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RESISTANCE TO BT IN ARKANSAS POPULATIONS OF COTTON BOLLWORM

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Abstract

Laboratory colonies of the cotton bollworm, *Helicoverpa zea* were established from field populations of larvae collected from Bt and conventional crops in Arkansas and surrounding states during 2002 and 2003. Progeny, usually those from the 1st to 3rd generations following colonization, were exposed to a range of Cry1Ac endotoxin protein in a standard diet incorporation assay. Concentration-response regressions were compared to those obtained with similar procedures at Mississippi State University during 1992 and 1993. Resulting LC50s for several colonies, especially those established from larvae surviving on Bt crops, were higher than those measured prior to the commercial release of Bt cotton and Bt corn. Assays with progeny from field colonies crossed and backcrossed to a laboratory susceptible colony illustrated dominant or incompletely dominant inheritance with a maternal influence. Trends in survivorship on the treated diet were confirmed in assays with larvae fed Bt cotton leaf tissue. Results suggest that field selection for Bt resistance may be measurable by collecting larvae from the field environment and conducting traditional assays of the progeny in the laboratory. Genetic variability in *H. zea* populations to Cry1Ac appears to be a component of field control problems, but lower LC50s in colonies from conventional crops suggest that susceptible insects from non-Bt crop hosts often dilute frequencies of Bt resistance genes in *H. zea*. The higher LC50s in colonies from Bt crops and a few late-season colonies from conventional cotton illustrate the genetic potential for resistance and the need to continue to manage selection pressure.

Introduction

Transgenic cotton and corn expressing insecticidal proteins derived from *Bacillus thuringiensis* provide unprecedented control of several important insect pests. *Helicoverpa zea* is suppressed on these insecticidal crops, but survivors on Bt corn are common and Bt cotton is routinely sprayed with insecticide to prevent damage from high densities of *H. zea* (Bacheler and Mott 1997, Mahaffey et al. 1995, Layton et al. 1997, Smith 1997, Williams 2003). Reduced expression of insecticidal protein late in the growing season, as a result of various plant stresses, has been implicated as a reason for observed *H. zea* survival on Bt cotton (Greenplate et al. 1998, Greenplate 1999). Variability in expression among different varieties (Adamczyk and Sumerford 2001) and variable expression in different plant structures (Adamczyk et al 2001, Stewart et al. 2001.) have been associated with observations of *H. zea* survival on Bt cotton in the field environment. These "windows" of reduced protection seem to be a weak point in the overall plant defense against high densities of *H. zea* that may oviposit throughout the plant canopy, including structures with reduced expression. Oviposition on flowers or bloom tags late in the growing season is commonly perceived as a major influence on subsequent damage to Bt cotton (Gore et al. 2001).

The genetic capacity of insects to evolve resistance to Bt insecticidal proteins is well established, including the genetic capacity of the heliothines (Stone et al. 1989, Gould et al. 1995, Luttrell et al. 1999, Jackson et al. 2002, Burd et al. 2003, Akhurst et al. 2003). Monsanto scientists were the first to describe genetic variability in heliothine populations to endotoxin proteins (Stone et al. 1989, Stone and Sims 1993). This work was done several years in advance of the commercialization of Bt cotton, and has been corroborated several times (Gould et al. 1995, Luttrell et al. 1999, Hardee et al. 2001, Ali et al. 2003). Resistance management efforts to preserve the useful life of these insecticidal proteins have been major aspects of our scientific research and our regulatory policies over the past decade. Different perspectives on resistance management and the value of resistance management policies, especially those associated with mandated refugia, have dominated much of the national attention on cotton insect management for the past few years.

In 1992 and 1993, we were involved in a study to benchmark susceptibility to Cry1Ac and Cry1Ab proteins targeted for use in commercial Bt cottons (Luttrell et al. 1999). This study was supported by Monsanto Company and was intended as a point of comparison for future resistance problems and as a confirmation of the work already conducted and published by Monsanto scientists (Stone and Sims 1993). Results indicated high variability among heliothine populations in susceptibility to the endotoxin proteins and a genetic capacity of *H. zea* to develop resistance to Cry1Ac under laboratory selection. Susceptibility of *H. zea* was especially variable. Several of the long-time laboratory colonies were more susceptible than those studied by Monsanto. A few of the field colonies had LC50s higher than those reported by Stone and Sims (1993).

Ten years later, we resumed this work at the University of Arkansas (Ali et al. 2003). This paper reports the results of Bt resistance monitoring activities in 2002 and 2003 as compared to the earlier benchmark work at Mississippi State University (Luttrell et al. 1999).

Materials and Methods

Procedures used in 2002 and 2003 at the University of Arkansas were those described by Ali et al. (2003) and were essentially the same as those used at Mississippi State University in 1992 and 1993 (Luttrell et al. 1999). Larvae collected from conventional and Bt crops in Arkansas and surrounding states were used to establish laboratory colonies of *H. zea*. A few additional colonies were established from the offspring of crosses between field-collected insects, usually male moths captured in pheromone traps, and insects from laboratory susceptible colonies. Most of these colonies were obtained from the USDA, ARS SIMRU (United States Department of Agriculture, Agricultural Research Service, Southern Insect Management Research Unit, Stoneville, MS). One was a cross between 2002 field colonies and a laboratory susceptible colony. Progeny of all colonies, usually those from the first to third generations in laboratory culture, were exposed to a range of Cry1Ac protein in a standard diet incorporation assay.

Sources of Cry1Ac varied from the 1992-1993 studies and were a potential component of overall experimental variability. Purified Cry1Ac protein provided by Monsanto Company for 1992-1993 studies was the same source of protein used by Stone and Sims (1993). A liquid and a powdered (freeze dried) preparation of MVP II were used as Cry1Ac sources in 2002 and 2003. The powdered (freeze dried) preparation proved to be more uniform and stable in 2002 studies (Ali et al. 2003) and was chosen as the material for subsequent studies. It is the same source of CryIAc used by Monsanto Company and the USDA,ARS SIMRU at Stoneville, MS. All experiments included laboratory susceptible strains as a comparative reference to standardize the variability from year to year and across slight deviations in experimental procedures.

Our overall examination of *H. zea* susceptibility utilized five separate datasets with concentration-mortality regressions on a total of 53 different *H. zea* populations: (1) seven populations from 1992-1993 studies, (2) 13 populations from 2002 studies with liquid MVP II, (3) nine populations from 2002 studies with powdered MVP II, (4) seven populations from laboratory cross insects in 2002, and (5) 17 populations collected from field crops in 2002. Each concentration-mortality regression included observations of 270-560 insects (3-4 replicates of 5-7 doses with 18-20 test insects per dose).

Populations that did not survive in the laboratory colonization process or those where concentration-mortality regressions were not significant were eliminated from this overall examination of *H. zea* susceptibility to Cry1Ac. Most field colonies included collections of 50 to 200 individuals. Detailed records were retained on the survival of insects in the laboratory colonization process and will be the topic of future reports. Information on the different colonies can be found in Ali et al. (2003) and in a report by Ali et al. in this volume of the Beltwide Proceedings.

Progeny from the UA0234 colony were crossed with a laboratory susceptible colony to study inheritance patterns. The F1 generation was assayed for susceptibility to Cry1Ac and backcrossed to the parent field and laboratory colonies. Progeny from the backcrosses were also assayed using the standard assay procedures. UA0234 was a colony established from larvae surviving on Bt cotton in Ashley County, Arkansas during August 2002. The field was considered a problem field with unacceptable levels of boll damage.

Neonate larvae from the UA0234 and UA0233 colonies were also exposed to upper leaves from conventional (SureGrow 521R), Bollgard I (Monsanto Company) (SureGrow 215 BG/RR), and Bollgard II (Monsanto Company) (DeltaPine 424 BGII/RR) cottons grown in experimental plots at the University of Arkansas Research and Extension Center, Fayetteville, Arkansas in 2003. The UA0233 colony was from a problem field in Leflore County, Mississippi, and represents a geographic source of insects close to those from the 1992-1993 studies. By the time of these plant assays, the insects had been in laboratory culture for seven or more generations.

For the comparative purposes of this paper, resistance levels were estimated by LC50 values corrected for laboratory reference strain variability (LC50 of field colony/average LC50 of laboratory susceptible strain for the particular dataset). Differences in LC50 values were estimated by comparisons of overlapping 95% confidence intervals. Overall results were not adjusted for experiment-wise error and some portion of the differences noted may be due to expected random error.

Results

LC50s corrected for laboratory strain variability ranged from less than 1 ug/ml of diet in 1992-1993 studies to more than 100 ug/ml of diet in 2002-2003 studies (Figure 1). More variability was noted in 2003 studies because more colonies were from insects collected on Bt crops. It is important to remember that the 1992-1993 studies were from insects collected on non-Bt crops prior to the commercial deployment of Bt cotton and Bt corn.

Average LC50s for the laboratory susceptible strains varied 3-fold (0.56 ug/ml to 1.52 ug/ml) (Figure 2). Differences among the LC50s were not statistically significant. The maximum average LC50 observed for a laboratory susceptible colony (1.52 ug/ml) was included as a point of comparison in other data summaries (Figures 2-6).

LC50s obtained for *H. zea* field colonies in 1992-1993 (Luttrell et al. 1999) are summarized in Figure 2 as background benchmarks for data collected in 2002 and 2003. Four of these colonies had LC50s numerically less than that of the MAX LAB colony. The highest adjusted LC50 for *H. zea* in the 1992-1993 studies was 10.66 ug/ml of diet for colony 9315Z that originated from a collection of larvae on 'DES 119' cotton in Edgecomb County, NC in 1993. This LC50 was the only one in 1992-1993 studies significantly greater than the MAX LAB LC50. It was also included as a point of comparison for 2002-2003 studies (MAX 92-93 in Figures 3-6).

Initial experiments in 2002 were conducted with the liquid preparation of MVP II. Thirteen concentration-mortality regressions were obtained (Figure 3). Eleven of the resulting LC50s were significantly greater than MAX LAB LC50. Two were significantly greater than MAX 92-93 LC50. Both were colonies established from large larvae feeding in bolls of commercial Bollgard (Monsanto Company) cotton (UA0233 and UA0234). None of the colonies collected from conventional crops had LC50s greater than MAX 92-93.

Later in 2002, the powdered preparation of MVP II was used as the assay material. Significant regressions were obtained on response data from 9 colonies (Figure 5). Some of the colonies were the same as those tested earlier in 2002 with the liquid preparation of MVP II. Seven of the nine colonies had LC50s greater than MAX LAB. The two colonies with LC50s greater than MAX 92-92 were the same as those observed with the liquid MVP II studies (UA0233 and UA0234).

Laboratory cross colonies were established from crosses between field insects and laboratory susceptible strains in 2003. Significant concentration-mortality regressions were obtained for 7 of these colonies. Three of the colonies from the USDA, ARS SIMRU (USDA-NC, USDA-FL, USDA-MS) had LC50s greater than MAX LAB, but none had LC50s greater than MAX 92-93 (Figure 6). Interestingly, colonies from Texas and New Mexico had numerically smaller, but statistically similar, LC50s than MAX 93-93 and those from North Carolina, Florida, and Mississippi.

Significant regressions were obtained for 17 field colonies from Arkansas and surrounding states in 2003 (Figure 6). Eleven of the colonies were collected from Bt crops including Bt corn, Bollgard I cotton, Bollgard II cotton, and Vip Cotton (Syngenta Crop Protection) plots. Most of these collections were made in August. Adjusted LC50s in the 2003 field data ranged from a low of 1.53 ug/ml of diet for colony UA0319 collected from corn early in the growing season to a high of 95.77 for colony UA0322 collected from Bollgard I cotton late in the growing season. Of the 17 colonies studied, 13 had LC50s greater than MAX LAB. Four (UA0330, UA0323, UA0320, and UA0322) had LC50s greater than MAX 92-93. Three of the four (UA0323, UA0322, and UA0322) were from Bt-corn or Bt-cotton. UA0330 was from conventional cotton plots growing adjacent to Bt-corn and Bt-cotton in Tillar, Arkansas.

Results of the cross and backcross experiments with a susceptible laboratory colony (LAB) and colony PO (UA0234 from Bollgard cotton in Ashley County, AR in 2002) suggest dominant or incomplete dominant inheritance (Figures 7 and Figure 8) similar to that suggested by Burd et al. (2003). LC50s for the progeny of crosses between LAB and PO were intermediate between those for the parent LAB and PO colonies. Slightly different patterns of response were observed between regressions based on mortality plus stunting (Figure 7) and regressions based on mortality (Figure 8). Both datasets suggest a maternal effect on inheritance of Bt resistance genes in colony PO. The maternal effect is more stable in the backcross between males from the PO female X Lab male cross when backcrossed to PO females (i.e. maximized maternal contribution) in the mortality data than in the mortality plus stunting data. Both response datasets confirm an insect genetic component in the higher resistance levels observed for colony PO (UA0234) originating from larvae surviving on Bollgard I cotton.

Nearly all larvae from a laboratory susceptible strain (Lab Zea), colony UA0234 (from Bollgard cotton in Ashley County, Arkansas), and colony UA0233 (from Bollgard cotton in Leflore County, Mississippi) survived on upper leaves of conventional cotton. By 96 hr post exposure to leaves from Bollgard I cotton, 100% of Lab Zea insects had died. Only 48% of the UA0233 insects had died, and 93% of the UA0234 insects had died. On Bollgard II cotton, insects from the two field colonies were just as susceptible as those from the laboratory susceptible strain. Mortality of UA0234 and UA0233 insects was 100% after 48 hr of exposure to the Bollgard II cotton leaves.

Discussion and Implications for Resistance Management

This research illustrates the genetic capacity of *H. zea* to survive exposure to Cry1Ac insecticidal proteins in the field environment. This is nothing new or unexpected. In our 1992-1993 studies, we were able to select for resistance in the FZ strain at levels comparable to that observed in the 2002-2003 field strains after five to seven generations of selection (Luttrell et al. 1999). Burd et al. (2003) reported that resistant gene frequencies may be as high as 1/2332 in insects collected from light traps in North Carolina. Certainly, the insecticidal activity of Bollard cotton would eliminate susceptible genotypes and further concentrate the frequency of resistant individuals in survivors.

LC50s corrected for laboratory strain variability ranged from less than 1 ug/ml of diet in 1992-1993 studies to more than 100 ug/ml of diet in 2002-2003 studies (Figure 10). More variability was noted in 2003 studies because more colonies were from

insects collected on Bt crops. It is important to remember that the 1992-1993 studies were from insects collected on non-Bt crops prior to the commercial deployment of Bt cotton and Bt corn.

Figure 11 provides a summary of the 53 regressions obtained in our collective dataset sorted from lowest LC50 to highest LC50. Of the 53 colonies studied, 47 had LC50s numerically greater than LAB MAX. Thirty of the 53 had LC50s numerically greater than MAX 92-93. Only nine of these colonies had LC50s significantly greater than MAX 92-93, and only one of the nine was from a conventional crop. Eight of the nine were from colonies established from larvae surviving on Bt-corn or Bt-cotton.

The overall adjusted average LC50 for colonies from Bt crops (Figure 12) was 37.88 $\pm$ 6.69 (mean $\pm$ SEM) ug/ml of diet or 3.3-fold that for colonies from conventional crops (11.33 $\pm$ 1.79 ug/ml of diet). Recall that all of these values were corrected for laboratory variability. Uncorrected differences would be in the range of 9- to 10-fold. Average LC50s for colonies from conventional crops was almost identical to that for LAB MAX. Both were significantly less than the average for Bt crops.

Survival of the more resistant colonies on Bollgard cotton confirms the phenotypic expression of the resistant trait. Inheritance patterns resulting from assays of progeny from crosses between UA0234 and Lab Zea and backcrosses of resulting progeny with the parent colonies illustrate the genetic component of the survival mechanisms in colony UA0234 from Bollgard cotton in Ashley County, Arkansas. Resistance seems to be inherited as a dominant or incompletely dominant trait with a strong maternal contribution. Differences in patterns of inheritance between datasets based on mortality and mortality plus stunting may indicate the influence of several genes. Jackson et al. (2002) reported a substantial quantitative component in genetic variation of *H. zea* to Cry1Ac and Cry2Ab.

The significance of this work is its empirical contribution to our understanding of selection in the field environment. Given the time and energy to collect insects, establish colonies, and carefully assay progeny from the first few generations in laboratory culture, one should be able to follow selection bouts in the field and unravel some of the current debate about the value of refugia and mandated resistance management. This is difficult because of the inherent variability associated with sampling and rearing insects, but clearly doable.

If the suggestion of a strong maternal inheritance is correct, consideration should be given to the current practice of monitoring heliothine populations for Bt resistance by assaying progeny from crosses of laboratory susceptible females with wild males from pheromone traps. Recessively inherited resistance would also be lost in this process.

The general susceptibility of *H. zea* on conventional crops suggests that factors are working in the system to dilute selection for Bt resistance genes. Given the polyphagous nature of this insect and its historical tendency to slowly evolve insecticide resistance, this appears to be a logical conclusion. The situation may be quite different for less polyphagous pests like *Heliothis virescens*.

The higher LC50s for colonies from Bt crops is a reminder of the genetic capacity of the insect to evolve resistance and a caution that we should avoid creating environments that foster multiple selection bouts. The rare but observed higher LC50s in a few populations from conventional cotton warrant continued prudence and concern for resistance evolution.

Acknowledgements

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References Cited

Adamczyk, J. J., Jr., and D. V. Sumerford. 2001. Potential factors impacting season-long expression of Cry1Ac in 13 commercial varieties of Bollgard cotton. J. Insect Science 1:13. (6 pp.)

Adamczyk, J. J., Jr., D. D. Hardee, L. C. Adams, and D. V. Sumerford. 2001. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A© delta-endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 94:284-290.

Akhurst, R. J., W. James, L. J. Bird, and C. Beard. 2003. Resistance to the Cry1Ac delta-endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 96:1290-1299.

Ali, M. I., R. G. Luttrell, S. Y. Young, III, K. C. Allen, and L. T. Luttrell. 2003. Monitoring insect resistance in Arkansas to chemical insecticides and Bt-endotoxins. Proc. Beltwide Cotton Conf., pp. 1138-1149.

Bacheler, J. S., and D. W. Mott. 1997. Efficacy of grower-managed Bt cotton in North Carolina. Proc. Beltwide Cotton Conf., pp. 858-861.

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

Gore, J., B. R. Leonard, and J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on "Bollgard" and "Bollgard II" cotton flower bud and flower components. J. Econ. Entomol. 94:1445-1451.

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. 88:1545-1559.

Greenplate, J. T., G. P. Head, S. R. Penn, and V. T. Kabuye. 1998. Factors potentially influencing the survival of *Helicoverpa zea* on Bollgard cotton. Proc. Beltwide Cotton Conf. Pp. 1030-1033.

Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92:1377-1383.

Hardee, D. D., L. C. Adams, W. L. Solomon, 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.

Jackson, R. E., J. R. Bradley, Jr., F. Gould, and J. W. Van Duyn. 2002. Genetic variation for resistance to Cry1Ac and Cry2Ab in bollworm, *Helicoverpa zea*, in North Carolina. Proc. Beltwide Cotton Conf. 6 pp.

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.

Mahaffey, J. S., J. R. Bradley, Jr., and J. W. VanDuyn. 1995. Bt cotton field performance in North Carolina under conditions of unusually high bollworm populations. Proc. Beltwide Cotton Conf., pp. 795-798.

Layton, M. B., M. R. Williams, and S. Stewart. 1997. Bt cotton in Mississippi: the first year. Proc. Beltwide Cotton Conf., pp. 861-863.

Smith, R. H. 1997. An extension entomologist's 1996 observations of Bollgard (Bt) technology. Proc. Beltwide Cotton Conf., pp. 856-858.

Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94:752-760.

Stone, T. B., and S. R. Sims. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*. J. Econ. Entomol. 86:989-994.

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

Williams, M. R. 2003. Cotton insect losses 2002. Proc. Beltwide Cotton Conf., pp. 101-109.

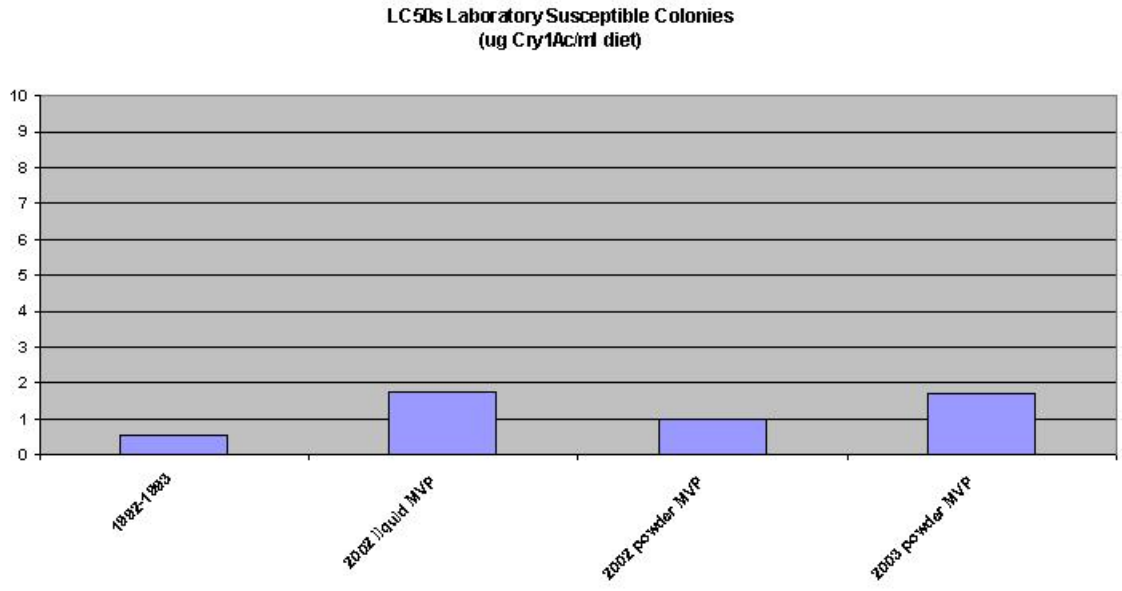

Figure 1. Average LC50 for laboratory susceptible colonies exposed to Cry1Ac in diet incorporation assays (mortality response).

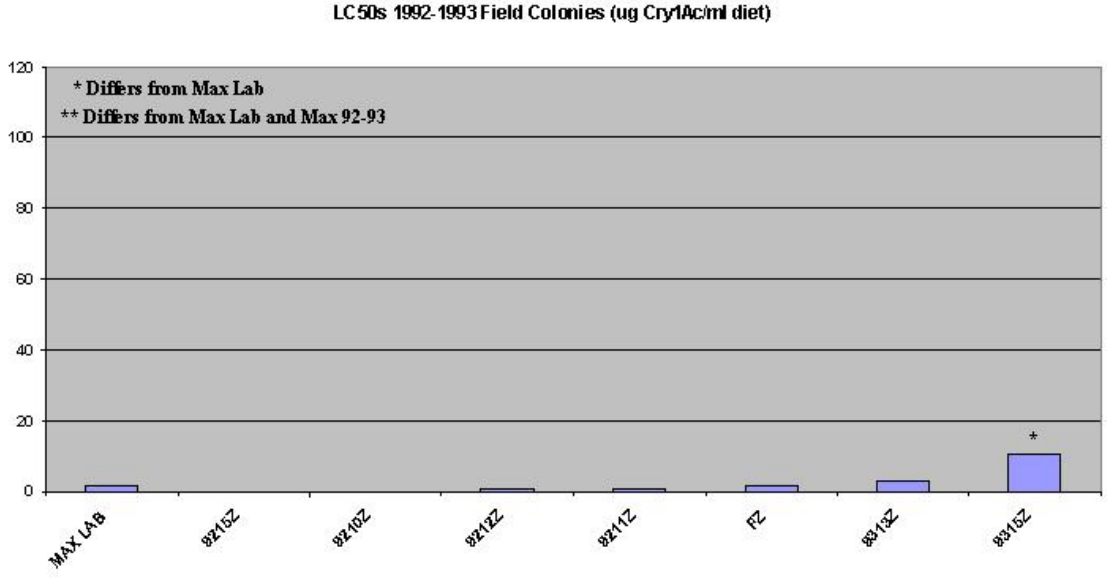

Figure 2. LC50s for 1992-1993 studies (Luttrell et al. 1999) compared to MAX LAB.

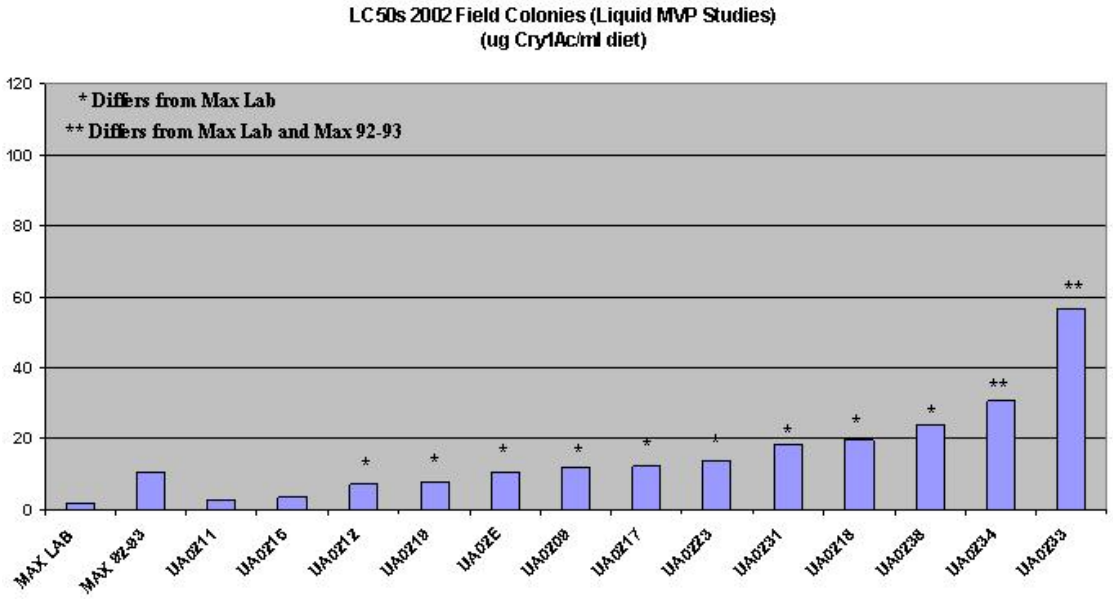

Figure 3. LC50s from 2002 studies with a liquid preparation of MVP II compared to MAX LAB and MAX 92-93.

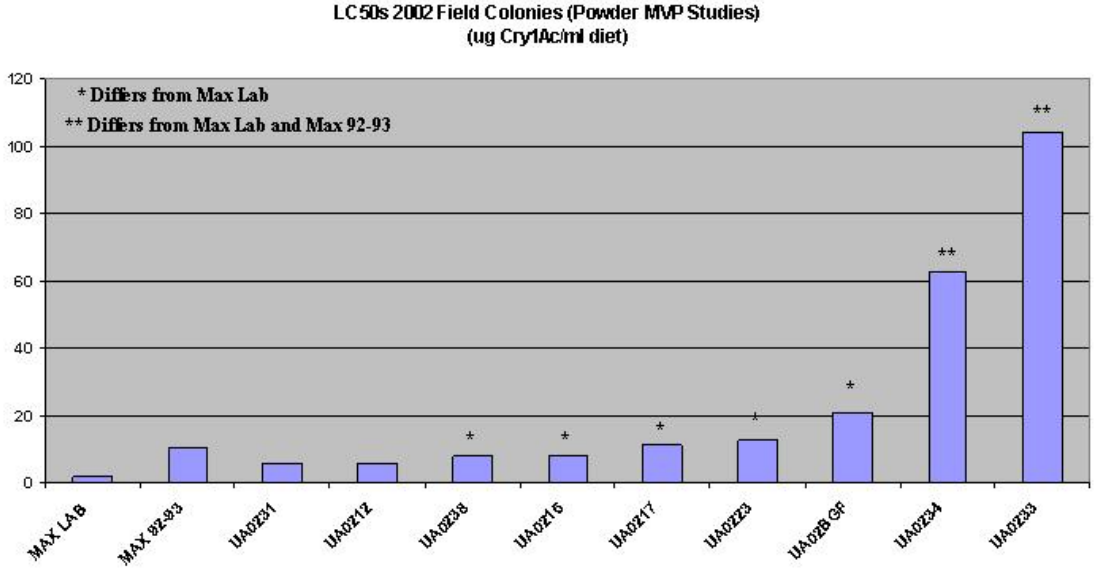

Figure 4. LC50s from 2002 studies with a powdered preparation of MVP II compared to MAX LAB and MAX 92-93.

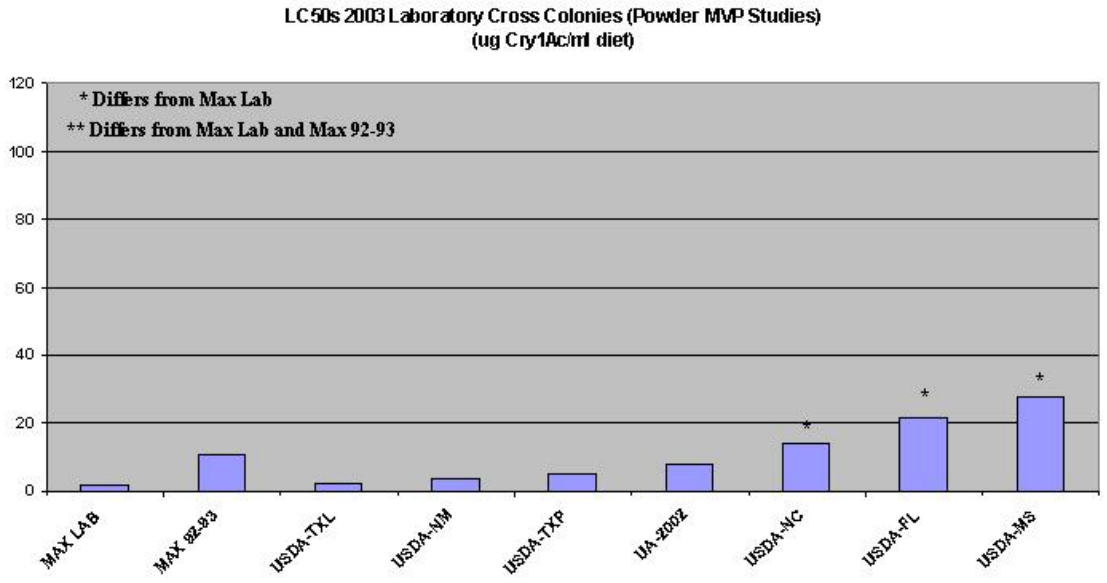

Figure 5. LC50s from 2003 studies of laboratory cross colonies compared to MAX LAB and MAX 92-93.

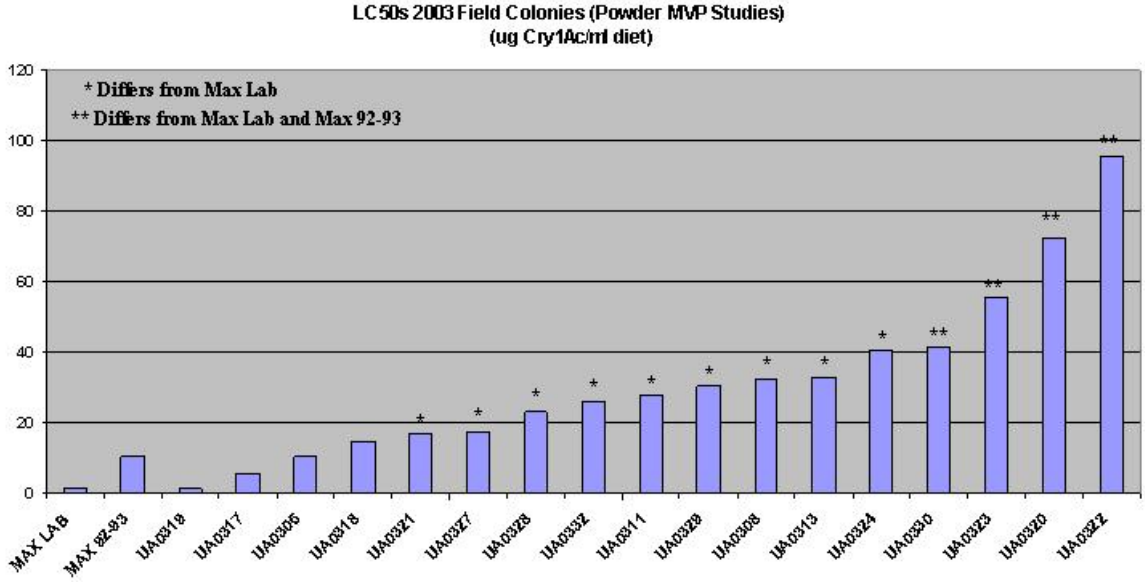

Figure 6. LC50s from 2003 studies of field colonies compared to MAX LAB and MAX 92-93.

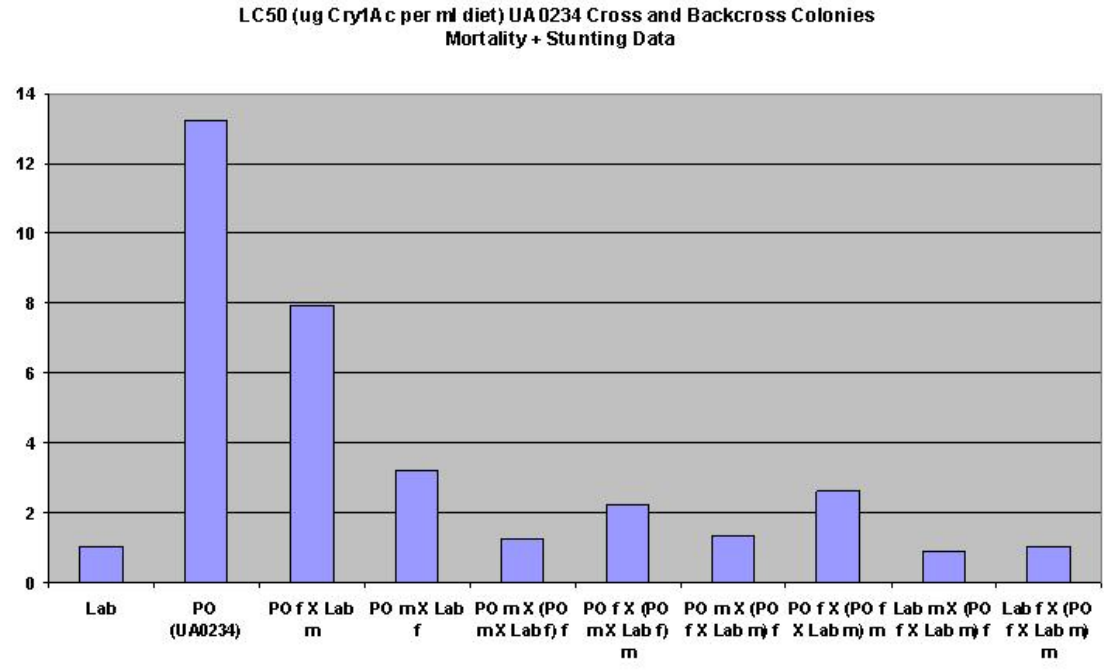

Figure 7. LC50s of progeny from crosses of laboratory susceptible (LAB) and field resistant (PO) colonies of *H. zea* exposed to Cry1Ac in diet incorporation assays (mortality plus stunting response).

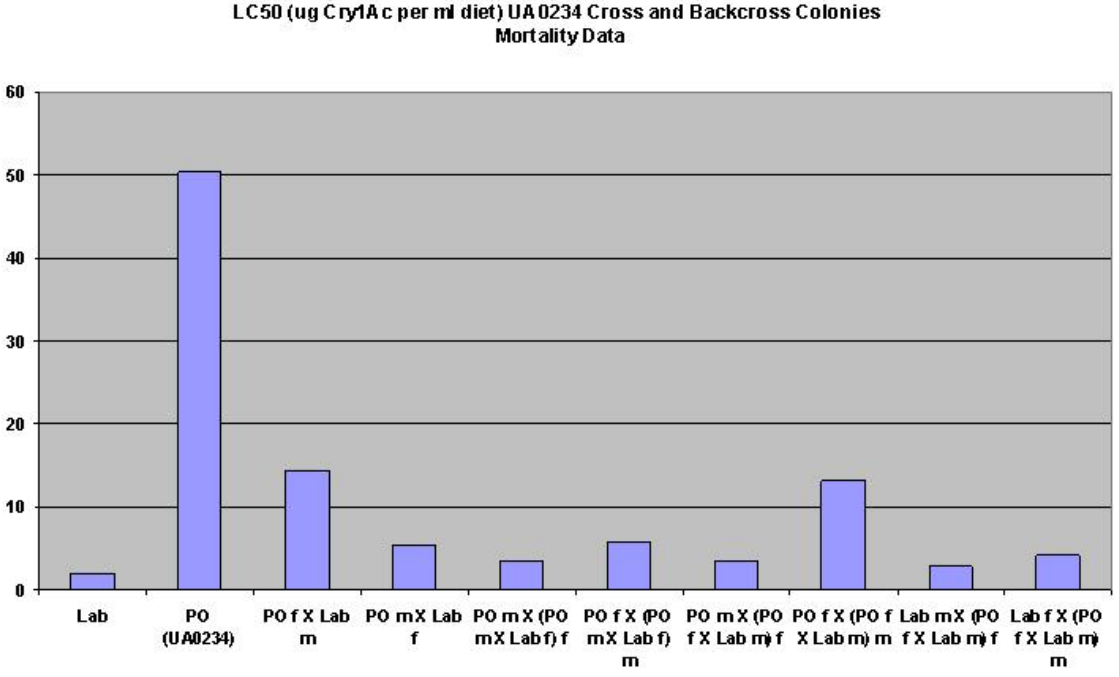

Figure 8. LC50s of progeny from crosses of laboratory susceptible (LAB) and field resistant (PO) colonies of *H. zea* exposed to Cry1Ac in diet incorporation assays (mortality response).

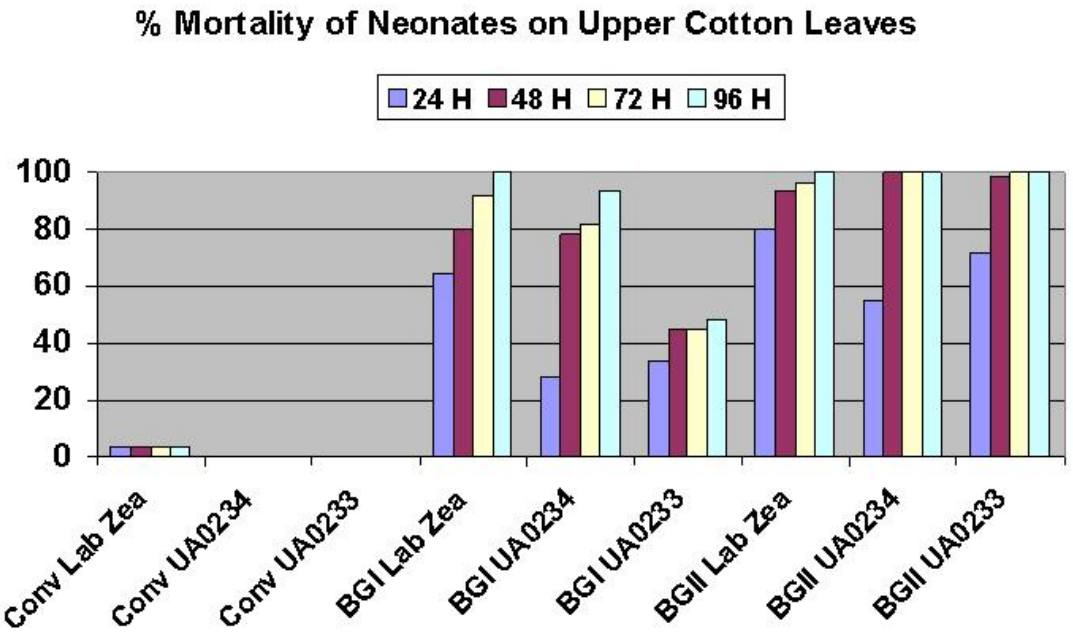

Figure 9. Mortality of laboratory susceptible (Lab Zea) and field resistant (UA0233 and UA0234) colonies of *H. zea* exposed to upper leaves of convention, Bollgard I, and Bollgard II cottons.

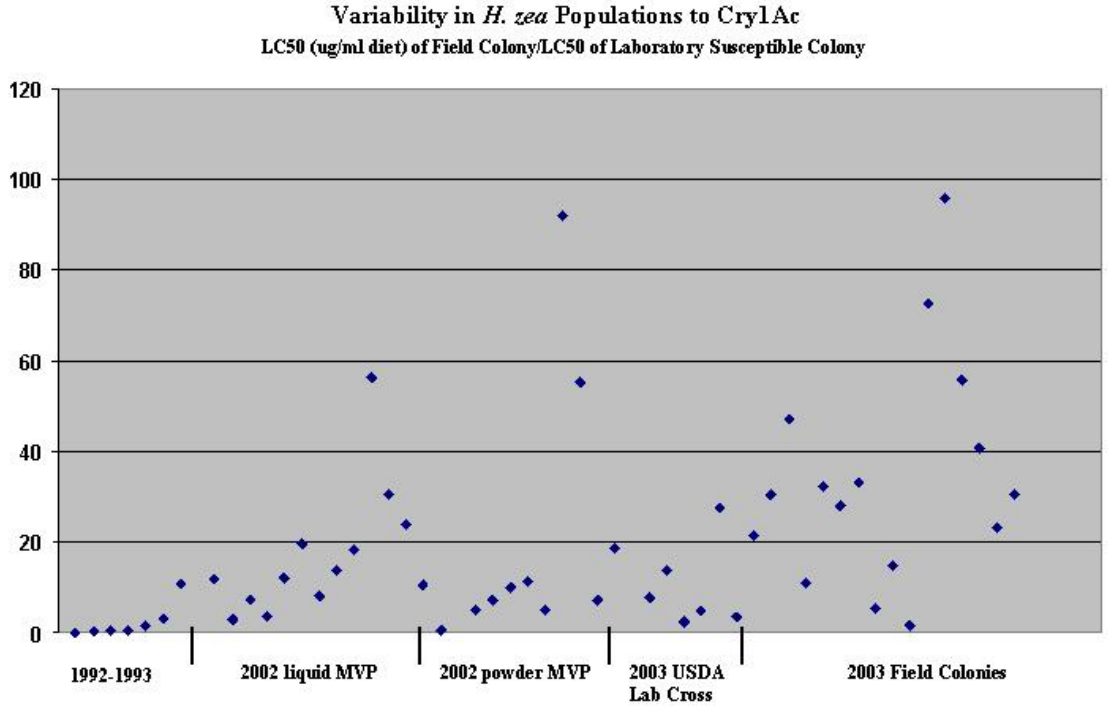

Figure 10. Variation in LC50s from concentration-mortality regressions describing mortality of neonate *H. zea* larvae exposed to Cry1Ac protein in diet incorporation assays over a 10 year period.

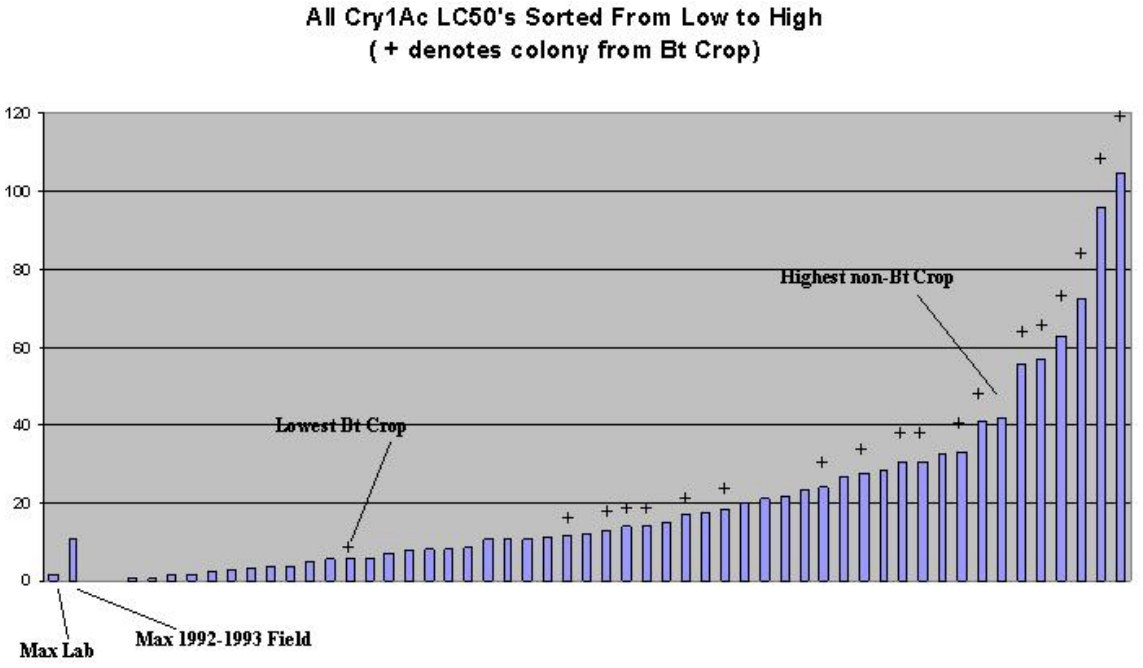

Figure 11. Range of LC50s obtained in 1992-1993 and 2002-2003 diet incorporation assays with *H. zea* exposed to Cry1Ac.

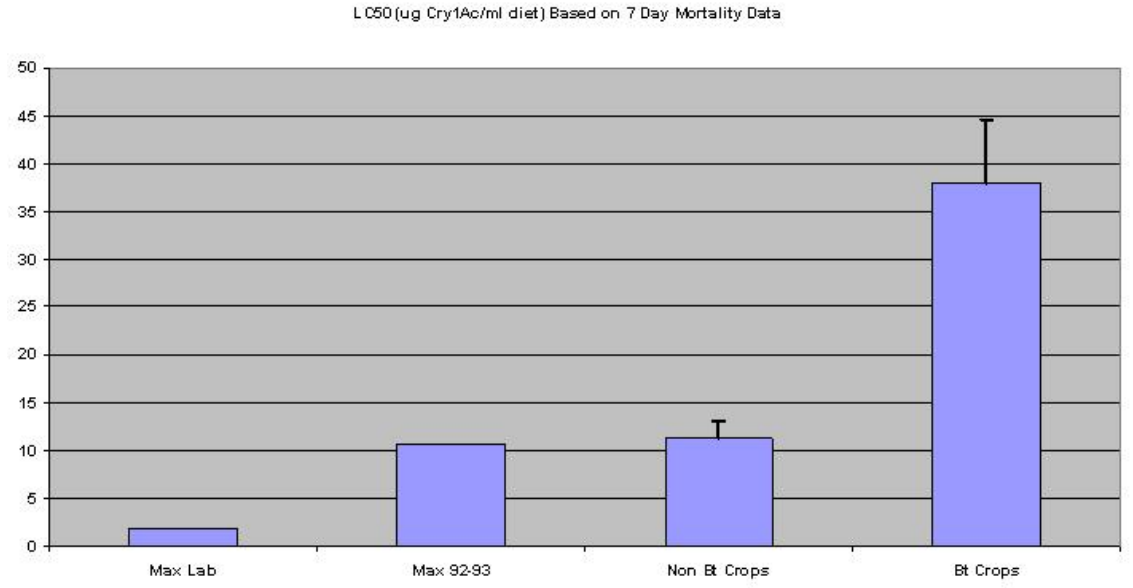

Figure 12. Average LC50 for colonies of *H. zea* from conventional and Bt crops exposed to Cry1Ac in diet incorporation assays.