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MEASURING BT SUSCEPTIBILITY IN HELIOTHINE POPULATIONS IN ARKANSAS: RESULTS OF THIRD YEAR STUDIES

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Abstract

Twelve tobacco budworm, *Helicoverpa virescens* and 31 bollworm, *Helicoverpa zea* laboratory and field colonies were assayed for susceptibility to Cry1Ac and Cry2Ab proteins at the University of Arkansas in 2004. In comparison to a University of Arkansas laboratory susceptible population, mean susceptibilities of field populations of bollworm were 3- to 6-fold and 8- to 10-fold higher than colonies collected from conventional and transgenic Bt crops, respectively. Susceptibilities of laboratory cross and field populations of bollworm to Cry1Ac and Cry2Ab were 3- to 4-fold higher than the laboratory susceptible colony. Susceptibility of laboratory cross and field populations of tobacco budworm to both Cry1Ac and Cry2Ab varied up to 2 fold as compared to a University of Arkansas laboratory colony.

Introduction

Transgenic crops expressing Bt toxins have been widely adapted in commercial crop production in the southern U.S. This has resulted in a reduction in insecticide use in cotton (Layton et al. 2003) and a major shift in insect management systems with more emphasis placed on fixed costs of production. Bollgard II® cotton expressing multiple insecticidal proteins has been commercially released and other transgenic crops expressing different proteins will soon be commercially available. A majority of the current cotton acreage in Arkansas is planted to Bt cotton resulting in significant potential selection for resistance and cross-resistance. This selection may be further enhanced by increased acreage of Bt corn that also serves as a source of food of bollworm, *Helicoverpa zea* (Boddie).

Research has shown that the bollworm and the tobacco budworm, *Heliothis virescens* (F.), have the genetic capacity to evolve resistance to Bt toxins in the laboratory (Stone et al. 1989, Gould et al. 1992, 1995, Burd et al. 2003). Even though no control failure has been reported, heliothine larvae are found surviving on Bt cotton (Burd et al. 2003, Tabashnik et al. 2003, Akhurst et al. 2003) and Bt cotton is routinely sprayed for control of bollworm (Layton et al. 2003). Susceptibility of heliothines to Bt may vary widely among geographically diverse populations (Stone and Sims 1993, Luttrell et al. 1999, Hardee et al. 2001). During 2002 and 2003, we have found that susceptibility of field collected bollworm and tobacco budworm to Cry1Ac and Cry2Ab varies within Arkansas (Ali et al. 2003, 2004, Luttrell et al. 2004). There is less variability within tobacco budworm. Variability in field populations of bollworm seems to be associated with within season selection. Higher LC_{50}s arte usually associated with larvae collected from Bt crops and those collected late in the season. With the increasing Bt cotton acreage in Arkansas, the potential for resistance development to Bt toxin by bollworm or tobacco budworm is a major concern for producers and technology regulators. To better understand the potential for Bt resistance in heliothines in Arkansas, we initiated a resistance-monitoring program in 2002 to monitor shifts in concentration-mortality responses associated with field collected insect pests. This report describes the third year of our results (Ali et al. 2003, 2004).

Materials and Methods

Bt Endotoxin Studies

Susceptibilities of laboratory, laboratory cross and field populations of bollworm and tobacco budworm to Bt endotoxin proteins were studied by establishing laboratory colonies from field collections and exposing progenies (1-4 generation) to Cry1Ac and Cry2Ab endotoxin proteins in a diet incorporation bioassay (Luttrell et al. 1999, Ali et al. 2003, 2004). Over 1300 3rd to 5th stage bollworm and 200 tobacco budworm larvae were collected on several dates and different crops from field locations in Arkansas during May to August 2004. Six tobacco budworm and 26 bollworm colonies were established (Table 1). Additionally, one field and five laboratory cross populations (progeny of crosses between field males captured in pheromone traps and laboratory susceptible females) of bollworm and four laboratory cross populations of tobacco budworm were obtained from the USDA-ARS, Southern Insect Management Research Unit, Stoneville, MS. One laboratory colony of bollworm and one colony of tobacco budworm were received from Monsanto Company. St. Louis, MO. University of Arkansas (UA) laboratory

susceptible colonies of bollworm and tobacco budworm were used as references in all Bt assays. Colonies were maintained on a pinto bean artificial diet (Burton, 1969) in the Margaret McClendon Insect Rearing Facility, Department of Entomology, University of Arkansas, Fayetteville, AR 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.

Neonate bollworm or tobacco budworm were individually exposed to Bt-endotoxin in wells of bioassay trays (C-D International) containing appropriate amounts of lyophilized MVPII (Cry1Ac) or corn leaf powder (Cry2Ab) (provided by Monsanto Company) 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 those that failed to molt to second instar were recorded after 7 days of exposure to the treated diet. Concentration-mortality regressions were developed on all data using SAS (Probit Procedures).

Results and Discussions

The number of bollworm larvae collected during 2004 varied among crops. Highest numbers of larvae were collected on Bt corn (N=607), followed by corn (N=306), conventional cotton (N=225), soybean (N=137), pigeon pea (N=90), Bt cotton (N=42) and wild geranium (N=28). Among the tobacco budworm, the number of larvae collected from several non-crop hosts was similar and ranged from 50 to 70 on paulownia, geranium, velvet leaf and pigeon pea. No tobacco budworm was collected on Bt crop.

Table1. Laboratory, laboratory cross and field colonies of bollworm and tobacco budworm established for Bt assay during 2004.

Colony	Date of Collection	Place of Collection	Colony Type	Host Plant/Source
H. virescens				
MonLab	NA	NA	Lab	Monsanto Lab
UALab	NA	NA	Lab	UA Lab
C4204	NA	NA	Lab-Cross	USDA-ARS-Stoneville, MS
C4304	NA	NA	Lab-Cross	Florida
C4604	NA	NA	Lab-Cross	Texas
C4704	NA	NA	Lab-Cross	Texas
F0404	05/19/04	Tillar, AR	Field	Paulownia
F0504	05/28/04	Tillar, AR	Field	Geranium
F2604	08/02/04	Foreman, AR	Field	Velvetleaf
F3904	09/02/04	Tillar, AR	Field	Paulownia
F4004	09/02/04	Tillar, AR	Field	Paulownia
F4504	09/22/04	Fayetteville, AR	Field	Pigeon pea
H. zea				
Lab	NA	NA	Lab	UA Lab
Lab	NA	NA	Lab	Monsanto Lab
C4404	NA	NA	Lab-Cross	Florida
C4804	NA	NA	Lab-Cross	Texas
C4904	NA	NA	Lab-Cross	Texas
C5304	NA	NA	Lab-Cross	Florida
C5004	NA	NA	Lab-Cross	Arkansas
F0104	05/16/04	Tillar, AR	Field	Corn
F0204	05/19/04	Tillar, AR	Field	Corn
F0304	05/19/04	Tillar, AR	Field	Geranium
F0704	06/06/04	Foreman, AR	Field	Corn
F0904	06/08/04	Tillar, AR	Field	Bt corn

F1004	06/10/04	Tillar, AR	Field	Cotton
F1104	06/20/04	Tillar, AR	Field	Corn
F1204	06/21/04	Foreman, AR	Field	Bt corn
F1304	06/30/04	Tillar, AR	Field	Bt corn
F1404	06/29/04	Foreman, AR	Field	Bt corn
F1504	06/23/04	Tillar, AR	Field	Bt corn
F1604	06/30/04	Tillar, AR	Field	Bt corn
F1704	06/30/04	Tillar, AR	Field	Bt corn
F1804	07/14/04	Foreman, AR	Field	Soybean
F2004	07/20/04	Foreman, AR	Field	Soybean
F2104	07/20/04	Foreman, AR	Field	Corn
F2204	07/23/04	Monticello, AR	Field	Bt corn
F3004	08/10/04	Stoneville, MS	Field	Bt corn
F3104	08/11/04	Tillar, AR	Field	Cotton
F3304	08/13/04	Fayetteville, AR	Field	Cotton
F3404	07/22/04	Tillar, AR	Field	Cotton
F3704	07/22/04	Pickens, AR	Field	Cotton
F3804	07/22/04	Tillar, AR	Field	Cotton
F4104	09/22/04	Fayetteville, AR	Field	Pigeon pea
F5104	06/21/04	Foreman, AR	Field	Bt corn
F5204	06/21/04	Foreman, AR	Field	Bt corn

Field colonies of bollworm were established from larvae collected on Bt corn (N=11), cotton (N=6), corn (N=5) and soybean (N=2). One colony was established from pigeon pea and one was established from larvae collected on wild geranium. Three field colonies of tobacco budworm were established from larvae collected on paulownia (a suspected refuge crop for tobacco budworm). One colony was established from larvae collected on velvetleaf, geranium and pigeon pea (Table 1).

Studies with Cry1Ac

Susceptibility of laboratory, laboratory cross and field populations of bollworm to Cry1Ac varied among and between populations (Figure 1). The LC_{50}s for UA laboratory and Monsanto laboratory colonies were 3.02 and 19.83 µg/ml, respectively. The mean LC_{50}s for laboratory cross, Bt corn, corn and cotton populations were 10.35 ± 4.06 µg/ml, 32.91 ± 5.82 µg/ml, 10.27 ± 3.37 µg/ml and 20.48 ± 6.04 µg/ml (mean ± S.E.), respectively. As compared to UA lab colony, the mean LC_{50} of laboratory cross, Bt corn, corn and cotton were 3-10 fold higher. However, as compared to the Monsanto laboratory colony, the mean LC_{50} of insects from Bt corn was only 1.6 fold higher. Luttrell et al. (1999) reported wide variation in susceptibilities of laboratory and field populations of bollworm in the 1990's. In our first year report (Ali et al. 2003), we reported that LC_{50}s of 14 field colonies ranged from 4 to 85 µg of toxin/ml of diet. Based on overlapping fiducial limits, 10 of these colonies had significantly higher LC_{50}s than laboratory colonies. In our second year report (2004), we indicated that field colonies from Bt crops in Arkansas and Mississippi were up to 25-fold less susceptible than the most susceptible laboratory colony. This year we did not establish field colonies from Bollgard I®, Bollgard II® or Vipcot® cottons. Results of assays with colonies established from other crops were generally within the range of those reported last year (Ali et al. 2004). We are still working with a colony of bollworm (colony F3704) collected as eggs on conventional cotton near Pickens, AR The site of collection was surrounded by Bt crops. Preliminary results are showing elevated LC_{50}s, but we need more investigation. The insects are being studied by scientists of other laboratories to confirm our preliminary work.

As compared to the Monsanto laboratory bollworm colony, the UA bollworm laboratory colony was over 6-fold more susceptible to Cy1Ac in 2004 studies. Susceptibility of the UA laboratory colony to Cry1Ac was similar to other laboratory colonies reported by Sims et al. (1993) for another Monsanto laboratory colony (1.35 µg/ml), Luttrell et al. (1999) for several laboratory colonies (0.02 to 8.82 µg/ml), Burd et al. (2002) for a laboratory control colony (2.78 to 3.00 µg/ml) and Ali et al. (2003) for a USDA-ARS, Stoneville, MS laboratory colony (1.75 µg/ml).

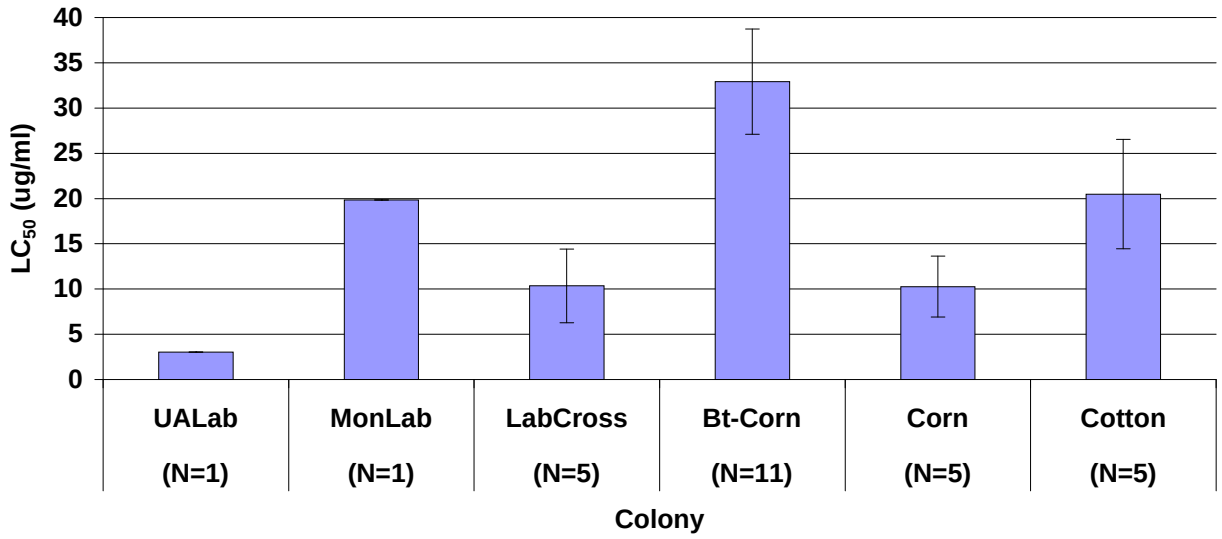


Fig. 1. Average mortality LC_{50} estimates of laboratory, laboratory cross and field populations of bollworm exposed to Cry1Ac in diet-incorporated assays. Figures in parentheses are number of colonies included in the overall average.

The LC_{50}s of UA and Monsanto laboratory colonies of tobacco budworm were 1.45 and 1.34 µg/ml, respectively. The mean LC_{50}s (± S.E.) of laboratory cross and field populations of tobacco budworm were 2.91 ±0.66 µg/ml and 1.62 ±0.55 µg/ml, respectively. In reference to the UA laboratory colony, LC_{50S} of laboratory cross colonies were up to 2 fold higher (Figure 2). Luttrell et al. (1999) and Ali et al. (2003) both reported more variation among laboratory and field populations of tobacco budworm. Tobacco budworm susceptibility to Cry1Ac appear to be rather stable and much less variable than bollworms susceptibility.

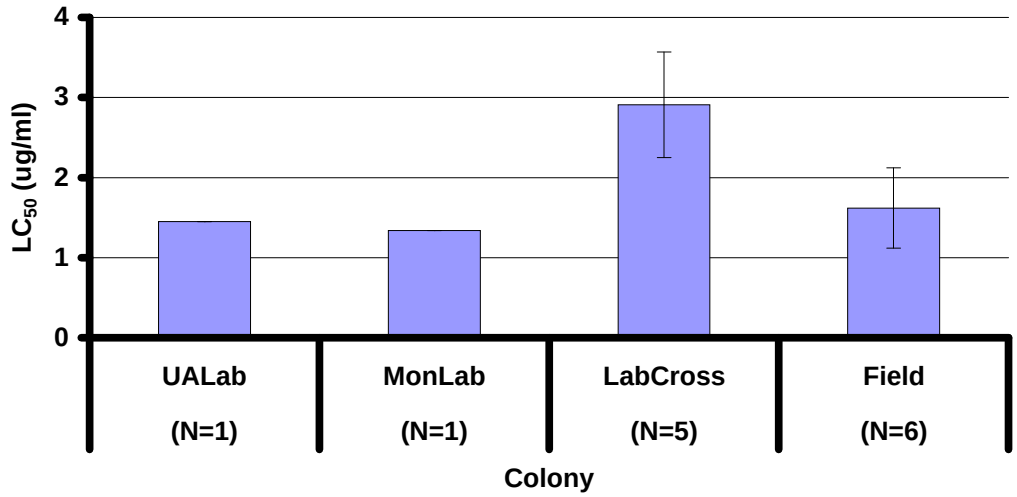


Fig. 2. Average mortality LC_{50} estimates of laboratory, laboratory cross and field populations of tobacco budworm exposed to Cry1Ac in diet-incorporated assays. Figures in parentheses are number of colonies included in the overall average.

LC_{50} estimates for mortality and mortality plus stunting were positively correlated in bollworm (R^2 = 299, N = 30, P > 0.0018) (Figure 3) but not with tobacco budworm (R^2 = 0.075, N = 12, P > 0.3907) (Figure 4) exposed to Cry1Ac.

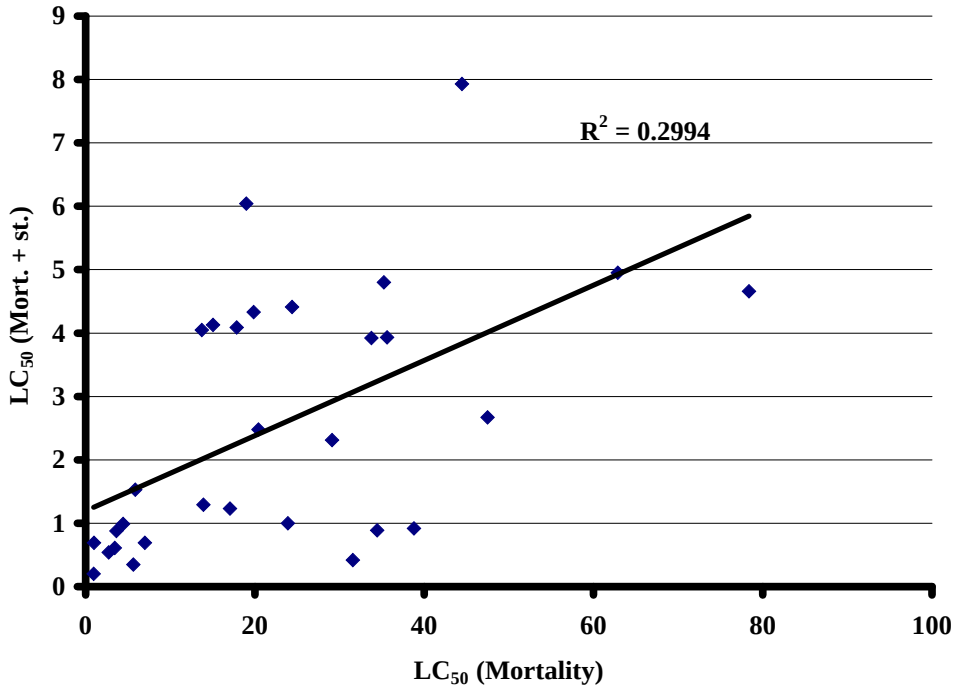


Figure 3. Correlation of LC_{50} (µg/ml) estimates for mortality and mortality plus stunting for larvae of bollworm exposed to Cry1Ac in 2004 studies.

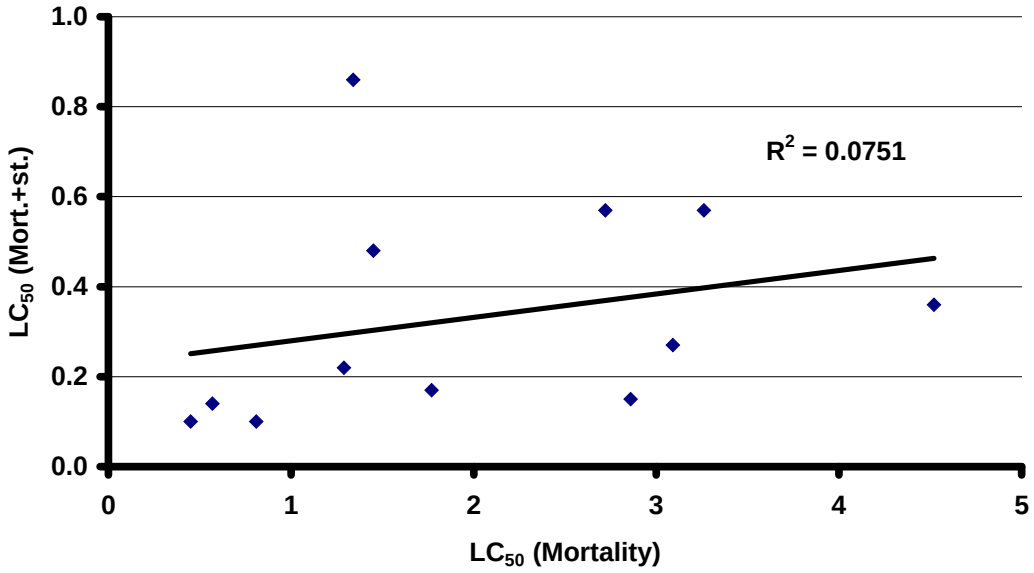


Fig. 4. Correlation of LC_{50} (µg/ml) estimates for mortality and mortality plus stunting for larvae of tobacco budworm exposed to Cry1Ac in 2004 studies.

Studies with Cry2Ab

Susceptibility of laboratory, laboratory cross and field populations of bollworm to Cry2Ab varied among and between populations (Figure 5). The LC_{50} for the Monsanto laboratory colony (96.98 µg/ml) and the UA laboratory (5.98 µg/ml) varied up to 16 fold. LC_{50} (mean ± S.E.) for laboratory cross, Bt corn, corn and cotton populations were 23.69 ± 11.72 µg/ml, 49.33 ± 7.49 µg/ml, 21.52 ± 6.38 µg/ml and 47.64 ± 8.32 µg/ml, respectively. Susceptibilities of laboratory cross and field populations showed up to 8-fold variation as compared to the UA laboratory colony. All laboratory and field populations had lower LC_{50}s than the Monsanto laboratory colony. In our first year study, we (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. In our second year study (Ali et al. 2004), we reported that with the exception of a 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 a colony established from larvae on BG II cotton was 18-fold higher than the laboratory susceptible colony. During 2004, we did not establish field colonies from Bollgard I®, Bollgard II® or VipCot® cottons.

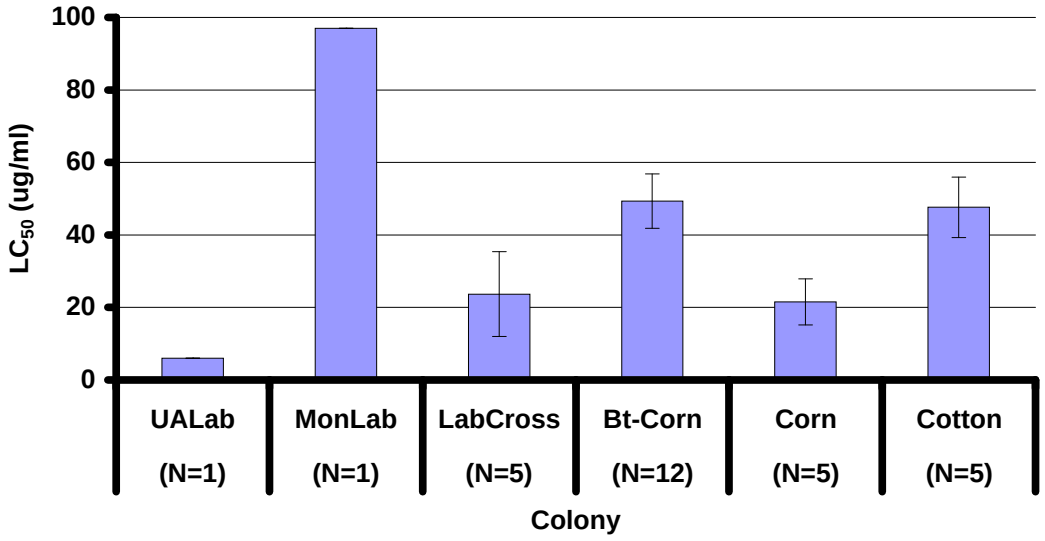


Fig. 5. Average mortality LC_{50} estimates of laboratory, laboratory cross and field populations of bollworm exposed to Cry2Ab in diet-incorporated assays. Figures in parentheses are number of colonies included in the overall average.

As compared to the Monsanto laboratory bollworm colony, the UA bollworm laboratory colony was over 16-fold more susceptible to Cry2Ab endotoxins. Susceptibility of the UA laboratory colony to Cry2Ab was similar to that measured for the USDA-ARS, Stoneville, MS laboratory colony in 2002 (Ali et al. 2003).

LC_{50}s of the UA and the Monsanto laboratory colonies of tobacco budworm were 1.45 and 5.41 µg/ml, respectively. The mean LC_{50}s (± S.E.) of laboratory cross and field populations of tobacco budworm were 2.85 ±0.11 µg/ml and 1.92 ±0.66 µg/ml, respectively. In reference to the UA laboratory colony, the LC_{50} of laboratory cross colonies were up to 1.9 fold higher (Figure 6).

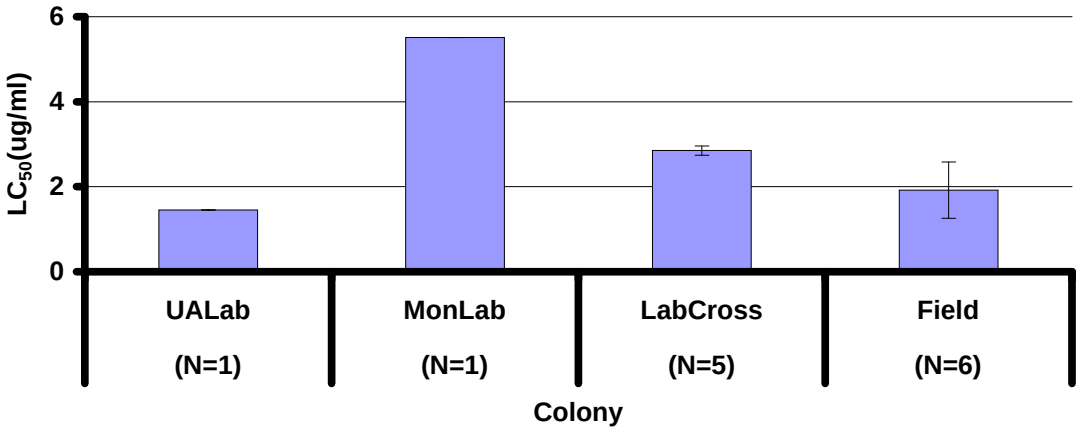
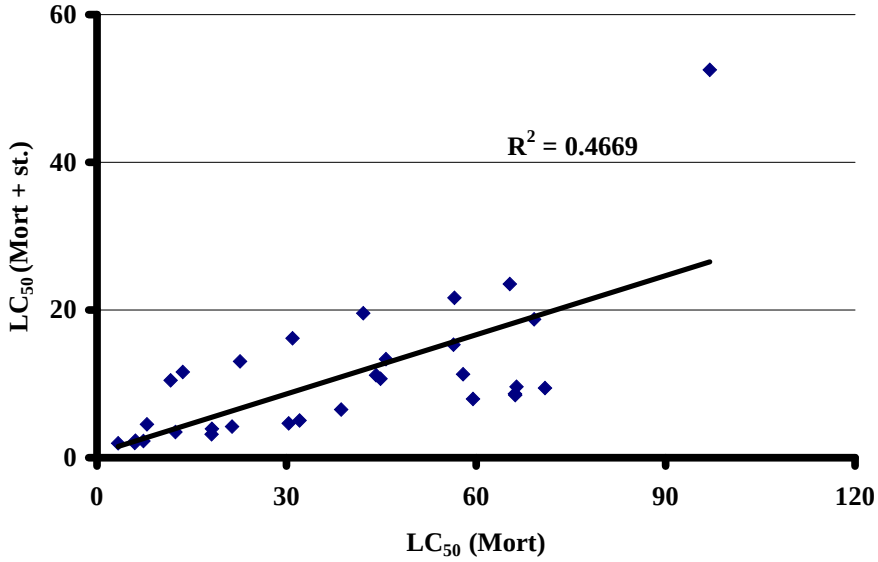


Fig. 6. Average mortality LC_{50} estimates of laboratory, laboratory cross and field populations of tobacco budworm exposed to Cry2Ab in diet-incorporated assays. Figures in parentheses are number of colonies included in the overall average.

LC_{50} estimates for mortality and mortality plus stunting showed significant positive relationship in bollworm (R^2 = 467, N = 31, P > 0.0001) (Figure 7) and tobacco budworm (R^2 = 0.632, N = 12, P > 0.0020) (Figure 8) exposed to Cry2Ab.



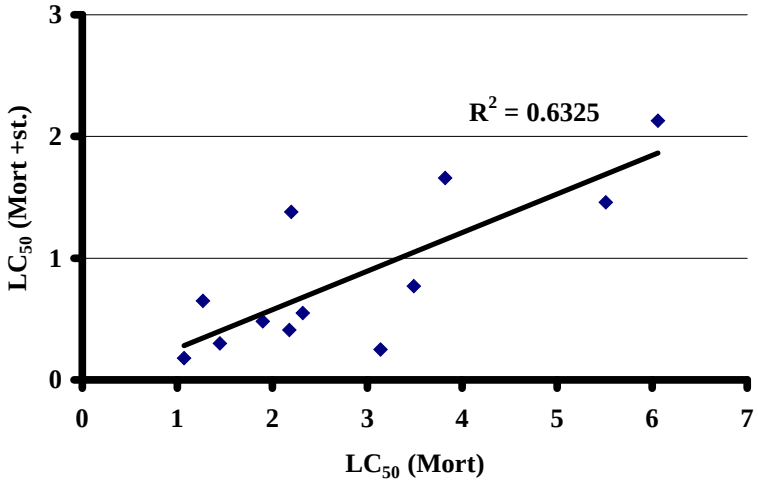


Figure 7. Correlation of LC_{50} (µg/ml) estimates for mortality and mortality plus stunting for larvae of bollworm exposed to Cry2Ab in 2004 studies.

Figure 8. Correlation of LC_{50} (µg/ml) estimates for mortality and mortality plus stunting for larvae of tobacco budworm exposed to Cry2Ab in 2004 studies.

Summary and Conclusions

Results showed that susceptibilities of bollworm to Cry1Ac and Cry2Ab endotoxins varied among field colonies. These results support findings of Luttrell et al (1999), Hardee et al. (2002), and Ali et al. (2003, 2004), where variation in susceptibilities of tobacco budworm and bollworm were observed in field populations exposed to Cry1Ac proteins.

Like our two previous reports (Ali et al. 2003, 2004), higher LC_{50}s were associated with colonies established from collections of larvae from Bt crops and those larvae collected later in the season. This suggests ongoing natural selection in the field environment. Our assay results seem to be sensitive enough to detect these seasonal shifts in susceptibilities. The genetic base of these differences is uncertain at this time.

Except for tobacco budworm exposed to Cry1Ac, LC_{50}s for mortality and mortality plus stunting were significantly related. However, R^2 values ranged from 0.2994 to 0.6325 for the different proteins and species studied. This indicates that more than 27-70% of the variation among colonies in LC_{50}s based on mortality was not explained by variation within LC_{50}s for mortalities plus stunting. Previously we have reported similar relationships among LC_{50}s for mortality and mortality plus stunning for larvae of bollworm and tobacco budworm exposed to Cy1Ac and Cry2Ab proteins (Ali et. al. 2004). This difference in response among the assay procedures and laboratory colonies with different levels of susceptibility is the focus of our ongoing research.

As compared to the Monsanto laboratory bollworm colony, the UA bollworm laboratory colony was over 6-fold and 16-fold more susceptible to Cy1Ac and Cry2Ab endotoxins, respectively. The UA laboratory bollworm colony has been maintained on a pinto bean and wheat-germ based artificial diet (Burton, 1969) for 20 years with no expose to external genetic materials. It is considered inbred and highly susceptible. The Monsanto laboratory strain and the most recent USDA-ARS at Stoneville, MS have been infused with genetic materials from the field (Sakuntala Sivasupramaniam and Carlos Blanco, personal communication). This probably increases the relative vigor and health of the colonies, but it may also be a source for introduction of rather common genetic traits. Future studies should consider this variability in response of laboratory strains.

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