ARTHOPOD MANAGEMENT

Laboratory and Field Evaluations of Insecticide Toxicity To Stink Bugs (Heteroptera: Pentatomidae)

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ABSTRACT

Stink bugs have become more common pests in cotton (Gossypium hirsutum L.), and the frequency of insecticides applied for their control has increased; therefore, establishing baseline insecticide mortality data is important for future resistance monitoring programs. A series of laboratory and field studies were used to characterize the susceptibility of stink bug species and selected development stages to pyrethroid and organophosphate insecticides. Stink bugs collected in the field were exposed to technical grade insecticides using the adult vial test (AVT) and to formulated products applied to cotton bolls and foliage. In the AVT, acephate was more toxic than dicrotophos to brown stink bug \( [Euschistus servus \ (\text{Say})] \) adults. Brown stink bug and southern green stink bug \( [Nezara viridula \ (L.)] \) adults were equally sensitive to dicrotophos. Generally, brown stink bug adults were most sensitive to the pyrethroid, bifenthrin (1.8- to 6.5-fold), compared with other pyrethroids. Brown stink bug adults were significantly less susceptible than southern green stink bug adults to cyfluthrin (3.9-fold), cypermethrin (2.9- to 33.8-fold), and \( \lambda \)-cyhalothrin (7.6- to 66.5-fold). The LC\(_{50}\) (µg/vial) for pyrethroids in the AVT ranged from 0.27 to 2.55, 0.06 to 0.40, and 0.02 to 0.58 for brown stink bug adults, late-instar nymphs (of all species), and southern green stink bug adults, respectively. The order of susceptibility of stink bug species and developmental stages to insecticides from least to most susceptible was adult \( Euschistus \) spp., late-instar nymphs, and southern green stink bug adults. In field studies, acephate, dicrotophos, and high rates of bifenthrin, cypermethrin, cyfluthrin, \( \beta \)-cypermethrin, and \( \lambda \)-cyhalothrin-treated plant tissue produced significant levels of brown stink bug adult mortality (52.5 to 89.2%) compared with non-treated controls (\( P \leq 0.01)\).

The occurrence of injury from stink bugs (Heteroptera: Pentatomidae) and subsequent need for chemical control has increased in mid-south and southeastern cotton producing states. Brown stink bug \( [Euschistus servus \ (\text{Say})] \), southern green stink bug \( [Nezara viridula \ (L.)] \), and green stink bug \( [Acrosternum hilare \ (\text{Say})] \), are the predominant species in a phytophagous stink bug complex. In cotton, stink bugs can induce abscission of small bolls, decrease seed cotton yield, reduce lint quality, and affect seed germination (Wene and Sheets, 1964; Barbour et al., 1990; Greene et al., 1999). During 1995 to 2001, the number of acres infested with stink bugs increased from 3.53 to over 6.18 million across the United States Cotton Belt (Williams, 1996; 2002). In Louisiana, stink bug-infested acreage increased from 8,367 to over 700,000 acres (Williams, 1996; 2002). During 2001 in Louisiana, an average of 1.5 insecticide treatments per acre was applied to control stink bugs (Williams, 2002).

Insecticides are the primary tool used to manage stink bug infestations in cotton. Proper species identification and characterization of life stages are necessary because differential susceptibility to insecticides has been reported to vary among species and life stages (McPherson et al., 1979). McPherson et al. (1979) demonstrated morningglory stink bug \( [Edessa bifida \ (\text{Say})] \), adults had a significantly higher LD\(_{50}\) to methyl parathion than adults of other stink bug species. The LD\(_{50}\) of methyl parathion for fifth instar nymphs of the southern green stink bug, green stink bug, and brown stink bug were higher than for their corresponding adults. Insecticide efficacy trials in soybean \( [Glycine max \ (L.)] \) Merrill indicate brown stink bug is more difficult to control with products recommended for southern green stink bug (Fitzpatrick et al., 2001).
In 2001, Louisiana’s insecticide recommendations for cotton IPM were refined to distinguish between brown stink bug (Euschistus spp.) and southern green stink bug/green stink bug (Bagwell et al., 2001). Only the organophosphates (acephate, dicrotophos, and methyl parathion) are recommended for control of Euschistus spp.; however, both organophosphates and pyrethroids are recommended for control of southern green stink bug and green stink bug. Several other states across the southeastern United States have similar recommendations for stink bug pest management in cotton (Patrick and Lentz, 2001; Anonymous, 2002; Bagwell et al., 2002; Boyd and Phipps, 2003; Johnson et al., 2002; Bachelor and Van Duyne, 2003; Roberts et al., 2003).

The incidence of stink bug problems in cotton will likely increase as broad-spectrum insecticide applications for key lepidopteran pests decline (Stewart et al., 2001). Therefore, defining the differences in insecticide susceptibility among species and life stages will become a critical issue for selecting an insecticide. Additionally, it is important to continually evaluate the field performance of insecticides for future insect control recommendations in cotton. This is particularly significant for Euschistus spp. Fewer insecticides are effective against these species compared with the southern green stink bug and green stink bug.

Specific studies were designed to modify the AVT for stink bugs and establish dose-mortality data for selected insecticides among species and life stages. Additionally, insecticide performance at field application rates was evaluated for control of the brown stink bug adults using treated plant tissue.

**MATERIALS AND METHODS**

**Insect collections.** Brown stink bug, southern green stink bug, and green stink bug, adults and nymphs were obtained early-season (May and June) from mustard (Brassica spp.), and corn (Zea mays L.) and late-season (August and September) from soybean in Northeast Louisiana (Franklin Parish) during 2000, 2001, 2002, and 2003. Another brown colored stink bug species (Euschistus quadator Rolston), was collected as adults from soybean in south Louisiana (Livonia, LA., Pointe Coupee Parish) during 2002. Insects were collected using a standard 38.1 cm diameter sweep net or removed by hand from plants. Collections were made ca. 24 h prior to field and laboratory studies. 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 peanut (Arachis hypogae L.) seeds.

**Laboratory studies.** AVT procedures similar to those described by Plapp et al. (1987; 1990) and Snodgrass (1996) were used to evaluate the activity of organophosphate (dicrotophos and acephate) and pyrethroid (bifenthrin, λ-cyhalothrin, cyfluthrin, and cypermethrin) insecticides against stink bug adults and nymphs (4-5th instars) during 2001, 2002, and 2003. 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), cyfluthrin (94.9% w/w, Bayer Crop Protection, Kansas City, MO), cypermethrin (59% w/w cis, 39% w/w trans, Chem Service, West Chester, PA), and λ-cyhalothrin (98.7% w/w, Syngenta Crop Protection, Greensboro, NC) were developed by dissolving technical grade samples in acetone. Dilutions were made from each stock solution to yield the desired insecticide concentrations. Insecticide concentrations (six to ten/compound/test) of organophosphate and pyrethroid insecticides ranged from 0.05 to 7.5 µg/vial and 0.001 to 10.0 µg/vial, respectively. The interior surface of 20 ml glass scintillation vials was coated with an insecticide by pipetting 0.5 ml of the appropriate insecticide solution into the vials. These vials were then 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 introduced into insecticide-treated or non-treated vials (one adult or nymph/vial). A minimum of 10 insects for each species and/or life stage were subjected to each dose within a bioassay. No food source 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 within 5 s after being dislodged from the vial. Bioassays conducted within a 3-wk 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 Abbott's formula (Abbott, 1925). Corrected mortality data was subjected to probit analysis using Polo PC (LeOra Soft-
ware, Berkeley, CA), and LC$_{50}$ and 95% confidence intervals were estimated. The LC$_{50}$ values were considered significant if 95% confidence intervals did not overlap (Robertson and Preisler, 1992).

**Field studies.** Field trials were conducted during the summers of 2001 and 2003 at the LSU AgCenter Macon Ridge Research Station (Franklin Parish). Plots were planted to the cotton cultivars ‘Suregrow 747’ (Deltapine and Land Co., Scott, MS), ‘Stoneville 4691B’ (Stoneville Pedigreed Seed Co., Memphis, TN), ‘Deltapine 458BR’ (Deltapine and Land Co., Scott, MS), ‘Stoneville 4793R’, and ‘Fiber Max 989BR’ (Bayer Crop Sciences, Research Triangle Park, NC) in trial 2001-A, 2001-B, 2001-C, 2003-A and C, and 2003-B and D, respectively. Cotton was managed using agronomic practices and pest control strategies as recommended by the LSU AgCenter. Plots were four rows on 101.6-cm centers and 15.2 m in length. Treatments were arranged in a randomized block design with four replications.

Insecticide treatments were applied to cotton plots at a growth stage of four to seven nodes above first position white flower (NAWF) and 10 to 14 nodes above the mainstem cotyledon during 2001 and 2003, respectively. Treatments included the following: acephate (Orthene 90S, 90.0% ai wt/wt, Valent USA Corporation, Walnut Creek, CA), dicrotophos (Bidrin 8EC, 82.0% ai wt/wt, Amvac Chemical Corporation, Newport Beach, CA), bifenthrin (Capture 2EC, 25.1% ai wt/wt, FMC Corporation, Philadelphia, PA), λ-cyhalothrin (Karatase Z 2.08CS, 22.8% ai wt/wt, Syngenta Crop Protection, Greensboro, NC), cypermethrin (Ammo 2.5EC, 30.6% ai wt/wt, FMC Corporation, Philadelphia, PA), z-cypermethrin (Mustang Max 0.8EC, 9.6% ai wt/wt, FMC Corporation, Philadelphia, PA), and cyfluthrin (Baythroid 2EC, 25% ai wt/wt, Bayer Crop Science, Research Triangle Park, NC).

In trials 2001-A and 2001-B, treatments were applied on 7 Aug and 14 Aug, respectively, with a high-clearance sprayer calibrated to deliver 56.1 L/ha (6.0 GPA) using TX-8 hollow cone nozzles (Spraying Systems Company; Wheaton, IL) (two/row) at 276 kPa (40 psi). In trial 2001-C, treatments were applied on 22 Aug with a hand-held CO$_2$ sprayer calibrated to deliver 131.8 L/ha (14.1 GPA) using TeeJet 8002 flat fan nozzles (Spraying Systems Company; Wheaton, IL) (two/row) at 207 kPa (30 psi). In trials 2003-A, B, C, and D, treatments were applied on 3, 10, 18, and 25 Jun, respectively, with a tractor mounted sprayer calibrated to deliver 93.48 L/ha (10 GPA) using TeeJet AI1100015VS flat fan nozzles (Spraying Systems Company; Wheaton, IL) (two/row) at 207 kPa (30 psi). At 2 to 3 h after application, 10 bolls (uppermost, first position quarter-size, one/plant) and 10 to 15 leaves (first fully expanded leaf below the last fully expanded terminal leaf) were collected per plot during 2001 and 2003, respectively. No rainfall occurred between insecticide application and removal of plant tissue in each of the trials. Each boll was placed in a 0.09 L (3.0 oz) plastic specimen vial and each leaf was placed in a Petri dish (100 x 15 mm) supplied with a moistened disk (8.9 cm) of filter paper. Vials and Petri dishes were transported to the laboratory and infested with one brown stink bug. Specimen vials and Petri dishes were stored in the laboratory under ambient conditions (ca. 26.7°C). Percentage mortality was determined at 48 h after infestation. The criterion for mortality was the inability of the insect to assume an upright posture within 5 s after being dislodged from the vial. Data were analyzed with ANOVA and treatments were compared with the non-treated control in each trial using a Dunnett’s one-tailed test (SAS Institute, Version 8.0, Cary, NC).

**RESULTS AND DISCUSSION**

**Laboratory (AVT) studies.** For brown stink bug adults exposed to organophosphate insecticides, LC$_{50}$s ranged from 0.17 to 1.26 µg/vial (Table 1). Acephate was significantly more toxic (6.4- and 7.4-fold) to brown stink bug adults than dicrotophos. The responses of southern green and brown stink bug adults to dicrotophos were not significantly different.

The LC$_{50}$s for brown stink bug adults exposed to pyrethroid insecticides ranged from 0.27 to 2.55 µg/vial (Table 1). Brown stink bug adults were most sensitive to bifenthrin and cyfluthrin, and least sensitive to λ-cyhalothrin and cypermethrin. Toxicity of λ-cyhalothrin to brown stink bug adults and *E. quadrator* adults was not significantly different in 2002.

The LC$_{50}$s for southern green stink bug adults exposed to pyrethroid insecticides ranged from 0.02 to 0.58 µg/vial. The pyrethroid, λ-cyhalothrin (5.3-, 4.8-, and 5.0-fold), was significantly more toxic than bifenthrin to southern green stink bug adults during each year. Cypermethrin toxicity to southern green stink bug adults in 2000 and in 2002 was similar to bifenthrin and λ-cyhalothrin, respectively.

Toxicity of bifenthrin was generally similar between brown stink bug and southern green stink bug.
adults; however, in 2002, brown stink bug adults were less sensitive (2.7-fold) than southern green stink bug adults. Similarly, *E. quadrator* adults were 8.1-, 17.8-, and 44.5-fold less sensitive to λ-cyhalothrin compared with southern green stink bug adults. Southern green stink bug adults were significantly more sensitive than brown stink bug adults to cyfluthrin (3.9-fold), cypermethrin (2.9- to 33.8-fold), and λ-cyhalothrin (7.6- to 66.5-fold).

The LC50s for brown stink bug and southern green stink bug nymphs exposed to pyrethroid insecticides ranged from 0.06 to 0.29 and 0.18 to 0.40 µg/vial, respectively (Table 2). Brown stink bug nymphs were significantly more sensitive to λ-cyhalothrin than bifenthrin. There were no significant differences among pyrethroids in the responses of southern green stink bug nymphs. The response of brown stink bug, green stink bug, and southern green stink bug nymphs to λ-cyhalothrin was similar during 2002. The response of brown stink bug and southern green stink bug nymphs to bifenthrin was not different.

### Table 1. Response of stink bug adults to insecticides at 4 h after exposure in the adult vial test

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Year</th>
<th>N°</th>
<th>Slope ± SE</th>
<th>LC50 (95% CL)</th>
<th>Year</th>
<th>N°</th>
<th>Slope ± SE</th>
<th>LC50 (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acephate</td>
<td>2001</td>
<td>210</td>
<td>3.14 ± 0.37</td>
<td>0.17 (0.12-0.26)</td>
<td>Southern green stink bug</td>
<td>2001</td>
<td>270</td>
<td>2.00 ± 0.22</td>
</tr>
<tr>
<td>Dicrotophos</td>
<td>2001</td>
<td>495</td>
<td>1.35 ± 0.14</td>
<td>1.09 (0.61-2.54)</td>
<td>2002</td>
<td>270</td>
<td>1.85 ± 0.21</td>
<td>0.63 (0.40-0.94)</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>2000</td>
<td>330</td>
<td>2.65 ± 0.32</td>
<td>0.47 (0.38-0.59)</td>
<td>2001</td>
<td>675</td>
<td>0.93 ± 0.11</td>
<td>0.39 (0.33-0.46)</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>2001</td>
<td>667</td>
<td>1.55 ± 0.11</td>
<td>0.59 (0.33-0.46)</td>
<td>2002</td>
<td>240</td>
<td>1.18 ± 0.15</td>
<td>0.58 (0.31-1.18)</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>2000</td>
<td>245</td>
<td>3.37 ± 0.42</td>
<td>0.92 (0.80-1.06)</td>
<td>2001</td>
<td>270</td>
<td>0.87 ± 0.11</td>
<td>0.39 (0.26-0.55)</td>
</tr>
<tr>
<td>λ-cyhalothrin</td>
<td>2000</td>
<td>335</td>
<td>1.97 ± 0.19</td>
<td>0.84 (0.71-0.99)</td>
<td>2002</td>
<td>240</td>
<td>2.52 ± 0.35</td>
<td>0.32 (0.21-0.42)</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>559</td>
<td>1.27 ± 0.15</td>
<td>2.55 (1.39-9.33)</td>
<td>2002</td>
<td>240</td>
<td>3.40 ± 0.58</td>
<td>0.10 (0.07-0.14)</td>
</tr>
</tbody>
</table>

### Table 2. Response of stink bug nymphs to insecticides at 4 h after exposure in the adult vial test

| Insecticide | Year | N° | Slope ± SE | LC50 (95% CL) | Southern green stink bug | 2001 | 240| 0.96 ± 0.14 | 0.18 (0.08-0.34) |
|-------------|------|----|------------|--------------|--------------------------|      |    |               |               |
| Bifenthrin  | 2003 | 230| 1.62 ± 0.17 | 0.29 (0.18-0.47) | 2001 | 360| 0.73 ± 0.13 | 0.40 (0.26-0.68) |
| Cypermethrin| 2002 | 200| 0.71 ± 0.12 | 0.06 (0.02-0.14) | 2002 | 200| 1.44 ± 0.15 | 1.33 (0.56-4.52) |

* Total number of insects tested including controls.
* Concentrations reported in µg insecticide per vial; LC50 values are significantly different if 95% confidence limits did not overlap.
* Response of *E. quadrator* adults to λ-cyhalothrin in 2002 [n = 222; Slope ± SE: 2.56 ± 0.37; LC50 (95% CL): 0.89 (0.67-1.14)].
The response of adults and nymphs within a species to insecticides in the AVT was significantly different (Tables 1 and 2). Brown stink bug nymphs were significantly more sensitive (22.2-fold) to \( \lambda \)-cyhalothrin compared with brown stink bug adults. Southern green stink bug nymphs were less sensitive than adults to cypermethrin and \( \lambda \)-cyhalothrin. Bifenthrin was equally toxic to brown stink bug and southern green stink bug, regardless of life stage.

Similar results have been obtained in Mississippi using the AVT (G.L. Snodgrass, personal communication). Brown stink bug adults were observed to be more tolerant to organophosphate and pyrethroid insecticides compared with green stink bug adults and southern green stink bug adults. The LC50s for brown stink bugs exposed to organophosphate and pyrethroid insecticides were 1.0- to 11.6-fold and 0.9- to 13.9-fold greater, respectively, than LC50s for southern green stink bugs and green stink bugs.

**Field studies.** In trials 2001-A, B, and C, mortality (53.5 to 85.0%) of brown stink bug adults exposed to bolls treated with acephate or dicrotophos was significantly greater than non-treated bolls \( (P \leq 0.05) \) (Table 3). Mortality of brown stink bug exposed to bifenthrin was significantly greater than on non-treated bolls \( (P \leq 0.05) \), and was similar to that of acephate and dicrotophos. Mortality of brown stink bug on bolls treated with \( \lambda \)-cyhalothrin was not significantly different from non-treated bolls.

In trial 2003-A, there was a positive relationship between the rate of \( \lambda \)-cyhalothrin applied to leaf tissue and brown stink bug mortality (Table 3). Folage treated with \( \lambda \)-cyhalothrin at 0.034 kg/ha (0.03 lb ai/acre) and 0.045 kg/ha (0.04 lb ai/acre) produced mortality of brown stink bug that was significantly greater than on non-treated foliage at \( P \leq 0.01 \) and \( P \leq 0.05 \), respectively. Mortality at lower rates of \( \lambda \)-cyhalothrin was not significantly different from non-treated foliage \( (P>0.05) \). In trials 2003-B, C, and D, all of the insecticides tested produced greater mortality of brown stink bug than non-treated foliage \( (P \leq 0.01) \).

The organophosphate insecticides, acephate and dicrotophos are currently recommended for control of brown stink bug in Louisiana. These insecticides provided mortality at 48 h ranging from 53.3 to 89.2% on bolls and foliage. The highest labeled rate of \( \lambda \)-cyhalothrin \([0.045 \text{ kg ai/ha (0.04 lb ai/acre)}]\) provided mortality of brown stink bug adults comparable to the recommended insecticides (76.7%).

High rates of other pyrethroids (bifenthrin, cypermethrin, \( \lambda \)-cypermethrin, and cyfluthrin) demonstrated mortality (52.5 to 74.7%) of brown stink bug adults comparable to acephate and dicrotophos.

Field trials in cotton and soybean have provided evidence that organophosphate insecticides, including acephate, dicrotophos, and methyl parathion, provide consistent and satisfactory control of green stink bug, southern green stink bug, and brown stink bug (McPherson et al., 1999a; 1999b; Willrich et al., 2000; Fitzpatrick et al., 2001). Greene et al. (2001) demonstrated with topical application techniques that acephate \([0.56 \text{ kg ai/ha (0.5 lb ai/acre)}]\) and dicrotophos \([0.28 \text{ and } 0.56 \text{ kg ai/ha (0.25 and 0.5 lb ai/acre)}]\) provided 69 to 100% mortality of adult and fifth instar nymphs of southern green stink bug and brown stink bug. Although McPherson et al. (1979) determined the laboratory response (LC50) of stink bug nymphs (brown stink bug, green stink bug, and southern green stink bug) was greater than their corresponding adults to methyl parathion, field control failures with recommended organophosphate insecticides have not been reported in the United States.

Results from field studies comparing the efficacy of insecticides between southern green stink bug adults and nymphs support results from the AVT in the present research. Southern green stink bug nymphs exposed to bolls treated with \( \lambda \)-cyhalothrin \([0.034 \text{ kg ai/ha (0.03 lb ai/acre)}]\) within 4 h after application resulted in 65% mortality after exposure for 24 h (Willrich et al., 2003). Southern green stink bug adults exposed to bolls at 24 h after treatment resulted in 97.5% mortality after exposure for 24 h. Based on these studies, southern green stink bug adults are highly sensitive to insecticides, particularly \( \lambda \)-cyhalothrin.

These data and that in other reports suggest that pyrethroids as a class are not equally toxic to all stink bug species. Topical applications of pyrethroid insecticides (bifenthrin, cypermethrin, \( \lambda \)-cypermethrin, and cyfluthrin) resulted in 77 to 98% mortality of southern green stink bug adults and nymphs (Greene et al., 2001). In contrast, mortality of brown stink bug adults and nymphs ranged from 20 to 65%. Of the pyrethroids tested, bifenthrin, provided 65 and 63% mortality of brown stink bug adults and nymphs, respectively (Greene et al., 2001). Emfinger et al. (2001) demonstrated that bifenthrin applied at 0.056 and 0.078 kg ai/ha \((0.05 \text{ and } 0.07 \text{ lb ai/acre)}\) controlled brown stink...
Based on these results, Alabama, Arkansas, Georgia, Louisiana, Missouri, North Carolina, and Tennessee are justified in differentiating insecticide recommendations between brown stink bug species and southern green stink bug/green stink bug in cotton (Patrick and Lentz, 2001; Anonymous, 2002; Bagwell et al., 2002; Boyd and Phipps, 2003; Johnson et al., 2002; Bachelor and Van Duyn, 2003;
Roberts et al., 2003). In the future, initiating control measures against stink bugs in cotton will require more than detection of the stink bug pest complex and determination of the infestation level. Proper identification of species and developmental stages will be necessary because insecticide susceptibility varies among species and life stages.

Numerous products are recommended for management of southern green stink bug and green stink bug. Insecticides representing the organophosphate class have provided the most consistent control of brown stink bugs. Tolerance re-assessment and re-registration is currently in progress for organophosphates, as directed under the Food Quality Protection Act of 1996. As additional restrictions for organophosphate use are implemented, it will be critical to evaluate the toxicity of registered and experimental insecticides against the complete spectrum of stink bug species found in cotton fields.

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