Pest Manag Sci 60:680–684 (online: 2004)
DOI: 10.1002/ps.848

Changes in susceptibility to conventional insecticides of a Cry1Ac-selected population of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

Kongming Wu* and Yuyuan Guo

Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China

Abstract: The changes in the susceptibility of the cotton bollworm, *Helicoverpa armigera* (Hübner) to three insecticides (lambda-cyhalothrin, phoxim and endosulfan) commonly used for control of this pest in China were monitored by bioassays at various generations. The insects were originally collected from Bt cotton fields and selected with Cry1Ac over 44 generations. In comparison with a susceptible strain, the larval resistance of the Bt-selected populations to Cry1Ac toxin increased 106-fold. Simultaneously, the resistance levels to lambda-cyhalothrin, phoxim and endosulfan declined dramatically. The results indicated no positive cross-resistance between Cry1Ac toxin and the insecticides. Evidence of the lack of cross-resistance to three commonly used synthetic insecticides in our laboratory-derived Cry1Ac-resistant population may suggest that growers can confidently use these insecticides if and when resistance to Cry1Ac cotton does occur.

Keywords: *H armigera*; Bt toxin; insecticide; resistance management

1 INTRODUCTION

Helicoverpa armigera (Hübner) is one of the more important agricultural pests in Asia, Africa and Australia.[1] Its ability to develop resistance to various groups of insecticides, including the synthetic pyrethroids and organophosphates, is partially responsible for its pest status.[2–5] In China, both synthetic pyrethroids and organophosphate insecticides have been used over the past 20 years for control of *H armigera*. Since the mid-1980s, because of the gradual acquisition of insecticide resistance by this species, frequent outbreaks of this pest have been observed.[6–9]

Transgenic cotton, engineered to continuously express δ-endotoxin from the *Bacillus thuringiensis* Berliner (Bt) gene, holds great promise for controlling this insect pest. In China, commercial cultivation of Bt cotton expressing a *Cry1Ac* gene began in 1997 on about 10 000 ha, and rapidly increased to 1.067 million ha in 2000, with the proportion of Bt cotton planting reaching over 90% of the total cotton planting area in northern China.[10–12]

However, like conventional insecticide resistance, Bt resistance in *H armigera* could seriously diminish the utility of the technique. Recently, several insect pests, including *H armigera*, have shown the potential to develop resistance to Bt in the laboratory,[13–19] and resistance has also been detected in field populations of the diamondback moth, *Plutella xylostella* L.[14] Bt resistance management has consequently been made a pre-requisite for approval for cultivation of many Bt transgenic crops.[20–23] Bt cotton cultivation has led to a decline in insecticide use from about 20 to seven applications per season in the areas in China in which it has been most intensively used, such as Hebei and Shandong Provinces.[11] This decreased insecticide usage in Bt cotton is likely to be an important factor in the evolution of the resistance of pest insects to insecticides.[9,24] In order to assess the potential impact of Bt cotton cultivation on the evolution of insect resistance to insecticides, we conducted studies in which the stability of insecticide resistance of a field population of *H armigera* was evaluated over a period in which the population was selected with Cry1Ac protein in the laboratory.

2 MATERIALS AND METHODS

2.1 Insect source and rearing methods

About one hundred of third- to sixth-instar larvae of *H armigera* were collected from Bt cotton plants in mid August 1998 in Langfang, Hebei Province, where insecticides had been used regularly to control

* Correspondence to: Kongming Wu, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China
E-mail: wkm@caascose.net.cn

Contract/grant sponsor: Ministry of Science and Technology of China; contract/grant number: Projects Grant 863 (2001 AA212271); Projects Grant 973 (G2000016208)
(*Received 30 January 2003; revised version received 28 September 2003; accepted 26 November 2003*)
Published online 12 February 2004

 Pest Manag Sci 1526–498X/2004/$30.00

H armigera for two decades before Bt cotton was planted in 1997. The larvae were fed individually on a soybean + maize artificial diet.[9,25] Pupae were kept separately in jars until adult emergence. About fifty pairs of adults were placed in cages (50 × 50 × 50 cm) and fed with 5% honey solution. The top of each cage was covered with white gauze for oviposition. Eggs were collected every 24 h during the oviposition period. The gauze sheets, with eggs, were placed individually in clear glass cups (250 ml) containing soybean + maize artificial diet. Single neonate larva was reared to pupation in a 20-ml glass tube covered with a cotton plug. The Yuzhou strain, originally collected in 1989 from Henan Province, had been in culture in the laboratory without insecticide exposure for more than 10 years, and was used as the susceptible control strain in the experiment. Environment conditions in the insect culture process were kept at 28 (±2) °C and a photoperiod of 14:10 h light : dark with a relative humidity of 90 (±5)%.

2.2 Cry1Ac protein and insecticides

The endotoxin protein used for susceptibility tests was Cry1Ac contained in MVPII® (a commercial Bioinsecticide formulation supplied by Mycogen, San Diego, CA). The MVPII was freeze-dried, powdered, and assayed for Cry1Ac purity as per the procedure described by Greenplate.[26] Three major (commonly used) insecticides, phoxim (84.6%; Hebei Agrochemicals, Shijiazhuang, China), lambda-cyhalothrin (43%; Zeneca Agrochemicals, UK), and endosulfan (95%; Hoechst, Germany), were used for the topical bioassays.

2.3 Selection of resistance to Cry1Ac

Adults of the field strain (F_0) were transferred to the mating cage. Neonates belonging to the F_1 generation were divided into two groups. The first group was reared on a normal artificial diet for bioassays for establishing baselines. The second group (about 1000 individuals) was reared on an artificial diet which contained 0.3 μg ml^{-1} Cry1Ac protein. After 7 days of feeding, about 200 of the individuals, which developed most rapidly, were transferred to a normal artificial diet until moth emergence. The moths were transferred to the mating cage for production of the next generation, and about 1000 of the resulting larvae were used for further selection in each generation. A concentration of 0.3 μg ml^{-1} Cry1Ac protein was used for resistance screening until the F_9 generation. The concentration was then increased to 0.6 μg ml^{-1} for the F_{10} to F_{25} generations, 1.0 μg ml^{-1} Cry1Ac toxin from F_{26} to F_{33}, 3.0 μg ml^{-1} from F_{34} to F_{38}, and finally 5.0 μg ml^{-1} Cry1Ac toxin form the F_{39} to F_{44} generations. The procedure for all bioassays was the same except for the changes in the selecting Cry1Ac doses.

2.4 Change in susceptibility to the insecticides

Susceptibility of the insects selected with Cry1Ac to the conventional insecticides was determined by bioassays in the 5th, 8th, 9th, 17th, 29th, 33rd, 38th and 44th generations (depending upon the availability of insects in these generations). Hatched larvae were first transferred to artificial diet without Cry1Ac protein and allowed to develop up to the third instar. All the insecticide evaluation bioassays were done using third-instar larvae.

2.6 Bioassay procedures

The larval growth-inhibition assays with Cry1Ac protein were performed as described by Sims *et al*.[27] An aliquot of Cry1Ac solution was mixed with warm liquid agar-based insect diet, blended in a mixer, poured into 24-well insect assay trays and allowed to solidify as it cooled. Each well contained about 2 ml of treated diet. One neonate larva was added to each well. Each bioassay consisted of five to six concentrations with three replicates. Assays were incubated at 26 (±0.5) °C and evaluated after 6 days by the number of larvae that reached the third instar.

Insecticide bioassays were conducted with larvae weighing 20–30 mg using procedures of Armes *et al*.[3] One microlitre of solution of each test concentration was applied onto the dorsum of third-instar larva. Three to four replicates of 10 larvae were treated with each of five or more insecticide concentrations. Control larvae were treated with 1 μl of acetone. After treatment, the larvae were kept singly in 30-ml clear plastic cups and fed on fresh artificial diet at 26(±0.5) °C. Larval mortality was assessed after 24 h of treatment. Moribund insects unable to move in a coordinated (normal) manner when prodded with a blunt probe were counted as dead.

2.7 Data analysis

The median lethal doses (LD_{50}) of insecticides and median growth inhibition concentration (IC_{50}) of Cry1Ac were estimated by probit analysis using the software package POLO-PC.[28,29] Mortality was corrected using Abbott's formula for each probit analysis.[30] Resistance factors were calculated as the ratio of IC_{50} or LD_{50} at a particular generation compared with that of the susceptible strain.

3 RESULTS

3.1 Resistance to Cry1Ac Toxin

The bioassay showed that Cry1Ac susceptibility of the original field strain (IC_{50} : 0.040 μg ml^{-1}) before selection was only slightly higher than that in the susceptible strain (Yuzhou). During the early stages of selection, its resistance slowly developed until a 6-fold factor was reached at F_{15}. Its IC_{50} value then increased from 0.169 μg ml^{-1} in F_{15} to 0.436 μg ml^{-1} in F_{30}. It then entered a stage of rapid increase, from 16-fold in F_{30} to 106-fold in F_{44} (Table 1).

3.2 Change in susceptibility to the insecticides

The LD_{50} values in the susceptible strain (Yuzhou) were 0.066 μg g^{-1} to lambda-cyhalothrin, 0.525 μg g^{-1}

Table 1. Cry1Ac susceptibility changes in the strain of *Helicovepa armigera* originally collected from Langfang, during the selection process with Cry1Ac protein

Strain (generation)	IC_{50} ($\mu g\,ml^{-1}$)	95% EL		IC_{90} ($\mu g\,ml^{-1}$)	Slope (± SE)	Resistance factor
		Lower	Upper			
Yuzhou (s)	0.028	0.012	0.047	0.141	1.840 (±0.219)	—
F_1	0.040	0.014	0.085	0.421	1.247 (±0.115)	1
F_5	0.067	0.050	0.088	0.817	1.178 (±0.142)	2
F_9	0.104	0.078	0.137	1.019	1.292 (±0.151)	4
F_{10}	0.131	0.101	0.167	1.137	1.366 (±0.131)	5
F_{13}	0.122	0.105	0.142	0.404	2.462 (±0.226)	4
F_{14}	0.115	0.071	0.176	6.398	0.734 (±0.130)	4
F_{15}	0.169	0.138	0.203	0.709	2.060 (±0.191)	6
F_{16}	0.263	0.098	1.110	6.231	0.933 (±0.119)	9
F_{18}	0.276	0.197	0.421	2.351	1.377 (±0.122)	10
F_{25}	0.349	0.267	0.486	3.312	1.311 (±0.150)	12
F_{30}	0.436	0.351	0.566	2.464	1.704 (±0.208)	16
F_{33}	0.753	0.420	2.393	7.509	1.283 (±0.245)	27
F_{36}	0.802	0.322	4.956	19.765	0.921 (±0.116)	29
F_{38}	1.416	1.039	2.102	8.858	1.609 (±0.207)	51
F_{44}	2.979	2.059	4.252	15.047	1.822 (±0.180)	106

to phoxim and $1.834\,\mu g\,g^{-1}$ to endosulfan. In comparison with the susceptible strain, the LD_{50} values increased 26-fold for lambda-cyhalothrin-, 24-fold for phoxim- and 9-fold for endosulfan-treated field strain larvae (F_1). Over the Cry1Ac selection period there was a decreasing trend in the level of resistance to all three chemicals, with a sharp decline of resistance in the first 10 generations and thereafter a gradual decrease in the level of resistance up to 38th generation in all treatments. (Table 2).

Finally, by F_{38} the LD_{50} values had decreased from 1.742 to $0.082\,\mu g\,g^{-1}$ in lambda-cyhalothrin, 12.693 to $0.546\,\mu g\,g^{-1}$ in phoxim and 16.803 to $1.062\,\mu g\,g^{-1}$ in endosulfan; they then stayed in a stable range in the later generations. In respect of susceptibility of lambda-cyhalothrin, phoxim and endosulfan, the level of the insect resistance to these insecticides gradually came down to a level similar to that of the susceptible strain. On the basis of these results, no positive cross-resistance between Cry1Ac toxin and these insecticides was observed.

4 DISCUSSION

As part of the effort to manage insect resistance to *Bacillus thuringiensis* toxins, it is important to develop a resistant strain by selection with Bt toxin in the laboratory, so as to advance the understanding of resistance mechanisms before insect resistance becomes more widespread in the field. Development of resistance to Bt with selection pressure was first demonstrated in Indianmeal moth, *Plodia interpunctella* (Hübner).[31] Since 1986, through laboratory selection, various insect species have developed high levels of resistance to different Bt toxins.[32,33] The rate of development of insect resistance depends on many factors, such as selection pressure, toxin type,[34,35] insect species,[36] selection methods and the condition and origin of the test population.[37] Laboratory selection of a strain of *Spodoptera littoralis* Boisduval with spore crystal preparations of Cry1C toxin for 14 generations resulted in >500-fold resistance, using two different methods of selection.[37] Colonies of *P interpunctella* of different origins have evolved resistance at different rates.[13] Studies on the Australian strain of *H armigera* for resistance to Cry1Ac indicated that the resistance was first detected after 16 generations of continuous selection, which peaked to approximately 300-fold at generation 21, and first-instar larvae were able to complete their larval development on transgenic cotton expressing *Cry1Ac* and produce fertile adults. Our results showed 106-fold development of resistance in *H armigera* after selection with *Cry1Ac* over 44 generations. On the basis of these findings, it is strongly suggested that this insect may be expected to develop field resistance to Bt cotton if an appropriate resistance-management strategy is not adopted.

Many investigations have been conducted for understanding the pattern of evolution of resistance by *H armigera* to insecticides in fields.[3–10,39] In general, resistance frequencies of *H armigera* to insecticides at the start of the cotton growing season are relatively low, and usually increase sharply until 2–3 weeks after cessation of insecticide applications during a cotton season. In the final stages of cotton growth, resistance frequencies begin to decline, and by the beginning of the following cotton season resistance frequencies have usually returned to relatively low levels.[5,6] There are two main hypotheses in relation to this pattern of *H armigera* resistance evolution, which are not mutually exclusive. The first hypothesis is that unsprayed refugia of host plants produce susceptible (or at least less-resistant) insects which decrease resistance frequencies by immigration and subsequent

Table 2. Susceptibility changes of the test strain of *Helicoperva armigera* originally collected from Langfang to lambda-cyhalothrin, phoxim and endosulfan during the selection process with Cry1Ac protein

Insecticide	Strain (generation)	LD_{50} ($\mu g\,g^{-1}$)	95% EL		LD_{90} ($\mu g\,g^{-1}$)	Slope (±SE)	Resistance factor
			Lower	Upper			
Lambda-cyhalothrin	Yuzhou (s)	0.066	0.042	0.095	0.505	1.449 (±0.250)	—
	F_1	1.742	1.246	2.666	10.678	1.627 (±0.267)	26
	F_5	1.037	0.525	1.543	7.301	1.512 (±0.329)	16
	F_8	0.949	0.593	1.370	7.421	1.435 (±0.252)	14
	F_9	0.881	0.515	2.070	23.664	0.897 (±0.183)	13
	F_{17}	0.436	0.312	0.583	1.817	2.068 (±0.351)	7
	F_{29}	0.066	0.044	0.095	0.430	1.578 (±0.311)	1
	F_{33}	0.049	0.034	0.065	0.201	2.084 (±0.359)	1
	F_{38}	0.082	0.047	0.120	0.579	1.511 (±0.320)	1
	F_{44}	0.048	0.031	0.067	0.260	1.753 (±0.333)	1
Phoxim	Yuzhou (s)	0.525	0.328	0.766	4.457	1.380 (±0.245)	—
	F_1	12.693	8.640	19.907	92.766	1.484 (±0.306)	24
	F_5	11.024	3.063	20.577	68.145	1.620 (±0.319)	21
	F_8	4.793	3.243	7.030	31.591	1.565 (±0.309)	9
	F_9	3.422	1.246	6.023	30.620	1.350 (±0.272)	7
	F_{17}	3.170	2.210	4.677	27.352	1.369 (±0.204)	6
	F_{29}	1.443	0.990	2.053	8.437	1.671 (±0.315)	3
	F_{33}	0.182	0.132	0.252	0.843	1.926 (±0.313)	0
	F_{38}	0.546	0.286	0.807	3.792	1.522 (±0.325)	1
	F_{44}	0.380	0.289	0.508	1.402	2.262 (±0.361)	1
Endosulfan	Yuzhou (s)	1.834	1.134	2.574	10.035	1.736 (±0.333)	—
	F_1	16.803	12.588	23.045	68.993	2.089 (±0.344)	9
	F_5	16.683	9.128	35.237	67.991	2.100 (±0.347)	9
	F_8	11.693	7.106	16.231	54.151	1.925 (±0.369)	6
	F_9	12.247	8.325	18.797	107.29	1.360 (±0.237)	7
	F_{17}	5.134	2.334	8.559	105.553	0.976 (±0.225)	3
	F_{29}	7.294	4.873	12.834	54.571	1.466 (±0.271)	4
	F_{33}	4.521	3.025	6.750	39.596	1.360 (±0.238)	2
	F_{38}	1.062	0.679	1.438	4.181	2.154 (±0.465)	1
	F_{44}	1.201	0.898	1.606	4.726	2.155 (±0.351)	1

breeding with populations from heavily insecticide-treated crops.[4,5,40] The second hypothesis involves a biological cost of resistance or 'fitness deficit', which confers some disadvantage to resistant insects in the absence of selection pressure.[5] Wu and Guo[24] found that after rearing in the laboratory for 17 generations without insecticide treatment, the susceptibility of a field strain of *H armigera* from China to lambda-cyhalothrin, phoxim and endosulfan decreased from 14.96, 30.04 and 30.79 $\mu g\,g^{-1}$ to 0.21, 3.08 and 7.09 $\mu g\,g^{-1}$, respectively, suggesting an instability of insecticide resistance. The present study did not show cross-resistance between Cry1Ac protein and these insecticides, which means that large-scale planting of Bt cotton is likely to reduce resistance levels to conventional insecticides over time, as their use decreases in the field.

The susceptibly (LD_{50}) of *H armigera* to lambda-cyhalothrin, phoxim and endosulfan in the populations collected from 16 locations in northern China from 1994 to 1997 ranged from 4.58–30.94, 8.94–79.11 and 22.90–105.29 $\mu g\,g^{-1}$, respectively.[24] The susceptibilities (LD_{50}) of two Langfang populations collected in June and August in 1997, from the same site as in present study, to lambda-cyhalothrin, phoxim and endosulfan were in the ranges from 4.58–7.65, 31.76–33.75 and 24.88–58.26 $\mu g\,g^{-1}$, respectively as against LD_{50} values of 1.742 (lambda-cyhalothrin), 12.693 (phoxim) and 16.803 $\mu g\,g^{-1}$ (endosulfan) for the test population collected in August, 1998 from Bt cotton, showing significant increases in insect susceptibility to insecticides, probably as a result of the reduction in insecticide applications on Bt cotton.

ACKNOWLEDGEMENTS

We thank Dr Derek Russell (NRI, UK), Dr GT Gujar (Indian Agricultural Research Institute, New Delhi), and Dr K Ahmed (Institute of Field and Horticultural Crops, Pakistan Agricultural Research Center, Islamabad) for critical review and discussion of the manuscript. This research was supported by Projects Grant No 863 (2001AA212271) and Projects Grant No 973 (G2000016208) of the Ministry of Science and Technology of China.

REFERENCES

1 Fitt G, The ecology of *Heliothis* species in relation to agroecosystems. *Annu Rev Entomol* **34**:17–52 (1989).

2 Gunning RV, Easton CS, Greenup LR and Edge VE, Pyrethroid resistance in *Heliothis armigera* (Lepidoptera: Noctuidae) in Australia. *J Econ Entomol* 77:1283–1287 (1984).

3 Armes NJ, Jadhav DR and DeSouza KR, A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. *Bull Entomol Res* **86**:499–514 (1996).

4 Armes NJ, Jadhav DR, Bond GS and King ABS, Insecticide resistance in *Helicoverpa armigera* in South India. *Pestic Sci* **34**:355–364 (1992).

5 Souza KD, Holt J and Colvin J, Diapause, migration and pyrethroid-resistance dynamics in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Ecol Entomol* **20**:333–342 (1995).

6 Wei C, Investigation on the resistance levels of the cotton bollworm *Heliothis armigera* to four kinds of pyrethroid pesticides in China in 1991. *Resist Pest Manag* **4**:17–21 (1992).

7 Guo Y, Progress in the researches on migration regularity of cotton bollworm and relationships between the pest and its host plants. *Acta Entomol Sin* **40**:(Suppl): 1–6 (1997).

8 Wu K and Guo Y, The influences of gene flow between geographical populations on the evolution of insecticide resistance in *Helicoverpa armigera*. *Acta Entomol Sin* **40**:(Suppl): 30–34 (1997).

9 Wu K, Liang G and Guo Y, Phoxim resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in China. *J Econ Entomol* **90**:868–872 (1997).

10 Wu K, Guo Y and Wang W, Field resistance evaluations of Bt transgenic cotton GK series to cotton bollworm. *Acta Phytophyl Sin* **27**:317–321 (2000).

11 Pray C, Huang J, Ma D and Qiao F, Impact of Bt Cotton in China. *World Develop* **29**:813–825 (2001).

12 Qu X, Jiang Y and Zhang R, Current status and strategies of utilization of Bt cotton. *Plant Prot Tech Exten* **21**:37–39 (2001).

13 McGaughey WH and Beeman RW, Resistance to *Bacillus thuringiensis* in colonies of Indian meal moth and almond moth (Lepidoptera: Pyralidae). *J Econ Entomol* **81**:28–33 (1988).

14 Tabashnik BE, Cushing NL, Finson N and Johnson MW, Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J Econ Entomol* **83**:1671–1676 (1990).

15 Tabashnik BE, Schwartz JM, Finson N and Johnson MW, Inheritance of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J Econ Entomol* **85**:1046–1055 (1992).

16 Tabashnik BE, Finson N, Groeters FR, Moar WJ, Johnson MW, Luo K and Adang MJ, Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Proc Natl Acad Sci USA* **91**:4120–4124 (1994).

17 Gould F, Anderson A, Reynolds A, Bumgarner L and Moar W, Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *J Econ Entomol* **88**:1545–1559 (1995).

18 Chaufaux J, Müller-cohn J, Buisson C, Sanchis V, Lereclus D and Pasteur N, Inheritance of resistance to the *Bacillus thuringiensis* Cry1c toxin in *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J Econ Entomol* **90**:873–878 (1997).

19 Liang G, Tan W and Guo Y, Study on screening and inheritance mode of resistance to Bt transgenic cotton in cotton bollworm. *Acta Entomol Sin* **43**:(Suppl.): 57–62 (2000).

20 McGaughey WH and Whalon ME, Managing insect resistance to *Bacillus thuringiensis* toxins. *Science (Washington)*. **258**:1451–1455 (1992).

21 Tabashnik BE, Evolution of resistance to *Bacillus thuringiensis*. *Annu Rev Entomol* **39**:47–79 (1994).

22 Alstad DN and Andow DA, Managing the evolution of resistance to transgenic plants. *Science (Washington)* **268**:1894–1896 (1995).

23 Peck SL, Gould F and Ellner SP, Spread of resistance in spatially extended regions of transgenic cotton: implications for management of *Heliothis virescens* (Lepidoptera:Noctuidae). *J Econ Entomol* **92**:1–16 (1999).

24 Wu K and Guo Y, The coordinated development and analysis of contributing factors of cotton bollworm resistance to insecticides in round-bohai bay region. *Acta Phytophyl Sin* **27**:173–178 (2000).

25 Zhou L, Fang Y and Yang J, Investigation on artificial diet in *Heliothis armigera*. *Acta Entomol Sin* **24**:108–110 (1981).

26 Greenplate JT, Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard® cotton fruit and terminals. *J Econ Entomol* **92**:1377–1383 (1999).

27 Sims SR, Greenplate JT, Stone TB, Caprio MA and Gould FL, Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins, *Molecular genetics and evolution of pesticide resistance*, ed by Brown TM, *ACS Symposium Series* No 645, American Chemical Society, Washington, DC, pp 229–242 (1996).

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

29 Russell R, Robertson JL and Savin NE, POLO: a new computer program for probit analysis. *Bull Entomol Soc Am* **23**:209–213 (1977).

30 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).

31 McGaughey WH, Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science (Washington)* **229**:193–195 (1985).

32 Stone TB, Sims SR and Marrone PG, Selection of tobacco budworm for resistance to a genetically engineered *Pseudomonas fluorescens* containing the δ-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*. *J Invertebr Pathol* **53**:228–234 (1989).

33 Gould F, Martinez-Ramirez A, Anderson A, Ferre J, Silva FJ and Moar WJ, Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. *Proc Natl Acad Sci USA* **89**:7986–7988 (1992).

34 Sneh B and Schuster S, Effect of exposure to sublethal concentration of *Bacillus thuringiensis* Berliner ssp *Entomocidus* on the susceptibility to the endotoxin of subsequent generations of the Egyptian cotton leafworm *Spodoptera littoralis* Boisd (Lep: Noctuidae). *Z Angew Entomol* **96**:425–428 (1983).

35 Heckel DG, The complex genetic basis of resistance to *Bacillus thuringiensis* toxin in insects. *Biocontrol Sci Technol* **4**:405–417 (1994).

36 Salama HS and Matter MM, Tolerance level to *Bacillus thuringiensis* Berliner in the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *J Appl Entomol* **111**:225–230 (1991).

37 Müller-cohn J, Chaufaux J, Buisson C, Gilois N, Sanchis V and Lereclus D, *Spodoptera littoralis* (Lepidoptera: Noctuidae) resistance to Cry1C and cross-resistance to other *Bacillus thuringiensis* crystal toxins. *J Econ Entomol* **89**:791–797 (1996).

38 Akhust RJ, James W, Bird LJ and Beard C, Resistance to the Cry1Ac δ-endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J Econ Entomol* **96**:1290–1299 (2003).

39 Daly JC, Insecticide resistance in *Heliothis armigera* in Australia. *Pestic Sci* **23**:165–176 (1988).

40 Comins HN, The management of pesticide resistance. *J Theor Bio* **65**:399–420 (1977).