CONTINUOUS MONITORING OF THE SUSCEPTIBILITY OF *HELICOVERPA ZEA* IN THE SOUTHERN U.S. TO DIFFERENT BT PROTEINS

Fei Yang
David Kerns
Texas A&M University
College Station, TX
Jeffrey Gore
Donald Cook
Mississippi State University
Stoneville, MS
Angus Catchot
Fred Musser
Mississippi State University
Starkville, MS
Gus Lorenz
University of Arkansas
Lonoke, AR
Nick Seiter
University of Arkansas
Monticello, AR
Scott Stewart
University of Tennessee
Jackson, TN
Sebe Brown
Louisiana State University
Winnsboro, LA

Abstract

The cotton bollworm, *Helicoverpa zea* (Boddie), is one of the most important insect pests of cotton, *Gossypium hirsutum* L. Transgenic cotton plants expressing one or more *Bacillus thuringiensis* (Bt) proteins have been widely planted for control of cotton bollworms since 1996. However, extensive and constant exposure of cotton bollworms to Bt proteins may result in a shift towards a reduction in susceptibility. To estimate the susceptibility of the field populations of cotton bollworm to Bt proteins, we compared the survivorship of F1 or F2 generations of field-derived cotton bollworms collected from Mid-South region in the U.S. to four individual Bt proteins (Cry1Ac, Cry1F, Cry2Ab2, and Vip3a) with that of a susceptible counterpart using diet overlay bioassays. Results generated from this study would provide essential information for Bt resistance management in the Southern U.S.

Introduction

The cotton bollworm (CBW), *Helicoverpa zea* (Boddie), is one of the most important insect pests of cotton, *Gossypium hirsutum* L. Cry1Ac was the first Bt protein used in transgenic Bt cotton for control of cotton insect pests since 1996. Currently, Bt proteins used in transgenic cotton plants in the U.S. are categorized into three groups: Cry1, which contains Cry1Ab, Cry1Ac, and Cry1F; Cry2, which includes Cry2Ab and Cry2Ae; and Vip3a. The corresponding cotton varieties include: Bollgard 1 (Cry1Ac), Bollgard 2 (Cry1Ac + Cry2Ab), Widestrike (Cry1Ac + Cry1F), TwinLink (Cry1Ab + Cry2Ae), Widestrike 3 (Cry1Ac + Cry1F + Vip3a), Bollgard 3 (Cry1Ac + Cry2Ab + Vip3a) and TwinLink Plus (Cry1Ab + Cry2Ae + Vip3a). However, extensive and constant exposure of cotton bollworms to these Bt proteins not only in transgenic cotton plants but also in Bt corn plants may result in a shift towards a reduction in susceptibility. To estimate the susceptibility of the field populations of cotton bollworm to these Bt proteins, we compared the survivorship of F1 or F2 generations of field-derived cotton bollworms collected from Southern regions in the U.S. to four individual Bt proteins (Cry1Ac, Cry1F, Cry2Ab2, and Vip3a) with that of a susceptible counterpart using diet overlay bioassays.

Materials and Methods

Bacillus thuringiensis toxins

Susceptibility of *H. zea* was determined against four Bt proteins: Cry1Ac, Cry1F, Cry2Ab2, and Vip3a. Cry1Ac protein was provided by Monsanto Company as lyophilized MVPII powders with 20.0% AI. Cry1F protein was provided by Dow Agrosciences as lyophilized powders with 53.0% AI. Cry2Ab2 protein was provided by Monsanto in the form of lyophilized (freeze-dried) Bt-corn leaf powder expressing ~4 mg of Cry2Ab2 protein/g. Syngenta Biotechnology Inc. provided the Vip3Aa19 protein with a purity of 77.8%.

Insect sources

A total of 14 field populations of *Helicoverpa zea* were collected from across the central and eastern cotton belt in 2017. F_1 or F_2 generations of these field-collected populations were used for the bioassays described below. Detailed collection information was listed in Table 1. In addition, a susceptible colony was also used in the current study, which was obtained from a commercial source Benzon Research Inc., Carlisle, PA.

Diet-overlay bioassays

For each population, a diet-overlay bioassay, described by Anilkumar et al (2009), was used to evaluate the larval susceptibility of *H. zea* to Cry1Ac, Cry2Ab2, Cry1F and Vip3A toxins. Each bioassay included 7-8 concentrations plus one untreated control. Diet-overlay concentrations for Cry1Ac and Cry2Ab2 ranged from 0, 0.01, 0.0316, 0.1, 0.316, 1.0, 3.16, to 10.0 µg/cm^2; diet-overlay concentrations for Vip3a were from 0, 0.01, 0.0316, 0.1, 0.316, 1.0, to 3.16 µg/cm^2; and diet-overlay concentrations for Cry1F ranged from 0, 0.02, 0.04, 0.2, 0.4, 2.0, 4.0, to 8.0 µg/cm^2. We used repeater pipets to dispense 0.8 ml per well of liquid diet (Southland Product, Inc. Lake Village, AR) into 128-well bioassay trays (C-D International, Pitman, NJ). Once the diet cooled and solidified, Bt protein solution suspended in 0.1% Triton-X100 was overlaid onto the diet surface of each well and allowed to air dry. A constant volume of 40 µl Bt protein solution was overlaid into each well for Cry1Ac, Cry1F, and Vip3a proteins, while a volume of 200 µl Bt protein solution per well was used for Cry2Ab2 protein. One neonate (< 24 h) of *H. zea* was released on the diet surface in each well. After larval inoculation, wells were covered with vented lids (C-D International, Pitman, NJ). Each combination of insect population by Bt protein concentration was replicated four times with 16-32 larvae in each replication. The bioassay trays were placed in an environmental chamber maintained at 26 ± 1 ˚C, 50% RH, and a 14:10 (L:D) h photoperiod. Larval mortality, and larval instar were recorded on the 7th day after inoculation.

Data analysis

Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar (mortality = dead+L1) divided by the total number of insects assayed, and was used to determine LC_{50} values and the corresponding 95% confidence limit (CL). Larval mortality at each concentration was first corrected based on the control mortality using the method of Abbott (1925), followed by Probit analysis (SAS Institute, 2010) to determine the LC_{50} that caused 50% mortality and the corresponding 95% confidence limit (CL). Where the LC_{50} 95% CLs for each field collected population did not overlap with the LC_{50} 95% CL of the BZ-SS susceptible colony, resistance ratio was calculated using the LC_{50} of a field population divided by the LC_{50} of the BZ-SS susceptible strain. In some cases, the LC_{50} value of an insect population 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 assayed. If the LC_{50} value of an insect population was smaller than that of the BZ-SS, a negative sign was assigned to the resistance ratio.

In addition, because the probit analysis mentioned above was not used to analyze the dose-response data of the insect colonies due to low mortalities, and some of the bioassay data didn't fit the probit model well, larval mortality data for each Bt protein at individual concentration were also analyzed using a one-way analysis of variance (ANOVA) with insect strain as the main factor (SAS Institute, 2010). For ANOVA,

original data on the percentage of larval mortality were transformed using arcsine ($\chi 0.5$) to meet the assumptions of normality. Treatment means were separated using Tukey's HSD test at $\alpha = 0.05$ level (SAS Institute, 2010).

Results and Discussion

LC_{50} values of different populations of *H. zea* to Bt proteins.

Detailed data of LC_{50} values for four Bt proteins were listed in **Table 2**. The LC_{50} value of the BZ-SS on Cry1Ac diet was 0.091 μg/cm^2 with a 95% CL of 0.077-0.108 μg/cm^2 (**Table 2**). Compared to BZ-SS, all 14 field populations exhibited significantly lower susceptibility to Cry1Ac protein. The LC_{50} values of LA-WB, AR-RH, and MS-BE were 6.259, 2.771, and 5.645 μg/cm^2, respectively, resulting in the resistance ratios from 30.5-68.8-fold. The LC_{50} values of the remaining 11 field populations were all ≥ 10.0 μg/cm^2, because the highest Cry1Ac concentration (10.0 μg/cm^2) used in the bioassays cannot kill more than 50% of the insects, resulting in the corresponding resistance ratios ≥109.8-fold.

The LC_{50} value of the BZ-SS on Cry2Ab2 diet was 0.20 μg/cm^2 with a 95% CL of 0.17-0.24 μg/cm^2 (**Table 2**). Compared to BZ-SS, LC_{50} values for Cry2Ab2 of the field populations of AR-RH, and LA-DLT were 1.21, and 0.65 μg/cm^2, respectively, resulting in the resistance ratios from 3.3 to 6.1-fold, suggesting that these two field populations were susceptible to Cry2Ab2 protein. However, for the remaining 11 field populations, the LC_{50} values were ≥ 2.28 μg/cm^2, with the corresponding resistance ratios were ≥ 11.4-fold.

The LC_{50} value of the BZ-SS on Vip3A diet was 0.96 μg/cm^2 with a 95% CL of 0.86-1.12 μg/cm^2 (**Table 2**). Compared to BZ-SS, all 12 field populations were susceptible to Vip3A protein, with the LC_{50} values ranging from 0.03 to 1.25 μg/cm^2 (**Table 2**).

The LC_{50} value of the BZ-SS on Cry1F diet was 0.73 μg/cm^2 with a 95% CL of 0.55-0.97 μg/cm^2 (**Table 2**). Compared to BZ-SS, the LC_{50} values for Cry1F protein of all seven tested populations were > 4.00 μg/cm^2 (**Table 2**).

ANOVA test for larval mortality of different populations of *H. zea* to Bt proteins.

The effect of insect strain on mortality was significant for the Cry1Ac concentrations of 0.1-10.0 μg/cm^2 ($F \geq 9.67$; df =14, 42; $P < 0.0001$) (**Table 3**). ANOVA showed that the larval mortality of the field populations was significantly ($P < 0.05$) lower than that of BZ-SS at the Cry1Ac concentrations from 0.1 to 10.0 μg/cm^2, especially for MS-SE, MS-SK, LA-DLT and TN-JN (**Table 3**). For instance, at the highest Cry1Ac concentration of 10.0 μg/cm^2, larval mortality for BZ-SS was 100.0%, while the values or MS-SE, MS-SK, LA-DLT and TN-JN were 4.7, 15.6, 12.5, and 4.7%, respectively.

The effect of insect strain on mortality was also significant for the Cry2Ab2 concentrations of 0.1-10.0 μg/cm^2 ($F \geq 7.46$; df =13, 39; $P < 0.0001$) (**Table 4**). In general, ANOVA showed that the larval mortality of the field populations was significantly ($P < 0.05$) lower than that of BZ-SS at the Cry2Ab2 concentrations from 0.1 to 10.0 μg/cm^2, with only five exceptions (**Table 4**). Notably, the field populations of TX-CS, TX-WT, MS-SE, and LA-JV showed significantly lower susceptibility against Cry2Ab2 protein at all tested concentrations. For example, at the highest Cry2Ab2 concentration of 10.0 μg/cm^2, the mortality for BZ-SS was 100%, while the mortality for TX-CS, TX-WT, MS-SE, and LA-JV were 35.4, 32.8, 36.5, and 14.3%, respectively.

The effect of insect strain on mortality was also significant for all the Vip3A concentrations of 0.1-3.16 μg/cm^2 ($F \geq 18.99$; df =12, 36; $P < 0.0001$) (**Table 5**). ANOVA showed that the larval mortality of all field populations was either similar or significantly ($P < 0.05$) higher than that of BZ-SS, indicating that the field population of *H. zea* were very susceptible to Vip3A protein.

The effect of insect strain on mortality was significant for Cry1F concentrations of 0.4 -4.0 μg/cm^2 ($F \geq 6.90$; df =7, 24; $P \leq 0.0002$) (**Table 6**). Overall, ANOVA tests showed that the larval mortality of all field

populations was significantly ($P < 0.05$) higher than that of BZ-SS at the Cry1F concentrations ranging from 0.4 to 4.0 μg/cm^2 with only one exception of LA-DLT at the concentration of 0.4 μg/cm^2 (**Table 6**).

Table 1. Insect sources used in the study in 2017.

Insect colony	Collected Site	Collected date	Host	No. Pupae
BZ-SS	Benzon	/	/	/
LA-WB	Winnsboro, LA	06/13/2017	Non-Bt corn	186
LA-DLN	Alexandria, LA	07/16/2016	Non-Bt cotton	131
LA-DLT	Alexandria, LA	07/16/2016	TL cotton	107
LA-JV	Jonesville, LA	08/02/2017	BG2 cotton	121
TX-CS	College Station, TX	06/21/2017	WS cotton	77
TX-WT	Wharton, TX	06/23/2017	TL cotton	20
MS-SE	Stoneville, MS	07/11/2017	VT2P corn	111
MS-SK	Starkville, MS	07/21/2017	VT2P & Non-Bt corn	97
MS-SC	Silver City, MS	07/20/2017	BG2 cotton	75
MS-BE	Benoit, MS	07/25/2017	BG2 cotton	69
AR-RH	Rohwer, AR	07/18/2017	Grain sorghum	157
AR-PB	Pine Bluff, AR	08/23/2017	Bt cotton	62
TN-ML	Milan, TN	07/27/2017	Obsession corn	135
TN-JN	Jackson, TN	08/23/2017	Obsession corn	131

Table 2. LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to four Bt proteins in 2017.

Bt protein	Insect strain	N[a]	LC_{50} (95% CL) (µg/cm²)[b]	Slope ± SE	X^2	df	Resistance ratio[c]
Cry1Ac	BZ-SS	958	0.091 (0.077, 0.108)	1.55 ± 0.09	28.4	26	1.0
	LA-WB	948	6.259 (2.000, 70107)	1.38 ± 0.59	46.2	26	68.8 *
	TX-CS	958	> 10.000	/	/	/	> 109.8 *
	TX-WT	945	> 10.000	/	/	/	> 109.8 *
	MS-SE	952	> 10.000	/	/	/	> 109.8 *
	LA-DLN	512	> 10.000	/	/	/	> 109.8 *
	MS-SK	512	> 10.000	/	/	/	> 109.8 *
	AR-RH	512	2.771 (0.368, 13033)	1.57 ± 0.72	53.0	26	30.5 *
	MS-SC	512	> 10.000	/	/	/	> 109.8 *
	LA-DLT	512	> 10.000	/	/	/	> 109.8 *
	LA-JV	512	> 10.000	/	/	/	> 109.8 *
	MS-BE	512	5.645 (3.621, 10.141)	0.94 ± 0.10	32.0	26	62.0 *
	TN-ML	512	> 10.000	/	/	/	> 109.8 *
	TN-JN	512	> 10.000	/	/	/	> 109.8 *
	AR-PB	512	> 10.000	/	/	/	> 109.8 *
Cry2Ab2	BZ-SS	960	0.20 (0.17, 0.24)	1.61 ± 0.09	30.7	26	1.0
	LA-WB	953	> 10.00	/	/	/	> 50.0 *
	TX-CS	959	> 10.00	/	/	/	> 50.0 *
	TX-WT	960	> 10.00	/	/	/	> 50.0 *
	MS-SE	512	> 10.00	/	/	/	> 50.0 *
	LA-DLN	512	> 10.00	/	/	/	> 50.0 *
	MS-SK	512	9.22 (2.88, 9313)	1.31 ± 0.53	49.5	26	46.1 *
	AR-RH	512	1.21 (0.72, 2.22)	1.19 ± 0.17	71.8	26	6.1
	MS-SC	512	2.28 (1.25, 4.94)	1.18 ± 0.20	66.7	26	11.4 *
	LA-DLT	512	0.65 (0.38, 1.54)	1.28 ± 0.18	69.3	26	3.3
	LA-JV	512	> 10.00	/	/	/	> 50.0 *
	MS-BE	512	2.43 (1.17, 7.22)	0.65 ± 0.10	46.2	26	12.1 *
	TN-ML	512	6.18 (3.53, 14.27)	1.05 ± 0.15	44.4	26	30.9 *
	TN-JN	512	6.46 (4.05, 12.08)	0.91 ± 0.10	25.0	26	32.3 *
Vip3A	BZ-SS	895	0.96 (0.86, 1.12)	2.81 ± 0.28	22.2	22	1.0
	LA-WB	895	0.33 (0.18, 0.61)	2.08 ± 0.43	75.0	22	-2.9
	TX-CS	896	0.03 (0.02, 0.04)	2.08 ± 0.43	75.0	22	-32.0
	TX-WT	895	0.82 (0.73, 0.90)	5.67 ± 0.87	11.0	22	-1.2
	MS-SE	894	0.06 (0.04, 0.07)	1.84 ± 0.18	48.0	22	-16.0
	LA-DLN	448	0.10 (0.08, 0.12)	2.37 ± 0.22	10.9	22	-9.6
	MS-SE	448	0.04 (0.03, 0.05)	2.62 ± 0.27	9.6	22	-24.0
	AR-RH	448	1.25 (1.00, 1.57)	5.24 ± 1.23	38.8	22	1.3

	MS-SC	448	0.05 (0.04, 0.06)	3.20 ± 0.34	15.2	22	-19.2
	LA-DLT	448	0.33 (0.19, 0.61)	1.70 ± 0.31	15.2	22	-2.9
	LA-JV	448	0.15 (0.12, 0.18)	2.05 ± 0.18	18.7	22	-6.4
	MS-BE	448	0.04 (0.03, 0.06)	1.29 ± 0.15	35.2	22	-24.0
	TN-ML	448	0.12 (0.10, 0.14)	2.30 ± 0.21	22.3	22	-8.0
Cry1F	BZ-SS	512	0.73 (0.55, 0.97)	1.68 ± 0.15	28.9	26	1.0
	TX-CS-WS	512	> 8.00	/	/	/	> 10.9 *
	LA-DL-NBt cotton	448	> 4.00	/	/	/	> 5.4
	LA-DL-TL	448	> 4.00	/	/	/	> 5.4
	LA-JE-BG2	512	> 8.00	/	/	/	> 10.9 *
	AR-Roher-GS	448	> 4.00	/	/	/	> 5.4
	MS-Silver City-BG2	448	> 4.00	/	/	/	> 5.4
	MS-Stoneville-VT2P	448	> 4.00	/	/	/	> 5.4

[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. Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar (mortality = dead+L1) divided by the total number of insects assayed.

[c] Resistance ratio for Bt protein were calculated by dividing the LC_{50} value of an insect population by that of the susceptible strain (BZ-SS). If the LC_{50} an insect population was smaller than that of the BZ-SS, a negative sign was assigned to the resistance ratio.

* indicates significant resistance ratios that were ≥ 10-fold.

Table 3. Larval mortality of different populations of *Helicoverpa zea* on Cry1Ac-diet at different concentrations.

Insect strain	Larval mortality (%)				
	Cry1Ac concentration (µg/cm^2)				
	0.1	0.316	1.0	3.16	10
BZ-SS	42.9 ± 6.0 c	79.6 ± 2.7 c	96.6 ± 1.4 c	100.0 ± 0.0 g	100.0 ± 0.0 f
LA-WB	0.8 ± 0.8 ab	1.6 ± 0.9 ab	6.3 ± 2.2 ab	48.8 ± 1.2 ef	51.6 ± 4.7 cd
TX-CS	0.8 ± 0.8 ab	7.1 ± 1.5 ab	15.6 ± 2.2 b	29.2 ± 3.8 cde	56.3 ± 8.1 cde
TX-WT	3.1 ± 1.8 ab	2.3 ± 1.5 ab	3.6 ± 2.6 ab	7.8 ± 3.7 abc	48.4 ± 8.2 cd
MS-SE	0.8 ± 0.8 ab	1.6 ± 0.9 ab	0.8 ± 0.8 a	4.7 ± 2.7 ab	4.7 ± 3.0 a
LA-DLN	1.2 ± 1.2 ab	1.2 ± 1.2 a	4.0 ± 2.6 ab	11.1 ± 2.6 abcd	27.0 ± 6.1 bc
MS-SK	3.1 ± 1.8 ab	1.6 ± 1.6 ab	1.6 ± 1.6 a	4.7 ± 3.0 ab	15.6 ± 3.1 ab
AR-RH	2.8 ± 2.8 ab	1.2 ± 1.2 a	9.9 ± 5.2 ab	66.7 ± 6.5 f	82.5 ± 4.8 e
MS-SC	0.0 ± 0.0 a	0.0 ± 0.0 a	14.1 ± 3.9 ab	23.4 ± 6.9 bcde	26.6 ± 3.0 bc
LA-DLT	4.7 ± 3.0 ab	4.7 ± 3.0 ab	3.1 ± 1.8 ab	6.3 ± 4.4 ab	12.5 ± 2.6 ab
LA-JV	4.8 ± 1.6 ab	1.6 ± 0.9 ab	6.4 ± 1.9 ab	19.4 ± 1.9 bcde	60.0 ± 4.8 de
MS-BE	6.3 ± 2.6 ab	12.5 ± 2.6 b	10.9 ± 5.3 ab	39.1 ± 3.9 def	68.8 ± 5.7 de
TN-ML	10.9 ± 1.6 b	12.5 ± 2.6 b	17.2 ± 1.6 b	21.9 ± 9.7 bcde	35.9 ± 1.6 bcd
TN-JN	1.6 ± 1.6 ab	0.0 ± 0.0 a	1.6 ± 1.6 a	1.6 ± 1.6 a	4.7 ± 3.0 a
AR-PB	0.0 ± 0.0 a	2.8 ± 2.8 ab	19.0 ± 3.0 b	36.5 ± 3.7 def	55.6 ± 10.9 cde
ANOVA	$F_{14,42} = 9.67$ $P < 0.0001$	$F_{14,42} = 26.81$ $P < 0.0001$	$F_{14,42} = 25.05$ $P < 0.0001$	$F_{14,42} = 26.62$ $P < 0.0001$	$F_{14,42} = 31.81$ $P < 0.0001$

Table 4. Larval mortality of different populations of *Helicoverpa zea* on Cry2Ab2-diet at different concentrations.

Insect strain	Larval mortality (%)				
	Cry2Ab2 concentration (µg/cm^2)				
	0.1	0.316	1.0	3.16	10
BZ-SS	26.6 ± 3.3 c	60.9 ± 4.1 e	89.1 ± 3.7 e	97.7 ± 1.5 e	100.0 ± 0.0 f
LA-WB	0.0 ± 0.0 a	2.7 ± 1.1 ab	7.3 ± 2.5 abc	29.1 ± 3.0 ab	48.1 ± 5.4 bcd
TX-CS	0.0 ± 0.0 a	0.6 ± 0.6 ab	21.3 ± 6.7 abcd	5.5 ± 2.2 a	35.4 ± 3.0 ab
TX-WT	3.1 ± 1.8 ab	1.0 ± 1.0 ab	2.2 ± 1.1 ab	7.2 ± 2.9 a	32.8 ± 8.5 ab
MS-SE	1.2 ± 1.2 a	5.6 ± 4.1 ab	1.2 ± 1.2 a	14.3 ± 6.6a	36.5 ± 7.8 abc
LA-DLN	1.2 ± 1.2 a	5.6 ± 4.1 ab	7.9 ± 1.8 abc	19.0 ± 4.8 ab	49.2 ± 8.6 bcd
MS-SK	0.0 ± 0.0 a	0.8 ± 0.5 ab	4.1 ± 3.6 ab	18.0 ± 7.8 ab	65.6 ± 9.0 cd
AR-RH	26.6 ± 10.6 bc	14.1 ± 6.9 bcd	29.7 ± 8.2 bcd	70.3 ± 6.4 cd	93.8 ± 2.6 ef
MS-SC	3.2 ± 1.8 ab	3.1 ± 1.8 ab	40.6 ± 17.0 cd	64.1 ± 9.3 cd	75.0 ± 4.4 de
LA-DLT	5.0 ± 3.2 ab	28.3 ± 3.2 d	48.3 ± 11.0 d	88.3 ± 5.7 de	98.3 ± 1.7 f
LA-JV	1.2 ± 1.2 a	0.0 ± 0.0 a	5.2 ± 2.3 ab	7.9 ± 1.8 a	14.3 ± 4.1 a
MS-BE	20.0 ± 5.6 bc	26.2 ± 4.9 cd	29.5 ± 4.1 bcd	45.9 ± 5.6bc	78.7 ± 6.8 de
TN-ML	4.7 ± 3.0 ab	12.5 ± 2.6 bcd	7.8 ± 3.0 abc	25.0 ± 6.8 ab	75.0 ± 5.7 de
TN-JN	6.3 ± 2.6 abc	6.3 ± 2.6 abc	17.2 ± 3.9 abcd	46.9 ± 7.4 bc	56.3 ± 5.7 bcd
ANOVA	$F_{13,39} = 7.46$	$F_{13,39} = 14.45$	$F_{13,39} = 13.32$	$F_{13,39} = 27.87$	$F_{13,39} = 24.84$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Table 5. Larval mortality of different populations of *Helicoverpa zea* on Vip3A-diet at different concentrations.

Insect strain	Larval mortality (%)			
	Vip3A concentration (µg/cm^2)			
	0.1	0.316	1.0	3.16
BZ-SS	2.9 ± 2.2 abc	1.3 ± 0.8 a	5.9 ± 1.4 a	88.2 ± 2.9 a
LA-WB	10.9 ± 2.0 bcd	31.3 ± 4.9 b	94.5 ± 2.0 c	100.0 ± 0.0 b
TX-CS	77.3 ± 6.3 gh	74.2 ± 4.5 c	100.0 ± 0.0 c	100.0 ± 0.0 b
TX-WT	0.0 ± 0.0 a	1.6 ± 0.9 a	68.8 ± 2.2 b	100.0 ± 0.0 b
MS-SE	62.5 ± 4.9 fgh	95.3 ± 1.6 de	100.0 ± 0.0 c	100.0 ± 0.0 b
LA-DLN	52.4 ± 7.6 fg	87.3 ± 2.6 cd	100.0 ± 0.0 c	100.0 ± 0.0 b
MS-SK	85.9 ± 3.0 h	98.4 ± 1.6 de	100.0 ± 0.0 c	100.0 ± 0.0 b
AR-RH	1.2 ± 1.2 ab	5.2 ± 2.3 a	31.7 ± 16.6 a	98.4 ± 1.6 b
MS-SC	82.8 ± 6.4 h	100.0 ± 0.0 e	100.0 ± 0.0 c	100.0 ± 0.0 b
LA-DLT	20.3 ± 8.2 cde	31.3 ± 5.7 b	85.9 ± 5.3 bc	100.0 ± 0.0 b
LA-JV	32.3 ± 5.6 def	74.2 ± 7.0 c	96.8 ± 1.9 c	100.0 ± 0.0 b
MS-BE	51.6 ± 5.3 fg	92.2 ± 3.0 cde	100.0 ± 0.0 c	100.0 ± 0.0 b
TN-ML	41.9 ± 7.0 ef	87.1 ± 2.6 cd	96.8 ± 3.2 c	100.0 ± 0.0 b
ANOVA	$F_{12,36} = 40.04$	$F_{12,36} = 81.07$	$F_{12,36} = 35.86$	$F_{12,36} = 18.99$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Table 6. Larval mortality of different populations of *Helicoverpa zea* on Cry1F-diet at different concentrations.

Insect strain	Larval mortality (%)		
	Cry1F concentration (µg/cm^2)		
	0.4	2.0	4.0
BZ-SS	28.1 ± 5.4 c	76.6 ± 5.3 b	93.8 ± 2.6 b
TX-CS	0.0 ± 0.0 a	7.1 ± 4.1 a	1.8 ± 1.8 a
LA-DLN	0.0 ± 0.0 a	4.7 ± 4.7 a	10.9 ± 4.7 a
LA-DLT	20.1 ± 7.8 bc	1.6 ± 0.9 a	7.3 ± 5.2 a
LA-JV	5.6 ± 4.1 ab	2.4 ± 1.4 a	7.9 ± 3.2 a
AR-RH	4.0 ± 2.6 ab	1.2 ± 1.2 a	9.5 ± 3.0 a
MS-SC	1.2 ± 1.2 ab	8.3 ± 3.8 a	11.1 ± 4.5 a
MS-SE	1.2 ± 1.2 ab	2.4 ± 1.4 a	8.3 ± 2.8 a
ANOVA	$F_{7,24} = 6.90$	$F_{7,24} = 17.79$	$F_{7,24} = 25.76$
	$P = 0.0002$	$P < 0.0001$	$P < 0.0001$