# MONITORING FOR TOLERANCE TO CRYIAc IN POPULATIONS OF TOBACCO BUDWORM AND COTTON BOLLWORM: SUMMER 1998

**D. V. Sumerford and W. L. Solomon**
**USDA-ARS-SIMRU**
**Stoneville, MS**

## Abstract

In an examination of a laboratory colony of *Helicoverpa zea* (Boddie), we found heritable variation to the Bt toxin, CryIAc. Based on this preliminary finding, we used a similar bioassay to monitor field populations of *H. zea* and *Heliothis virescens* F. There was significant variation in the log weights (mg) (measure of tolerance to CryIAc both within and between field colonies for both species). The average log weights (mg) and % mortality of colonies were negatively correlated in both species. Additional testing of one colony of *H. virescens* found that susceptibility to CryIAc could be transmitted to offspring. We are currently conducting follow up tests on the remaining field colonies to determine if the variation in tolerances has a heritable basis.

## Introduction

The conservation of susceptibility to the delta endotoxin proteins of *Bacillus thuringiensis* (*Bt*) in field populations of insect pests of cotton has received considerable interest. First, resistance to *Bt* proteins has been documented in laboratory and field populations of several insect pests (Stone et al. 1989, Gould et al. 1992, 1995, Moar et al. 1995, Tabashnik et al. 1990, 1997). In addition, the recent registration and deployment of transgenic cotton expressing the *Bt* protein, CryIAc, and the development of resistance to many conventional insecticides by Lepidopterous pests of cotton have made the preservation of susceptibility to CryIAc an important goal of pest management in cotton-growing areas. To effectively manage resistance it is necessary to monitor insect pests of cotton for changes in their tolerances of CryIAc. Tolerances to CryIAc in field populations of *Heliothis virescens* F. (tobacco budworm, TBW) and *Helicoverpa zea* (Boddie) (cotton bollworm, CBW) were monitored via of a bioassay using doses of CryIAc designed to detect quantitative differences in tolerance. We present the results of these bioassays in this paper.

## Methods and Materials

A study was conducted to determine if heritable variation was present in a laboratory strain of CBW ("WP-Hz"). Individuals from WP-Hz were fed on artificial diet containing 0.5 µg/ml CryIAc for 7-days, weighed, and then transferred to non-toxic diet to finish larval development ("Parental" generation). At the pupal stage, survivors were placed into two groups based on the median of the 7-day log weights (mg) (weights above median = "Large" group, below median = "Small" group. Adults from these two groups were mated *inter se* and their offspring were tested on both 0.5 µg/ml CryIAc and non-toxic diet. Weights of larvae on non-toxic diet were used as an indicator of general vigor. If larvae from the Small group develop significantly slower on the non-toxic diet than larvae from the Large group, then slower growth on toxic diet by the Small group is more indicative of less vigorous larvae rather than a difference in tolerance to CryIAc. Based on the preliminary results of this study, we used a similar bioassay for work with field populations of CBW and also modified the bioassay for use with TBW.

During the 1998 field season, entomologists and consultants from seven states within the U. S. cotton belt collected eggs or larvae produced by field populations of TBW and CBW. The collected individuals ($F_1$'s) were shipped to USDA-ARS, Stoneville, MS, reared to pupation on artificial diet (29 ± 3 °C, 55-60% RH, 14:10 (L:D) h photoperiod) and subsequent generations of these $F_1$ adults were evaluated for tolerance to CryIAc. Field populations producing fewer than 50 $F_1$ adults were discarded to diminish the likelihood of inbreeding effects.

In this report, we summarize the efforts of one part of the resistance-monitoring program for the Southern Insect Management Research Unit, Stoneville, MS. Larvae from submitted colonies were evaluated via a bioassay using 7-day and 10-day weights for CBW and TBW, respectively. Weights were used as a quantitative measure of tolerance to CryIAc (29 ± 3 °C, 55-60% RH, 14:10 (L:D) h photoperiod). Doses for these tests were 0.05 and 0.5 µg/ml for TBW and CBW, respectively. To make the toxic diet, a concentrated solution of freeze-dried MVPII was incorporated into 300ml of artificial diet to obtain the desired concentration of CryIAc. Neonates were placed in 30-ml cups containing approx. 5 ml of the artificial diet. In addition to the tests on CryIAc diet, some larvae were also tested on non-toxic diet. Larvae were weighed to the nearest hundredth of a milligram. All analyses of weights were performed on log transformed data. Mortality was also recorded at the time of weighing.

## Results

### Preliminary Analysis of WP-Hz

Preliminary work with a lab strain (WP-Hz) of CBW found that there was heritable variation for tolerance to CryIAc. The average log weight (±SE) of the parental generation was 0.940 (0.055). There was also a great deal of variation in log weights within the lab strain (CV = 67.7%). Figure 1 presents the mean weights of the "Large" and "Small" groups on CryIAc and non-toxic diet. When challenged on the CryIAc diet, CBW larvae from the Large group were

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1237-1239 (1999)
National Cotton Council, Memphis TN

significantly larger than larvae from the Small group ($P$ = 0.0017). There was a trend towards larger larvae from the Small group on the non-toxic diet ($P$ = 0.064). The larger weights of larvae from the Small group on the non-toxic diet suggest that the initial variation in the log weights of the parental generation on CryIAc diet was not related to general vigor. Therefore, heritable variation for tolerance to CryIAc seems to be the best explanation for the observed differences between large and small groups.

**Analysis of Field Colonies of CBW and TBW**

There were significant differences among CBW colonies for both their average 7-d log weight ($P$ < 0.0001, REML ANOVA) and mortality ($P$ < 0.0001, G test) on diet containing CryIAc (Figure 2). The average log weight of CBW colonies was negatively correlated with their mortality on diet containing CryIAc ($r$ = -0.595, $P$ = 0.032). The correlation between the average log weight (mg) of submitted colonies on CryIAc and non-toxic diet was not significantly different from zero ($r$ = 0.455, $P$ = 0.121).

Comparisons of frequency distributions found considerable overlap in the log weights of CBW colonies and also considerable variation within CBW colonies. The intraclass correlation was calculated as 1.41%. Therefore, most of the variation in log weights was found within colonies (98.59%). Coefficients of variation ranged from 42.36 - 287.189% and averaged (±SE) 114.68±18.12%.

There were significant differences among TBW colonies for both their average 10-d log weight ($P$ < 0.0001, REML ANOVA) and mortality ($P$ < 0.0001, G test) on diet containing CryIAc (Figure 3). The average log weight of TBW colonies was negatively correlated with their mortality on diet containing CryIAc ($r$ = -0.752, $P$ = 0.051). The correlation between the average log weight (mg) of submitted colonies on CryIAc and non-toxic diet was not significantly different from zero ($r$ = -0.056, $P$ = 0.905).

Comparisons of frequency distributions found considerable overlap in the log weights of TBW colonies and also considerable variation within TBW colonies. The intraclass correlation was calculated as 12.97%. Therefore, most of the variation in log weights was found within colonies (87.03%). Coefficients of variation ranged from 89.94 - 333.09% and averaged (±SE) 169.91±38.25%.

To further investigate the nature of the variation in log weights, individuals from the WC colony (Washington Co., MS) were placed into four size groups ("SG") based on their weights as follows: SG1, =30$^{th}$ percentile (i. e., smallest 30% of larvae); SG2, 31$^{st}$-60$^{th}$ percentile; SG3, 61$^{st}$-90$^{th}$ percentile; SG4 >90$^{th}$ percentile (largest 10% of larvae). Adults from these groups were allowed to randomly mate and their offspring were tested for 10 d on both non-toxic diet and 0.05 µg/ml CryIAc. SG3 did not produce enough larvae to test. There were no significant differences among the size groups for their 10-d log weights (mg) on non-toxic diet ($P$ = 0.635). Larvae from SG1 grew significantly slower ($P$ = 0.042) on the CryIAc diet and also experienced significantly more mortality ($P$ < 0.0001) on the diet containing CryIAc than larvae from SG2 and SG4 (Figure 4). There were no significant differences between SG2 and SG4.

## Discussion

WP-Hz and field colonies of CBW had comparable amounts of variation for tolerances to CryIAc. Most of the variation in log weights was found within individual colonies of CBW. We are currently conducting analyses based on size-based matings with the field colonies to determine if some of this variation has a genetic basis. The only change for the analyses of field colonies is that individuals were split into four weight groups based on the 1$^{st}$ - 3$^{rd}$ quartiles. It should be noted that this technique is better for detecting quantitative inheritance and is less sensitive for tolerances that are expressed by a major, recessive gene. More work is necessary to determine the importance of this variation under field conditions.

Gould et al. (1992) found resistance to CryIAc in TBW to be governed by a major, recessive gene with a moderately low frequency in the field (Gould et al. 1997). Such a trait would be difficult to detect with the method used above. Our results with the WC colony of TBW found that less tolerant individuals produce offspring who are also more intolerant of CryIAc. This is most likely not the same trait as described by Gould et al. (1992). It appears that the variation found within WC is not the result of a major gene and it may be better viewed as heritable variation for susceptibility to CryIAc instead of tolerance of CryIAc. We are continuing to do more research with this strain, as well as the remaining TBW colonies.

## Acknowledgements

We would like to thank M. Mullins and A. Combest for their technical assistance during this study. We would also like to thank L. Adams for receiving and maintaining the submitted field colonies.

## References

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

Gould, F, A. Anderson, A. Reynolds, L. Bumgarner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88:1545-59.

Gould, F., A. Anderson, A. Jones, D. Sumerford, D. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. Proc. Natl. Acad. Sci. USA 94:3519-23.

Moar, W. J., M. Pustzi-Carey, H. Van Faassen, D. Bosch, R. Frutos, C. Rang, K. Luo, and M. J.Adang. 1995. Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). Appl. Environ. Microbiol. 61: 2086-2092.

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

Tabashinik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83: 1671-1676.

Tabashnik, B. E., Y-B. Liu, N. Finson, L. Masson, and D. G. Heckel. 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proc. Natl. Acad.Sci. USA 94: 1640-1644.

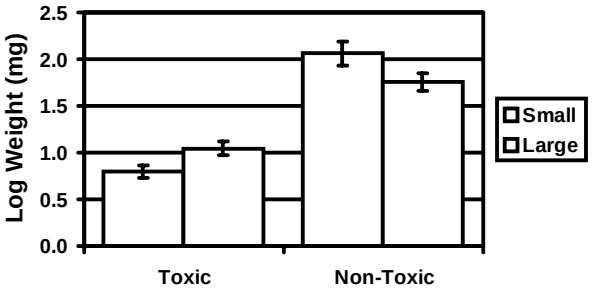


Figure 1. Mean log weight of CBW larvae after 7 days of feeding on 0.5 µg/ml CryIAc and non-toxic diets. "Small" and "Large" refer to the 7-day larval performance of parents on diet containing 0.5 µg/ml CryIAc.

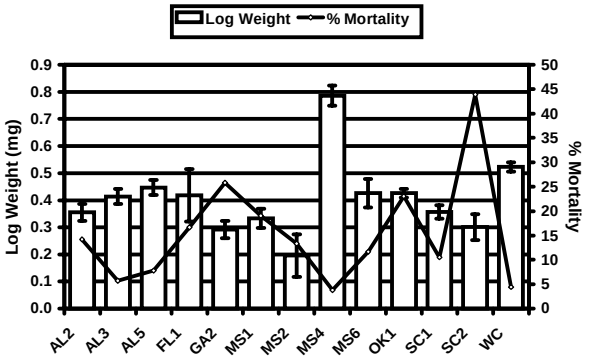


Figure 2. Mean log weight (mg) and the % mortality of CBW strains after feeding for 7 days on a diet containing 0.5 µg/ml CryIAc.

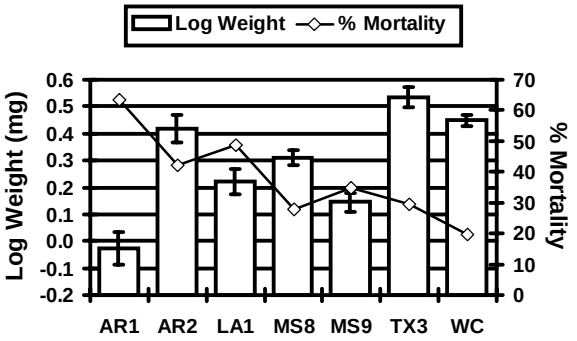


Figure 3. Mean log weight (mg) and % mortality of TBW colonies after feeding on an artificial diet containing 0.05 µg/ml CryIAc.

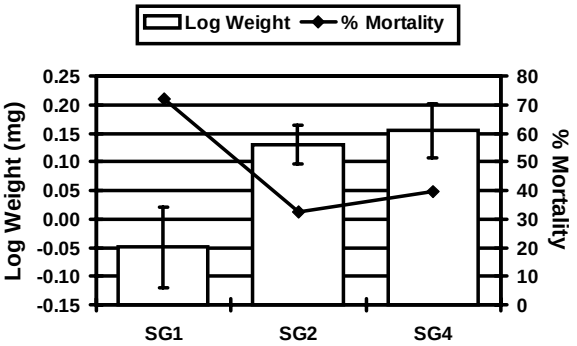


Figure 4. Mean log weight and % mortality of TBW larvae from the WC colony after 10 d of feeding on 0.05 µg/ml CryIAc and non-toxic diets. SG1, SG2, and SG4 refer to the 10-d larval performance of parents on diet containing 0.05 µg/ml CryIAc (see text).