UNDERSTANDING THE RISKS ASSOCIATED WITH INHERITANCE AND CROSS-RESISTANCE IN CRY AND VIP3A RESISTANCE OF *HELICOVERPA ZEA*

Fei Yang
David Kerns
Texas A&M University
College Station, TX

Abstract

The cotton bollworm or corn earworm, *Helicoverpa zea*, is a major target of Bt cotton and pyramided Bt corn in the U.S. In recent years, field-evolved resistance of *H. zea* to Cry1 and Cry2 proteins has been widely reported in the U.S., especially in the southern states. In addition, a recent study provided strong evidence that *H. zea* has developed field-evolved resistance to Vip3Aa protein in the southern U.S. that meets the criteria for early warning of resistance but not practical resistance. Currently, high dose/refuge and gene-pyramiding are the two main IRM strategies implemented for planting Bt crops in the U.S. Information on the genetic basis and cross-resistance of Cry and Vip3Aa resistance in *H. zea* can help evaluate the risks associated with the resistance in the field. In this study, we characterized the Cry2Ab and Vip3Aa resistance in *H. zea* to test whether it meets the assumptions of IRM as recessive resistance and no cross-resistance.

Introduction

Crops genetically engineered to produce Cry and Vip proteins from the bacterium *Bacillus thuringiensis* (Bt) have been planted for control of some major lepidopteran and coleopteran pests for more than two decades.[1] Environmental and economic benefits associated with the adoption of Bt crops, which include pest suppression, reduced use of conventional insecticides, and increased yields, have been documented in numerous studies.[2-7] However, widespread adoption of Bt crops has placed intense selection pressure on the pest populations and challenged the long-term sustainability of the Bt technology.[8, 9] To date, field-evolved resistance to Bt crops with practical consequences has been globally reported in at least 23 cases.[10-15] Thus, implementing effective insect resistance management (IRM) strategies is essential to combat evolution of insect resistance and ensure the sustainable use of Bt crop technology. Currently, the high dose/refuge and gene-pyramiding are the two main IRM strategies implemented for Bt crops globally.[16, 17]

The high dose/refuge strategy is based on the concept that rare homozygous resistant individuals originating from Bt plants will mate with susceptible insects developed from non-Bt refuge plants and produce heterozygous offspring. The 'high dose/refuge' strategy is expected to work most effectively when resistance is inherited as a functionally recessive trait so that heterozygous progeny can be killed by the high dose Bt proteins expressed in the Bt plants; when resistance is not recessive and heterozygous offspring can survive on the Bt plants, the strategy is less effective.[16] Therefore, understanding the genetic basis of Bt resistance is essential in developing IRM strategies for the sustainable use of Bt crop technology.

Pyramided Bt crops are primarily adopted to delay insect resistance development by producing multiple distinct Bt proteins to kill the same insect pest. A key assumption favoring the success of pyramiding strategy is that insects resistant to one Bt protein are still susceptible to other Bt proteins.[17, 18] However, the efficacy of pyramiding strategy for resistance management could be jeopardized by cross-resistance, which is defined as insects resistant to one Bt protein also exhibit resistance against other Bt proteins.[18, 19] Therefore, understanding the cross-resistance patterns of Bt resistance in pyramided Bt crops is also critical in developing IRM strategies for the sustainable use of Bt crop technology.

The corn earworm/cotton bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), is a major target pest of both Bt cotton and Bt corn in North America. Currently, field-evolved resistance of *H. zea* to Cry1 and Cry2 proteins has been widely reported in the U.S., especially in the Southern states.[15, 20-23] The widespread occurrence of Cry1 and Cry2 resistance challenges the long-term efficacy of pyramided Bt crop technologies against *H. zea*. In the lab, we have successfully developed the *H. zea* strains showing highly resistance to Cry2Ab2 and Vip3Aa proteins, respectively, using F_2 screen methods with populations collected from the Southern U.S. The availability of these resistant strains offered a valuable opportunity to understand the genetic basis of Cry2Ab2 and Vip3Aa resistance in *H. zea*, and its impact on the efficacy of other Bt proteins used in current Bt crops. In this study, we characterized the

genetic bases and cross-resistance of Cry2Ab2 and Vip3Aa resistance in *H. zea* to test whether it meets the assumptions of IRM as recessive resistance and no cross-resistance.

Materials and Methods

Insect Sources

In this study, we used a Cry2Ab2-resistant strain (Cry-RR), a Vip3Aa-resistant (Vip-RR) strain and a susceptible (SS) strain of *H. zea* as the original insect sources. SS was obtained from a commercial source, Benzon Research Inc., Carlisle, PA. Study has shown that SS is susceptible to the Cry1Ac, Cry2Ab2, and Vip3Aa proteins in diet-overlay bioassays.[24] The Cry-RR strain of *H. zea* was established using an F_2 screen method with populations collected from Genuity VT Double Pro corn fields in Snook, TX in 2018.[25] Compared to SS, the Cry2Ab2-resistant *H. zea* strain exhibited a significant level of resistance to Cry2Ab2 corn-leaf powder protein with a resistance ratio of more than 400-fold.[25] The Vip-RR strain was isolated through F_2 screening with insects light-trapped in Texas, U.S. in 2019.[26] Vip-RR has demonstrated more than 588.0-fold resistance to Vip3Aa39 purified protein compared to the SS strain.[26] Prior to the inheritance study, backcross and reselection between RR (Cry-RR and Vip-RR) and SS had been conducted twice to ensure a similar genetic background for the tested strain. The backcrossed re-selected resistant strains were used in this study.

To characterize the Cry2Ab2 and Vip3Aa resistance in *H. zea*, we first conducted reciprocal crosses between the SS and RR (Cry-RR and Vip-RR) strains to generate F_1 strains (F_{1a}: RR ♀*SS ♂, and F_{1b}: RR ♂*SS ♀) to evaluate for dominance and sex linkage. Then we crossed the two F_1 strains to generate F_2 strains (F_{2a}: F_{1a}*F_{1a}, and F_{2b}: F_{1b}*F_{1b}) to determine whether the resistance is controlled by a single gene. Finally, we crossed the F_1 strains with RR to further determine if the resistance is controlled by a single gene.

Bt Proteins

Bayer CropScience (St. Louis, MO) provided the Cry1Ac, Cry1A.105 and Cry2Ab2 proteins, and Dr. Juan-Luis Jurat-Fuentes, University of Tennessee supplied the Vip3Aa39 protein. The Cry1Ac protein was lyophilized MVPII powder with 20.0% AI. The Cry1A.105 protein was a liquid formulation with a concentration of 1.116 mg/ml. The Cry2Ab2 protein was lyophilized (freeze-dried) Bt corn leaf powder expressing ~5.2 mg of Cry2Ab2 protein/g. The Vip3Aa39 protein was a liquid formulation with a concentration of 0.9 mg/ml.

Dose-Response Bioassay

Larval susceptibility of *H. zea* to the four Bt proteins described above was determined using a diet-overlay bioassay as described in Yang et al.[26] For each Bt protein, a full-range bioassay consisting of 6-9 protein concentrations plus a negative control lacking Bt protein was conducted using 128-well bioassay trays (C-D International, Pitman, NJ). The concentrations ranged from 0.01 to 31.6 µg/cm^2 for Cry1A.105, 0.01 to 100 µg/cm^2 for Cry1Ac, 0.0316 to 20 µg/cm^2 for Cry2Ab2, and 0.0316 to 10 µg/cm^2 for Vip3Aa39. For each Bt concentration, 40 µl of protein solution was overlaid onto the surface of each cell for Cry1A.105, Cry1Ac, and Vip3Aa39 proteins, and 200 µl of protein solution was overlaid on the surface of each cell for the Cry2Ab2 protein. Once the protein solution was dry, each cell was infested with one *H. zea* neonate (< 24 h) and covered with vented lids (C-D International, Pitman, NJ). Each combination of insect genotype and Bt protein concentration was replicated four times with 16 neonates per replication. After infestation, the insects were maintained under conditions of 50% RH, 26 ± 1 â€C, and a photoperiod of 16h (L):8h (D). Larval instar and mortality were recorded on the 7th day after inoculation.

Data Analysis

Larval mortality was calculated as mortality (%) = 100* number of dead larvae plus number of surviving larvae still in the first instar divided by total number of insects assayed.[26] Larval mortality for each Bt protein concentration was corrected based on the control mortality according to the method of Abbott.[27] The median lethal concentration (LC_{50}) that caused 50% mortality and the corresponding 95% confidence limit (CL) were calculated using probit analysis.[28] Resistance ratio for each Bt protein was calculated using the LC_{50} of an insect genotype divided by the LC_{50} of SS. Differences among LC_{50} of insect genotypes were tested by comparing the 95% confidence interval of the LC_{50} for each insect genotype. If the confidence intervals overlap, the differences between LC_{50}s are not significant, otherwise the differences are significant.

Effective dominance (D_{ML}) was calculated using the equation: $D_{ML} = (ML_{RS} - ML_{SS}) / (ML_{RR} - ML_{SS})$, where ML_{SS}, ML_{RR}, and ML_{RS} are the mortality of the SS, RR, and RS at a given Bt concentration, respectively. D_{ML} ranges from

0 to 1, where D_{ML}=0 indicates completely recessive resistance and D_{ML}=1 means resistance is completely dominant.[29] Maternal effects of resistance in *H. zea* was evaluated by comparison of the LC_{50} values and mortality between F_{1a} and F_{1b}.[30] To determine whether the resistance is monogenic or polygenic, we used the Chi-square (Ç2) tests for goodness of fit based on a Mendelian monogenic.[30]

Results and Discussion

Resistance to Cry2Ab2 Protein in Diet Bioassay

On Cry2Ab2 corn-leaf powder, the LC_{50} values for SS and Cry-RR were 0.70 µg/cm^2 and 286.35 µg/cm^2, respectively (Table 1). The difference in the LC_{50}s of SS and Cry-RR was significant based on the non-overlapped 95% CLs, resulting in a resistance ratio of 409.1-fold for Cry-RR (Table 1). The results indicate that the Cry-RR strain was resistant to Cry2Ab2 corn-leaf powder. The LC_{50} values of the two F_1 strains were not different based on the overlapped 95% CLs and ranged from 14.96 to 15.73 µg/cm2, which was 21.4 to 22.5-fold greater than the SS strain. These results suggest that maternal effects and sex linkage were not present in the Cry2Ab2 resistance, and thus the inheritance of the Cry2Ab2 resistance was considered to be autosomal.

Based on the mortality at each Cry2Ab2 concentration in the concentration-response bioassays, the effective dominance level (D_{ML}) of the resistance varied depending on the concentration of both Cry2Ab2 corn-leaf powder (Table 2). At the concentrations of 1.0-10.0 µg/cm^2 of Cry2Ab2 corn-leaf powder, D_{ML} ranged from 0.80 to 0.98, suggesting that the resistance was functionally incompletely to nearly completely dominant (Table 2). However, at the highest concentration of 31.6 µg/cm^2 of Cry2Ab2 corn-leaf powder, D_{ML} was 0.42, indicating that the resistance was incompletely recessive (Table 2). The direct test for a monogenic (single-gene) mode of inheritance of Cry2Ab2 resistance showed significantly greater mortality ($P < 0.001$) than expected at concentrations of 1.0-31.6 µg/cm2 for the F_2 strains (except for 3.16 µg/cm^2), and backcrosses between F_1 and RR (Table 3). These results suggested that more than one locus is involved in conferring Cry2Ab2 resistance in *H. zea*.

SS was susceptible to all three Bt proteins with an estimated LC_{50} value of 0.027 µg/cm2 against Cry1Ac, 0.025 µg/cm^2 against Cry1A.105, and 0.112 µg/cm^2 against Vip3Aa39 (Table 4). Cry-RS and Cry-RR were also susceptible to Vip3Aa39 protein with estimated LC_{50} values of 0.173 and 0.044 µg/cm$_2$, respectively (Table 4). In contrast, both Cry-RS and Cry-RR exhibited significant levels of resistance against Cry1Ac, and Cry1A.105 relative to SS based on the non-overlapping of 95% CLs (Table 4). Relative to SS, the resistance ratio for Cry-RS was 128.1-, 56.1-, and 1.5-fold against Cry1Ac, Cry1A.105 and Vip3Aa, respectively (Table 4). Similarly, the resistance ratio for Cry-RR was 779.4-, 4476.7-, and 0.4-fold against Cry1Ac, Cry1A.105 and Vip3Aa, respectively (Table 4).

Based on these results, the risks of Cry2 resistance in *H. zea* may be relatively high because the resistance is non-recessive, autosomal, controlled by multiple single genes, and showing resistant to other Cry1 Bt proteins.

Resistance to Vip3Aa Protein in Diet Bioassay

The resistance ratio was 45,194.1-fold for Vip-RR relative to SS (Table 5). The LC_{50} value for F_{1a} and F_{1b} was 0.14 and 0.10 µg/cm^2, respectively, which was similar according to the overlapped 95% CLs (Table 5). Compared to SS, the resistance ratio for F_{1a} and F_{1b} was 0.8- and 0.6-fold, respectively (Table 5). These results indicate that maternal effects and sex linkage were absent in the Vip3Aa resistant strains of *H. zea*, and thus the inheritance of the Vip3Aa resistance in *H. zea* was autosomal.

Effective dominance (D_{ML}) calculated based on the equation showed that D_{ML} was d 0.0 for Vip3Aa39 concentrations ranging from 0.1-31.6 µg/cm^2, suggesting that the Vip3Aa resistance in *H. zea* was functionally completely recessive (Table 6). The Ç2 test showed that the observed mortality was not different ($P > 0.05$) from the expected mortality at Vip3Aa39 concentration of 3.16 and 10.0 µg/cm^2 for the pooled F_2, and pooled backcross between Vip-RR and F_1 (Table 7). These results indicate that the Vip3Aa resistance in *H. zea* was likely controlled by a single gene.

LC_{50} value of SS was 0.11 µg/cm2 against Cry1Ac, and 0.70 µg/cm^2 against Cry2Ab2 (Table 8). LC_{50} value of Vip-RR was 0.17 µg/cm2 against Cry1Ac, and 0.34 µg/cm^2 against Cry2Ab2 (Table 8). Relative to SS, the resistance ratio for Vip-RR was 1.5-, and 0.49-fold against Cry1Ac, and Cry2Ab2, respectively (Table 8).

Based on these results, the risks of Vip3Aa resistance in *H. zea* may be relatively low because the resistance is recessive, autosomal, controlled by a single gene, and not cross-resistant to other Cry1 and Cry2 Bt proteins.

References

1. James C, Global status of commercialized biotech/GM crops: 2017. Brief No. 53. ISAAA: Ithaca, NY, USA. (2018).
2. Carpenter JE, Peer-reviewed surveys indicate positive impact of commercialized GM crops. *Nat Biotechnol* **28:** 319-321 (2010).
3. Edgerton MD, Fridgen J, Anderson Jr JR, Ahlgrim J, Criswell M, Dhungana P, *et al.*, Transgenic insect resistance traits increase corn yield and yield stability. *Nat Biotechnol* **30**: 493-496 (2012).
4. Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, *et al.*, Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science* **330:** 222-225(2010).
5. Kathage J, and Qaim M, Economic impacts and impact dynamics of Bt (*Bacillus thuringiensis*) cotton in India. *Pro Nat Acad Sci USA* **109:** 11652-11656 (2012).
6. Lu YH, Wu KM, Jiang YY, Guo YY, and Desneux N, Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* **487**: 362-365 (2012).
7. Wu KM, Lu YH, Feng HQ, Jiang YY, and Zhao JZ, Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. *Science* **321:** 1676-1678 (2008).
8. Gould F, Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu Rev Entomol* **43**: 701-726 (1998).
9. Tabashnik BE, Brévault T, and Carrière Y, Insect resistance to Bt crops: lessons from the first billion acres. *Nat Biotechnol* **31**: 510-521 (2013).
10. Tabashnik BE, and Carrière Y, Surge in insect resistance to transgenic crops and prospects for sustainability. *Nat Biotechnol* **35**: 926-935 (2017).
11. Grimi DA, Parody B, Ramos ML, Machado M, Ocampo F, Willse A, Martinelli S, and Head G, Field-evolved resistance to Bt maize in sugarcane borer (*Diatraea saccharalis*) in Argentina. *Pest Manag Sci* **74**: 905-913 (2018).
12. Smith JL, Lepping MD, Rule DM, Farhan Y, and Schaafsma AW, Evidence for field-evolved resistance of *Striacosta albicosta* (Lepidoptera: Noctuidae) to Cry1F *Bacillus thuringiensis* protein and transgenic corn hybrids in Ontario, Canada. *J Econ Entomol* **110**: 2217-2228. (2017).
13. Chandrasena DI, Signorini AM, Abratti G, Storer NP, Olaciregui ML, Alves AP, and Pilcher CD, Characterization of field-evolved resistance to *Bacillus thuringiensis*-derived Cry1F ´-endotoxin in *Spodoptera frugiperda* populations from Argentina. *Pest Manag Sci* **74**: 746-754 (2018).
14. Gassmann AJ, Shrestha RB, Kropf AL, St Clair CR, and Brenizer BD, Field-Evolved Resistance by Western Corn Rootworm to Cry34/35Ab1 and other *Bacillus thuringiensis* Traits in Transgenic Maize. *Pest Manag Sci* **DOI**: 10.1002/ps.5510 (2019).
15. Yang F, González JCS, Williams J, Cook DC, Gilreath RT, Kerns D, Occurrence and ear damage of *Helicoverpa zea* on transgenic *Bacillus thuringiensis* maize in the field in Texas, US and its susceptibility to Vip3A protein. *Toxins* **11**: 102 (2019).
16. US-EPA (US Environmental Protection Agency), Report of subpanel on *Bacillus thuringiensis* (Bt) plant-pesticides and resistance management. Available at: https://archive.epa.gov/scipoly/sap/meetings/web/pdf/finalfeb.pdf (1998).
17. Carrière Y, Crickmore N and Tabashnik BE, Optimizing pyramided transgenic Bt crops for sustainable pest management. Nat Biotechnol 33: 161-168 (2015).
18. Carrière Y, Fabrick JA and Tabashnik BE, Can pyramids and seed mixtures delay resistance to Bt crops? Trends Biotechnol 34: 291-302 (2016).
19. Brévault T, Heuberger S, Zhang M, Ellers-Kirk C, Ni X, Masson L, Li X, Tabashnik BE and Carrière Y, Potential shortfall of pyramided transgenic cotton for insect resistance management. Proc Natl Acad Sci 110: 5806-5811 (2013).
20. Dively GP, Venugopal PD, and Finkenbinder C, Field-evolved resistance in corn earworm to Cry proteins expressed by transgenic sweet corn. *PloS One* **11:** e0169115 (2016).
21. Reisig DD, Huseth AS, Bacheler JS, Aghaee MA, Braswell L, Burrack HJ, Flanders K, Greene JK, Herbert DA, Jacobson A, and Paula-Moraes SV, Long-term empirical and observational evidence of practical *Helicoverpa zea* resistance to cotton with pyramided Bt toxins. *J Econ Entomol* **111**: 1824-1833 (2018).
22. Bilbo TR, Reay-Jones FPF, Reisig DD, and Greene JK, Susceptibility of corn earworm (Lepidoptera: Noctuidae) to Cry1A.105 and Cry2Ab2 in North and South Carolina. *J Econ Entomol* **112**: 1845-1847 (2019).

23. Kaur G, Guo J, Brown S, Head GP, Price PA, Paula-Moraes S, Ni X, Dimase M, and Huang F, Field-evolved resistance of *Helicoverpa zea* (Boddie) to transgenic maize expressing pyramided Cry1A. 105/Cry2Ab2 proteins in northeast Louisiana, the United States. *J Invertebr Patho* **163:** 11-20 (2019).
24. Kerns DL, Yang F, Cook D, Gore J, Stewart S, Jurat-Fuentes JL, Lorenz G, Catchot A, Brown S, 2020. Bt resistance and Bt technology performance for Texas and the Mid-South in 2019. In Proceedings of the 2020 Beltwide Cotton Conferences, Austin, TX, USA (2020).
25. Yang F, Head GP, Price PA, González JCS, Kerns DL, Inheritance of Bacillus thuringiensis Cry2Ab2 protein resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae). Pest Manag Sci DOI, 10.1002/ps.5916 (2020).
26. Yang F, González JCS, Little N, Reisig D, Payne G, Dos Santos RF, et al., First documentation of major Vip3Aa resistance alleles in field populations of *Helicoverpa zea* (Boddie)(Lepidoptera: Noctuidae) in Texas, USA. Sci Rep 10: 5867 (2020).
27. Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18:** 265-267 (1925)
28. SAS Institute, *SAS/ STAT User's Third Edition*, SAS Institute Inc, Cary, NC, USA (2010).
29. Bourguet D, Genissel A, Raymond M, Insecticide resistance and dominance levels. J. Econ. Entomol. 93, 1588-1595 (2000).
30. Roush RT, and Tabashnik BE, Pesticide Resistance in Arthropods. Chapman and Hall, New York (1990).

Table 1. Mortality response (LC_{50}) of different genotypes of *Helicoverpa zea* to Cry2Ab2-corn leaf powder in diet-overlay bioassays.

Strain	N[#]	LC_{50} (95% CL) (µg/cm^2)[§]	Slope ± SE	Ç²	df	Resistance ratio[£]
SS	512	0.70 (0.51, 0.97)	1.53 ± 0.15	40.2	26	-
Cry-RR	512	286.35 (65.44, 5243)	0.41 ± 0.07	32.6	26	409.1
F_1a: Cry-RR *SS	512	14.96 (6.06, 105.08)	1.26± 0.37	43.3	26	21.4
F_1b: Cry-RR *SS	512	15.73 (8.88, 37.33)	1.18 ± 0.20	33.7	26	22.5

[#] 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 for an insect genotype was calculated using its LC_{50} value divided by the LC_{50} of SS.

Table 2. Effective dominance level (D_{ML}) of Cry2Ab2 resistance in *Helicoverpa zea* based on the larval mortality observed in diet-overlay bioassays.

Concentration (µg/cm^2)	Dominance (D_{ML})	Conclusion
1	0.87	Incompletely dominant
3.16	0.98	Incompletely dominant
10	0.80	Incompletely dominant
31.6	0.42	Incompletely recessive

Table 3. Test for monogenic inheritance for resistance to Cry2Ab2 by comparing expected and observed mortalities of the F_2 backcross populations between F_1 and SS and between F_1 and Cry-RR at different Cry2Ab2 concentrations.

Strain	Concentration (µg/cm²)	N#	Observed dead (O)	Expected dead (E)	Ç²	*P*-value
Pooled BCS: F_1*SS	1	256	13.8	92.7	105.15	< 0.05
	3.16	256	50.9	116.7	68.16	< 0.05
	10	256	124.4	174.7	45.62	< 0.05
	31.6	256	251.9	223.5	28.45	< 0.05
Pooled BCR: F_1*Cry-RR	1	256	5.9	14.8	5.74	< 0.05
	3.16	256	8.96	23.6	9.96	< 0.05
	10	256	49.9	72.7	9.97	< 0.05
	31.6	256	204.0	145.4	54.71	< 0.05
Pooled F_2: F_1*F_1	1	128	12.2	26.9	10.20	< 0.05
	3.16	128	27.4	35.1	2.32	0.13
	10	128	49.8	61.9	4.56	< 0.05
	31.6	128	60.9	92.2	38.00	< 0.05

Total number of neonates assayed.

Table 4. Mortality response (LC_{50}) of different genotypes of *Helicoverpa zea* to Bt proteins in diet-overlay bioassays.

Bt protein	**Insect***	**N#**	**LC_{50} (95% CL) (µg/cm²) §**	**Slope ± SE**	**X^2**	**df**	**Resistance ratio£**
Cry1Ac	SS	576	0.027 (0.020, 0.035)	1.58 ± 0.16	26.5	30	1.0
	Cry-RS	512	3.459 (2.586, 4.636)	1.13 ± 0.09	26.6	26	128.1
	Cry-RR	512	21.043 (12.393, 41.715)	0.61 ± 0.07	28.2	26	779.4
Cry1A.105	SS	576	0.025 (0.020, 0.030)	2.56 ± 0.29	23.0	30	1.0
	Cry-RS	512	1.402 (0.990, 1.999)	0.88 ± 0.08	30.4	26	56.1
	Cry-RR	512	111.918 (25.350, 2780.000)	0.32 ± 0.07	36.6	26	4476.7
Vip3Aa	SS	448	0.112 (0.094, 0.133)	3.23 ± 0.35	12.9	22	1.0
	Cry-RS	448	0.173 (0.130, 0.229)	2.11 ± 0.25	39.9	22	1.5
	Cry-RR	448	0.044 (0.032, 0.049)	3.13 ± 0.46	4.4	22	0.4

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 for an insect genotype was calculated using its LC_{50} value divided by the LC_{50} of SS.

Table 5. Mortality response (LC_{50}) of different genotypes of *Helicoverpa zea* to Vip3Aa39 protein in diet-overlay bioassays.

Insect genotype	**N#**	**LC_{50} (95% CL) (µg/cm²) §**	**Slope ± SE**	**X^2**	**df**	**Resistance ratio£**
SS	512	0.17 (0.14, 0.21)	2.84 ± 0.29	10.5	26	-
RR	512	7683.00	1.01 ± 0.73	5.0	25	45194.1
F_{1a}: RR *SS	512	0.14 (0.11, 0.16)	2.89 ± 0.31	9.1	26	0.8
F_{1b}: RR *SS	512	0.10 (0.08, 0.12)	3.36 ± 0.38	11.1	26	0.6

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 for an insect genotype was calculated using its LC_{50} value divided by the LC_{50} of SS.

Table 6. Effective dominance level (D_{ML}) of Vip3Aa resistance in *Helicoverpa zea* based on the larval mortality observed in diet-overlay bioassays.

Vip3Aa39 concentration (µg/cm^2)	Dominance (D_{ML})	Description
0.1	-1.2	Completely recessive
0.316	-0.2	Completely recessive
1	0.0	Completely recessive
3.16	0.0	Completely recessive
10	0.0	Completely recessive
31.6	0.0	Completely recessive

Table 7. Test for fitting a Mendelian monogenic model for Vip3Aa resistance in *Helicoverpa zea*.

Vip3Aa39 concentration	Insect Strain	N[#]	Observed dead (O)	Expected dead (E)	Ç^2	*P*-value
3.16 µg/cm^2	Pooled Backcross	256	119.8	128.0	1.049	0.306
	Pooled F_2	128	94.7	96.0	0.068	0.794
10 µg/cm^2	Pooled Backcross	256	130.3	129.7	0.006	0.938
	Pooled F_2	128	91.8	96.4	0.907	0.341

[#] Total number of neonates assayed.

Table 8. Mortality response (LC_{50}) of different genotypes of *Helicoverpa zea* to Bt proteins in diet-overlay bioassays.

Bt protein	**Insect***	**N[#]**	**LC_{50} (95% CL) (µg/cm^2) [§]**	**Slope ± SE**	**X^2**	**df**	**Resistance ratio[£]**
Cry1Ac	SS	512	0.11 (0.08, 0.14)	1.39 ± 0.11	31.6	26	1.0
	Vip-RR	512	0.17 (0.12, 0.23)	1.13 ± 0.10	24.9	26	1.5
Cry2Ab2	SS	512	0.70 (0.51, 0.67)	1.53 ± 0.15	40.2	26	1.0
	Vip-RR	512	0.34 (0.26, 0.44)	1.40 ± 0.11	31.9	26	0.49

[#] 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 for an insect genotype was calculated using its LC_{50} value divided by the LC_{50} of SS.