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MONITORING INSECT RESISTANCE IN ARKANSAS TO CHEMICAL INSECTICIDES AND BT-ENDOTOXINS

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Abstract

A resistance-monitoring program was established at the University of Arkansas in 2002 to annually gauge changes in major pest susceptibilities to insecticidal proteins in transgenic crops and shifts in regional and temporal susceptibility to major modes of action common to critical conventional insecticides. Nineteen colonies of Lepidoptera were collected and assayed for susceptibility to Cry1Ac and Cry2Ab proteins. The bollworm, *Helicoverpa zea*, exhibited more variability in susceptibility then other species tested. Variation among field colonies was 15-to 22-fold. Laboratory susceptible colonies were 5-to 80-fold more susceptible then some field colonies. Susceptibilities of field collected insects to acephate, cypermethrin, malathion, and spinosad were measured by a treated glass vial technique. Base-line data were collected for several species of noctuid moths and important hemipteran pests. An overview of first year results and benchmark data are presented in this paper.

Introduction

Transgenic Bt cotton lines expressing Bollgard® genes are important pest management tool for cotton farmers throughout the world (Cannon 2000, Edge et al. 2001). The widespread adoption of Bollgard cotton in the US has been associated with a significant decrease in broad-spectrum insecticide use (ReJesus et al. 1997, Gianess and Carpenter 1999, Fernandez-Cornejo et al. 2000). These benefits could be diminished if the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), the two key pests of cotton, develop resistance to Bt toxins (Stone et al. 1989, Gould et al. 1992, 1995, Moar et al. 1995, Burd et al. 2000, Jackson et al. 2001). Conventional insecticides remain an integral component of total cotton insect management.

Prompt detection of resistance evolution and implementation of appropriate management responses has been (Plapp et al. 1987, Roush and Luttrell 1989, Leonard et al. 1987, Elzen et al. 1992, Brown et al. 1998, Ottea and Holloway 1998, Holloway et al. 1998, Mascarenhas et al. 1998, Bagwell et al. 2000, Moulton et al. 2000) and will continue to be a critical component of profitable cotton production in the US. In addition, some lepidopteran and sucking pests of cotton have reportedly developed resistance against some major conventional chemical insecticides

The level of Bt susceptibility may vary widely among geographically diverse populations (Stone and Sims 1993, Luttrell et al. 1999). Over the past few years, Hardee et al. (2001) monitored the status of bollworm and tobacco budworm resistance to Bt cotton in the US. They reported that tolerance of tobacco budworm to Bt toxin did not change during the period 1996 to 1998; however, tolerance of bollworm to Bt cotton appeared to increase. They suggested that areas producing higher amounts of Bt cotton would be areas for greater chance of resistance development to the insecticidal proteins. Luttrell et al. (1999) reported that through selection, bollworm has the capability to develop resistance to Bt toxin. Likewise, the susceptibility of bollworm to pyrethroid insecticides has been changing over time (Bagwell et al. 2000). With the increasing Bt cotton acreage in Arkansas, the potential for resistance development to Bt toxin by bollworm or tobacco budworm is very high. Changing insecticide use patterns are shifting the focus of conventional insecticides to the sucking pest complex. There is a growing need for management of insecticide resistance in these pests previously classified as secondary pests. To address these concerns, we initiated a resistance-monitoring program in Arkansas to monitor the shifts in resistance levels in important agronomic pests. During 2002, we conducted research to: (1) refine procedures and collect base-line information on the status of Bt resistance in major Lepidoptera associated with the ecologically diverse agricultural systems of Arkansas, and (2) establish assay procedures to monitor evolving resistance to conventional insecticides in polyphagous pest species that frequent a diversity of different crops across our agricultural landscape.

Materials and Methods

Bt Endotoxin Studies

Relative susceptibilities of field populations of noctuid pests to Bt endotoxin proteins were studied by establishing laboratory colonies from field collections and examining progenies (1-4 generation) to Cry1Ac and Cry2Ab endotoxin proteins in a diet incorporation bioassay (Luttrell et al. 1999). Thirty-six colonies of eggs and larvae of variable ages (neonates to 4th stage) were collected from 12 locations in Arkansas, Missouri and Mississippi from corn, Bt-corn, sorghum, soybean and cotton, and Bt-cotton during the period of May to September 2002. Of these, one colony of soybean looper (*Pseudoplusia includens*), three colonies of tobacco budworm, and 15 colonies of bollworm were established in the laboratory (Table1). Lar-

vae were collected and placed in 30-ml Solo plastic cups containing artificial diets (Burton, 1969). These collections were brought to the Insect Rearing Facility, Department of Entomology, University of Arkansas, Fayetteville, AR and maintained until pupation in a walk-in temperature-controlled room at 26°C, 70% RH and 14:10 (L:D) photoperiod. Progenies of resulting colonies were used for bioassays. Laboratory colonies of bollworm, tobacco budworm and soybean looper that had been maintained in the rearing facility for many generations were used as reference strains for susceptibility. Additional laboratory colonies of bollworm and tobacco budworm were obtained from the USDA-ARS Southern Fields Crop Insect Management Research Unit, Stoneville, MS and used as the reference strain.

For Cry1Ac bioassays, a liquid formulation of MVP II (20% δ-endotoxin of *Bacillus thuringiensis* var *kurstaki*) provided by Mycogen Corporation, San Diego, CA and lyophilized MVP II toxin provided by Monsanto Company, St. Louis, MO were used. For Cry2Ab bioassays, lyophilized Bt corn-leaf tissue provided by Monsanto Company was used. All diet-incorporated bioassays included six to seven concentrations. For Cry1Ac, liquid MVP II toxin was diluted in distilled water to obtain desired dosages. For Cry2Ab, the appropriate amount of lyophilized corn leaf powder was weighed for each concentration. Toxins and freshly prepared diet (Burton, 1969) were poured into 140 ml Nalgene plastic bottles, vortexed and dispensed (ca. 1 ml/well) into the wells of assay trays (C-D International, 30 Edgemoor Av, Pitman, NJ). Once the diet was dry, one neonate test insect was placed in each well. The trays were then covered with plastic ventilated covers and incubated at 27°C, 70% R.H and 14:10 photoperiod, for seven days. There were 48 to 112 larvae used for each concentration and 3 to 8 replications conducted on different days. Larval mortality was recorded after 7 days.

As the season progressed and initial mortality response data were obtained, we decided to use lyophilized powder MVP II instead of the liquid formulation. For these later bioassays, data on developmental stages of the larvae were recorded in addition to mortality. Mortality data were analyzed by probit procedures (SAS, 1998) to estimate LD_{50}, fiducial limits and slopes. In developmental bioassays used later in the season with lyophilized MVP II powders, larvae that failed to molt to second instar were considered dead and were added to the mortality data. Results are shown in Table 4.

Insecticide Studies

Susceptibilities of field-collected insects to a range of contact-active insecticides were measured by exposure to treated scintillation vials. Most of the insects collected and assayed for response to chemical insecticides were from three geographically and ecologically diverse production systems in Arkansas: Wildy Farms, a concentrated cotton production in northeastern Arkansas; Tillar and Company, a diversified agricultural planting company in southeastern Arkansas; and Matteson Farms, a diversified grain farm in southwestern Arkansas. Cotton research plots and pheromone traps for major lepidopteran pests were located at each study site. These study areas are the proposed sites for future community management programs that approach insect management problems from area-wide or community perspectives. Understanding the seasonal and spatial distribution of insecticide selection can be an important component of these evolving management systems.

The insecticides tested were acephate, cypermethrin, malathion, and spinosad. They were chosen as benchmark insecticides because at their widespread use in Arkansas and the historical data available from previous studies. Technical grade cypermethrin, acephate, spinosad, and malathion were supplied by FMC Corporation (Philadelphia, PA), Valent USA (Germantown, TN), Dow Agro Sciences (Indianapolis, IN), and American Cyanamid Co (Princeton, NJ), respectively. Doses used for all insecticides except spinosad were 0.1, 0.3, 1, 3, 10 and 30 µg per vial. Spinosad doses were 3, 10 and 15 µg per vial. Insecticides were dissolved in analytical grade acetone to obtain desired concentration. New 20-ml scintillation vials were treated individually as described by Luttrell et al. (986). Doses of chemical (250 µl/vial) were pipetted into each vial and rolled on a "hot dog" roller. This insured that the interior of the vial was coated with the desired chemical. The vials were stored at room temperature. Fresh vials were prepared at monthly intervals

Study insects were collected directly from field plots or from pheromone traps and placed in treated scintillation vials similar to the methods previously described to measure tobacco budworm resistance to pyrethroid insecticides Luttrell et al. (986). Insects were placed directly in the vials in the field for some studies and care was taken to minimize exposure of the vials to direct sunlight and heat. Observations of mortality were made at numerous intervals post-exposure to the vials, but exposures for 1 day (~24 hours) were used as the standard unit in this paper. Vials with insects were stored at room temperature during the course of assay and mortality observations Inability to move when probed with forceps was the critical assessment of mortality.

Insects were collected and tested (a single dose of a single insecticide) throughout the season when populations were high enough to collect about 20 insects per observation. A total of 3719 field collected insects were assayed during 2002 including bollworm adults (n=1002), green stink bug (*Acrosternum hilare* (Say)) adults and late instar nymphs (n=678), beet armyworm (*Spodoptera exigua* Hübner) moths (n=535), cotton fleahopper (*Pseudatomoscelis seriatus*) adults (n=345), three cornered alfalfa hopper adults (n=320), tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois) adults (n=270), tobacco budworm adults (n=269), rice stink bug (*Lissorhoptrus oryzophilus* Kuschel) adults (n=210), cabbage looper adults (n=29), soybean looper adults (n=24), brown stink bug (*Euschistus servus* (Say)) adults (n=15), and fall armyworm (*Spodoptera fu-*

giperda (J. E. Smith)) adults (n=13). A total of 177 independent observations (one dose of one insecticide) were made of field or pheromone trapped insects (average of 21 insects per observation) over the 2002 growing season.

All assays included appropriate untreated controls (vials treated with acetone alone), and resulting data were corrected for mortality observed in the untreated controls. Mortality in the untreated controls varied widely since study insects were field collected. Data were by descriptive statistics summarized across all study sites and all dates since the purpose of 2002 studies was the establishment of base-line susceptibilities for future reference. General trends in the seasonal responses of the different species are summarized in Figures 1-6.

Results and Discussion

Bt Endotoxin Studies

The relative susceptibilities of laboratory and field colonies to Cry1Ac based on seven-day mortality are presented in Table 2. Susceptibility of tobacco budworm laboratory colonies to Cry1Ac ranged from an LC_{50} of 0.22 to an LC_{50} of 0.43 µg of toxin/ml of diet. The USDA laboratory colony was 1.95-fold less susceptible than the UA laboratory colony. Luttrell et al. (1999) reported over 3-fold variation in susceptibilities of five laboratory colonies in the 1990's.

Susceptibilities of field populations of tobacco budworm to Cry1Ac differed significantly from those of laboratory susceptible colonies. LC_{50}s for the three field colonies of tobacco budworm ranged from 1.20 to 2.78 µg of toxin/ml of diet, or about 5- to 12-fold higher than the LC_{50} for laboratory colonies. Previously, Luttrell et al. (1999) reported a 5-fold level of variation among 11 field populations of tobacco budworm. However, susceptibilities of laboratory and field colonies were similar when larval development was included as a criterion of response (Table 3). The LC_{50}s for the UA laboratory tobacco budworm colony was 0.21 µg of toxin/ml of diet, as compared to 0.26 to 0.27 µg of toxin/ml of diet for the field colonies.

Susceptibility of bollworm colonies to Cry1Ac varied among laboratory and field colonies. LC_{50}s for UA and USDA laboratory colonies of bollworm were 1.04 and 1.75 µg of toxin/ml of diet, respectively, indicating the USDA laboratory colony was slightly less susceptible than the UA laboratory colony. Earlier, Luttrell et al. (1999) reported a wide variation in susceptibility of different laboratory colonies. Susceptibility of field populations of bollworm to Cry1Ac varied significantly. The LC_{50}s for 14 field colonies ranged from 4 to 85 µg of toxin/ml of diet. Based on overlapping fiducial limits, 10 of our field colonies had significantly higher LC_{50}s than both laboratory colonies. Considering larval developmental effects in the observed response (Table 3), field colonies were up to 33-fold less susceptible than the most susceptible laboratory colony. In a similar study, Luttrell et al. (1999) reported about 300-fold variation in susceptibility using a mortality-based assay.

The LC_{50} for UA laboratory soybean looper colony exposed to Cry1Ac was 5.02 µg/ml. A similar response was observed to the field colony (4.70 µg of toxin/ml of diet).

Results of Cry2Ab bioassays are presented in Table 4. Susceptibility of tobacco budworm to Cry2Ab did not vary significantly among the field colonies, however, significant variation in susceptibilities of field and laboratory colonies were observed. LC_{50}s for laboratory colonies of tobacco budworm ranged from 0.002 to 0.004 percent of diet with toxin among the laboratory colonies, while this range among field colonies was 0.01 to 0.08 percent of diet with toxin. Field colonies were 2.5- to 40-fold less susceptible than the laboratory colonies.

Susceptibility of bollworm to Cry2Ab did not vary among laboratory colonies. LC_{50}s for laboratory colonies ranged from 0.10 to 0.14 percent of diet with toxin. Susceptibilities of the nine colonies varied widely, LC_{50}s ranged from 0.16 to 0.62 percent of diet with toxin. Relative to the laboratory colonies, field colonies were 1.6 to 6.2-fold less susceptible, and most field colonies were significantly less susceptible than the laboratory colonies.

Insecticide Studies

Corrected mortalities summarized across 2002 for all study sites for beet armyworm, bollworm, tobacco budworm, cotton fleahopper, green stink bug, and tarnished plant bug generally illustrated dose responses (Figures 1-6). Average mortalities for beet armyworm were less than 100% for all doses studied. At the 30 µg/vial dose, the highest dose tested, corrected mortalities for beet armyworm were 78.8, 67.3, and 83.7% for acephate, cypermethrin and malathion, respectively. Mortality at a spinosad dose of 15 µg/vial was 66.7%. For beet armyworm acephate, cypermethrin, malathion, and spinosad mortalities at a dose of 10 µg/vial were 36.8, 6.8, 44.4, and 29.2%, respectively.

More assays were conducted with bollworm adults from pheromone traps than with other insects. The bollworm is generally more susceptible to insecticides than the beet armyworm. No survival of bollworm was observed at doses of 10 and 30 µg/vial with acephate, cypermethrin, and malathion treated vials (Figure 2). Average mortalities at 3 µg/vial were 75.0, 72.3, and 33.8 percent for acephate, cypermethrin, and malathion, respectively.

Population densities of tobacco budworm were much lower than those observed in the 1980's and early 1990's prior to wide-spread adoption of Bt cotton. Average mortality at the diagnostic dose of 10 µg of cypermethrin/vial was 51% across all sample sites and dates (Figure 3); however, variability among samples was high and ranged from 0 to 100% mortality. Mortality at a 10 µg/vial dose of spinosad was 82.4%. A few samples were recorded at the 10 µg/vial dose of malathion with an average corrected mortality of 50%. However, the sample size was less than 10 individuals and data were not included in the overall seasonal analyses.

Lepidoptera monitored were all adults from pheromone trap captures at Wildy Farms, Tillar and Company, and Matteson Farms. Non-lepidopteran species were collected from cotton or from weeds, soybean, corn and grain sorghum adjacent to cotton research plots. We assumed that these populations would have been exposed to over sprays of acephate, cypermethrin, and malathion made to the research area because of the close proximity of the collection sites to cotton. Cotton fleahoppers were collected from cutleaf evening primrose and wooly croton (Luttrell et al. 2002) on field margins. Mortalities of cotton fleahopper generally followed a dose response for cypermethrin and malathion (Figure 4), but a dose response was not observed with acephate. Overall mortalities with cotton fleahopper were lower than those expected and those observed with other species. This needs further investigation. Collecting and handling fleahoppers is difficult and the research methodologies may need some refinement for cotton fleahopper assays. Corrected mortalities ranged from 50 to 67% at the 10 µg/vial doses of the different insecticides (Figure 4).

Green stinkbug exhibited clear dose-mortality responses at the 1-day observation interval for all three insecticides tested (Figure 5). Doses of 30 µg/vial with acephate and cypermethrin killed all bugs in all samples season-long. Some survival at the malathion 30 µg/vial dose was observed. Corrected mortalities for acephate, cypermethrin, and malathion treated vials at the 10 µg/vial dose were 66.7, 78.4, and 60.2%, respectively. Only a few brown stinkbugs were assayed during 2002 and mortality was observed at doses of all insecticides at 10 µg/vial and higher. In general, little or no mortality of brown stinkbug was observed at doses less than 3 µg/vial with any of the insecticides. Mortalities of green stinkbugs ranged from 22 to 65% at the 3 µg/vial doses and from 0 (with acephate) to 35.7% (with cypermethrin) at the 1 µg/vial doses. No insecticide caused mortality of brown stinkbug at doses of 1 µg/vial. Another stinkbug species, the rice stinkbug, was assayed from grain sorghum growing adjacent to cotton research plots. Although data were not summarized for this cotton report, the rice stinkbug appears to be relatively susceptible to insecticide treated vials. Dose responses were observed with all three insecticides, and corrected mortalities for acephate, cypermethrin, and malathion at the 1 µg/vial dose were 77.8, 33.3, and 33.3%, respectively.

The tarnished plant bug is known to have varied responses to conventional insecticides, especially the pyrethroids (Holloway et al. 1998). In our 2002 studies, dose mortality responses were obvious with the seasonal data summarized for acephate, cypermethrin, and malathion (Figure 6). At lower doses, the tarnished plant bug appeared to be more susceptible to malathion than acephate and cypermethrin. Corrected mortalities at the 10 µg/vial dose ranged from 70 to 85%. Those at the 3 µg/vial dose ranged from 20.8% (with acephate) to 85.0% (with malathion).

Summary and Overall Conclusions

Results showed that susceptibilities of tobacco budworm and bollworm to Cry1Ac and Cry2Ab endotoxins varied widely among field colonies. These results support findings of Luttrell et al (1999) and Hardee et al. (2002), who also reported wide variation in susceptibilities of tobacco budworm and bollworm field populations to Cry1Ac proteins.

The higher LC_{50}s seem to be associated with collections of larvae from Bt cotton. This may be somewhat expected given the wide-variability observed among bollworm populations prior to wide-spread commercial use of Bt crops (Stone and Sims 1993, Luttrell et al 1999) and the known ability of bollworm to survive on some Bollgard I cottons (Mahffey et al., 1995). Hardee et al (2001) reported significant increases in the percentages of bollworm larvae scored as tolerant after five days of feeding on Cry1Ac diet from 1997 to 1998. Larvae collected from Alabama, Florida, Mississippi Delta, Georgia, and South Carolina exhibited higher survival in 1998. An observed increase in survival rates from larvae collect in Arkansas was not significant, and no increase was observed in collections from Texas. The range in percentages of larvae classified as tolerant was 10-fold or greater over the two years and higher percentages of the tolerant larvae were associated with collections from Bt Cotton. Our observed variability in susceptibilities among field colonies of bollworm ranged from 15- to 22-fold depending upon which assay material and criteria for response were used. As with Hardee et al. (2001), our higher LC_{50}s were associated with collections from Bt cotton.

During 2002 studies, we used both liquid MVP II and lyophilized powder of MVP II from Monsanto as source for Cry1Ac. Later in the season, we observed that the liquid formulation exhibited slightly lower potency than the lyophilized powder. Accordingly lyophilized powder was thereafter used as the source of Cry1Ac. A comparison of results reported in Table 2 and Table 3 the potential differences in activity of the two sources of Cry1Ac and illustrate the importance of standardization

in comparing results among different research groups. Of the seven colonies tested by exposure to both Cry1Ac sources, the lyophilized powder was 1.3- to 10.3-fold more active. Average activity was 3.8-fold more than that of the liquid MVP II formulation. Since those tests were conducted against different generations of the various colonies, we cannot be sure that some of the potency difference may be associated with loss of genetic variability in the colonies over time. We are fairly confident that the differences were primarily due to overall potencies and not major variability in samples of the two sources. The range in susceptibilities of field-collected bollworm was 15.3-fold with the liquid formulation and 22.3-fold with the lyophilized powder.

To better understand this potency issue and standardize our ability to compare results among laboratories, researchers at North Carolina State University, Auburn University, USDA-ARS Southern Field Crop Insect Management Research Unit, and Monsanto and Company, have agreed to conduct comparative assays with the same endotoxin sources and share reference colonies of insects. Results of these comparative assays should be available next year.

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Table 1. Information on laboratory and field colonies of bollworm, tobacco budworm and soybean looper assayed for relative susceptibility Cry1Ac and Cry2Ab tolerance monitoring.

Date (clony #)	**Location**	**Source**	**Number**
H. virescens	UA	Lab	250
H. virescens	USDA	Lab	250
H. zea	UA	Lab	250
H. zea	USDA	Lab	250
P. includens	UA		200
Corn borer			
5/07/02 (1)	Tillar, AR	Weeds	5
5/30/02 (2)	Tillar, AR	Corn	4
5/30/02 (3)	Tillar, AR	Corn	10
6/05/02 (4)	Tillar, AR	Corn	15
6/05/02 (5)	Tillar, AR	Corn	15
H. virescens			
6/06/02 (6)	Foreman, AR	Early corn	31
6/11/02 (7)	Foreman, AR	Silk corn	30
6/11/02 (8)	Foreman, AR	Mid silk corn	60
H. zea			
6/20/02 (9)	Winchester, AR	Mid silk corn	38
6/21/02 (10)	Lockesburg, AR	Sweet corn	30
6/21/02 (11)	Matteson Farm, AR	Late silk corn	30
6/21/02 (12)	Matteson Farm, AR	Ear corn	31
6/21/02 (13)	Matteson Farm, AR	Sweet corn	60
6/27/02 (14)	Matteson Farm, AR	Ear corn	60
6/27/02 (15)	Winchester, AR	Corn	32
7/4/02 (16)	Matteson Farm, AR	Sorghum	150
7/8/02 (17)	Matteson Farm, AR	Bt-corn	119
7/9/02 (18)	Tillar, AR	Corn	114
7/9/02 (19)	Tillar, AR	Sorghum	118
7/9/02 (20)	Tillar, AR	Bt-cotton	17
7/15/02 (21)	Kiser, AR	Sorghum	60
7/17/02 (22)	Winchester, AR	Cotton	15
7/17/02 (23)	Wichester, AR	Bt-cotton	60
7/17/02 (24)	Winchester, AR	Sorghum	30
7/23/02 (25)	Tillar, AR	Corn	30
7/23/02 (26)	Tillar, AR	Grain sorghum	73
H. virescens			
7/26/02 (26)	Missouri	Cotton	87
7/26/02 (27)	Missouri	Pyr. Cotton	15
H. zea			
7/26/02 (29	Leachville, AR	Refuge cotton	20
8/05/30 (30)	Tillar, AR	Cotton	33
8/05/02 (31)	Wildy Farms, AR	Cotton	24
8/15/02 (32)	Tillar, AR	Soybean	56
8/15/02 (33)	Mississippi	Bt-cotton	200+
8/24/02 (34)	Portland, AR	Bt-cotton	204
8/24/02 (35)	Portland, AR	Bt-cotton	6 (eggs)
8/24/02 (36)	Portland, AR	Cotton	5 (eggs)
P. includens			
8/23/02 (37)	Tillar, AR	Soybean	205
H. zea			
9/20/02 (38)	Fayetteville, AR	Corn	60

Table 2. Susceptibility of bollworm, tobacco budworm and soybean looper to liquid MVP II (Cry1Ac) endotoxin in diet incorporated bioassays (Based on mortality data).

Colony	Source	LC_{50} (µl/ml of diet)	Fiducial limit		Slope
			Lower	Upper	
H. virescens					
UA	Lab	0.22	0.15	0.31	0.98
USDA	Lab	0.43	0.21	0.76	0.87
#6-8	Corn	2.78	1.25	25.72	0.75
#27	Cotton	1.13	0.75	1.54	1.57
#28	Cotton	1.20	0.83	1.73	1.02
H. zea					
UA	Lab	1.04	0.44	2.63	1.20
USDA	Lab	1.75	0.95	3.86	0.50
#9	Corn	17.80	7.06	164.15	0.58
#11, 13 & 14	Corn	4.54	0.39	11.77	0.93
#12	Sweet corn	10.82	7.06	15.76	1.54
#16	Sorghum	5.59	2.25	9.63	1.31
#17	Bt-corn	18.31	1.0	245.85	1.13
#18	Corn	29.78	20.19	47.42	1.47
#19	Sorghum	12.05	8.54	17.58	1.95
#23	Bt-cotton	20.82	10.35	46.83	0.81
#31	Cotton	27.90	10.47	137.30	1.28
#33	Bt-cotton	85.45	46.70	288.81	1.07
#34	Bt-cotton	46.22	26.76	155.48	1.02
#38	Corn	36.30	23.14	71.56	1.66
Escapee		15.93	9.01	30.27	1.27
P. includens					
UA	Lab	5.02	0.91	31.30	2.13
#37	Soybean	4.70	2.31	11.96	1.57

Table 3. Susceptibility of bollworm and tobacco budworm to liquid and lyophilized MVP II (Cry1Ac endotoxin) in diet incorporated bioassays (Based on mortality and developmental success).

Insect	Source	LC_{50} (µl/ml of diet)	Fiducial Limits		Slopes
			Lower	Upper	
H. virescens					
Lab	UA	0.21	0.07	0.72	1.36
#6-8	Field	0.26	0.18	0.36	1.64
#27	Field	0.27	0.12	0.46	0.98
H. zea					
UA	Lab	0.66	0.46	0.93	1.67
USDA	Lab	1.35			1.02
#12	Field	2.74	1.89	3.79	1.91
#16	Field	4.30	1.54	10.67	2.05
#17	Field	1.80	1.02	2.70	1.02
#23	Field	7.03	0.63	43.86	1.64
#31	Field	5.21	3.98	6.64	3.36
#33	Field	22.29	16.74	32.85	1.72
#34	Field	13.73	1.44	621.00	1.38

Table 4. Tolerance of bollworm, tobacco budworm and soybean looper to Cry2Ab endotoxin in diet incorporated bioassays (Based on mortality data).

Colony	Source	LC_{50} (%.of diet)[1]	Fiducial Limit		Slope
			Lower	Upper	
H. virescens					
Lab	UA	0.004	0.00	0.02	0.70
Lab	USDA	0.002	0.00	0.01	0.72
#6-8	Corn	0.08	0.03	0.31	1.11
#27	Cotton	0.01	0.00	1.54	1.16
#28	Cotton	0.03	0.01	0.08	1.10
H. zea					
Lab	UA	0.14	0.08	0.23	1.49
Lab	USDA	0.10	0.06	0.14	1.16
#9	Corn	0.59	0.26	3.58	1.69
#11, 13 & 14	Corn	0.55	0.41	0.69	1.96
#16	Sorghum	0.45	0.24	0.81	0.91
#17	Bt-corn	0.46	0.35	0.56	2.43
#18	Corn	0.51	0.33	0.80	1.33
#23	Bt-cotton	0.32	0.23	2.94	2.36
#31	Cotton	0.16	0.02	0.32	1.49
#34	Bt-cotton	0.92	0.76	1.12	2.41
#38	Corn	0.62	0.50	0.77	1.95
Escapee		0.33	0.28	0.38	3.19
P. includens					
Lab	UA	0.03	0.00	0.12	1.42
#37	Soybean	0.04	0.01	0.08	0.85

[1]Corn leaf powder (source of Cry2Ab) was mixed with diet as weight-by-weight basis. For example, for 1% of CryAb/ ml of diet, 1 g of corn leaf powder was mixed with 99 ml of diet.

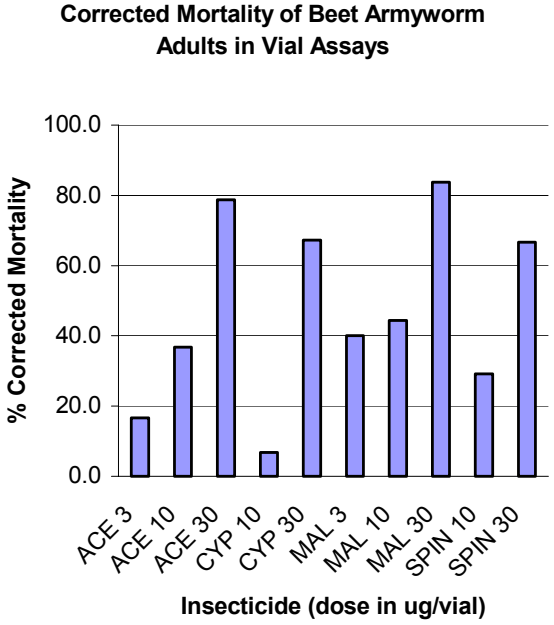

Figure 1. Mortality of beet armyworm adults exposed for ~24 hours to scintillation vials treated with acephate (ACE), cypermethrin (CYP), malathion (MAL), and spinosad (SPIN).

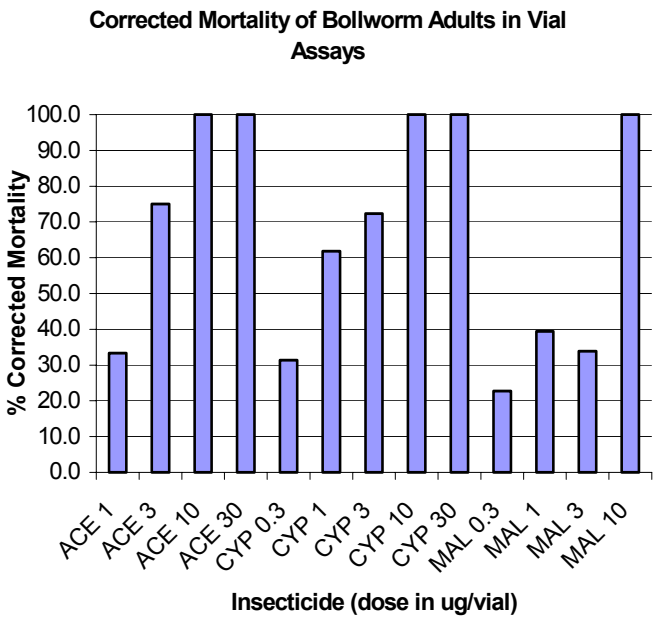

Figure 2. Mortality of bollworm adults exposed for ~24 hours to scintillation vials treated with acephate (ACE), cypermethrin (CYP), and malathion (MAL).

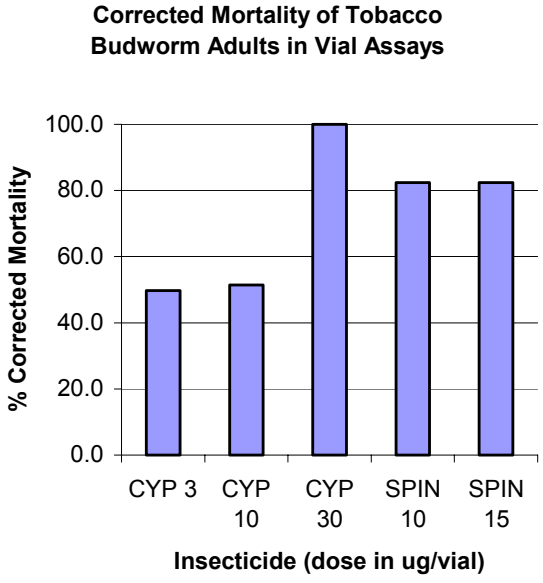

Figure 3. Mortality of tobacco budworm adults exposed for ~24 hours to scintillation vials treated with cypermethrin (CYP) and spinosad (SPIN).

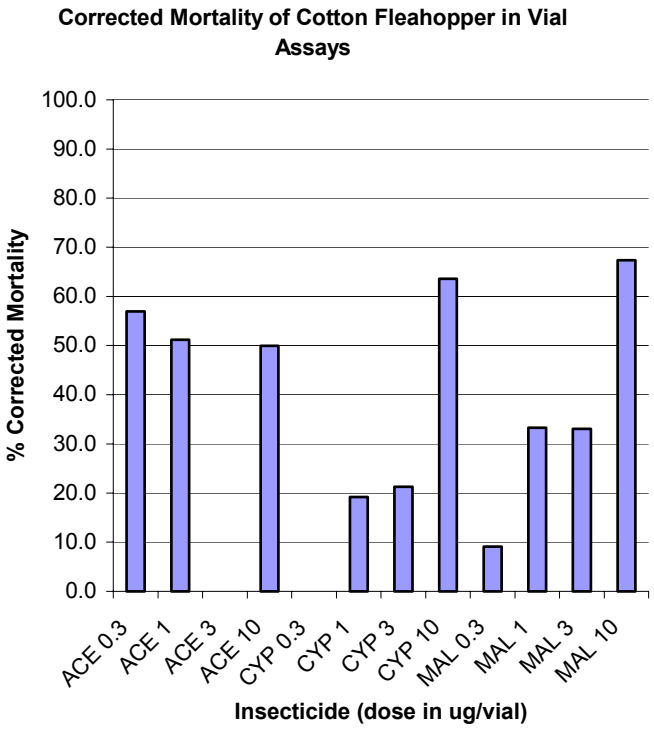

Figure 4. Mortality of cotton fleahopper exposed for ~24 hours to scintillation vials treated with acephate (ACE), cypermethrin (CYP), and malathion (MAL).

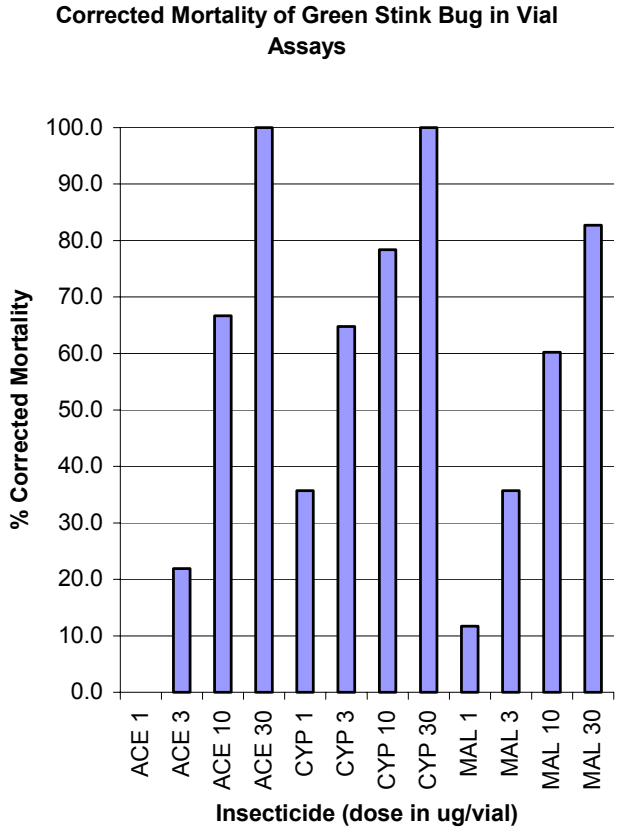

Figure 5. Mortality of green stink bug exposed for ~24 hours to scintillation vials treated with acephate (ACE), cypermethrin (CYP), and malathion (MAL).

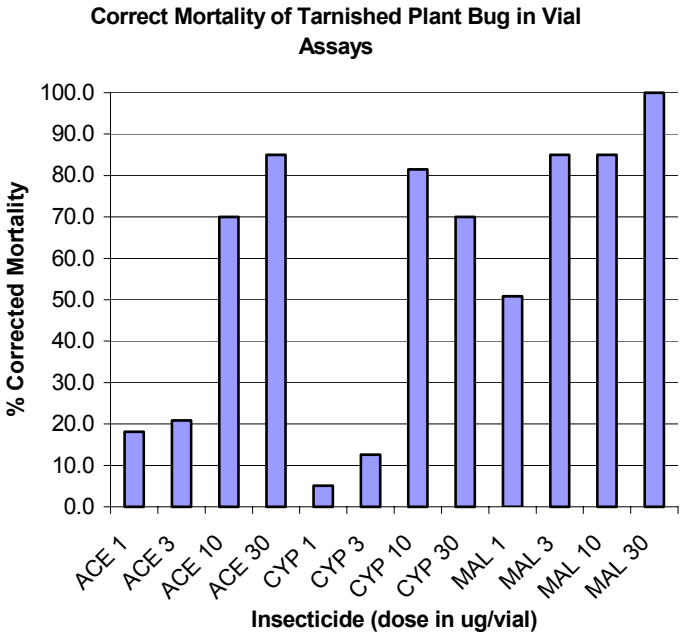

Figure 6. Mortality of tarnished plant bug exposed for ~24 hours to scintillation vials treated with acephate (ACE), cypermethrin (CYP), and malathion (MAL).