporated, and Louisiana's cotton producers for their financial support.

References

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Archer, T.L., E.D. Bynum, Jr., and F.W. Plapp, Jr. 1994. Chlorpyrifos resistance in greenbugs (Homoptera: Aphididae): cross-resistance and synergism. J. Econ. Entomol. 87: 1437-1440.

Bachelor, J.S. and D.W. Mott. 2000. Small plot and large-scale comparison of stink bug damage to Bollgard and conventional cotton in North Carolina, pp. 1117-1119. *In* Proceedings 2000 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Bagwell, R.D., J.W. Barnett, B.R. Leonard, G. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2001. Cotton Insect Control, 2001. Louisiana Cooperative Extension Service. Pub. no. 1083.

Baldwin, J.L., D.J. Boethel, and B.R. Leonard. 2001. Control Soybean Insects, 2001. Louisiana Cooperative Extension Service. Pub. No. 2211.

Campanhola, C. and F.W. Plapp, Jr. 1989. Pyrethroid resistance in the tobaco budworm (Lepidoptera: Noctuidae): insecticide bioassays and field monitoring. J. Econ. Entomol. 82: 22-28.

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, pp. 1159-1161. *In* Proceedings 2001 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Fitzpatrick, B.J., M.M. Willrich, D.J. Boethel, and B.R. Leonard. 2001. Evaluation of insecticides against stink bug on soybean, 2000. Arthropod Management Tests. Vol. 26 F96, http://frontpage.entsoc.aa.psiweb.com/Protected/AMT/AMT26/F96.asp.

Greene, J.K. and G.A. Herzog. 1999. Management of stink bugs using symptoms of boll injury as a monitoring tool, pp. 1041-1044. *In* Proceedings 1999 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Leonard, B.R., J.B. Graves, and P.C. Ellsworth. 1999. Insect and mite pests of cotton. *In* Cotton: origin, history, technology, and production C.W. Smith [ed.]. John Wiley and Sons, Inc: New York.

LeOra Software. 1987. POLO-PC a user's guide to Probit or Logit analysis. LeOra Software, Berkeley, CA.

McPherson, R.M., J.B. Graves, and T.A. Allain. 1979. Dosage-mortality responses and field control of seven pentatomids associated with soybean, exposed to methyl parathion. Environ. Entomol. 8: 1041-1043.

Mink, J.S., D.J. Boethel, and B.R. Leonard. 1993. Monitoring permethrin resitance in soybean looper (Lepidoptera: Noctuidae) adults. J. Entomol. Sci. 28: 43-50.

Plapp, F. W., G. M. McWhorter, and W.H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm in Texas-1986, pp. 324-326. *In* Proceedings 1987 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Roberts, P. 1999. Observations of emerging pests in low spray environments, p. 1034. *In* Proceedings 1999 Beltwide Cotton Conf., National Cotton Council, Memphis, TN

Roof, M.E. and F. Arnette. 2000. Monitoring stink bugs in cotton in South Carolina, pp. 1122-1123. *In* Proceedings 2000 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

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

Williams, M.R. 1996. Cotton insect loss estimates, 1995, pp. 670-689. *In* Proceedings 1996 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Williams, M.R. 2001. Cotton insect loss estimates, 2000, pp. 774-776. *In* Proceedings 2001 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Table 1. Responses of adult brown stink bug and southern green stink bug to bifenthrin in the AVT for early and late-season collections (LC_{50} values significantly different if 95% confidence intervals did not overlap).

	Southern Green Stink Bug		
Date of collection	**n[1]**	**LC_{50}**	**95% CL**
13 June	90	0.24	0.17-0.34
21-22 Aug	280	0.24	0.09-0.55
	Brown Stink Bug		
31 May	315	0.25	0.17-0.34
12-20 Aug	270	0.28	0.09-0.55

LC values expressed in µg insecticide per vial.
[1]Number of adults tested including controls.

Table 2. Responses of adult brown stink bug, and southern green stink bug adults and nymphs to *lambda*-cyhalothrin in the AVT (LC_{50} values significantly different if 95% confidence intervals did not overlap).

Date of collection/Stage	**Southern Green Stink Bug**		
	n[1]	**LC_{50}**	**95% CL**
8-21 Aug -adult	251	0.05	0.02-0.08
6-15 Sept -nymph	320	0.40	0.26-0.68
	Brown Stink Bug		
14 June –adult	270	2.27	1.56-5.07
1-13 Aug -adult	289	2.05	1.05-9.22

LC values expressed in µg insecticide per vial.
[1] Number of insects tested including controls.

Table 3. Response of adult brown stink bug to dicrotophos in the AVT for early and late-season collections (LC_{50} values significantly different if 95% confidence intervals did not overlap).

Date of collection	**n[1]**	**LC_{50}**	**95% CL**
24-25 June	255	0.72	0.42-1.30
22-23 Aug	240	1.68	0.82-8.28

LC values expressed in µg insecticide per vial.
[1]Number of adults tested including controls.

Table 4. Response of adult brown stink bug to acephate and cypermethrin in the AVT (LC_{50} values significantly different if 95% confidence intervals did not overlap).

	Acephate			**Cypermethrin**		
Date of collection	**n[1]**	**LC_{50}**	**95% CL**	**n[1]**	**LC_{50}**	**95% CL**
6-21 June				270	0.87	0.68-1.10
19-28 June	210	0.17	0.12-0.26			

LC values expressed in µg insecticide per vial.
[1]Number of insects tested including controls.

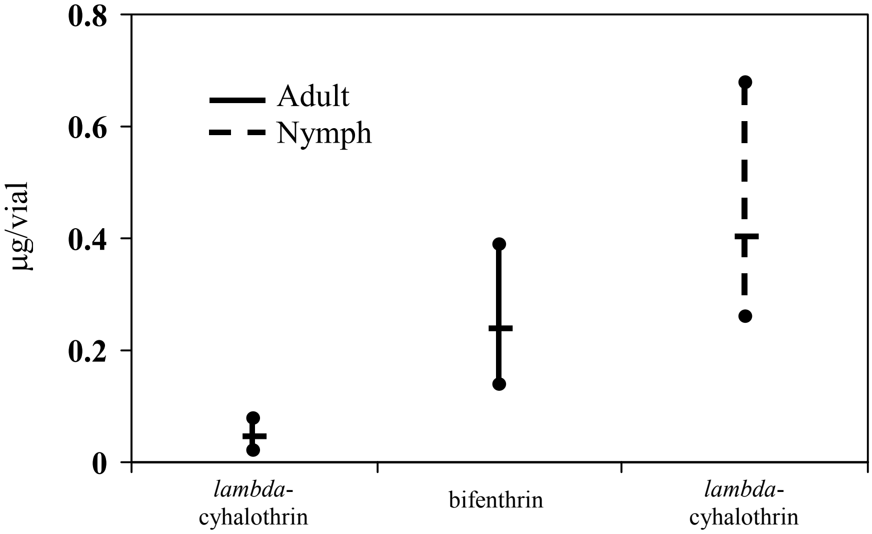

Figure 1. LC_{50} (95% CL) of southern green stink bug adults and nymphs to selected insecticides in the AVT.

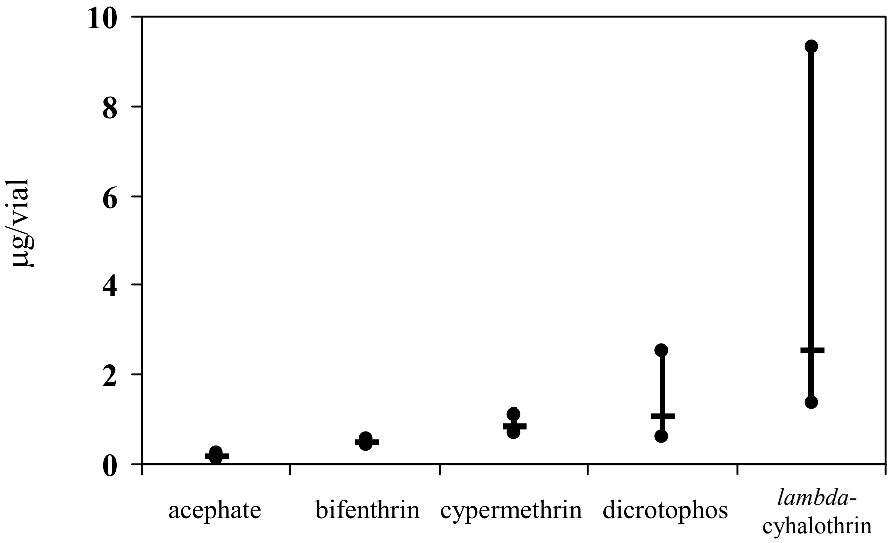

Figure 2. LC_{50} (95% CL) of brown stink bug adults to selected insecticides in the AVT.