2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

BASELINE SUSCEPTIBILITIES OF COTTON INSECTS TO TRANSGENIC INSECTICIDAL PROTEINS AND CHEMICAL INSECTICIDES IN ARKANSAS

M.I. Ali, R.G. Luttrell, Gabriel Horn, and S.Y. Young III
Department of Entomology
University of Arkansas
Fayetteville, AR

Abstract

Four laboratory, eight laboratory cross and twenty field populations of bollworm (BW), *Helicoverpa zea* or tobacco budworm (TBW), *Heliothis virescens* (F.) were assayed for susceptibility to Cry1Ac and Cry2Ab proteins at the University of Arkansas in 2003. The susceptibility of BW to Cry1Ac varied among and between the laboratory, laboratory cross and field populations. In comparison with laboratory susceptible populations, LC_{50}s were 4- to 7-fold higher in colonies collected from conventional crops and 12- to 25-fold higher in colonies collected from Bt crops. Susceptibilities of the laboratory cross and field populations of BW to Cry2Ab were 4- to 18-fold higher than the laboratory susceptible colony. Susceptibilities of field collected noctuids, mirids and pentatomids to acephate, cypermethrin, malathion, spinosad, cyfluthrin and bifenthrin were measured by a treated glass vial technique. Base-line data were established for future references and comparison to first year data reported last year.

Introduction

Bollgard® cottons expressing Cry1Ac insecticidal protein (Bt-cotton) have been widely adopted in commercial cotton production in the southern U.S. This represents a major technical advancement in insect pest management, and insecticide use has declined as a result (Williams, 2003). Potential resistance to Cry1Ac and similar Bt toxins in targeted pest populations remains a major concern for the US cotton industry. Research has shown that the bollworm (BW), *Helicoverpa zea* (Boddie), and the tobacco budworm (TBW), *Heliothis virescens* (F.), have the genetic capacity to evolve resistance to Bt toxins (Stone et al., 1989, Gould et al., 1992, 1995, Luttrell et al., 1999, Burd et al., 2003). BW consistently survives and damages Bt-cotton (Burd et al., 2003), and Bt-cotton is often sprayed for control of BW (Williams, 2003). Susceptibility of heliothines to Bt varies widely among geographically diverse populations (Stone and Sims, 1993, Luttrell et al., 1999, Hardee et al., 2001). During 2002, we (Ali et al., 2003) found that susceptibility of field collected BW and TBW to Cry1Ac and Cry2Ab varied widely in Arkansas and Mississippi.

Conventional insecticides remain an integral component of cotton IPM programs in Arkansas. Thus, detection of resistance evolution and implementation of appropriate management responses has been (Plapp et al., 1990, Roush and Luttrell, 1989, Leonard et al., 1987, Bagwell et al., 2000) and will continue to be a critical component of profitable cotton production. In addition to the concerns for pest resistance to Bt insecticidal proteins, numerous lepidopteran and sucking pests of cotton have repeatedly developed resistance to most classes of insecticide. Managing this evolving process through timely detection and altered strategies is still an important component of contemporary IPM systems. Concerns for resistance to the pyrethroids are of particular interest because of their economic values for the control of BW on Bt-cotton. These sprays directed at BW may indirectly impact other species present in the cotton system.

With increasing Bt-cotton and Bt-corn acreage in Arkansas, the potential for resistance development to Bt toxin by BW or TBW is high. Changing insecticide use patterns are shifting the focus of conventional insecticides to the sucking pest complex and over sprays of Bt-cotton for BW control. This adds to the potential selection pressure for broad-spectrum insecticide resistance. To address these concerns and gather critical data for changing strategic management options, we established a resistance-monitoring program in Arkansas in 2002 (Ali et al., 2003). This is a report of second year activities and results.

Materials and Methods

Cry1AC and Cry2Ab Bioassays

Over 1100 3rd to 5th stage BW and 22 TBW larvae were collected on several dates and different crops from field locations in Arkansas during May to August 2003 (Figure 1). Additionally, one field and five laboratory cross populations (progeny of crosses between field males captured in pheromone traps and laboratory susceptible females) of BW, and two laboratory and two laboratory cross populations of TBW were obtained from USDA-ARS SIMRU, Stoneville, MS. Laboratory susceptible colonies of BW and TBW were maintained at the University of Arkansas and used as references in the Bt assays.

Neonates of BW or TBW were individually exposed to Bt-endotoxin proteins (Cry1Ac and Cry2Ab) in wells of bioassay trays (C-D International) containing appropriate amounts of lyophilized MVPII (Cry1Ac) (19.1 ± 0.60 active ingredient) or corn leaf powder (Cry2Ab) (5.6 mg/g active ingredient) (provided by Monsanto Company, St. Louis, MO) incorporated with pinto bean diet. There were 48 to 112 larvae used for each concentration and 3 to 8 replications were conducted on different

days. Larval mortality and mortality plus stunting (including larvae that failed to molt to second instars in the response data) were recorded after 7 days and regressions were developed on all data using SAS (Probit Procedures). Relationships between LC_{50}s for mortality and mortality plus stunting data across the different colonies were studied by correlation and regression procedures (SAS). The colonies were grouped by source of collection for this paper.

Insecticide Studies

Field collected adult BW, TBW, beet armyworm (BAW), (*Spodoptera exigua* Hübner), and green stink bug (GSB) (*Acrosternum hilare* (Say)), tarnished plant bug (TPB) (*Lygus lineolaris* Palisot de Beauvois) and brown stink bug (BSB) (*Euschistus servus* (Say)) were placed in insecticide-treated 20-ml scintillation vials as descried by Luttrell et al. (1987) and Ali et al. (2003). Mortality (inability to move when probed) data were taken at 12-24 hrs of exposure. Dose-mortality regressions were developed for studies with sufficient dose variability, otherwise, resulting data were corrected for mortality observed in the untreated controls and reported for individual doses.

Results and Discussion

LC_{50}s for mortality of Bw and TBW exposed to Cry1Ac and Cry2Ab were correlated with those for mortality plus stunting (Figure 2, 3).

Susceptibility of laboratory cross and field populations of BW to Cry1Ac varied widely among and between populations. The mean mortality (± S.E.) plus stunting LC_{50} for laboratory cross populations was 1.59 ± 0.54 µg/ml which was 2-fold higher than that of the laboratory susceptible reference. Across the field populations, the LC_{50} for larvae collected from BG II cotton was the highest (26.29 ±13.05 µg/ml), followed by BG I cotton (12.25 ±3.78 µg/ml), Vipcot (Syngenta Crop Protection) (19.30 ±0.03 µg/ml), Bt-corn (13.84 ±3.24 µg/ml), conventional cotton (6.71 ±1.24 µg/ml) and corn (3.66 ±1.17 µg/ml). In comparison to the laboratory susceptible population, LC_{50} values for non-transgenic crops were 3- to 7-fold higher while those for transgenic crops were up to 25-fold higher (Figure 6). In similar studies prior to the commercial release of Bt crops, Luttrell et al. (1999) reported wide variation in susceptibilities of laboratory and field populations of BW. Previously, we (Ali et al. 2003) reported that field colonies from Bt crops in Arkansas and Mississippi were up to 33-fold less susceptible than the most susceptible laboratory colony.

The mean LC_{50}s of laboratory and laboratory cross populations of TBW were 0.40 ±0.16 µg/ml and 0.33 ±0.12 µg/ml, respectively. The LC_{50} of the field population was 0.85 µg/ml (Figure 5). Luttrell et al. (1999) and Ali et al. (2003) both reported more variation among laboratory and field populations of tobacco budworm. Our 2003 studies were limited to only one field colony and more data are needed to study TBW susceptibility to Bt proteins.

The LC_{50} for laboratory BW and laboratory cross populations to Cry2Ab were 3.58 µg/ml and 16.24 ±3.20 µg/ml, respectively. The LC_{50}'s for colonies from BG II cotton, BG I cotton, Bt-corn, cotton, corn and Vipcot were 63.43 ±21.78 µg/ml, 30.00 ±5.94 µg/ml, 20.21 ±7.44 µg/ml, 16.97 ±3.96 µg/ml, 12.56 ±5.83 µg/ml and 16.62 µg/ml, respectively. With the exception of the colony from BG II cotton, LC_{50}s for the laboratory cross and field populations were 4- to 8-fold higher than that for the laboratory reference. The LC_{50} for the colony from BG II cotton was 18-fold higher than the laboratory susceptible colony (Figure 4). Ali et al. (2003) reported that relative to the laboratory colonies, field colonies were 2- to 6-fold less susceptible, and most field colonies were significantly less susceptible than the laboratory colonies.

The LC_{50} of the laboratory and laboratory cross TBW population was 1.97 ± 0.62 µg/ml and 0.81 ±0.11 µg/ml, respectively. The LC_{50} for the single field colony was 1.31 µg/ml (Figure 7). We previously (Ali et al. 2003) reported that field colonies were 2.5- to 40-fold less susceptible than laboratory colonies.

Insecticide Bioassays

Susceptibility of adult noctuids, pentatomids and mirids to insecticides varied within and between species. LC_{50}s for cypermethrin ranged from 0.11 to 7.24 µg/vial. BAW was the least and BW was the most susceptible insects species studied. Among the noctuids, susceptibility to cypermethrin varied in the order of BAW < TBW < BW, while among the hemipteran pests, susceptibility to cypermethrin varied in the order of TPB < BSB < GSB. The LC_{50} for acephate ranged from 1.47 to 3.56 µg/vial, and BSB was the least and TPB was the most susceptible insect. No LC_{50} for acephate for BAW was calculated. LC_{50}s for malathion across all insect species ranged from 1.24 to 1.35 µg/vial. Among these, BW was the least susceptible and TPB was most susceptible (Figure 8).

Across common doses of insecticides, observations of mortality also varied. At 1 µg of bifenthrin per vial, percent corrected mortality ranged from 22.7 to 86.7% for the different species. TPB was the least and BW was the most susceptible insect. At 1 µg of cyfluthrin per vial, percent corrected mortality ranged from 21.2 to 100%. Again, TPB was the least and BW was most susceptible insects. At 15 µg of spinosad per vial, mortality of BW was 81.7%, mortality of TBW and BAW were 61.6 and 25.8%, respectively (Figure 9).

Conclusions

Arkansas populations of cotton insects vary in their response to Bt insecticidal proteins and conventional insecticides. This variation is measurable using traditional laboratory assays on insects from field collections. Routine assessment of changes in relative susceptibilities may be important information for improved management strategies.

LC50s for mortality and mortality plus stunting were significantly related, but R^2 values ranged from 0.5010 to 0.6915 for the different proteins and species studied. This indicates that more than 31-50% of the variation among colonies in LC_{50}s based on mortality was not explained by variability in LC_{50}s based on mortality plus stunting. Using stunting as a component of the Bt response has become a standard procedure (Sims et al., 1996). More research is needed to understand the biological nature of these different laboratory projections of field mortality.

Variation in the response of BW and TBW to Cry1Ac and Cry2Ab continues to illustrate the genetic capacity of both species to develop resistance to Bt insecticidal proteins. Higher LC_{50}s associated with insects from Bt crops illustrates field selection and our ability to actually measure field selection through detailed field sampling and traditional laboratory assay approaches. More data are needed for TBW. Variability in the laboratory cross data suggests that some resistance mechanisms may be inherited as dominant traits carried by adult males since the origin of these colonies were adult males from pheromone traps crossed with laboratory susceptible females.

Baseline data on the susceptibility of all cotton insects to the range of transgenic and traditional insecticides used in the cotton system will become increasingly important. Our preliminary work during 2002 (Ali et al., 2003) and 2003 coupled with information from our USDA and university colleagues in surrounding states will provide important benchmarks for future cotton IPM and resistance management programs.

Acknowledgements

This research was supported by a research initiative grant from the Arkansas Agricultural Experimental Station and a grant from Monsanto Company. Sakuntala Sivasupramaniam, Carlos Blanco, Jeff Gore, John Adamczyk, Dick Hardee, David Black, Clint Allen, Kaylee Luttrell, Bryce Blackman, John Smith, John Kihia, Awatef Akraby, Hana Akrabi, Steven Coy and Tina Gray Teague assisted with field collection or assay of insects.

References

Ali, M.I., Luttrell, R. G., Young, S. Y., Allen, C., and L. Luttrell. 2003. Monitoring insect resistance in Arkansas to chemical insecticides and Bt-endotoxins. Pp. 1138-1149 *In* Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Bagwell, R. D., Cook, D. C., Adamczyk, Leonard, B. R. and S. Micinski. 2000. Status of insecticides resistance in tobacco budworm and bollworm in Louisiana during 1999. Pp 914-918. *In* Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Burd, A.D., Gould, F. Bradley, J. R., Van Duyan, J. W. and W. J. Moar. 2003. Estimated frequency of nonrecessive Bt resistance gene in bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in Eastern North Carolina. J. Econ. Entomol. 96: 137-142.

Gould, F., Martinez-Ramirez, A., Anderson, A, Ferre, J., Silva, F. J. and W. J. Moar. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxin in *Heliothis virescens*. Proc. Natl. Acad. Sci. USA. 89: 7986-7990.

Gould, F., Anderson, A., Reynolds, A., Bumgarner, L. and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxin. J. Econ. Entomol. 88: 1545-1559.

Hardee, D. D., Adams, L. C., Solomon, W. L., and D. V. Sumerford. 2001. Tolerance to Cry1Ac in populations of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae): Three-year summary. J. Agric. Urban Entomol. 18: 187-197.

Luttrell, R. G., R. T. Roush, A. Ali. J. S. Mink, M.R. Reid and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of Heliothis virescens (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80:985-989.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92: 21-32.

Leonard, B. R., Graves, J. B., T. C. Sparks and A. M. Pavloff. 1987. Susceptibility of bollworm and tobacco budworm larvae to pyrethroid and organophosphate insecticides. pp 320-324. *In* Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Plapp, F. W., Jr., J. A. Jackson, C. Campanhola, R. E. Frisbie, J. B. Graves, R. G. Luttrell, W. F. Kitten, and M. Wall. 1990. Monitoring and management of pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. J. Econ. Entomol. 83: 335-341.

Roush, R. T. and R. G. Luttrell. 1989. Expression of resistance to pyrethroid insecticides in adults and larvae of tobacco budworm (Lepidoptera: Noctuidae): implications for resistance monitoring. J. Econ. Entomol. 82: 1305-1310

Sims, S. R., J. G. Greenplate, T. B. Stone, M. A. Caprio, and F. L. Gould. 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins, pp. 229-242. In Molecular genetics and evolution of pesticide resistance. ACS Symposium Series No. 645. American Chemical Society, Washington, DC.

Stone, T. B., Sim, S. R. and P. G. Marrone. 1989. Selection of tobacco budworm to a genetically engineered *Pseudomonas fluorescens* containing the ∂–endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*. J. Invert. Pathol. 53: 228-234.

Stone, T. B., and S.R. Sims. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 986-994.

Williams, M. R. 2003. Cotton insect loses 2002. pp 101 109. *In* Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

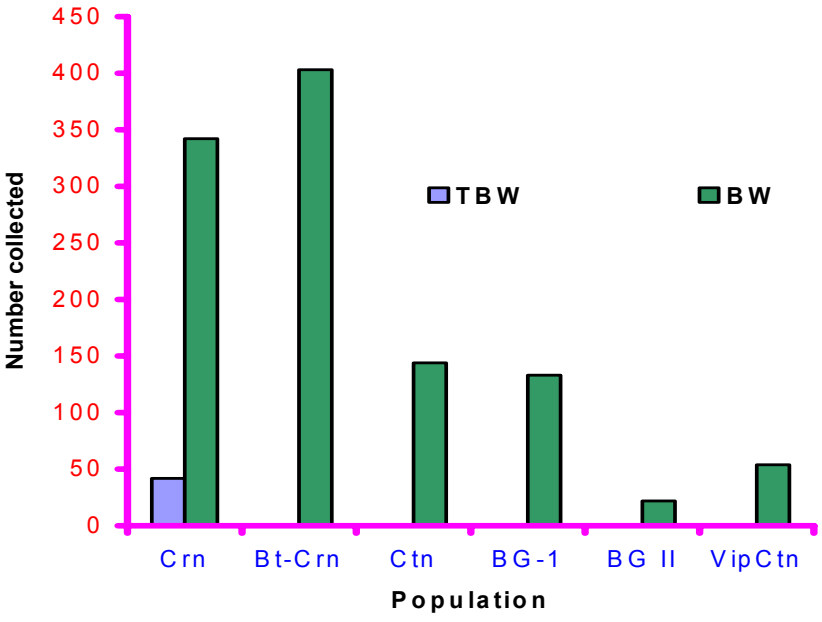

Figure 1. Number of heliothine larvae collected from different crops in Arkansas during 2003.

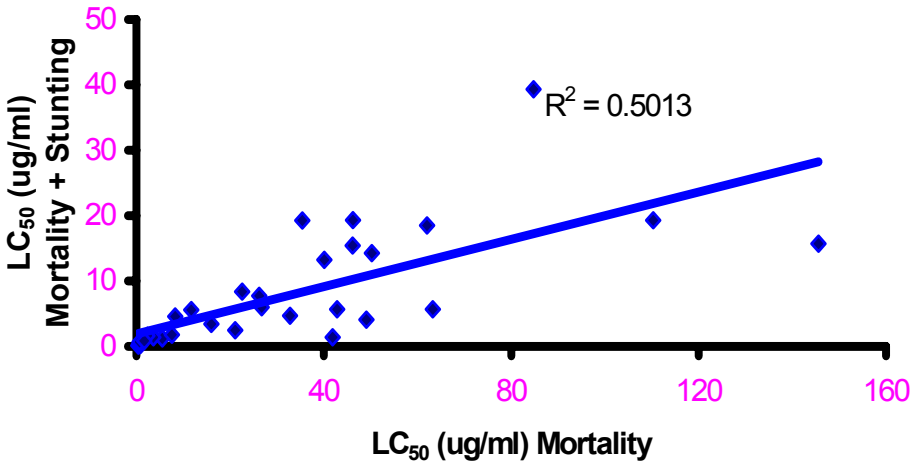

Figure 2. Relationships between LC_{50}s for mortality and mortality plus stunting for BW and TBW exposed to Cry1Ac.

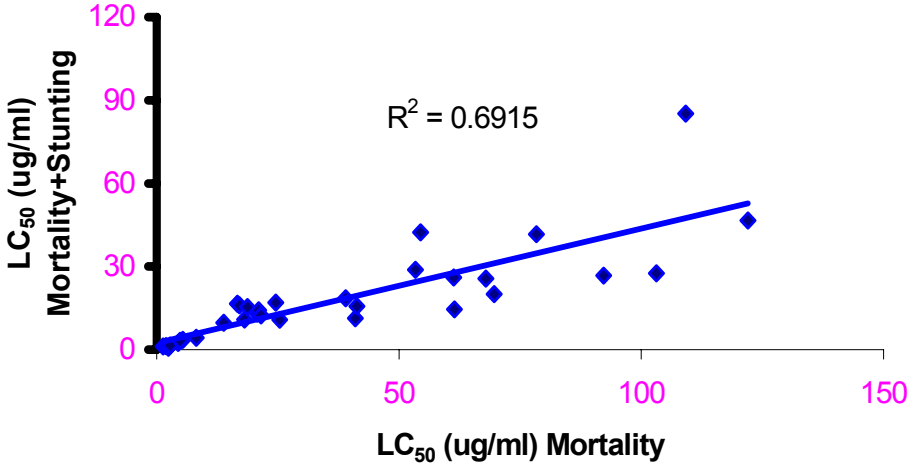

Figure 3. Relationships between LC_{50}s for mortality and mortality plus stunting for BW and TBW exposed to Cry2Ab.

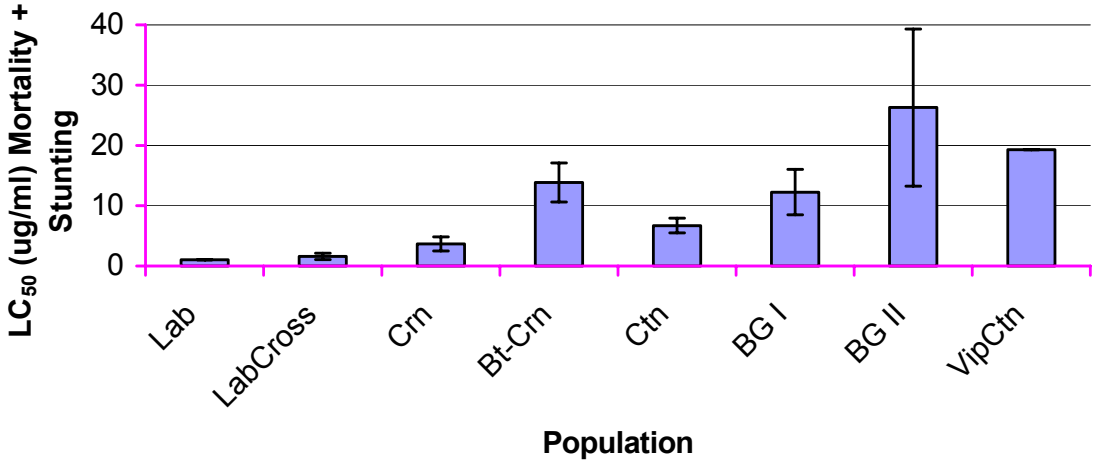

Figure 4. Mean Cry1Ac LC_{50} (±S.E.) for BW collected from corn (Crn), Bt-corn (Bt-Crn), conventional cotton (Ctn), Bollgard I (BG I), Bollgard II (BG II) and Vipcot (VipCtn) in Arkansas during 2003.

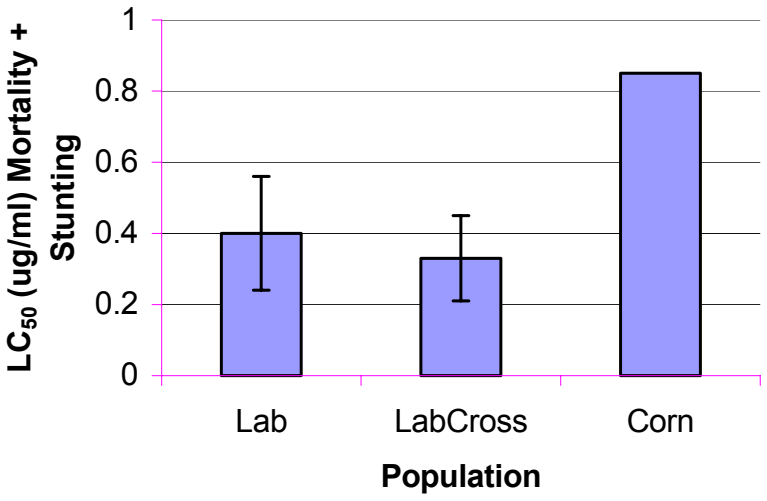

Figure 5. Mean Cry1Ac LC_{50} (± S.E.) for TBW collected from different crops in Arkansas during 2003.

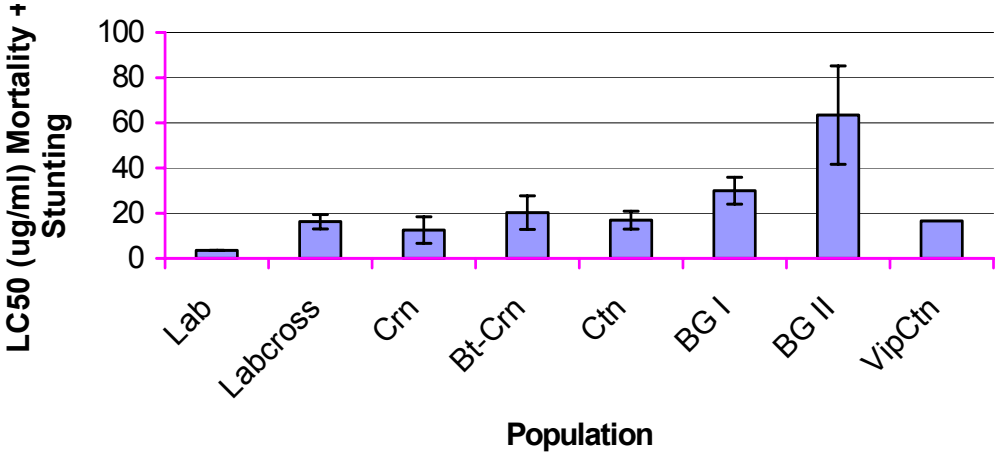

Figure 6. Mean Cry2Ab LC_{50} (± S.E.) for BW collected from corn (Crn), Bt-corn (Bt-Crn), conventional cotton (Ctn), Bollgard I (BG I), Bollgard II (BG II) and Vipcot (VipCtn) in Arkansas during 2003.

Figure 7. Mean Cry2Ab LC_{50} (± S.E.) for TBW collected from different crops in Arkansas during 2003.

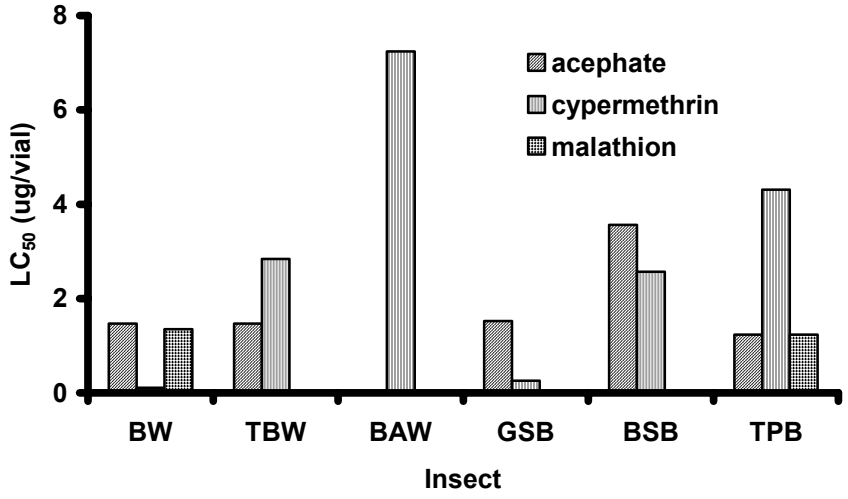

Figure 8. LC_{50} of adult noctuids, pentatomids and mirids exposed to insecticides in treated vial assays

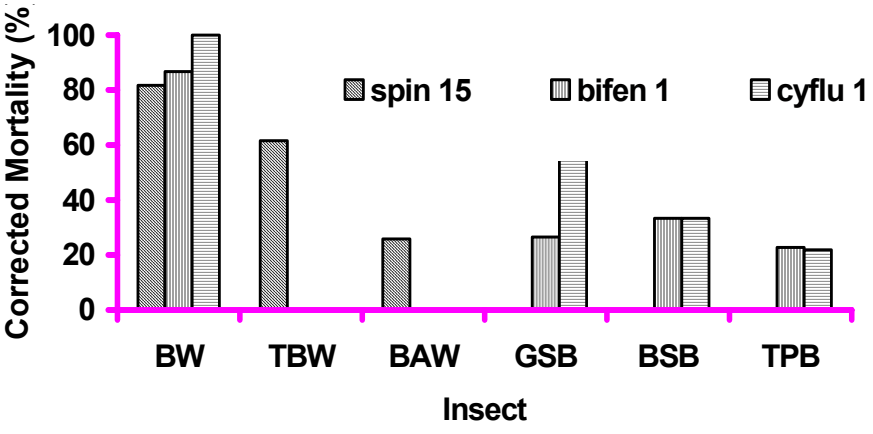

Figure 9. Corrected mortality (%) of noctuids, pentatomids and mirids exposed to spinosad (spin), bifenthrin (bifen) and cyfluthrin (cyflu) in treated vial assays.