

# A simulation model for adaptation of cotton bollworm to transgenic Bt cotton in northern China

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**Abstract:** The commercial use of transgenic cotton expressing an insecticidal protein gene from *Bacillus thuringiensis* (Bt) began in 1998 in northern China. Resistance management is a major concern for the sustainable use of Bt cotton. With our understanding of the cropping and ecological system in northern China, we developed a simulation model to forecast adaptation of the cotton bollworm, *Helicoverpa armigera* (Hübner), to Bt cotton. The model incorporated population genetics, biological and operational factors in a multiple cropping system. Variables of reproductive fitness on different host plants, dominance of resistance alleles, expression level of Bt toxin in cotton and use of insecticides in Bt cotton fields were included in the model. In the typical cropping system of northern China, the expected life of Bt cotton are seven years if all cotton is Bt cotton, and ten years if only spring planted cotton (about 70% total cotton area) is Bt cotton in northern China based on the model. The life expectancy decreases quickly with increases in initial frequency of resistance allele, dominance of the resistance gene, and the percent area of Bt cotton planted. The results also showed that supplemental control is essential on Bt cotton when the expression of Bt toxin declines. Because of the lack of a consistent high dose and the complexity of managing individual fields of Bt cotton by many different growers in the same region, the use of the high-dose/refuge strategy in northern China will be difficult to achieve.

**Key words:** *Helicoverpa armigera*; transgenic Bt cotton; resistance; adaptation; simulation model

The cotton bollworm, *Helicoverpa armigera* (Hübner), is the most important cotton pest in China. Long-term application of pyrethroids resulted in serious resistance problems and reduced control efficacy (Fan *et al.*, 1997). Transgenic cotton, developed in the US and China and expressing an insecticidal protein from *Bacillus thuringiensis* (Bt), provided a powerful new tool after commercial use began in northern China in 1998. The acreage of Bt cotton grown in China was about 80 000 ha in 1998 and over 0.3 million ha in 1999, and greater than 90% of the cotton fields in Shandong and Hebei provinces in northern China cotton belts were Bt cotton in 2000 (Zhao *et al.*, 2000). A survey of 282 Bt cotton farmers in northern China demonstrated significant increases of production efficiency and decreased health risks to farmers (Huang *et al.*, 2002). To preserve these benefits a resistance management program must be implemented so that insects do not rapidly become resistant to the Bt cotton since some insects have already demonstrated their

ability to develop resistance to Bt toxins (Shelton *et al.*, 2002).

The high-dose/refuge is the only resistance management strategy currently available for Bt transgenic crops, and the US growers must chose one of the structural refuge options as required by the US EPA (Shelton *et al.*, 2002). To use Bt plants expressing a high-dose of the toxin in combination with a refuge would conserve susceptible alleles within the population. The high dose is required through the entire crop's growth to ensure that homozygous and heterozygous susceptible insects are controlled and the refuge strategy must be considerate of the ability of insects in the refuge to mate with individuals carrying resistant alleles, thereby decreasing the probability of resistant phenotypes. Bt cotton provides excellent control of *Heliothis virescens* in the US (Gould and Tabashnik, 1998), but *Helicoverpa zea* in the US and *H. armigera* in China are less sensitive to the available Bt toxins and showed significant survival on Bt cotton in the

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fields (Stone and Sims, 1993; Zhao *et al.*, 1998, 2000). The survival of *H. zea* on Bt cotton in the US indicated that the "high-dose" approach is not valid for this species (Gould and Tabashnik, 1998).

Pest resistance management (PRM) strategies for transgenic crops have been examined in several models (Caprio, 1994; Tabashnik, 1994; Onstad and Gould, 1998). The fundamental goal is to minimize exposure of pests to the toxin in space and time. The models reported that expression level of Bt toxin and the insect's sensitivity are key factors in resistance development. Additionally, it is important to have sufficient susceptible alleles to dilute the frequency of resistance alleles, and that there be random mating of insects carrying each type of allele. Models and experimental data also showed that separate but adjacent refuges might be superior to other strategies for insects which can move between plants in their larval stage (Shelton *et al.*, 2002). We developed a simulation model that includes reproductive fitness of *H. armigera* on different host plants, dominance of resistance alleles, expression level of Bt toxin in cotton, and use of insecticides in Bt cotton fields, under ecological situations in the northern China cotton belt.

## 1 Materials and Methods

### 1.1 Model design and realization

Development of our model are based on the following key assumptions. (1) Resistance of *H. armigera* to Bt toxin is controlled by a single autosomal locus ( $G$ ) with two alleles ( $G_R$  and  $G_S$ ), and the degree of dominance is  $h$ . Thus, there are three genotypes ( $G_S G_S$ , susceptible;  $G_R G_S$ , heterozygous; and  $G_R G_R$ , resistant). The initial resistance allele frequency is  $p$ . (2) There are  $n$  types of host-plant fields planted. Wheat, cotton and corn are major host plants of *H. armigera*. Movement of larvae between different kinds of fields is negligible, and generations do not overlap. Mating occurs randomly among adults of different genotypes and different fields. Egg distribution depends on the preference of *H. armigera* adults on different host plants. (3) Population survival is assumed to be density-independent. Genotype survival rates are defined as  $L$  for  $G_R G_R$ ,  $K$  for  $G_S G_S$ , and  $hL + (1 - h)K$  for  $G_R G_S$  (Alstad and Andow, 1995). The

reproductive disadvantage of resistant genotypes on non-Bt host plants is negligible. (4) Survivors feeding on different types of non-Bt crops have the same fertility rate. (5) Proportion of each genotype in each generation can be calculated according to the Hardy-Weinberg equation.

### 1.2 Model developing progress from N generation to N + 1 generation

The three genotypes are designated as  $G_1$  ( $G_S G_S$ ),  $G_2$  ( $G_R G_S$ ) and  $G_3$  ( $G_R G_R$ ).  $F_i$  ( $i = 1 \sim n$ ) denotes the  $i$ th type of field. Assuming total crop area is denoted by  $A$ , the percentage of  $F_i$  is denoted by  $A_i$ .  $T_i$  denotes the adults oviposition probability upon the plants in  $F_i$ .  $D$  denotes average population density of all fields in the egg stage.  $D_i$  denotes population density on  $F_i$ . The frequencies of  $G_R$  and  $G_S$  are given by  $p$  and  $q$  where  $q = 1 - p$ . The frequency of  $G_j$  ( $j = 1 \sim 3$ ) is denoted by  $P_j$  ( $j = 1 \sim 3$ ). The population density of genotype  $G_j$  in  $F_i$  is denoted by  $D_{ij}$ .

$$D_{ij} = P_j (DA) (A_i T_i / \sum (A_i T_i)) / (AA_i) \\ = DP_j T_i / \sum (A_i T_i) \quad (i = 1 \sim n, j = 1 \sim 3)$$

$E_{ij}$  ( $i = 1 \sim n, j = 1 \sim 3$ ) denotes the natural survivorship of the genotype  $G_j$  in  $F_i$  as influenced by environmental factors.  $S_{ij}$  ( $i = 1 \sim n, j = 1 \sim 3$ ) denotes the relative survivorship of the genotype  $G_j$  in  $F_i$  as influenced by a supplemental insecticide and other operational factors (including light trap to kill moths).  $W_{ij}$  ( $i = 1 \sim n$ ) denotes the effective fitness of the genotype  $G_j$  in  $F_i$ .

$$W_{ij} = E_{ij} S_{ij} \quad (i = 1 \sim n, j = 1 \sim 3)$$

$N_{ij}$  ( $i = 1 \sim n, j = 1 \sim 3$ ) denotes the number of surviving adults of the genotype  $G_j$  in  $F_i$ .

$$N_{ij} = (AA_i D_{ij}) W_{ij} = DAP_j A_i T_i W_{ij} / \sum (A_i T_i) \\ (i = 1 \sim n, j = 1 \sim 3)$$

$p'$  denotes the frequency of the resistant allele in the egg stage of the next generation and is independent of  $D$  and  $A$ .

$$p' = \sum (N_{i3} + N_{i2}/2) / \sum (N_{ij}) \\ = \sum (P_3 A_i T_i W_{i3} + P_2 A_i T_i W_{i2}/2) / \\ \sum (P_j A_i T_i W_{ij}) \\ (i = 1 \sim n, j = 1 \sim 3)$$

### 1.3 Initializing parameters

In northern China, *H. armigera* can complete four

generations per year. The larvae of the first generation feed mainly on wheat. The larvae of the second generation feed on several crops planted in the spring, but the dominant host plant is cotton. The third and fourth generations feed mainly on crops planted in the summer, including cotton, corn, peanut, soybean, and other vegetables (Shi *et al.*, 1995). The percentage area of each crop is set according to survey data from Handan City, Hebei Province. We assume that Bt cotton is planted in the spring (about 70% of total cotton area) and non-Bt cotton is planted in the summer. Other crops (non-Bt) are considered as refuges of *H. armigera* because they are planted adjacent to cotton and most of the fields are very small (< 1 ha). The reproductive fitness of *H. armigera* on wheat and cotton planted in the spring was calculated according to the results of Dai *et al.* (1991). The reproductive fitness on corn, cotton planted in the summer and other crops was calculated according to the results of Shi *et al.* (1995). The oviposition performance on each crop

was calculated according to the results of Shi *et al.* (1995).

Because we do not have a *H. armigera* strain highly resistant to Bt, we assume that the characterization of Bt resistance in *H. armigera* was similar to that in *H. virescens* (Gould *et al.*, 1995). The dominance of resistance allele *h* was about 0.3. The initial frequency of resistance allele is assumed to be 0.001. Expression of Bt toxin in cotton begins to decline after flowering and some *H. armigera* larvae could survive on flowers and other tissues (Zhao *et al.*, 1998). The relative fitness of  $G_S G_S$  and  $G_R G_S$  are different in each generation. The details are listed on Table 1. The relative fitness of  $G_R G_R$  was 1.0 in any situation. Assuming larval mortality caused by chemical insecticides is 80% on non-Bt cotton, the relative fitness of each genotype was 0.2. In other non-Bt crop fields, each genotype has a relative fitness of 1.0 because *H. armigera* was unmanaged.

**Table 1** Initializing parameters of the typical cotton cropping system in northern China

Field type	Genotype	First generation				Second generation				Third generation				Fourth generation			
		$A_i$	$T_i$	$E_{ij}$	$S_{ij}$	$A_i$	$T_i$	$E_{ij}$	$S_{ij}$	$A_i$	$T_i$	$E_{ij}$	$S_{ij}$	$A_i$	$T_i$	$E_{ij}$	$S_{ij}$
F <sub>1</sub>	G <sub>1</sub>	0	-	-	-	0.06	0.84	0.00059	0	0.06	0.2	0.0031	0.01	0.06	0.22	0.0018	0.01
	G <sub>2</sub>			-	-			0.00059	0.0001			0.0031	0.31			0.0018	0.31
	G <sub>3</sub>			-	-			0.00059	1.0			0.0031	1.0			0.0018	1.0
F <sub>2</sub>	G <sub>1</sub>	0	-	-	-	0	0.84	0.00059	0.2	0	0.2	0.0031	0.2	0	0.22	0.0018	0.2
	G <sub>2</sub>			-	-			0.00059	0.2			0.0031	0.2			0.0018	0.2
	G <sub>3</sub>			-	-			0.00059	0.2			0.0031	0.2			0.0018	0.2
F <sub>3</sub>	G <sub>1</sub>	0	-	-	-	0	-	-	-	0	0.34	0.0025	0.002	0	0.4	0.0016	0.01
	G <sub>2</sub>			-	-			-	-			0.0025	0.3			0.0016	0.31
	G <sub>3</sub>			-	-			-	-			0.0025	1.0			9.9916	1.0
F <sub>4</sub>	G <sub>1</sub>	0	-	-	-	0	-	-	-	0.03	0.34	0.0025	0.2	0.03	0.4	0.0016	0.2
	G <sub>2</sub>			-	-			-	-			0.0025	0.2			0.0016	0.2
	G <sub>3</sub>			-	-			-	-			0.0025	0.2			0.0026	0.2
F <sub>5</sub>	G <sub>1</sub>	0	-	-	-	0	-	-	-	0.26	0.31	0.00055	1.0	0.26	0.24	0.00041	1.0
	G <sub>2</sub>			-	-			-	-			0.00055	1.0			0.00041	1.0
	G <sub>3</sub>			-	-			-	-			0.00055	1.0			0.00041	1.0
F <sub>6</sub>	G <sub>1</sub>	0.41	1.0	0.0007	1.0	0.02	0.16	0.00021	1.0	0.22	0.15	0.0005	1.0	0.22	0.14	0.00061	1.0
	G <sub>2</sub>			0.0007	1.0			0.00021	1.0			0.0005	1.0			0.00061	1.0
	G <sub>3</sub>			0.0007	1.0			0.00021	1.0			0.0005	1.0			0.00061	1.0

F<sub>1</sub>: Bt cotton planted in spring; F<sub>2</sub>: non-Bt cotton planted in spring; F<sub>3</sub>: Bt cotton planted in summer; F<sub>4</sub>: non-Bt cotton planted in summer; F<sub>5</sub>: corn planted in summer; F<sub>6</sub>: other field

### 1.4 Model programming and sensitivity analysis

The model is implemented as a C program for DOS. We assumed that Bt cotton would lose its efficacy when the frequency of resistance allele increased to 50%. The expected life of Bt cotton was defined as the number of years required for the frequency of resistance allele to increase to 50% from the initial value. Sensitivity analysis was made by running the program when the initial value of each of certain critical variables (initial resistance frequency, gene dominance, Bt cotton area, and chemical control) changed.

## 2 Results

### 2.1 Expected commercial life of Bt cotton and resistance development of *H. armigera*

In typical conditions as in Table 1, the model pre-

dicted that the frequency of resistant allele will rise to 0.5 after 38 generations, i. e., approximately 10 years of widespread use of Bt cotton (since 2000) in northern China (Fig. 1).

### 2.2 Sensitivity analysis of the model

Results of sensitivity analysis indicated that the expected life of Bt cotton will decrease quickly with the increase of initial frequency of resistance allele (Fig. 2A), gene dominance (Fig. 2B), and area percentage of Bt cotton (Fig. 3). If all cotton fields are Bt cotton, the expected life may be only 7 years (26 generations) (Fig. 3). Chemical insecticide applications in Bt cotton will prolong the life of Bt cotton and delay resistance development of the pest to Bt cotton (Fig. 4) because all genotypes, including homozygous resistant genotypes and heterozygotes, will be killed.

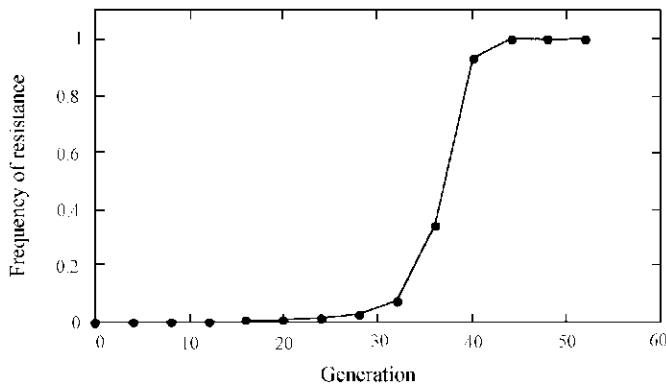


Fig. 1 Predicted development of Bt resistance of *H. armigera* after widespread use of Bt cotton in northern China

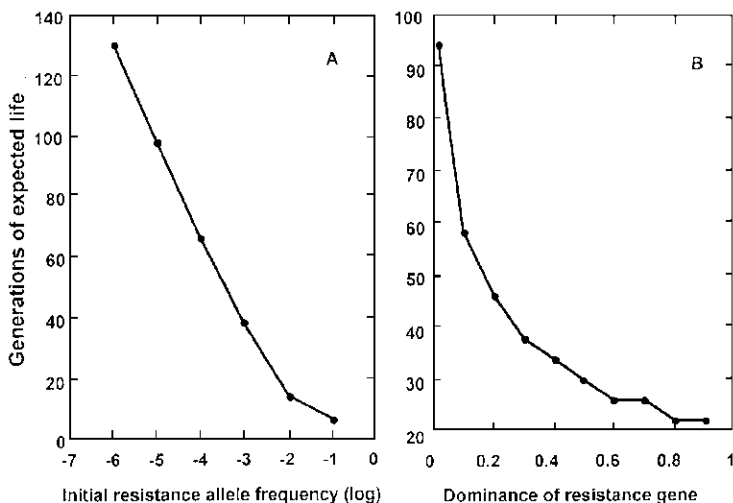


Fig. 2 Sensitivity analysis on initial frequency of resistance allele (A) and gene dominance (B) in *H. armigera*

### 2.3 Interaction between dominance of resistance allele and expressing level of Bt toxin

The expression level of Bt toxin in Bt cotton is presented as the relative fitness of heterozygotes on Bt cotton (Fig. 5). The arrow indicates the point at which all susceptible individuals are killed on Bt cotton when the relative fitness of heterozygotes was equal to  $h$  (Fig. 5). In general, there were many more susceptible individuals than resistant individuals or heterozygotes in the early stage. At a low-level expression of Bt toxin which is not

very harmful to any genotypes, a Bt cotton field with susceptible survivors could be considered an “inside refuge”. At a moderate expression level that kills all susceptible individuals, the expected life of Bt cotton will be the shortest, with loss of an “inside refuge” and large numbers of surviving resistant individuals and heterozygotes. At a high expression level that could kill all heterozygotes and susceptible individuals, the expected life of Bt cotton will increase with the increase in of heterozygotes mortality.

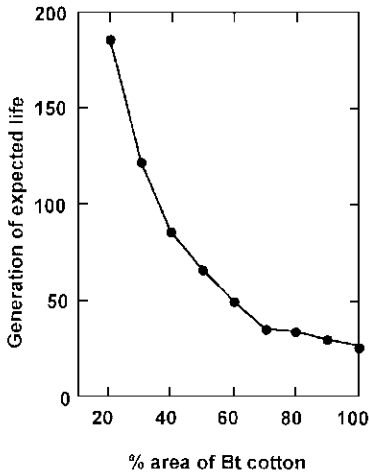


Fig. 3 Sensitivity analysis on the regional proportion of Bt cotton

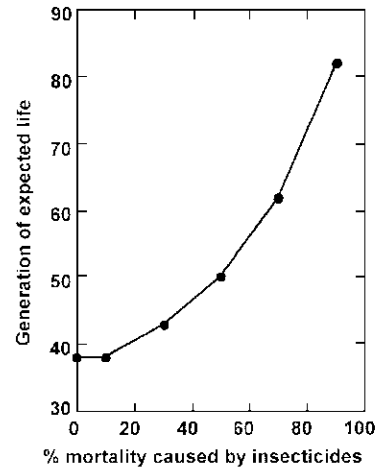


Fig. 4 Sensitivity analysis on the chemical control in Bt cotton field

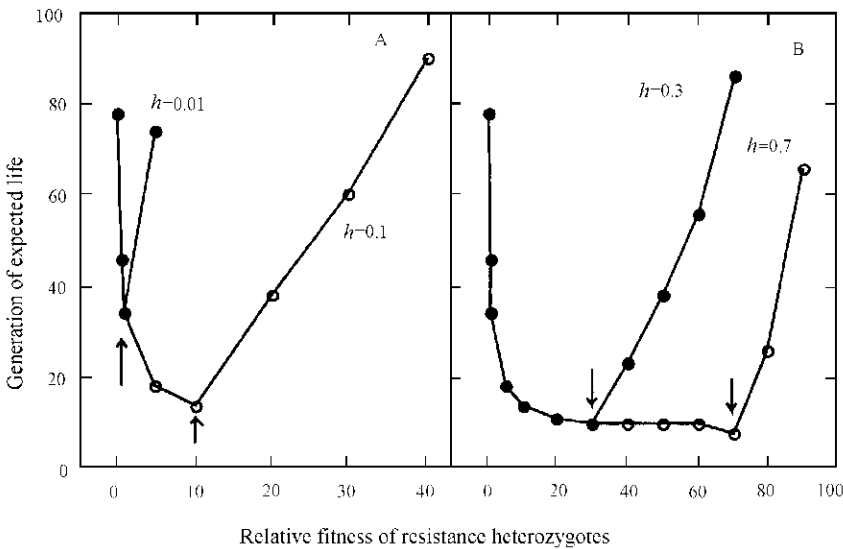


Fig. 5 Sensitivity analysis of expressing level of Bt toxin in cotton

### 3 Discussion

Due to the declining level of expression of the Bt toxin, when the third and fourth generation of *H. armigera* occur they will not be 100% controlled by Bt cotton (Zhao *et al.*, 1998). The lack of a "high dose" in current Bt cotton cultivars for *H. armigera* and the small scale production systems of cotton in northern China indicates that the "high dose/refuge" resistance management strategy is probably not feasible for Bt cotton in most cotton belts in China (Zhao *et al.*, 2000). There is also no mandatory requirement for a structured refuge by the regulatory authority in China because of complexity of managing individual small fields of Bt cotton by many different growers in the same region. The expected life of Bt cotton based on our modeling study is about 7 ~ 10 years if 70% ~ 100% cotton planted is Bt cotton, indicating that alternative resistance management strategies are needed for use by millions of cotton growers in northern China.

Theoretical models suggest that pyramiding two dissimilar toxin genes in the same plant has the potential to delay the onset of resistance much more effectively than single toxin plants released spatially or temporally (Roush, 1998). We have shown that transgenic tobacco expressing two insecticidal toxin (Bt and cowpea trypsin inhibitor, CpTI) genes could delay resistance development by *H. armigera* compared with Bt tobacco (Zhao *et al.*, 1999). Transgenic cotton expressing both Bt and CpTI genes developed in China (Guo *et al.*, 1999) went into commercial use in 2000 and the efficacy on *H. armigera* was significantly higher than one Bt gene cotton (Zhao *et al.*, 2000). Stewart *et al.* (2001) demonstrated that Bollgard II cotton that produce two Bt gene products (Cry1Ac and Cry2Ab) has greater insecticidal activity than the original Bt cotton expressing only the Cry1Ac toxin.

Supplemental chemical insecticides could be used to control more resistant genotypes and heterozygotes in Bt cotton fields when the Bt toxin expression declines. Our modeling results proved that the expected life of Bt cotton can be doubled if 90% *H. armigera* larvae on Bt cotton were killed by insecticides. Different management tactics are needed for different cotton regions in China because of

greatly different ecological situations among them. Model modification and validation for this study are needed based on new data of Bt resistance in *H. armigera*.

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## 华北地区棉铃虫对转 Bt 基因抗虫棉 抗性适应的模拟模型

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**摘要:** 通过对华北地区耕作制度和生态系统的了解, 在充分考虑种群遗传学、生物学和人为操纵因子等三大因素的基础上, 建立了一个预测棉铃虫对转 Bt 基因抗虫棉抗性适应的模拟模型。在华北地区典型的耕作制度下, 如果所有棉田均为 Bt 棉, 则 Bt 棉的预期寿命为 7 年; 如果只有春播棉为 Bt 棉 (约占棉田总面积的 70%), 则其寿命为 10 年。模型的灵敏度分析表明, Bt 棉的使用寿命随抗性基因的显性度、初始抗性频率、Bt 棉所占比例等因素的增长而迅速缩短。当 Bt 棉表达的杀虫蛋白量恰好全部杀死敏感基因型 ( $G_3G_3$ ) 个体时, Bt 棉的预期寿命最短。由于国外采用的“高剂量/庇护所”抗性治理策略不适用于棉铃虫及华北棉区的耕作制度, 我国需要加强对其它抗性治理措施 (如转双基因抗虫棉) 的研究与应用。

**关键词:** 棉铃虫; 转 Bt 基因; 抗虫棉; 抗性; 模型

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