Pak. J. Agri. Sci., Vol. 57(4), 1101-1106; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/20.9765
http://www.pakjas.com.pk

CHARACTERIZING THE MODE OF RESISTANCE INHERITANCEAND CROSS RESISTANCE IN PINK BOLLWORMAGAINST CRY1AC TOXIN AND ORGANOPHOSPHATE PESTICIDES IN PAKISTAN

Zunnu Raen Akhtar[1,*], Amer Rasul[1,*], Muhammad Sagheer[1], Asad Ali[2], Irfan Ashraf[3], Bushra Saddiq[3] and Mansoor-ul-Hasan[1]

[1]Department of Entomology, University of Agriculture Faisalabad, Pakistan;[2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China;[2]Department of Agriculture, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan; [3]University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. *Corresponding author's e-mail: zunnuraen.akhtar@uaf.edu.pk; amerrasul1212@gmail.com

In this study, resistance inheritance and cross resistance to Cry1Ac toxin and the insecticide triazophos in the pink bollworm (*Pectinophora gossypiella*) was investigated. F_1 progeny were simultaneously backcrossed with susceptible parents to obtainF_2 progenywithless genetic resistance against the Cry1Ac toxin and insecticide triazophos. In two sets of experiments, Cry1Ac/triazophos-resistant pink bollworms (Cry1Ac-R/Triazo-R)were selected through six generations and were reciprocally crossed with susceptible laboratory strains (Cry1Ac-S/Triazo-S). Resistance ratios for reciprocal crosses of *Cry1Ac* and triazophos were 16.25 and 27.11, respectively, while Resistant Parent RR values showing cross resistancewere10.51 and 6.50 folds, respectively. F_1 progeny were again back-crossed with susceptible parents to prove the phenomena of refuge strategy instantaneously reducing the resistance developeding pink bollworms. Parents, F_1 and F_2progenies were tested for their LC_{50} values. Results of cross resistance for*Cry1Ac*showed, dominance value of resistance was below 1, proving that resistance was completely recessive but autosomal in nature and was controlled by more than one gene. For triazophos, dominance was also autosomal but was dependent on concentration, i.e., at low concentration it was incompletely dominant, at middle concentration, partially recessive; while at higher concentration it was completely recessive. These findings are helpful for pink bollworm control in Bt cotton expressing *Cry1Ac* combined with insecticides spray.
Keywords: Pink bollworm, cross resistance, toxicity, resistance development.

INTRODUCTION

A pest population that exhibits a significantly lower mortality response to a pesticide than a normal population dose is considered resistant (Georghiou,1986). Resistance development to insecticides, in insects, was first reported by Melander (1914).Since, then, different strategies have been employed to manage insect pests including resistant hybrids, biological control using predators and parasitoids, and cultural control, in an effort to overcome resistance development. Likewise, transgenic crops were developed, which have achieved high levels of control against the target pests, thereby reducing pesticide applications and increasing profits (Mendelsohn *et al*., 2003; National Research Council, USA 2010).Today, more than 90% of countries have adopted biotech crops (James, 2017)

The evolution of resistance is a primary threat to the continuous success of transgenic crops (Tabashnik, 1994). Similar to pesticides, selection pressures on insects may result in resistance development to transgenic crops. Target pests have already developed resistance to transgenic crops under laboratory and field conditions (Huang *et al*., 1997; Bolin *et al*., 1999; Chaufaux *et al*., 2001; Ferre and van Rie, 2002; Gasteigeret *et al*., 2003; Siqueira *et al*., 2004; Ali *et al*., 2006; Li *et al*., 2007; Van Rensburg 2007; Tabashnik *et al*., 2008; Pereira *et al*., 2008). Lepidopteran pests have also developed resistance to Bt cotton in Pakistan (reviewed by Alvi *et al*., 2012; Akhtar *et al*., 2018).As a result, pests such as rice stem borers (Qu *et al*., 2003) have shown high levels of resistance against the organophosphate pesticide triazophos.

To control pink bollworm, different strategies including pesticides, sterile insect techniques, Bt cotton, cultural practices, and sex pheromones are used successfully (Hoy 2019). Among these techniques, transgenic cotton expressing Cry1Ac is the primary tool. Resistance to *Cry1Ac* expressing Bt cotton has been reported in China (Wan *et al*., 2012), USA (Tabashnik *et al*., 2000a), and Pakistan (Akhtar *et al*., 2016). However, pink bollworm has also developed resistance to organophosphate pesticides sprayed in fields of Bt cotton in Pakistan (Akhtar *et al*., 2018a;Akhtar *et al*., 2018b). Cross resistance was observed in the lepidopteran pest, cotton bollworm, in Pakistan, in which Cry1Ac was tested for cross resistance with spore forming λ-endotoxin (Ahmed *et al*., 2006).

Our hypothesis is also based on the assumption that, there is cross resistance among toxin resistant and insecticide resistant strains of pink bollworms. In order to develop an effective Integrated Resistance Management (IRM) strategy it was important to use Mendalian crosses to determine the resistance inheritance pattern or type, in laboratory selected target pest populations in which we crossed homozygous susceptible to homozygous resistant to achieve heterozygous populations on which to conduct bioassays. Through these crosses and using diet incorporated toxin bioassay, our target was to determine maternal sex linkage and dominance of resistance.

MATERIALS AND METHODS

***Insect collection and rearing conditions*:** Insects resistant and susceptible to Bt cotton were collected from fields, which were un-sprayed. While insects resistant and susceptible to insecticide were collected from fields sprayed with triazophos at the Research Farm of the University of Agriculture, Faisalabad, Pakistan. Cotton at boll formation was collected and these bolls were brought to the laboratory where larvae were removed and reared on artificial diet. Almost 2000 larvae were collected and were reared on artificial diet (Jothi *et al*., 2016), having Cry1Ac Bt toxin. Temperature was 27±1ºC, RH was above 70% and D:L period was kept at 16:8.

***Artificial dietand toxin*:** Diet was prepared as described by(Jothi *et al*., 2016) but was modified. Ingredients were: meshed seed meal, caeseine, sorbic acid, L-sorbic acid, and distilled water.Cry1Ac was added as a freeze dried formulation of MVPII containing approximately 20% Cry1Ac protoxin of Bt *kurstaki* encapsulated by transgenic *Pseudomonas fluorescence* (Mycogencorportation, San Diego, CA) (Alvi *et al*., 2012).

***Insecticide formulations*:** The recommended dose of Triazophos40% EC for cotton is 2 ml/litre, sprayedat 500-1000 ml/acre. Recommended active ingredients are 10 percent per litre.

***Insect strain selection*:** Larvae were collected from the field were feed artificial diet mixed with Cry1Ac at different concentrations for six generations. Formulations for selective experiments are as in Table. 1. Bioassays were conducted for surviving insect generations. Susceptible generations were also collected from the field and reared on artificial diet without the Bttox in for six generations.

For the triazophos selection experiment, field collected larvae were reared on non-Bt cotton sprayed with triazophos through sixth generations. Surviving larvae were used in the bioassays. Formulations for the experiments were as in Table. 2. Susceptible larvae were also collected from the field and were kept on non-Bt cotton for six generations and were also used for bioassays.

Table 1. History of selection to produce Cry1Ac selected strain of pink bollworm

Generation	Concentration µg/g	Number exposed	Number dead	%mortality
G1	28	2000	803	40.15
G2	30	1000	52	5.20
G3	35	1015	46	4.53
G4	38	1100	30	2.72
G5	42	1070	7	0.65
G6	45	1000	2	0.20

Table2. History of selection to produce triazophos selected strain of pink bollworm

Generation	Concentration µg/ml	Number exposed	Number dead	%mortality
G1	110	1500	577	38.46
G2	130	1090	56	5.13
G3	150	1000	35	3.50
G4	170	1200	21	1.75
G5	190	950	11	1.15
G6	210	1050	1	0.09

Bioassay: Concentrations used were lower for susceptible strains and higherfor resistant strains. Larvae surviving after seven days were recorded.

Statistical analysis

***Evolution of resistance*:**

Using POLO plus (LeOra1987), probit regression lines were drawn on concentration responses from each Mendalian cross. The results were produced in terms of LC_{50}values with 95%FL, Chi-Squared (X^2), slope with standard error, which were used to check data for reliability and RR ratio, which stands for 50% larvae killed in relation to a susceptible strain with a 95%CI.

***Maternal Sex linkage*:** F_1 and the slope of the LC_{50} of the F_1 progeny was considered in the context of maternal sex linkage of inheritance. Reciprocal crosses were made between susceptible and resistant strains of pink bollworms. To consider the better cross, pupae were separated for gender and kept in cages for crosses.

***Degree of dominance*:** Degree of dominance was calculated from the modified formula

D= (2XT-XRR-XSS)/(XRR-XSS)(Stone 1968)

D= Degree of dominance, XT= Phenotypic value of homozygote, XRR= Phenotypic value of heterozygote, XSS=Phenotypic value of other homozygote, log LC_{50} of reciprocal cross, Range of dominance from -1 (complete recessive resistance) to +1(complete dominant resistance).

***Effective dominance*:** Using the following formula, we calculated effective dominance *h*.

$h = (W_{12}-W_{22})/(W_{11}-W_{22})$

h= effective dominance, W_{11}= fitness of homozygous resistant parent, W_{12}= fitness of the heterozygous offspring, W_{22}=fitness of the homozygous susceptible parent, *h* varies from 0-1; 0.5 means co-dominance

Table3. Bioassay response of Cry1Ac and triazophos susceptible and resistant strains of pink bollworms to individual Cry1Ac and triazophos.

Strains	Bt toxin/pesticide	n[a]	LC_{50}/ LD_{50}(95%FL)(µg/g)	Slope ±SE	χ^2	df (χ^2)	RR[b]
Cry1Ac-S	Cry1Ac	864	1.70(1.51-1.91)	3.57±0.21	36.10	16	-
Cry1Ac-R	Cry1Ac	864	27.63(20.34-38.65)	2.63±0.23	92.04	16	16.25
Triazo-S	Triazophos	864	1.87 (1.65-2.09)	3.71±0.24	32.85	16	-
Triazo-R	Triazophos	864	50.71(41.93-59.75)	3.52±0.29	50.29	16	27.11

n= number of larvae tested, RR[b] Resistance ratio at LC50 having 95% confidence interval

Table4. Cross resistance of Cry1Ac and triazophos susceptible and resistant strains of pink bollworms to individual triazophos and Cry1Ac

Strains	Bttoxin/pesticide	n[a]	LC_{50}/ LD_{50}(95%FL)(µg/g)	Slope ±SE	χ^2	df (χ^2)	RR[b]
Cry1Ac-S	Triazophos	864	1.45(1.21-1.63)	5.36±0.47	66.6	16	-
Cry1Ac-R	Triazophos	864	15.25(13.1-17.83)	2.70±0.15	37.12	16	10.51
Triazo-S	Cry1Ac	864	1.55(1.34-1.78)	4.84±0.28	98.88	16	-
Triazo-R	Cry1Ac	864	10.09(8.80-11.44)	3.16±0.19	26.39	16	6.50

n= number of larvae tested, RR[b] Resistance ratio at LC50 having 95% confidence interval

Table5. Reciprocal crosses between Cry1Ac, triazophos resistant and susceptible strains of pink bollwormshowing dose response to individual toxins of Cry1Ie and Cry1Ab.

Strains	Cross	n	LC_{50}(95%FL) (µg/g)	Slope ±SE	χ^2	df (χ^2)
Cry1Ac-R	R♂ X S♀	864	8.12(6.27-10.74)	1.74±0.11	53.18	16
	S♂ X R♀	864	9.23(7.33-11.68)	2.14±0.16	48.92	16
Triazophos-R	R♂ X S♀	864	5.24(4.11-6.63)	2.31±0.13	63.54	16
	S♂ X R♀	864	4.89(4.43-4.76)	2.28±0.13	49.43	16

***Monogenic resistance test using Chi square*:**

$X^2 = (F-pn)^2/pqn$ (Georghiou, 1969)

F= Observed mortality, p= Expected mortality, n= number of adults, q= 1-p

Null hypothesis will be rejected in cases where the expected mortality is significantly different than the observed mortality.

RESULTS

***Evolution of resistance and selection*:** Pink bollworm larvae were selected with Cry1Ac and triazophos for six generations, separately. The mortality rate was decreased from 40.15 to 0.2% with Cry1Ac(Table. 1) and from 38.46 to 0.09 with triazophos (Table. 2).

***Sex linkage*:** For Cry1Ac and triazophos resistant larvae the RR value was 16.25 and 27.11, respectively (Table. 3).

In the cross resistance experiment, RR for Cry1Ac resistant larvae for triazophos and triazophos resistant larvae for Cry1Ac was 10.51 and 6.5, respectively (Table. 4).

***Dominance*:** Dominance was calculated in terms of h value. For both Cry1Ac and triazophos, *h* was higher at lower concentrations and lower at higher concentrations. It presents dominant to recessive resistance. Value varied from 0-1 while 0.5 was co-dominance (Tables 6&7).

Table6. Effective dominance of resistance to Cry1Ac in pink bollworms resistant, susceptible, and F1 progeny larvae.

Concentration	Strains	Survival%	Fitness	h[b]
2.5	Cry1Ac-S	18.75	0.19	0.14
	Cry1Ac-R	97.91	1.00	
	F1	52.08	0.53	
5	Cry1Ac-S	0.00	0.00	0.60
	Cry1Ac-R	52.08	1.00	
	F1	31.25	0.60	
25	Cry1Ac-S	0.00	0.00	0.00
	Cry1Ac-R	20.83	1.00	
	F1	0.00	0.00	
50	Cry1Ac-S	0.00	0.00	0.00
	Cry1Ac-R	4.16	1.00	
	F1	0.00	0.00	

Monogenic vs polygenic: The number of loci involved in controlling genetic resistance to Cry1Ac showed that among all calculated and expected mortalities, at all concentrations, there was no significance, so resistance is autosomal (Table. 8). In the case of triazophos, resistance was also autosomal (Table. 9).

Table 7. Effective dominance of resistance to Triazophos in resistant, susceptible and F1 progeny of pink bollworm larvae.

Concentration	Strains	Survival%	Fitness	h^b
2.5	Triazo-S	4.16	0.041	0.75
	Triazo-R	100	1	
	F1	83.33	0.83	
5	Triazo-S	0	0	0.60
	Triazo-R	100	1	
	F1	60.41	0.60	
50	Triazo-S	0	0	0
	Triazo-R	43.75	1	
	F1	0	0	
100	Triazo-S	0	0	0
	Triazo-R	0	1	
	F1	0	0	

Table8. Monogenic model for actual and expected mortality of pink bollworm using Cry1Ac.

Concentration µg/g	Actual mortality (%)	Expected mortality (%)	χ^2
F_1♀(S♀xR♂)xSS♂			
0.5	4.16	10.41	0.82
2.5	16.66	34.37	0.73
5	50.00	75.00	0.58
10	83.33	91.66	0.43
F_1♂(S♂xR♀)xSS♀			
0.5	2.08	7.29	0.92
2.5	16.66	30.20	0.68
5	41.66	70.83	0.29
10	77.08	88.54	0.53
SS♀xF_1♂(S♂xR♀)			
0.5	4.16	8.33	0.76
2.5	18.75	31.25	1.27
5	45.83	72.91	1.16
10	77.08	85.54	0.51
SS♂xF_1♀(S♀ xR♂)			
0.5	0.00	9.37	1.05
2.5	14.58	32.29	1.83
5	50.00	73.95	1.01
10	68.75	84.37	0.63

Table9. Monogenic model for actual and expected mortality of pink boll worms using triazophos.

Concentration µg/ml	Actual mortality (%)	Expected mortality (%)	χ^2
F_1♀(S♀xR♂)xSS♂			
0.5	12.50	18.75	0.60
2.5	37.50	50.00	0.26
5	83.33	91.66	0.36
10	100.00	100.00	0.27
F_1♂(S♂xR♀)xSS♀			
0.5	14.58	18.75	0.50
2.5	39.58	50.00	0.47
5	85.41	92.70	0.35
10	100.00	100.00	0.27
SS♀xF_1♂(S♂xR♀)			
0.5	18.75	19.79	0.33
2.5	41.66	52.08	0.46
5	87.50	93.75	0.33
10	100.00	100.00	0.27
SS♂xF_1♀(S♀ xR♂)			
0.5	16.66	20.83	0.48
2.5	41.66	52.08	0.46
5	83.33	91.66	0.36
10	100.00	100.00	0.27

DISCUSSION

Our results indicate that *h*, increased concentration; resulted in lower resistance, while lower concentrationsresulted ingreater resistance, resistance varied. Our results are in agreement with Wang *et al*. (2016; 2017), that dominance decreased with increased concentrations of Bt toxin.Crespo *et al*. 2009, studying European corn borer, also found that dominance was dependenton different levels of Bt toxin expression at different plant growth stages such as reproductive and vegetative. Tabashnik *et al*. (1990), found field developed resistance to Cry protein from maternal influence in diamond back moth, *Plutella xylostella.*

Refuge strategy is successfully used to delay resistance development in insects against Bt crops (Jin*et al*., 2015).Our studies, indicate that refuge strategy can delay resistance development in pink bollworm as backcross in the F1 progeny resulted in reduced resistance as compared to parent resistant. Our results also agree with those of Tabashnik *et al*. (2000), in which a Cry1Ac resistant strain was found to show substantial resistance against other Bt toxins; in our studies Cry1Ac resistant pink bollworms showed resistance against triazophos. Khalique *et al*. (2006), found cross resistance of Cry1Ac against various spore forming Bt toxins in cotton bollworms. Alvi *et al*. (2012), found cross resistance in Cry1Ac against different insecticides in cotton bollworms. Similarly, maternal influence in field strains was also found associated with inheritance of Cry protein in *Ostrinia nubilalis*; it was identified from concentration mortality curves of reciprocal crosses(Crespo *et al*., 2009).

Our experimental results show that the parents were homozygous resistant and susceptible while resulting F_1 progeny were a heterozygous population. These results are in agreement with other studies where F_1 progeny crosses showed that resistant alleles were expressed on susceptible laboratory reared population, while susceptible alleles were expressed on selected resistant laboratory reared population (Liu and Tabashnik, 1997; González-Cabrera *et al*., 2001; Tabashnik *et al*., 1997).

***Conclusion*:** The back-cross of resistant strains with susceptible ones show that refuge strategy can work well in the case of Bt cotton sprayed with triazophos, as developed resistance can be reversible. With resistance beingautosomal

these results support more production of Bt crops. The *h* value decreased with increased concentrations of triazophos suggesting that Bt cotton, with pesticide sprays, can be helpful in delaying resistance development in pink bollworm.We affirm that laboratory selected pink bollworm can develop minor levels of resistance against triazophos, and vice versa, which is at manageable levels if we follow a refuge strategy in Pakistan Bt cotton.

***Acknowledgments*:** We are thankful to the Higher Education Commission (HEC) for funding letter no. 21-570/SRGP/R&D/HEC/2014and Department of Entomology, University of Agriculture Faisalabad, Pakistan for providing technical support and laboratory facilities.

REFERENCES

Ahmed, K., K.M. Wu, G.M.Liang andY.Y.Guo.2006. Cross resistance of Cry1Ac resistant cotton boll worm, *Helicoverpaarmigera*to spore forming λ endotoxin of various *Bacillus thuringiensis* (Berliner). Pak.J.Biol. Sci. 9:1639-1649.

Akhtar, Z.R., M.J. Arif, Mansoor-ul-Hassan, B. Sadia, U. Irshad, M. Majid and Y.G. Yin, 2016. Genetic resistance in pink bollwormsagainst transgenic cotton: an evidence from Pakistan. Pak. Entomol. 38:153-157.

Akhtar, Z.R., A.A.Anjum, Z.Saeed and J.Khalid.2018. Resistance development in bollworms against Bt proteins deployed in genetically modified cotton. J. Entomol. Zool. St.6:1260-1264.

Akhtar, Z.R., M.Majid, U.Irshad, Z.Saeed, J.Khalid, H.Khan, A.A.Anjum, A.Noreen and Easha. 2018a. Resistance development of lepidopteran pests against insecticides in Pakistan: A case study of pink boll worm against different insecticides. J.Entomol. Zool. St. 6: 97-101.

Akhtar, Z.R., Z.Saeed, A.A.Anjum, H.Hira, A.Ihsan, A.NoreenandM.A.Salman. 2018b. Pink boll worm resistance evaluation against organophosphate in Cry1Ac expressing transgenic cotton. J.Entomol. Zool. St. 6: 821-824.

Ali, M.I., R.G. Luttrell andS.Y. Young.2006. Susceptibilities of *Helicoverpazea* and *Heliothisvirescens* (Lepidoptera: Noctuidae) populations to Cry1Ac insecticidal protein. J. Econ. Entomol. 99: 164-175.

Alvi, A.H.K., A.H.Sayyed, M.Naeemand M.Ali. 2012. Field Evolved Resistance in *Helicoverpaarmigera* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* Toxin Cry1Ac in Pakistan. PLoS ONE 7: e47309. doi:10.1371/journal.pone.0047309

Bolin, P.C., W.D. Hutchison and D.A. Andow.1999. Long-term selection for resistance to *Bacillus thuringiensis* Cry1Ac endotoxin in a Minnesota population of European corn borer (Lepidoptera: Crambidae). J. Econ. Entomol. 92: 1021–1030.

Chaufaux, J., M. Seguin, J.J. Swanson and B.D. Siegfried.2001. Chronic exposure of the European corn borer (Lepidoptera: Crambidae) to Cry1Ab *Bacillus thuringiensis* toxin. J. Econ. Entomol.94:1564-1570.

Crespo, A.L.B., T.A. Spencer, A.P.Alves, R.L. Hellmich, E.E. Blankenship, L.C. Magalhãesand B.D. Siegfried.2009. On-plant survival and inheritance of resistance to Cry1Ab toxin from *Bacillus thuringiensis* in a field-derivedstrain of European corn borer, *Ostrinianubilalis*. Pest Manag. Sci.65:1071-1081.

Ferre, Jand J. van Rie.2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 47:501-533.

Gasteiger, E., A. Gattiker, C. Hoogland, I. Ivanyi, R.D. Appel and A. Bairoch.2003. Expasy: The proteomics server for in-depth protein knowledge and analysis. Nucl. Acids Res. 31:3784-3788.

Georghiou, G.P. 1969. Genetics of resistance to insecticides in houseflies and mosquitoes.Exp. Parasitol. 26:224-255.

Georghiou, G.P. 1986. The magnitude of the resistance problem. pp.14-43. *In:* Pesticide resistance: strategies and tactics for management.

González-Cabrera, J., S.Herrero and J. Ferré.2001. High genetic variability for resistance to *Bacillus thuringiensis* toxins in a single population of diamondback moth. Appl.environ. microbial.67:5043-5048.

Huang, F.N., R.A. Higgins and L.L. Buschman.1997. Baseline susceptibility and changes in susceptibility to *Bacillus thuringiensis* subsp. kurstaki under selection pressure in European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol.90:1137-1143.

Hoy, M.A. 2019. Genetic modification of pest and beneficial insects for pest management programs. Chapter 4, insect molecular genetics, 4th edition (An introduction to principal and applications). Pp.563-620.

Jin, L., H.Zhang, Y.Lu, Y.Yang, K.Wu, B.E.Tabashnik andY.Wu.2015. Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. Nature Biotechnol. 33:169-173.

Jothi, D.B., V.C.B.Naik, S.Kranthi, K.R.Kranthiand R. Valarmathi.2016. Viable mass production method for cotton pink bollworm, *Pectinophoragossypiella* (Saunders). J. Basic Appl. Zool. 73:9-12.

James, C.2017. Global Status of Commercialized Biotech/GM Crops in 2017: ISAAA Briefs 53; ISAAA: Manila, Philippines.

Khalique, A., K.M.Wu, G.M.Liang and Y.Y. Guo.2006. Cross resistance to Cry1Ac resistant cotton boll worm, *Helicoverpa armigera*to spore a-endotxin of various *Bacillus thuringiensis* (Berliner). Pak.J. Biol.Sci. 9:1639-1649.

LeOra. POLO-PC: a user's guide to probit or logit analysis: LeOra Software Berkeley, CA; 1987.

Liu, Y. andB.E. Tabashnik.1997. Inheritance of Resistance to the *Bacillus thuringiensis* Toxin Cry1C in the Diamondback Moth. Appl.Environ.Microbiol. 63:2218-2223.

Li, G.P., K.M. Wu, F. Gould, J.K. Wang, J. Miaoi, X.W. Gao andY.Y. Guo.2007. Increasing tolerance to Cry1Ac cotton from cotton bollworm, *Helicoverpa armigera*, was confirmed in Bt cotton farming area of China. Ecol. Entomol. 32:366-375.

Melander, A.L. 1914. Can insects become resistant to sprays? J. Econ. Entomol.7:167-173.

Mendelsohn, M., J.Kough, Z.Vaituzis, andK.Matthews.2003. Are Bt crops safe? Nat. Biotechnol. 21:1003-1009.

National Research Council. 2010. The impact of genetically engineered crops on farm sustainability in the United States. National Academies Press.

Pereira, E.J.G., B.A. Lang, N.P. Storer and B.D. Siegfried.2008. Selection for Cry1F resistance in the European corn borer and cross-resistance to other Cry toxins. Entomol. Exp. Appl.126:115-121.

Qu, M., Z.Han, X.Xu and L.Yue. 2003. Triazophos resistance mechanisms in the rice stem borer (*Chilo suppressalis* Walker). Pestic.Biochem. Physiol. 77:99-105.

Siqueira, H.A.A., D. Moellenbeck, T. Spencer and B.D. Siegfried.2004. Cross-Resistance of Cry1Ab-Selected *Ostrinia nubilalis* (Lepidoptera: Crambidae) to *Bacillus thuringiensis* δ-Endotoxins. J. Econom. Entomol. 97:1049-1057.

Stone, B.F. 1968. A formula for determining degree of dominance in cases of monofactorial inheritance of resistance to chemicals. Bull. W.H.O. 38:325-326.

Tabashnik, B.E., Y.B.Liu, R.A.D.MaagdandT.J.Dennehy.2000.Cross-Resistance of Pink Bollworm (*Pectinophora gossypiella*) to *Bacillus thuringiensis* Toxins. Appl. Environ. Microbiol.66:4582-4584.

Tabashnik, B.E., A.L. Patin, T.J. Dennehy, Y.B. Liu, Y.Carriere, Y.M. Sims and L. Antilla. 2000a.Frequency ofresistance to *Bacillus thuringiensis* in field populations of pink bollworm. Proc. Natl. Acad. Sci. 97:12980-12984.

Tabashnik, B.E., N.L.Cushing, N.Finson, and M.W.Johnson.1990. Field Development of Resistance to *Bacillus thuringiensis* in Diamondback Moth (Lepidoptera: Plutellidae). J.Econom.Entomol. 83:1671-1676.

Tabashnik, B.E., Y.B.Liu, N.Finson, L.Masson and D.G. Heckel.1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proc. Natl. Acad. Sci. USA.94:1640-1644.

Tabashnik, B.E., A.J. Gassmann, D.W. Crowder and Y. Carriere.2008. Insect resistance to Bt crops: Evidence versus theory. Nat. Biotechnol.26:199-202.

Tabashnik, B.E.1994. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39:47-49.

Van Rensburg, J.B.J.2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. S. Afr. J. Plant. Soil. 24:147-151.

Wan, P., Y.Huang, H.Wu, M.Huang, S.Cong, B.E. Tabashnik and K. Wu.2012. Increased Frequency of Pink Bollworm Resistance to Bt Toxin Cry1Ac in China. PLoS ONE.7:e29975. doi:10.1371/journal.pone.0029975

Wang, Y.Q., J.Yang, Y.D.Quan, Z.Y.Wang, W.Z.CaiandK.L. He.2017. Characterization of Asian Corn Borer Resistance to Bt Toxin Cry1Ie. Toxins. 9:1-11. doi:10.3390/toxins9060186.

Wang, Y.Q., Y.D.Wang, Z.Y.Wang, A.Bravo, M.Soberon, K.L.He.2016. Genetic Basis of Cry1F-Resistance in a Laboratory Selected Asian Corn Borer Strain and Its Cross-Resistance to Other *Bacillus thuringiensis* Toxins. PLOS ONE.1-11. DOI:10.1371/journal.pone.0161189.

[Received 31 Oct 2019; Accepted 01 July 2020; Published (online) 17 July 2020]