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SPECIES COMPOSITION AND SEASONAL ABUNDANCE OF STINK BUGS IN COTTON IN THE LOWER TEXAS GULF COAST AND THE VIRULENCE OF *EUSCHISTUS* SPECIES TO COTTON

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Abstract

Stink bug damage has only recently been considered a serious problem in cotton production. For many decades, stink bugs were coincidentally controlled by insecticide applications made for the boll weevil and the tobacco budworm/bollworm complex. With the combination of boll weevil eradication programs, use of selective insecticides that have little to no effect on piercing/sucking insects, and wider adoption of Bt cotton cultivars, stink bugs have been allowed to establish themselves as annual mid- to late-season cotton pests.

A survey was conducted to determine the species composition, relative distribution, and seasonal abundance of stink bug species infesting cotton in the Lower Gulf Coast of Texas. Results indicate the stink bug complex in Lower Texas Gulf Coast cotton to be different than that of other states. Data suggest the brown stink bug, *Euschistus servus* (Say), and a complex of smaller *Euschistus* species, including *E. quadrator* (Rolston) and *E. obscurus* (Palisot), to be the dominant pests, along with some populations of southern green stink bug, *Nezara viridula* (L.), and green stink bug, *Acrosternum hilare* (Say).

A boll cage study was conducted to determine the virulence of *E. servus* and *E. quadrator*, including boll susceptibility, amount of boll damage, and overall yield/quality loss. Results indicated both species caused significant boll damage, but *E. servus* caused more yield and quality loss than *E. quadrator* and was able to damage larger bolls.

Studies were conducted to compare insecticide efficacy against *E. servus* and *E. quadrator* adults in cotton. Results indicated mortality of both species was greatest when exposed to Bidrin 8 EC (0.5 aia) as compared to the other insecticides. *Euschistus quadrator* were generally more susceptible to pyrethroids than *E. servus*, and Bifenthrin 2 EC (0.1 aia), Mustang Max 0.8 EC (0.0225 aia), and Prolex 1.25 EC (0.02 aia) caused the greatest mortality of the pyrethroids tested. Exposure to neonicotinoids resulted in low mortality of both species.

The Lower Texas Gulf Coast has a stink bug complex that differs somewhat from other areas of the Cotton Belt. While there is a difference in virulence between *E. servus* and *E. quadrator*, these data do not play a very important role in commercial management of stink bugs when sampling is conducted using evidence of internal feeding. These thresholds are based on the amount of damage that is present in the bolls, so, regardless of the species causing the damage, if a specific level of damage is reached, treatment is required. Bidrin will control the *Euschistus* species, but pyrethroids may have differing results. The important thing is to establish which species are present in order to determine which pesticide will be most efficacious due to differential stink bug toxicities.

Introduction

Stink bugs (Hemiptera: Pentatomidae) were reported as pests of cotton, *Gossypium hirsutum* (L.), during the early part of the 20th century (Morrill 1910, Cassidy and Barber 1939), but only recently has their damage to cotton been considered a serious problem. For many decades, stink bugs were controlled coincidentally by insecticide applications made for the boll weevil, *Anthonomous grandis* (Boheman), and the tobacco budworm/bollworm, *Heliothis virescens* (F.)/*Helicoverpa zea* (Boddie), complex (Barbour et al. 1988, Turnipseed et al. 1995, Turnipseed and Greene 1996). Because of the reduction in insecticide applications in cotton resulting from the eradication of the boll weevil, adoption of transgenic cotton cultivars utilizing Bollgard® technology, and the use of selective insecticides that have little to no effect on piercing/sucking insects, stink bugs have become annual mid- to late-season cotton pests (Roach 1988, Greene and Turnipseed 1996, Roberts 1999, Boethel 2000).

Based upon data presented herein, it appears that the stink bug complex in the lower Gulf Coast of Texas consists primarily of *Euschistus servus* (Say), *E. quadrator* (Rolston), and to a lesser extent, *Nezara viridula* (L.) and *Acrosternum hilare* (Say). This overall complex appears unique to this area. Although there are many studies that have established damage potential and economic thresholds for *E. servus*, *N. viridula*, and *A. hilare*, there has been very little work on *E. quadrator*. There is an urgent need to understand how the distribution, potential for damage, and susceptibility to pesticides of *E. quadrator* compares with these other dominant stink bug pests. The objectives of this research were to (i) determine the species composition, relative distribution, and seasonal abundance of stink bug species infesting cotton in the Lower Texas Gulf Coast, (ii) compare the virulence of *E. quadrator* to other stink bug species, including boll preference, boll susceptibility, boll damage, and impact on yield and fiber quality, and (iii) compare insecticide efficacy against *E. quadrator* and other stink bug species.

Materials and Methods

Survey, 2004-2005. Stink bug species infesting cotton and their seasonal abundance were determined by sampling commercial cotton fields throughout several counties along the Lower Texas Gulf Coast: Kleberg, Nueces, San Patricio, Refugio, Victoria, and Calhoun. Twenty-two fields were surveyed in 2004 and 20 fields in 2005. All fields selected for this survey were planted to cotton cultivars containing the lepidopteran-active toxin gene from the bacterium *Bacillus thuringiensis* (Bollgard® or Bollgard II®) to minimize use of broad-spectrum lepidopteran insecticides. Sampling was conducted using the drop-cloth method, with all samples taken approximately 25 m in from the field margin. A drop cloth (101.6 cm wide by 91.4 cm long) was placed between two adjacent rows of cotton and the plants on each row (total of 1.83 row-m) beside the cloth were shaken over it to dislodge stink bugs (Kogan and Pitre 1980, Ruesink and Kogan 1994).

A total of ten samples were taken per field. Fields were divided into quadrants; one sample was taken per field quadrant and the remaining samples were taken randomly within the field. Each paired sample was approximately 6-8 rows apart. Sampling was repeated on a weekly basis beginning at first bloom and ending at first open boll. The number of times each field was sampled varied between years due to weather conditions, physiological cut-out of cotton, and insecticidal applications made in survey fields. During times of heavy rainfall, fields could not be sampled due to running or standing water in the rows. If an insecticide application for stink bug control was made within one week prior to sampling, that field was not sampled that week.

Adult stink bugs collected from each individual field were placed into individual numbered, plastic containers, and subsequently identified to species using the keys of Rolston (1974) and McPherson and McPherson (2000).

Virulence Experiment. Only two species, *E. servus* and *E. quadrator*, were evaluated, and single stink bug adults were placed in individual boll cages holding bolls of one of three sizes. The experimental design was a no-choice, two factor factorial in a completely randomized design. The first factor consisted of three levels of stink bug infestation (untreated, *E. servus*, and *E. quadrator*) and the second factor three levels of boll diameter (1.8, 2.8, and 3.2 cm in diameter). These boll sizes correspond roughly to 7, 14, and 21 d-old bolls. Adults of *E. servus* and *E. quadrator* were collected from soybeans and held on green beans overnight to ensure the insects were not mortally wounded during capture. Bolls were randomly sampled for evidence of internal feeding prior to the study to ensure that they had suffered little/no previous damage at the start of the experiment. All bolls used for the study were located on first positions.

After bolls were selected using 1.8, 2.8, and 3.2 cm diameter templates, they were enclosed in individual cages. Boll cages were made of 12 oz. polystyrene cups, knee high nylon hose, and plastic wire ties, as described by Greene et al. (1999b). The bottom of the cup and the foot end of the hose were removed and the cup was placed inside the hose. The cup was placed over the boll, bottom end first, and one end of the hose was stretched around the branch of the cotton plant and wire-tied in place. The other end of the hose was twisted together and sealed with another wire-tie. Each cage was considered a replicate, and there were 20 replicates for each treatment. One stink bug was placed in each cage and left for 48 h. Upon removal, stink bugs were checked for mortality. Criterion for mortality was inability of the insect to assume an upright position when placed on a flat surface after removal from the cage. Ten cages per treatment were evaluated 48 h after stink bug removal for EIF and the number of punctures/warts per boll recorded. The weight of each boll was also recorded prior to internal inspection. The bolls from the remaining 10 cages were hand-harvested once they were open to determine yield and lint quality Seed-cotton was ginned with a laboratory roller gin and quality determined by Advanced Fiber Information System (AFIS) analysis.

Significant differences among treatments were determined by the general linear model (GLM) (PROC GLM: SAS Institute 2005). Treatments were separated by the least significant difference (LSD) post-hoc test. Treatments with heavy fire ant predation resulting in stink bug death, other stink bug mortality, or unhealthy stink bug activity (less than three punctures per boll) were treated as outliers and missing data for analyses. Voucher specimens from these studies were deposited in the Texas A&M University Insect Collection (#654), College Station, TX.

Insecticide Experiment. All stink bugs tested were collected from soybeans and held on green beans overnight to ensure the insects were not mortally wounded during capture. Plots consisted of one plant with 3 stink bugs of each species per insecticide treatment. Each caged cotton plat was a replication, and each treatment was replicated four times in a completely randomized design. Insecticides were applied using a CO_2 backpack sprayer calibrated to deliver 46.75 liters/ha through four hollowcone nozzles (TX2, Spraying Systems, Wheaton, IL) at 2.46 kg/cm^2. All insecticides were applied as above, with the exception of the second trial, in which the spray volume was increased to 93.5 liters/ha through four hollowcone nozzles (TX4, Spraying Systems, Wheaton, IL).

Significant differences among treatments were separated by using a chi-square test for homogeneity (SPSS, Inc. 2003). Voucher specimens from these studies were deposited in the Texas A&M University Insect Collection (#654), College Station, TX.

Results

Stink Bug Survey, 2004-2005. In 2004, fields were surveyed from 9 June through 21 August, and a total of 133 stink bugs were collected from the 22 fields. *Euschistus servus* was the most commonly collected stink bug (Table 1). When only cotton pests are considered, *E. quadrator* was the next most abundant, though much less abundant than *E. servus*. Three additional *Euschistus* species and *N. viridula* were also collected. The most common stink bugs collected that are not considered cotton pests were *Podisus* spp. and *Oebalus pugnax* (F.). The mean density of stink bugs per six row-feet was highest during 23 June 2004 to 21 July 2004, which corresponds to the middle and late weeks of bloom.

Mean stink bug densities were greatest in Refugio, Calhoun, and Victoria counties in 2004 (Table 1). Stink bugs were found in all counties except Kleberg. Stink bugs such as *E. quadrator* and *O. pugnax* were found in almost all counties, whereas *E. servus* and *Podisus* spp. were not found in the southern-most counties.

In 2005, the 20 survey fields were sampled from 8 June through 18 July, and a total of 127 stink bugs were collected. Again, *E. servus* was the most commonly collected stink bug in 2005 (Table 2), but densities of *E. quadrator* and *E. obscurus* were greater than those observed in 2004. A few other *Euschistus* spp. and *A. hilare* were also collected. *Oebalus pugnax* and *Podisus* spp. were the most commonly collected species that are not considered pests of cotton. As in 2004, the mean density of stink bugs was highest during 22 June 2005 to 6 July 2005, which corresponds to the middle and late weeks of bloom.

Mean stink bug densities were greatest in Refugio, Victoria, and Calhoun counties in 2005 (Table 2). Stink bugs were collected in all counties surveyed. *Euschistus quadrator* and *O. pugnax* were collected in all counties except Victoria, while *E. servus* and *Podisus* spp. were only collected in the northern-most counties of Victoria and Calhoun.

A total of 880 drop cloth samples were taken from 22 fields in 2004. The mean stink bug density per six row-feet was 0.15. In 2005, a total of 770 drop cloth samples were taken from 20 fields, resulting in a mean stink bug density per six row-feet of 0.17.

Virulence Experiment. *Euschistus servus* caused greater numbers of internal feeding punctures to medium and large bolls, and *E. quadrator* to small and medium bolls, relative to unexposed bolls (Table 3). *Euschistus servus* caused significantly more internal feeding punctures in large bolls than in small bolls, but *E. quadrator* caused the same amount of internal feeding punctures to all three ages of bolls.

There was no abscission of large or medium bolls, but *E. servus* induced 50% small boll abscission, and *E. quadrator* induced 22% small boll abscission, in the ten replicates that were evaluated for yield and quality (Table 4).

Euschistus servus significantly reduced lint weight in medium and large bolls relative to *E. quadrator* and in all boll sizes compared to unexposed bolls (Table 5). *Euschistus quadrator* significantly reduced lint weight in small and large bolls compared to unexposed bolls.

Euschistus servus reduced seed weight in medium and large bolls relative to *E. quadrator* and in all bolls compared to unexposed bolls (Table 6). *Euschistus quadrator* reduced seed weight in large bolls relative to unexposed bolls.

Insecticide Experiment. Mortality of *E. servus* was greater than the control for all treatments in the first trial (Figure 1). Mortality of *E. quadrator* was greatest when exposed to dicrotophos, bifenthrin and zeta-cypermethrin, and mortality was greater when exposed to these relative to cyfluthrin and the control. All treatments with the exception of cyfluthrin caused greater mortality than the control. Mortality of *E. servus* was greater than that of *E. quadrator* when exposed to cyfluthrin.

The second trial evaluated insecticide toxicity against only *E. quadrator* (Table 7). There were no other differences among treatments.

The last trial evaluated insecticide toxicity against *E. servus*, *E. quadrator*, and *N. viridula* adults (Figure 2). Mortality of *E. servus* and *E. quadrator* was greater than the control in all treatments except for the two rates of lambda-cyhalothrin alone and the rate of thiamethoxam alone. There were no differences in mortality among insecticide treatments on *N. viridula*, and all caused greater mortality than the control. Mortality of *E. servus* and *E. quadrator* was greater than that of *N. viridula* following exposure both rates of lambda-cyhalothrin, mortality of *E. servus* was greater than that of *N. viridula* when exposed to thiamethoxam alone.

Discussion

This research established that the Lower Texas Gulf Coast has a stink bug complex that differs from other areas of the Cotton Belt. *Euschistus servus* and lesser brown stink bugs, including *E. quadrator*, *E. obscurus*, *E. crassus*, and *E. ictericus*, made up the largest portion of this pest complex, and green/southern green stink bugs were less common than in other areas. Thresholds based on evidence of internal feeding are based on the percentage of bolls that are damaged. So, regardless of the stink bug species causing the damage, and their potential difference in virulence, if a specific level of damage is reached, treatment is recommended. Applications of dicrotophos resulted in highest mortality of the *Euschistus* species, but use of pyrethroids may result in different mortality rates. When treatment thresholds are reached, it is important to know which species are present in a field in order to determine which pesticide will be most efficacious in case of differential stink bug susceptibilities. Dynamic thresholds based on evidence of internal feeding thresholds may potentially be the best method for determining the need for stink bug control in cotton, but further research is necessary to develop such thresholds for Lower Texas Gulf Coast cotton.

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Table 1. Mean number of stink bug species collected per six row-feet of cotton, by county. Lower Texas Gulf Coast region, 2004.

Species	Kleberg	Nueces	San Patricio	Refugio	Victoria	Calhoun	**Season Total**
E. servus	0	0	0.006	0.100	0.145	0.140	**0.084**
Lesser Brown	0	0.025	0.033	0.100	0.007	0.030	**0.023**
E. quadrator	0	0.025	0.028	0.100	0.007	0.005	**0.016**
E. obscurus	0	0	0	0	0	0.015	**0.003**
E. ictericus	0	0	0	0	0	0.010	**0.002**
E. crassus	0	0	0.006	0	0	0	**0.001**
N. viridula	0	0	0.022	0	0	0	**0.005**
O. pugnax	0	0.017	0.011	0.033	0.003	0.020	**0.011**
Podisus spp.	0	0	0.006	0.033	0.021	0.070	**0.025**
P. acutissimus	0	0	0.006	0	0	0	**0.001**
E. bifida	0	0	0	0	0.003	0	**0.001**
P. punctulatus	0	0.008	0	0	0	0	**0.001**
TOTAL	**0.000**	**0.050**	**0.084**	**0.266**	**0.179**	**0.260**	**0.151**

Table 2. Mean number of stink bug species collected per six row-feet of cotton, by county. Lower Texas Gulf Coast region, 2005.

Species	Kleberg	Nueces	San Patricio	Refugio	Victoria	Calhoun	**Season Total**
E. servus	0	0	0	0	0.193	0.089	**0.058**
Lesser Brown	0.067	0.057	0.011	1.033	0	0.022	**0.066**
E. quadrator	0.067	0.043	0.011	0.067	0	0.006	**0.022**
E. obscurus	0	0.007	0	0.967	0	0.011	**0.416**
E. crassus	0	0.007	0	0	0	0.006	**0.003**
A. hilare	0	0	0.011	0	0	0.006	**0.004**
T. custator	0.011	0	0	0	0	0	**0.001**
O. pugnax	0.011	0.014	0.050	0.033	0	0.011	**0.020**
Podisus spp.	0	0	0	0	0.020	0.011	**0.006**
E. bifida	0	0	0	0	0.047	0	**0.009**
TOTAL	**0.089**	**0.071**	**0.072**	**1.066**	**0.260**	**0.139**	**0.165**

Table 3. Mean number of punctures for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June, 2005.

Boll Size		*Euschistus servus* ± SD		*Euschistus quadrator* ± SD		Untreated Control ± SD	
Small	1.8 cm	18.3 ± 11.3	bc	25.5 ± 14.4	ab	0.0 ± 0.0	c
Medium	2.8 cm	38.1 ± 16.7	ab	15.9 ± 17.4	bc	0.0 ± 0.0	c

Large	3.2 cm	47.6 ± 25.0	a	24.8 ± 24.5	ab	0.0 ± 0.0	c

Means followed by different letters are significantly different (P < 0.05, LSD)

Table 4. Percent abscission for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.

Boll Size		*Euschistus servus* ± SD		*Euschistus quadrator* ± SD		Untreated Control ± SD	
Small	1.8 cm	50.0 ± 53.7	a	22.2 ± 44.1	ab	0.0 ± 0.0	b
Medium	2.8 cm	0.0 ± 0.0	b	0.0 ± 0.0	b	0.0 ± 0.0	b
Large	3.2 cm	0.0 ± 0.0	b	0.0 ± 0.0	b	0.0 ± 0.0	b

Means followed by different letters are significantly different (P < 0.05, LSD)

Table 5. Mean lint weight (g) for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.

Boll Size		*Euschistus servus* ± SD		*Euschistus quadrator* ± SD		Untreated Control ± SD	
Small	1.8 cm	0.49 ± 0.5	B,b	0.92 ± 0.5	B,b	1.55 ± 0.4	A,b
Medium	2.8 cm	0.92 ± 0.5	B,a	1.50 ± 0.5	A,a	1.71 ± 0.3	A,b
Large	3.2 cm	1.33 ± 0.4	C,a	1.81 ± 0.4	B,a	2.29 ± 0.3	A,a

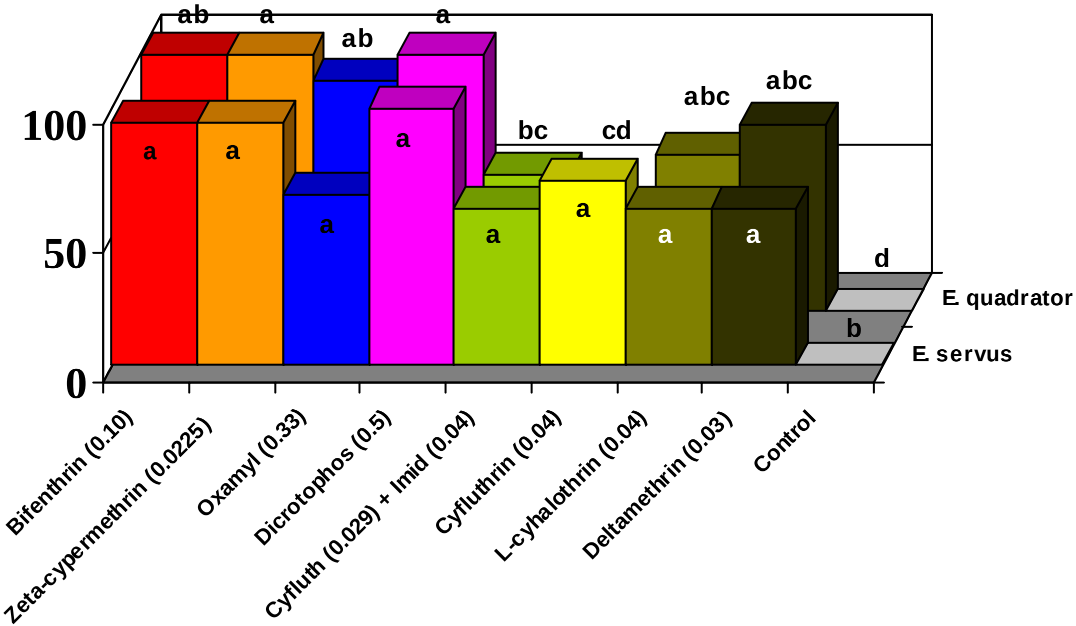
Means followed by different lower case letters within columns are significantly different (P < 0.05). Means followed by different upper case letters within rows are significantly different (P < 0.05).

Table 6. Mean seed weight (g) for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.

Boll Size		*Euschistus servus* ± SD		*Euschistus quadrator* ± SD		Untreated Control ± SD	
Small	1.8 cm	0.0322 ± 0.02	B,b	0.0521 ± 0.03	AB,b	0.0769 ± 0.02	A,b
Medium	2.8 cm	0.0530 ± 0.01	B,a	0.0749 ± 0.01	A,a	0.0842 ± 0.01	A,b
Large	3.2 cm	0.0655 ± 0.01	C,a	0.0789 ± 0.01	B,a	0.0920 ± 0.01	A,a

Means followed by different lower case letters within columns are significantly different (P < 0.05). Means followed by different upper case letters within rows are significantly different (P < 0.05).

Figure 1. Percent mortality of adult *E. servus* and *E. quadrator* exposed to selected insecticides for 72 h in bloom cages a on cotton, 7 July 2005.



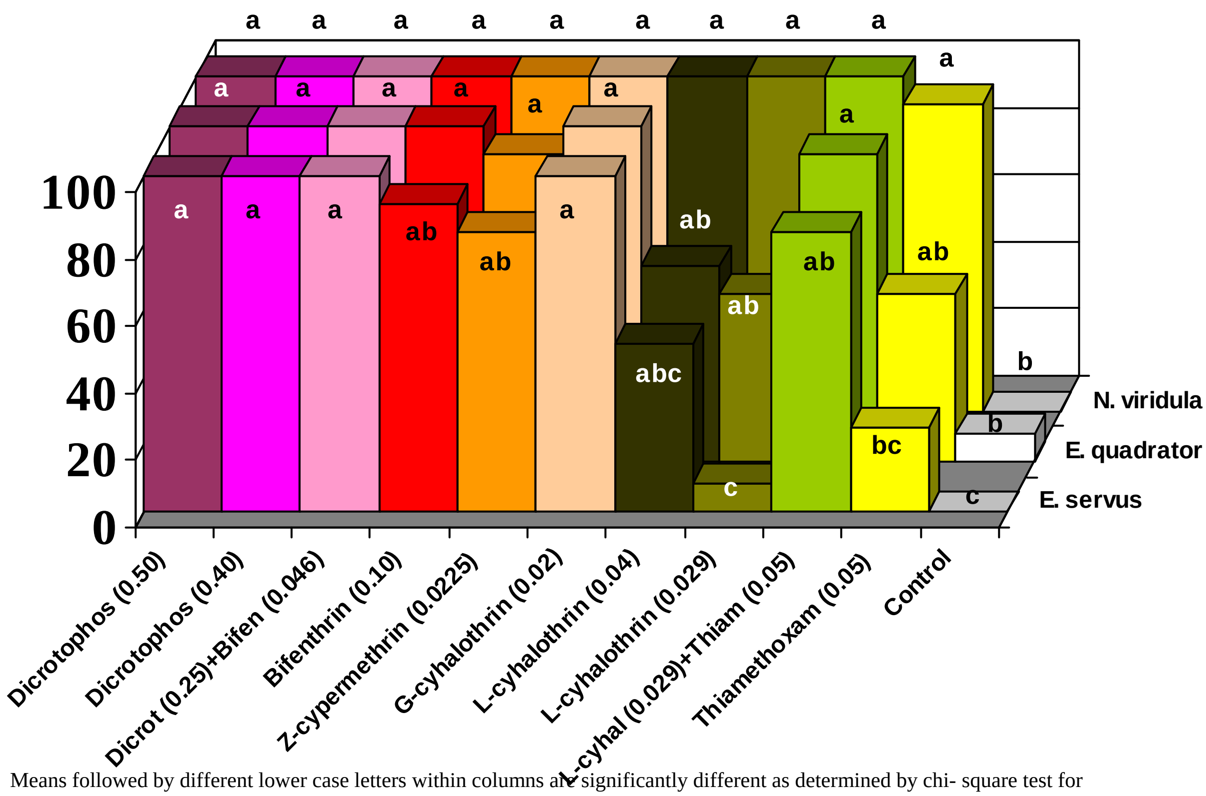
Means followed by different lower case letters within columns are significantly different as determined by chi-square test for homogeneity (P < 0.05). Means followed by different upper case letters within rows are significantly different as determined by chi-square test for homogeneity (P < 0.05).

Table 7. Percent mortality of adult *E. quadrator* exposed to selected insecticides for 72 h in bloom cages on cotton, 31 July 2005.

Treatment	Rate g (AI)/ha	*E. quadrator* mortality ± SD	
Thiamethoxam	56.0	25.0 ± 16.7	a
Acetamiprid	56.0	0.0 ± 0.0	a
untreated		0.0 ± 0.0	a

Means followed by different letters within columns are significantly different as determined by chi-square test for homogeneity (P < 0.05).

Figure 2. Percent mortality of adult *E. servus*, *E. quadrator*, and *N. viridula* exposed to selected insecticides for 72 h in bloom cages on cotton, 13 August 2005.



Means followed by different lower case letters within columns are significantly different as determined by chi- square test for homogeneity (P < 0.05). Means followed by different upper case letters within rows are significantly different as determined by chi-square test for homogeneity (P < 0. 05).