ESTIMATES OF SUSCEPTIBILITY OF BOLLWORM (*HELICOVERPA ZEA*) LARVAE TO *BACILLUS THURINGIENSIS* PROTEINS—2018

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Abstract

The principal objective of this project was to monitor the susceptibility of field-collected *Helicoverpa zea* (Bollworm/Corn earworm) larvae to the insecticidal *Bacillus thuringiensis* (*Bt)* Cry1Ac, Cry2Ab2, and Cry 2Ae proteins-2018. The susceptibility of field-collected bollworm (*Helicoverpa zea*) populations to *Bacillus thuringiensis* (*Bt*) Cry1Ac, Cry2Ae, and Cry2Ab2 proteins were evaluated. In general, Cry1Ac was less potent and less effective than either of the Cry2 proteins, and Cry2Ae was the most potent and effective protein evaluated.

Introduction

Although heliothine larvae are still considered major economically important pests of cotton in the U.S., the development and implementation of *Bt* cotton technologies have reduced cotton losses attributed to this pest complex. Unfortunately, due to the inherent tolerance of bollworm larvae to the *Bt* toxins, widespread adoption of the transgenic *Bt* technologies, varied expression of the *Bt* toxins within and among *Bt* crops, and noted variability in the susceptibility of bollworm larvae to the *Bt* toxins, increased selection for the development of resistance to the *Bt* proteins was expected and has been reported (Tabashnik et al. 2008, Tabashnik et al. 2013, Resig and Reay-Jones 2015, Dively et al. 2016). In order to sustain the use of this critical pest management tool, research efforts and agricultural practices devoted to the preservation of these technologies must be continued, and programs to monitor the susceptibilities of these key pests to insecticidal *Bt* proteins are of extreme importance to our industry.

Materials and Methods

Insects
Field populations of bollworm (CEW) were collected from Texas, South Carolina, and Georgia. Larvae were transferred to a pinto bean/wheat germ, agar-based diet (Southland Products, Inc., Lake Village, AR), and adults were placed in mating cages (15-20 :15-20) to produce adequate numbers of larvae for testing and fed a 10% honey/ molasses:water solution. Larvae and adults were maintained at 27˚C, a 14:10 (L:D) photoperiod, and ca. 50% RH. A laboratory, insecticide-susceptible CEW population was obtained from Benzon Research, Inc. (Carlisle, PA).

Bt Toxins
Cry1Ac (MVP II® lyophilized powder; 19.1% Cry1Ac protoxin) and Cry2Ab2 lyophilized corn leaf powder (4 mg Cry2Ab2/gm powder) were provided by Monsanto Corporation, St. Louis, MO. Purified Cry2Ae and Cry1Ab proteins were provided by Bayer CropScience/BASF Agricultural Solutions, Research Triangle, NC.

Bioassays
Larval susceptibilities to the *Bt* proteins were conducted using a standard diet overlay method (Anilkumar et al. 2009) in 128-well bioassay trays (C-D International, Pittman, NJ). One neonate larva was added to each well. Larval mortality was assessed on the 7th day of exposure and calculated based on the number of dead plus 1st instar survivors (mortality = dead + L1).

Analysis
Mortality data were analyzed by probit analysis (Finney 1971) using POLO-PC (LeOra Software, Petaluma, CA). Observed treatment responses were corrected for control mortality (Abbott 1925).

Results

The susceptibility of field-collected bollworm (*Helicoverpa zea*) populations to *Bacillus thuringiensis* (*Bt*) Cry1Ac, Cry2Ae, and Cry2Ab2 proteins were evaluated. In general, Cry1Ac was less potent (Mean LC_{50} = 10.5 µg/cm^2) and less effective (68.5% (± 5.7) at 31.6 µg/cm^2) than either of the Cry2 proteins, and Cry2Ae was the most potent (Mean LC_{50} = 0.18 µg/cm^2) and effective (97.7% (± 1.7) at 31.6 µg/cm^2) protein evaluated (Tables 1-3, Figures 1-3).

Table 1. Estimated LC_{50}s (µg/cm^2) for *Helicoverpa zea* (CBW) larvae following a 7-day exposure period to MVP II® (Cry1Ac) and Cry1Ab using a diet overlay bioassay. Larvae were scored as dead if they did not move when probed or had not developed to 2nd instar during the 7-day exposure period.

Treatment	Colony Host (Stage)	N	LC_{50}, ppm	95% C.I., ppm	Slope (S.E.)	§^{2}	*df*	RR
MVP II®	**Benzon-SS**	**724**	**0.14**	**0.09-0.24**	**1.70 (0.12)**	**129**	**28**	**1.0**
(Cry1Ac)	**College Sta.TX 2 non-*Bt* corn (L)**	**281**	**12.3**	**3.14-284**	**0.53 (0.09)**	**32.4**	**14**	**87.8***
	Amarillo TX 7 non-*Bt* corn (L)	**446**	**0.08**	**0.04-0.16**	**0.86 (0.08)**	**50.9**	**22**	**0.57**
	TX 8 non-Bt corn (L)	**144**	**2.01**	**0.62-11.2**	**0.45 (0.07)**	**3.94**	**6**	**14.4***
	Edisto-SC non-*Bt* corn (L)	**576**	**0.91**	**0.38-2.45**	**0.43 (0.07)**	**13.9**	**14**	**6.5**
	Carroll-GA non-*Bt* corn (L)	**144**	**0.06**	**0.01-0.20**	**0.46 (0.11)**	**2.31**	**6**	**0.43**
	Pike-GA non-Bt corn (L)	**238**	**> 31.6 (43%)**	**----**	**-----**	**-----**	**----**	**> 226***
	Sumter 1-GA non-*Bt* corn (L)	**432**	**3.51**	**0.72-56.4**	**0.52 (0.08)**	**38.0**	**12**	**25.1***
	Sumter 2-GA non-*Bt* corn (L)	**288**	**0.05**	**0.01-0.12**	**0.55 (0.09)**	**25.5**	**14**	**0.36**
	Tift 1-GA non-*Bt* corn (L)	**191**	**> 31.6 (24%)**	**-----**	**-----**	**-----**	**----**	**> 226***
	Tift 2-GA non-*Bt* corn (L)	**189**	**>31.6 (29%)**	**-----**	**-----**	**-----**	**----**	**> 226***
	Tift 3-GA non-*Bt* corn (L)	**82**	**1.32**	**0.53-8.56**	**1.08 (0.30)**	**3.47**	**4**	**9.43**
	Low LC_{50}	**0.05 µg/cm^2**						
	High LC_{50}	**> 31.6 µg/cm^2**						
	Mean LC_{50}	**10.5 µg/cm^2**						

[a]Total number of neonates assayed.
[b]The LC_{50} value of an insect strain was considered to be greater than the highest *Bt* protein concentration used in the bioassay if its larval mortality was < 50% at the highest concentration.
[c]Resistance ratios for *Bt* protein were calculated by dividing the LC_{50} of a field-collected insect population by the LC_{50} of the laboratory *Bt*-susceptible strain (Benzon-SS).
+The Ç2 value followed by "+" indicated poor fit of the data to the probit model (P > 0.05).
*Indicates resistance ratios e 10-fold.

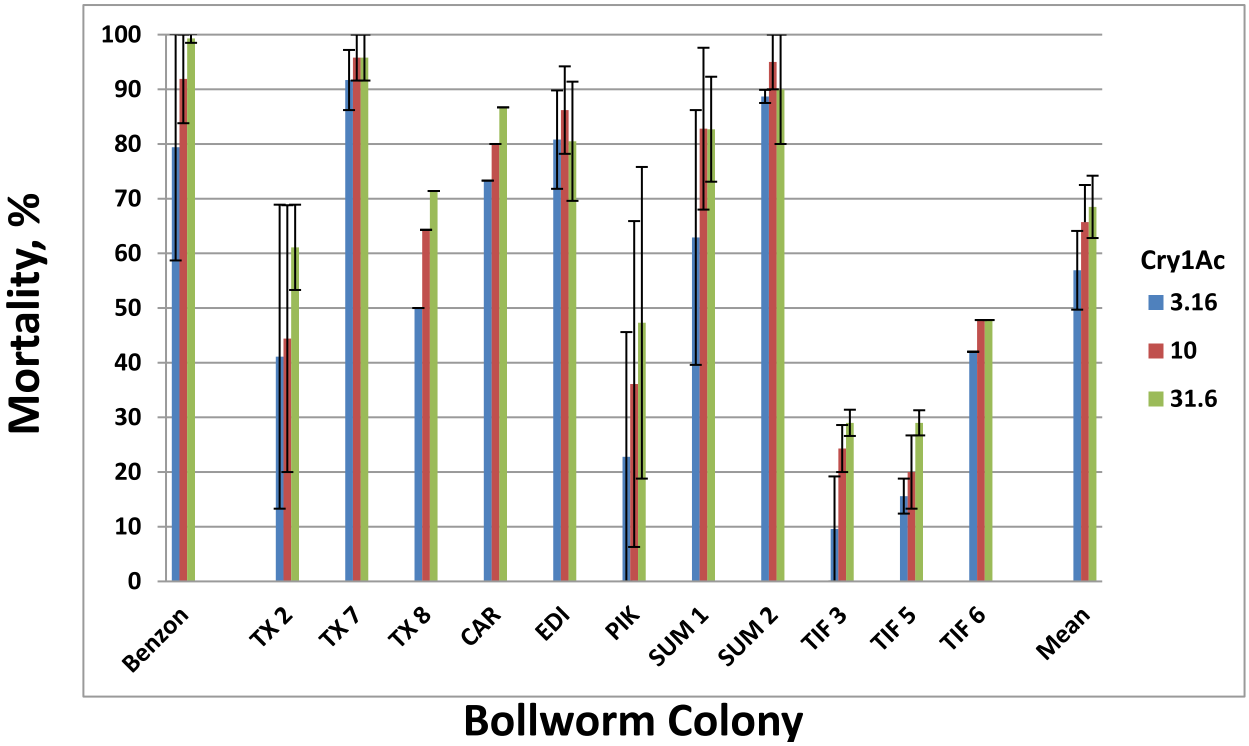

Figure 1. Response of field-collected bollworm (*Heliocoverpa zea*) larvae to discriminating concentrations of Cry1Ac (MVP II®) concentrations.

Table 2. Estimated LC_{50}s (µg/cm^2) for *Helicoverpa zea* (CBW) larvae following a 7-day exposure period to Cry2Ae using a diet overlay bioassay. Larvae were scored as dead if they did not move when probed or had not developed to 2nd instar during the 7-day exposure period.

Treatment	Colony Host (Stage)	N	LC_{50}, ppm	95% C.I., ppm	Slope (S.E.)	§^{2}	*df*	RR
Cry2Ae	**Benzon-SS**	**731**	**0.44**	**0.29-0.69**	**1.65 (0.12)**	**98.3**	**26**	**1.0**
	College Sta.TX 2 non-*Bt* corn (L)	**456**	**0.78**	**0.34-1.56**	**1.03 (0.09)**	**66.0**	**21**	**1.8**
	Port Lauaca TX 3 non-*Bt* corn (L)	**406**	**0.03**	**0.01-0.12**	**0.83 (0.12)**	**37.5**	**12**	**0.07**
	San Angelo TX 4 sorghum (L)	**413**	**0.30**	**0.20-0.47**	**1.40 (0.11)**	**41.3**	**24**	**0.68**
	Amarillo TX 7 non-*Bt* corn (L)	**723**	**0.07**	**0.02-0.16**	**1.04 (0.08)**	**97.2**	**27**	**0.16**
	TX 8 non-Bt corn (L)	**491**	**0.03**	**0.01-0.06**	**0.93 (0.09)**	**58.0**	**16**	**0.07**
	Edisto-SC non-*Bt* corn (L)	**429**	**0.04**	**0.02-0.07**	**0.96 (0.11)**	**24.6**	**17**	**0.09**
	Carroll-GA non-*Bt* corn (L)	**143**	**0.15**	**0.06-0.35**	**1.57 (0.24)**	**9.53**	**6**	**0.33**
	Pike-GA non-Bt corn (L)	**192**	**0.17**	**0.06-1.43**	**0.58 (0.13)**	**4.08**	**4**	**0.39**
	Sumter 1-GA non-*Bt* corn (L)	**506**	**0.15**	**0.11-0.21**	**1.38 (0.12)**	**23.4**	**23**	**0.34**
	Sumter 2-GA non-*Bt* corn (L)	**129**	**0.02**	**0.01-0.04**	**1.58 (0.36)**	**2.39**	**3**	**0.04**
	Tift 3-GA non-*Bt* corn (L)	**193**	**0.12**	**0.06-0.22**	**1.17 (0.21)**	**2.98**	**4**	**0.27**
	Tift 4-GA cotton (A)	**162**	**> 3.16 (97%)**	**-----**	**-----**	**-----**	**----**	**< 7.20**
	Tift 5-GA non-*Bt* corn (L)	**97**	**> 3.16 (90%)**	**-----**	**-----**	**-----**	**----**	**< 7.20**
	Tift 6-GA non-Bt corn (L)	**64**	**> 3.16 (94%)**	**-----**	**-----**	**-----**	**----**	**< 7.20**
	LSU	**605**	**0.10**	**0.06-0.15**	**1.27 (0.07)**	**252**	**61**	**0.23**
	Low LC_{50}	**0.02 µg/cm^2**						
	High LC_{50}	**0.78 µg/cm^2**						
	Mean LC_{50}	**0.18 µg/cm^2**						

[a]Total number of neonates assayed.
[b]The LC_{50}value of an insect strain was considered to be greater than the highest *Bt* protein concentration used in the bioassay if its larval mortality was < 50% at the highest concentration.
[c]Resistance ratios for *Bt* protein were calculated by dividing the LC_{50} of a field-collected insect population by the LC_{50} of the laboratory *Bt*-susceptible strain (Benzon-SS).
+The Ç^2 value followed by "+" indicated poor fit of the data to the probit model (P > 0.05).
*Indicates resistance ratios e 10-fold.

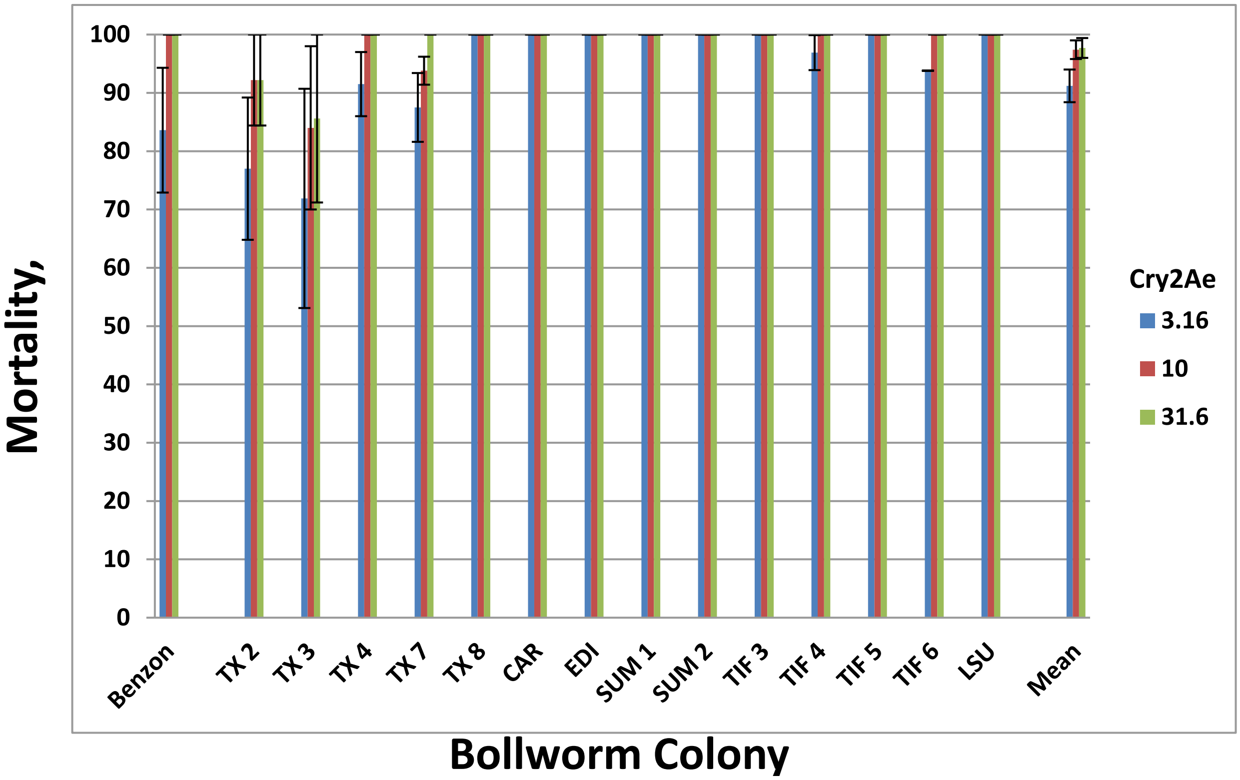

Figure 2. Response of field-collected bollworm (*Heliocoverpa zea*) larvae to discriminating concentrations of Cry2Ae concentrations.

Table 3. Estimated LC_{50}s (µg/cm^2) for *Helicoverpa zea* (CBW) larvae following a 7-day exposure period to Cry2Ab2 using a diet overlay bioassay. Larvae were scored as dead if they did not move when probed or had not developed to 2nd instar during the 7-day exposure period.

Treatment	**Colony Host (Stage)**	**N**	**LC_{50}, ppm**	**95% C.I., ppm**	**Slope (S.E.)**	**§^{2}**	***df***	**RR**
Cry2Ab2	**Benzon-SS**	**300**	**0.10**	**0.06-0.16**	**1.08 (0.08)**	**68.3**	**32**	**1.0**
	College Sta.TX 2 non-*Bt* corn (L)	**404**	**0.31**	**0.04-1.47**	**0.64 (0.07)**	**149**	**21**	**3.1**
	San Angelo TX 4 sorghum (L)	**177**	**> 31.6 (39%)**	**-----**	**-----**	**-----**	**-----**	**>316***
	Amarillo TX 7 non-*Bt* corn (L)	**425**	**0.56**	**0.20-1.53**	**0.68 (0.07)**	**71.6**	**21**	**5.6**
	Edisto-SC non-*Bt* corn (L)	**279**	**7.64**	**5.39-11.5**	**1.69 (0.20)**	**17.8**	**15**	**76.4***
	Pike-GA non-Bt corn (L)	**263**	**9.70**	3.48-64.1	**0.77 (0.11)**	**30.6**	**13**	**97.0***
	Sumter 1-GA non-*Bt* corn (L)	**560**	**4.09**	**2.05-10.0**	**0.82 (0.07)**	**91.1**	**30**	**40.9***
	Sumter 2-GA non-*Bt* corn (L)	**277**	**0.04**	**0.01-0.11**	**0.57 (0.09)**	**21.0**	**14**	**0.4**
	Tift 3-GA non-*Bt* corn (L)	**285**	**7.34**	**2.36-82.5**	**0.86 (0.14)**	**28.0**	**9**	**73.4***
	Tift 4-GA cotton (A)	**317**	**0.82**	**0.46-1.49**	**1.13 (0.11)**	**37.8**	**20**	**8.2**
	Tift 5-GA non-*Bt* corn	**143**	**1.41**	**0.41-6.78**	**0.71 (0.12)**	**8.41**	**6**	**14.1***
	Tift 6-GA non-*Bt* corn (L)	**358**	**6.65**	**2.30-40.2**	**1.36 (0.17)**	**46.6**	**9**	**66.5***
	Low LC_{50}	**0.05 µg/cm^2**						
	High LC_{50}	**> 31.6 µg/cm^2**						
	Mean LC_{50}	**10.5 µg/cm^2**						

[a]Total number of neonates assayed.
[b]The LC_{50}value of an insect strain was considered to be greater than the highest *Bt* protein concentration used in the bioassay if its larval mortality was < 50% at the highest concentration.
[c]Resistance ratios for *Bt* protein were calculated by dividing the LC_{50} of a field-collected insect population by the LC_{50} of the laboratory *Bt*-susceptible strain (Benzon-SS).
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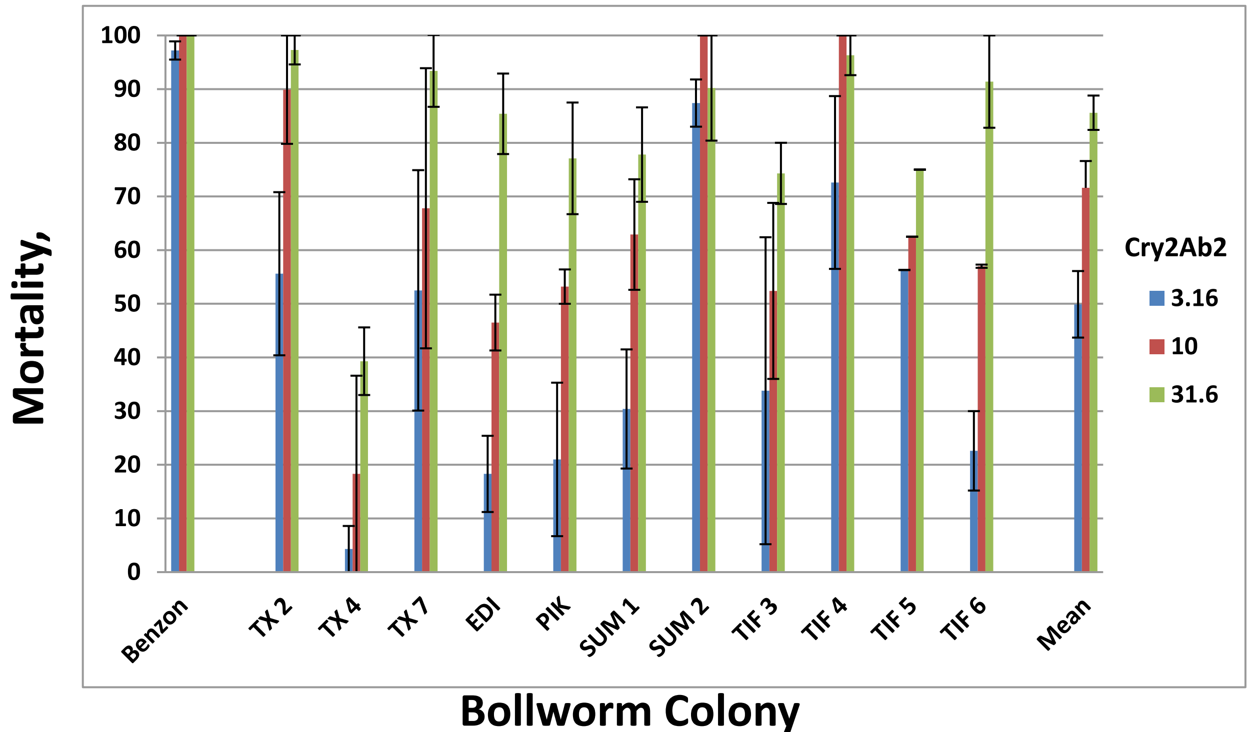

Figure 3. Response of field-collected bollworm (*Helicoverpa zea*) larvae to discriminating concentrations of Cry2Ab2 concentrations.

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