PERFORMANCE OF FIELD POPULATIONS OF *HELICOVERPA ZEA* AGAINST PYRAMIDED BT CORN AND PURIFIED VIP3Aa51 PROTEIN

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Abstract

The corn earworm, *Helicoverpa zea* (Boddie), is a major pest of Bt maize and cotton in the U.S. Reduced efficacy of Bt plants expressing Cry1 and Cry2 against *H. zea* has been reported in some areas of U.S. In this study, we evaluated the occurrence and ear damage of *H. zea* on transgenic Bt maize expressing Cry proteins or a combination of Vip3A and Cry proteins in the field in Texas in 2018. We found that occurrence of *H. zea* larvae and the ear viable kernel damage area were not different between non-Bt maize and Bt maize expressing Cry1A.105+Cry2Ab2 and Cry1Ab+Cry1F proteins. 67.5% of the pyramided Bt maize expressing Cry1Ab+Cry1F+Vip3A was damaged by 2nd - 4th instar larvae of *H. zea*. Diet bioassays showed that resistance ratio against Vip3Aa51 for *H. zea* obtained from Cry1Ab+Cry1F+Vip3A maize was 20.4 compared to a field population collected from Cry1F+Cry1A.105+Cry2Ab2 maize. Leaf tissue bioassays showed that 7-day survivorship on WideStrike3 (Cry1F+Cry1Ac+Vip3A) cotton leaves was significantly higher for the *H. zea* population collected from Cry1Ab+Cry1F+Vip3A maize than for a Bt susceptible laboratory population. Results generated from this study suggested that *H. zea* has evolved practical resistance to Cry1 and Cry2 proteins. Therefore, it is crucial to ensure the sustainable use of the Vip3A technology in Bt maize and cotton.

Introduction

Genetically engineered crops expressing *Bacillus thuringiensis* (Bt) proteins have been commercially planted for control of maize, cotton and soybean insect pests for more than two decades [1]. In 2017, global adoption of Bt crops reached over 100 million hectares [1]. Besides their high efficacy in controlling target insect pests, these Bt crops also offer environmental and economic benefits such as reduced chemical insecticide use and crop yield loss [2-7]. The widespread use of Bt technology places consistently strong selection pressure on target insect pest populations. Evolution of resistance becomes a primary threat to the durability of the Bt crops. From 1996 to 2005, field-evolved practical resistance to Bt crops was only reported in 3 cases worldwide [8]. However, the cumulative number of cases of field-evolved practical resistance to transgenic Bt crops reached 19 by 2018 [8-11].

Gene pyramiding that enables transgenic plants to express two or more dissimilar Bt proteins against the same insect pest is one of the major strategies currently adopted for insect resistance management in the U.S. [12] Relative to the single Bt protein crops, pyramided Bt crops are expected to be more effective in delaying evolution of resistance because when one Bt protein in the pyramids is ineffective, the remaining Bt proteins can kill the insects [13]. Currently, almost all Bt maize hybrids and cotton varieties on the U.S. market are pyramids [14]. The proteins targeting lepidopteran pest in Bt cotton include Cry1Ac, Cry1Ab, Cry1F, Cry2Ab, Cry2Ae and Vip3Aa19; and the proteins adopted in Bt maize contain Cry1Ab, Cry1A.105, Cry1F, Cry2Ab2 and Vip3Aa20. The very same or similar Bt proteins expressed in these two crops places strong selection pressure on target insect pests which feed on both crops in areas where maize and cotton are produced in the same landscape. For example, the corn earworm, also called the cotton bollworm, *Helicoverpa zea* (Boddie), is one of the most costly crop insect pests in North America [14, 15]. *H. zea* is a major target pest of pyramided Bt maize as well as Bt cotton in the U.S. Therefore, there is a high potential for multiple generations of *H. zea* exposure to different Bt crops. In addition, studies have indicated that *H. zea* has inherently low susceptibility to Cry1 and Cry2 proteins [17-19]. All of these factors could create favorable conditions for the fast evolution of resistance to Bt proteins in *H. zea*.

In recent years, *H. zea* control problems in fields of pyramided Bt maize and Bt cotton expressing Cry1/Cry2 have been reported in some areas of U.S. [20-23]. For example, Dively et al. [20] documented the field-evolved resistance of *H. zea* to Cry1Ab and Cry1A.105+Cry2Ab2 maize in Maryland. Reisig et al. [24] observed field-evolved practical resistance of *H. zea* to pyramided Bt cotton expressing Cry1Ac+Cry1F and Cry1Ac+Cry2Ab in North Carolina. Diet bioassays conducted by Yang et al. [21, 22] also confirmed high resistance ratios for Cry1Ac and Cry2Ab2 purified proteins of *H. zea* populations collected from Louisiana, Mississippi, Arkansas and Tennessee. Moreover, Cry1/Cry2 proteins are currently used in combination with Vip3A proteins in almost all recently released Bt maize and Bt cotton

products in the U.S. Previous studies have shown that there was no or very week cross-resistance between Vip3A and Cry proteins [25-30]. For example, Mahon et al. found that Vip3A resistant populations of *Helicoverpa armigera* and *Helicoverpa punctigera* had no cross-resistance to Cry1Ac and Cry2Ab proteins [25]. Wei et al. showed that *H. armigera* with high levels of resistance to Cry1Ac or Cry2Ab had no cross-resistance to Vip3A [26]. In addition, studies indicated that cross-resistance is absent among Cry1, Cry2 and Vip3A proteins in Cry1F-resistant, Cry2Ab2-resistant and Vip3A-resistant populations of *Spodoptera frugiperda* [28-30]. Because populations of *H. zea* in most U.S. fields are already resistant to the Cry1 and Cry2 proteins, this makes Vip3A the only protein in commercialized Bt crops that is consistently effective against *H. zea*, which markedly raises the risk of resistance [8]. In this study, we reported the occurrence and ear damage of *H. zea* on transgenic Bt maize expressing Cry proteins or a combination of Vip3A and Cry proteins in the field in Texas, USA and its susceptibility against Vip3A protein.

Materials and Methods

Source of Bt and Non-Bt Maize Hybrids

Plant injury and occurrence of *H. zea* in the field was evaluated against five maize hybrids including two non-Bt and three Bt maize hybrids. The three Bt maize hybrids included DKC 67-72VT2P, Genuity VT Double Pro (VT2P) (Monsanto Company, St. Louis, MO); P1637YHR, Intrasect; and Leptra, P1637VYHR (Pioneer Hi-Bred, Johnson IA). VT2P contains Cry1A.105 and Cry2Ab2 proteins; Intrasect expresses Cry1Ab and Cry1F proteins; Leptra contains Cry1Ab, Cry1F, and Vip3A proteins. The two non-Bt maize hybrids, DKC 67-70RR (Monsanto Company) and P1637R (Pioneer Hi-Bred), used in this study, were genetically closely related to one or two of the three Bt maize hybrids. We randomly sampled one maize ear from the middle two rows in each plot. These ears were brought back to the laboratory and were used for the qualitative ELISA tests. Expression/non-expression of the Cry and Vip3A proteins in the maize hybrids were confirmed using an ELISA-based technique (EnviroLogix, Quantiplate™ kits, Portland, ME). ELISA tests were conducted according to the protocol procedure manual (EnviroLogix, Quantiplate™ kits, Portland, ME).

Field Planting

A field trial was conducted on the Texas A&M University Farm in Snook, Texas on March 26, 2018 (34.999490° N; 101.918570° W). Each maize hybrid was planted on 3.1-wide × 9.1-long meter plots. Each plot contained four, 0.72-meter wide rows and each row contained about 43 plants. The distance between each plot was approximate 3 feet. These plots were arranged in a randomized complete block design with four plots (blocks) for each maize hybrid. Plant injury and occurrence of natural populations of *H. zea* were closely monitored and checked on the primary ears. In each plot, 20 plants per row were randomly sampled. Percentage of plants with live larvae, number of larvae per ear, larval development, percentage of plants with damaged ears and the area of viable kernel damage were recorded on June 22, 2018.

Dose Response Bioassays

Susceptibility to Vip3A of two F_1 populations of *H. zea* was evaluated using a diet-overlay bioassay as described in Anilkumar et al. and Yang et al. [30, 31] The first population was established from approximate 100, 2nd to 4th instar live larvae recovered from ears of Cry1Ab+Cry1F+Vip3A maize in the field on June 22, 2018. The second population consisted of ~150, 2nd to 5th instar larvae collected from ears of Cry1F+Cry1A.105+Cry2Ab2 maize plants at the same farm and the same day as the first population. These field-collected larvae were reared on the artificial diet (WARD'S Stonefly *Heliothis* diet, Rochester, NY) under 26 ± 1 °C, 50% RH and a 16:8 h (L: D) photoperiod until pupal stage. The Vip3Aa51 protein was provided by BASF Company (Research Triangle Park, NC) in solution at a concentration of 2.9 mg/ml. The protein was stored in 50 mM Caps pH 10.5, 10% glycerol, 1 mMDTT, and 10mM maltose. The sequence information of Vip3Aa51 protein could be achieved from NCBI with the GenBank Accession: KC156649.1. It shows 94.93% homology compared to the Vip3Aa19. Each bioassay consisted of seven concentrations ranging from 0-3.16 µg/cm^2. Repeater pipets were used 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, a volume of 40 µl Vip3A protein solution suspended in 0.1% Triton-X100 was overlaid onto the diet surface of each well and allowed to air dry. One neonate (< 24 h) 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 Vip3Aa51 protein concentration was replicated four times with 16 larvae in each replication. Bioassay trays were placed in an environmental chamber maintained at 26 ± 1 °C, 50% RH, and a 16:8 (L:D) h photoperiod. Larval mortality and larval weight were recorded on the 7th day after inoculation.

Cotton Leaf Tissue Bioassays

Performance of F_1 neonates (<24 h old) of *H. zea* collected from Cry1Ab+Cry1F+Vip3A maize plants, along with a laboratory susceptible population (SS) collected at the LSU AgCenter Macon Ridge Research Station in Franklin Parish on May 2016, were examined on the non-Bt, PHY 425RF and WideStrike 3, PHY 480W3FE (Dow AgroScience, Indianapolis, IN) (expressing Cry1Ac, Cry1F and Vip3A) cotton leaf tissues. Cotton leaf tissues were collected from field-grown plants on a Texas A&M University Farm in Snook, Texas in 2018. Expression/non-expression of the Cry and Vip3A proteins in the cotton varieties were confirmed using an ELISA-based technique (EnviroLogix, Quantiplate™ kits, Portland, ME). In the leaf tissue bioassays, one leaf of a cotton variety was placed in each well of 8-well trays (C-D International, Pitman, NJ), and five neonates of one of the two *H. zea* populations were then placed on the leaf tissue in the well. In each leaf tissue bioassay, there were four replications for each combination of cotton product and insect population, and each replication consisted of six wells each with 5 larvae (n = 4 x 30 = 120). Bioassay trays with larvae and leaf tissue were maintained at 26 ± 1 ˚C, 50% RH, and a 16:8 (L:D) h photoperiod. Leaves were replaced every two days. Larval survival and development were recorded on the 7th day after infestation.

Data Analysis

In the field assay and cotton leaf bioassay, data on number of larvae per ear, average instar and ear damage area were transformed to the log(x + 1) scale, while percentage of plants with live larvae, percentage of plants with damaged ears, and larval survivorship were transformed using arcsine of ($x^{0.5}$) to normalize treatment variances. The transformed data were then analyzed using one-way or two-way analysis of variance with maize hybrids, insect populations and cotton varieties as the main factors [32]. Treatment means were separated using Tukey's HSD test at $\alpha = 0.05$ level. Untransformed data are presented in tables.

In the protein bioassay, larval growth inhibition was calculated using the formula: growth inhibition (%) = 100 * (body weight of larvae feeding on control diet -body weight of larvae feeding on Vip3Aa51 protein-treated diet)/(body weight of larvae feeding on control diet). Larval mortality was calculated as mortality (%) = 100 * (number of dead larvae + number of surviving larvae that were still in the first instar) / total number of insects assayed, and larval mortality at each concentration was corrected based on the control mortality. Probit analysis was used to determine the median lethal concentration (LC_{50}) that caused 50% mortality and the corresponding 95% confidence limit (CL) [32]. Resistance ratio was calculated using the LC_{50} of the *H. zea* population collected from Cry1Ab+Cry1F+Vip3A maize, divided by the LC_{50} of the population collected from Cry1F+Cry1A.105+Cry2Ab2 maize. Moreover, larval mortality and growth inhibition was analyzed using a two-way ANOVA with insect population and protein concentration as the two main factors [32]. Original data on the percentage of larval mortality and growth inhibition were transformed using arcsine ($\chi^{0.5}$) to meet normality assumptions. Treatment means were separated using Tukey's HSD test at $\alpha = 0.05$ level [32]. Untransformed data are presented in figures.

Results and Discussion

Plant Injury and Occurrence of *H. zea* on Different Hybrids of Non-Bt and Bt Maize in the Field

The natural occurrence of H. zea was high in the field during the experimental period (Table 1). The effects of the treatment on percentage of plants with live larvae, number of larvae per ear, larval development, percentage of plants with damaged ears and ear damage area were all significant. For the two non-Bt maize hybrids, the mean percentage of plants with live larvae was 76.3% and the mean number of live larvae per ear was 0.95 (Table 1). Among Cry1Ab+Cry1F and Cry1A.105+Cry2Ab2 maize plants, 91.3% and 83.8%, respectively, were found with live larvae of H. zea, which was not significantly ($P > 0.05$) different from that of non-Bt plants (Table 1). An average of 1.85 and 1.30 larvae per ear was observed on the primary ears of Cry1Ab+Cry1F and Cry1A.105+Cry2Ab2 maize plants, respectively, which was similar ($P > 0.05$) as observed on the non-Bt maize. Larval development on Cry1Ab+Cry1F and Cry1A.105+Cry2Ab2 maize plants, as well as the two non-Bt maize plants were not different ($P > 0.05$) with an average instar of 4.71 (Table 1). All of the non-Bt, Cry1Ab+Cry1F and Cry1A.105+Cry2Ab2 maize plants suffered severe ear damage, and the damaged area per ear among these four maize hybrids were not different ($P > 0.05$) with an average of 17.0 cm^2-viable kernels (Table 1).

A high infestation of *H. zea* larvae were observed on Bt maize expressing Cry1Ab+Cry1F+Vip3A proteins (Table 1 and Figure 1). In general, 61.3% of Cry1Ab+Cry1F+Vip3A maize was found with an average of 0.79 larvae per ear, which did not differ ($P > 0.05$) from those observed on the non-Bt maize plants (Table 1). Larval development of *H. zea* on Cry1Ab+Cry1F+Vip3A maize ears was significantly ($P < 0.05$) delayed compared to that on non-Bt maize

ears. Average larval development index of larvae recovered from non-Bt plants was 4.90, while it was 3.19 for the larvae found on Cry1Ab+Cry1F+Vip3A maize ears (Table 1). In addition, 67.5% of Cry1Ab+Cry1F+Vip3A maize was found with ear damage and the damaged area per ear was estimated to be 1.3 cm^2 of viable kernels. These two values were significantly ($P < 0.05$) less than that of the non-Bt maize plants (Table 1).

Susceptibility of Two Different Field Populations of *H. zea* to Vip3A Protein in Diet Bioassays

Approximate 100, 2nd to 4th instar live larvae recovered from ears of Cry1Ab+Cry1F+Vip3A maize were collected and taken to the laboratory. Simultaneously, ~150 2nd to 5th instar larvae were collected from ears of Cry1F+Cry1A.105+Cry2Ab2 maize plants in another maize field at the same location. F_1 neonates (<24 h old) of these two populations were tested against purified Vip3Aa51 protein in diet overlay bioassays. Probit analysis showed that the LC_{50} value for *H. zea* populations collected from Cry1F+Cry1A.105+Cry2Ab2 maize was 0.041 µg/cm^2 (95% CL, 0.035-0.049 µg/cm^2) (Table 2). The LC_{50} value for *H. zea* populations collected from Cry1Ab+Cry1F+Vip3A maize was 0.838 µg/cm^2 with a 95% CL of 0.686-0.967 µg/cm^2 (Table 2). Therefore, the LC_{50} value to Vip3Aa51 protein was significantly higher ($P < 0.05$) for *H. zea* populations collected from Cry1Ab+Cry1F+Vip3A, compared to that obtained from Cry1F+Cry1A.105+Cry2Ab2 maize. The estimated resistance ratio for *H. zea* populations collected from Cry1Ab+Cry1F+Vip3A maize relative to *H. zea* populations collected from Cry1F+Cry1A.105+Cry2Ab2 maize was 20.4 (Table 2).

ANOVA tests showed that the main effects of insect populations, protein concentrations and their interactions on larval mortality and growth inhibition were all significant. Mortality for both populations at 0.01 µg/cm^2 was low and not significantly different ($P > 0.05$). At 3.16 µg/cm^2, the mortality for both populations were 100% (Figure 2). However, at each tested concentration from 0.0316-1.0 µg/cm^2, the mortality of *H. zea* populations collected from Cry1Ab+Cry1F+Vip3A was significantly ($P < 0.05$) lower than that of *H. zea* obtained from Cry1F+Cry1A.105+Cry2Ab2 maize (Figure 2). In addition, larval growth inhibition for *H. zea* collected from Cry1Ab+Cry1F+Vip3A was significantly less ($P < 0.05$) than for *H. zea* from Cry1F+Cry1A.105+Cry2Ab2 maize at each tested concentration from 0.01-0.1 µg/cm^2 (Figure 3).

Larval Survival and Development of *H. zea* Populations on Cotton Leaf Tissues

Performance of F_1 neonates (<24 h old) of *H. zea* collected from Cry1Ab+Cry1F+Vip3A maize plants, along with a laboratory susceptible population (SS), were examined on the non-Bt and WideStrike 3 (expressing Cry1Ac, Cry1F and Vip3A) cotton leaf tissues. Larval survival and development on non-Bt leaf tissues were not different ($P > 0.05$) between the two populations with an average survivorship of 80.4% and an average instar of 3.42 after 7 days (Table 3). Survivorship of SS on leaf tissues of WideStrike 3 cotton was low (3.3%) and the limited survivors were all 2nd instar (Table 3). However, 41.7% of *H. zea* larvae collected from Cry1Ab+Cry1F+Vip3A maize survived on leaf tissues of WideStrike 3, which was significantly higher ($P < 0.05$) than that of SS on WideStrike 3 cotton leaves. In addition, larval development of *H. zea* collected from Cry1Ab+Cry1F+Vip3A maize reached to 2.68 instar on leaf tissues of WideStrike 3 after 7 days, which was also significantly greater ($P < 0.05$) than that of SS (Table 3).

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Table 1. Plant injury and occurrence of *Helicoverpa zea* on different varieties of non-Bt and Bt maize in the field*.

Variety	Bt proteins	% plant with larvae[#]	Number of larvae/ ear	Average instar	% plant with damaged ear [§]	Damaged area per ear (cm^2)
DKC-NBt	/	77.5 ± 6.0 ab	0.96 ± 0.11 ab	4.86 ± 0.13 bc	100.0 ± 0.0 b	18.1 ± 0.9 b
P-NBt	/	75.0 ± 2.0 ab	0.93 ± 0.05 ab	4.94 ± 0.11 c	100.0 ± 0.0 b	19.1 ± 1.7 b
Intrasect	Cry1Ab+Cry1F	91.3 ± 4.3 b	1.85 ± 0.13 b	4.31 ± 0.06 b	100.0 ± 0.0 b	15.7 ± 1.5 b
VT2P	Cry1A.105+Cry2Ab2	83.8 ± 3.8 ab	1.30 ± 0.18 b	4.71 ± 0.11 bc	100.0 ± 0.0 b	15.0 ± 1.2 b
Leptra	Cry1Ab+Cry1F+Vip3A	61.3 ± 3.1 a	0.79 ± 0.04 a	3.19 ± 0.19 a	67.5 ± 1.4 a	1.3 ± 0.2 a
F-test	*F-value*	$F_{4,12} = 6.16$	$F_{4,12} = 13.30$	$F_{4,12} = 40.36$	$F_{4,12} = 1546.47$	$F_{4,12} = 224.56$
	P-value	0.0062	0.0002	< 0.0001	< 0.0001	< 0.0001

*Mean values within a column followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD test). [#] Percentage of plants with live larvae of *H. zea*. [§] Percentage of plants with damaged ears by larvae of *H. zea.*

Table 2. Mortality response (LC_{50}) of different populations of *Helicoverpa zea* to Vip3Aa51 protein in diet-overlay bioassays.

Insect population*	N[#]	LC_{50} (95% CI) (µg/cm^2) [§]	Slope ± SE	X^2	df	Resistance ratio[£]
CEW-TX-VT3P-2018	448	0.041 (0.035, 0.050)	2.87 ± 0.30	18.9	22	1.0
CEW-TX-Leptra-2018	448	0.838 (0.686, 0.966)	4.93 ± 1.02	19.0	22	20.4

*CEW-TX-VT3P-2018 refers to the *H. zea* population collected from ears of Cry1F+Cry1A.105+Cry2Ab2 maize plants, and CEW-TX-Leptra-2018 refers to the *H. zea* population recovered from ears of Cry1Ab+Cry1F+Vip3A maize. [#] Total number of neonates assayed. [§] Median lethal concentration (LC_{50}) that caused 50% mortality and the corresponding 95% confidence limit (CL). Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar divided by the total number of insects assayed. [£] Resistance ratio was calculated using the LC_{50} value of CEW-TX-Leptra-2018 divided by the LC_{50} of CEW-TX-VT3P-2018.

Table 3. Performance of two different populations of *Helicoverpa zea* on cotton leaf tissues*.

Cotton variety		Insect[§]	Survivorship (%)[£]	Average instar
Non-Bt		CEW-TX-Leptra-2018	78.3 ± 2.9 c	3.39 ± 0.01 c
		CEW-TX-SS	82.5 ± 3.2 c	3.45 ± 0.05 c
WideStrike 3		CEW-TX-Leptra-2018	41.7 ± 7.5 b	2.68 ± 0.03 b
		CEW-TX-SS	3.3 ± 1.4 a	2.00 ± 0.00 a
F-test	Insect	F-value	$F_{1, 12} = 19.83$	$F_{1, 11} = 161.46$
		P-value	0.0008	< 0.0001
	Cotton variety	F-value	$F_{1, 12} = 158.41$	$F_{1, 11} = 1483.01$
		P-value	< 0.0001	< 0.0001
	Insect *Cotton variety	F-value	$F_{1, 12} = 29.64$	$F_{1, 11} = 215.14$
		P-value	< 0.0001	< 0.0001

* Mean values within a column followed by the same letter are not significantly different at α = 0.05 (Tukey's HSD test). [§] CEW-TX-Leptra-2018 refers to the *H. zea* population recovered from ears of Cry1Ab+Cry1F+Vip3A maize, and CEW-TX-SS is a laboratory susceptible colony, which has been documented to be susceptible to Cry1Ac, Cry2Ab2, and Vip3A protein. [£] Larval survivorship was calculated based on the number of live larvae that were in the second instar and above divided by the total number of insects assayed [28].

Figure 1. Demonstration of occurrence and ear damage of *Helicoverpa zea* on Leptra maize containing Cry1Ab, Cry1F, and Vip3A proteins; and the Bt protein expression in kernels removed from ears of Leptra maize on QuickStix Combo ELISA test strips (EnviroLogix, ME, USA).

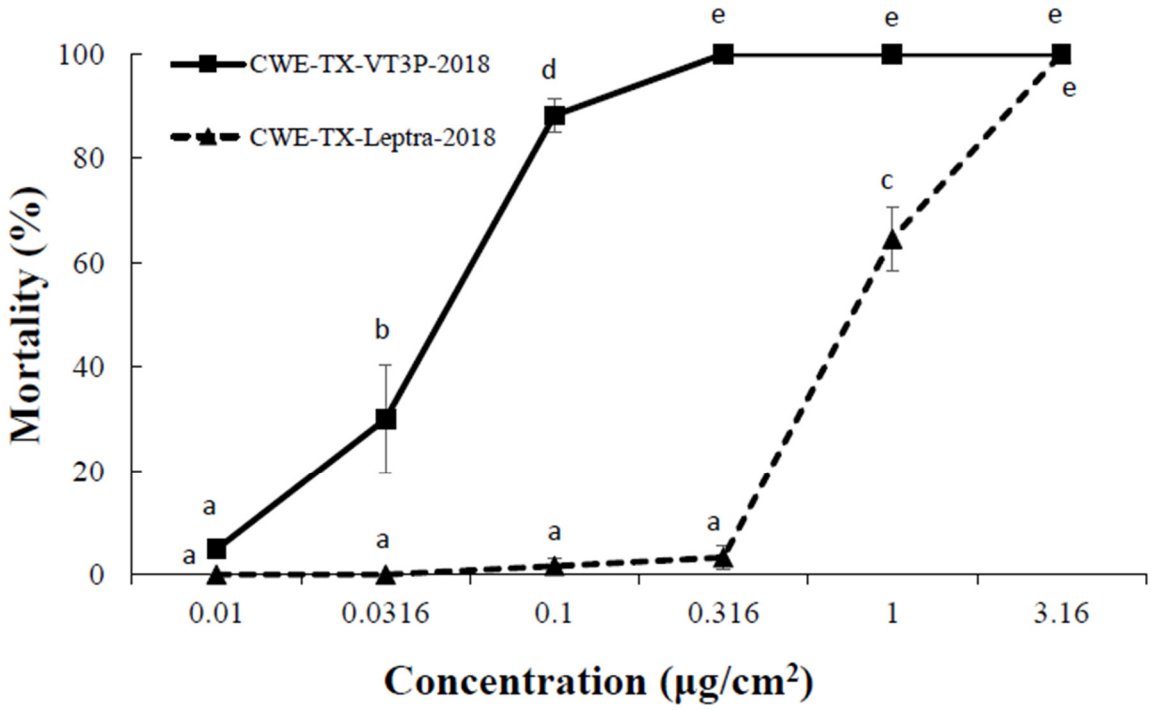

Figure 2. Mortality response of CEW-TX-VT3P-2018 and CEW-TX-Leptra-2018 to Vip3Aa51 protein in diet-overlay bioassays. CEW-TX-VT3P-2018 refers to the *H. zea* population collected from ears of Cry1F+Cry1A.105+Cry2Ab2 maize plants, and CEW-TX-Leptra-2018 refers to the *H. zea* population recovered from ears of Cry1Ab+Cry1F+Vip3A maize. Mean values followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD test).

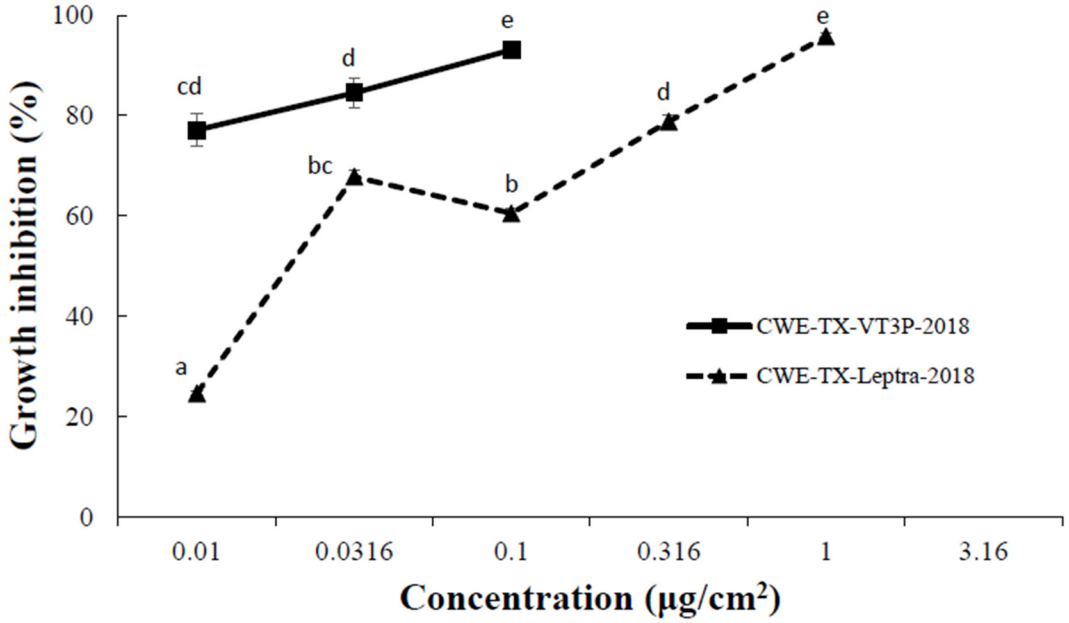

Figure 3. Growth inhibition response of CEW-TX-VT3P-2018 and CEW-TX-Leptra-2018 to Vip3Aa51 protein in diet-overlay bioassays. CEW-TX-VT3P-2018 refers to the *H. zea* population collected from ears of Cry1F+Cry1A.105+Cry2Ab2 maize plants, and CEW-TX-Leptra-2018 refers to the *H. zea* population recovered from ears of Cry1Ab+Cry1F+Vip3A maize. Mean values followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD test).