Modeling the evolution of resistance in cotton bollworm to concurrently planted Bt cotton and Bt maize in China

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Abstract

Background: Transgenic maize expressing toxins derived from the bacterium *Bacillus thuringiensis* (Bt) may be commercially planted in northern China where Bt cotton has been planted for more than two decades. While Bt maize brings additional benefits for insect control, it complicates the resistance management of cotton bollworm (CBW), *Helicoverpa armigera* (Lepidoptera, Noctuidae), a common target of Bt cotton and Bt maize.

Results: We developed population genetic models to assess the risk of resistance in CBW when Bt cotton and Bt maize are planted concurrently. Model simulations showed that when natural refuges are absent, the time to resistance (TTR) is less than 10 generations in the case of one-toxin Bt cotton and one-toxin Bt maize, but is more than 30 generations in the case of two-toxin Bt cotton and two-toxin Bt maize. The differences in the TTR between the two cases become greater as the proportion of natural refuge increases. Among the parameters we investigated, the fitness cost has a relatively smaller effect on the TTR, while the dominance of resistance and the proportion of natural refuge have a much greater effect.

Conclusions: We concluded that planting the first generation Bt cotton with Bt maize could significantly increase the risk of CBW resistance to Bt toxins as compared to planting a pyramid two-toxin Bt cotton. The strategies for reducing the risk of CBW resistance include replacing the one-toxin Bt cotton with a pyramid two-toxin Bt cotton, adopting a pyramid two-toxin Bt maize, and maintaining a sufficient proportion of natural refuges.

Keywords: Bt maize, Bt cotton, Cotton bollworm, Time to resistance, Model prediction
Background

Transgenic crops producing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have become a major strategy to fight key insect pests in agriculture during the past two decades [1-4]. By 2018, transgenic crops were planted on more than 190 million hectares worldwide [5]. Among all transgenic crops, the majority is cotton, maize and soybean.

One of the main threats to the long-term use of Bt crops is that target pests may evolve resistance to Bt toxin. To date, field resistance has been observed in the target pests of both Bt cotton and Bt maize [6-11]. Resistance can be affected by various ecological and genetic factors [12]. Among them, the most important factor is excessive planting of Bt plants [13], but other factors such as insect susceptibility to the Bt toxin, dominance of resistance, and strategy of resistance management are also very important [14-17].

The refuge strategy is one of the general approaches to managing resistance. With this strategy, a proportion of non-Bt host is planted as refuge to maintain susceptible insect populations. Because abundant susceptible insects from refuge can compete for mating with rare resistant ones and produce heterozygous offspring that cannot survive on Bt plants, evolution of resistance is delayed [18]. The applications of the refuge strategies have been documented in literatures, in the cases of structured refuges [14], natural refuges [19,20], and seed mixture [4].

In China, Bt cotton is the only Bt crop that has been commercially planted so far [21]. One of the main targets of Bt cotton is cotton bollworm (CBW), *Helicoverpa armigera* (Lepidoptera, Noctuidae), a highly polyphagous insect pest that can feed on a number of different agricultural crops. In northern China, CBW has 4 generations per year. The host crops of CBW include cotton, maize, wheat, soybean, peanut, vegetables, and the availability of host crops vary among different generations [22]. For the first generation, wheat is the primary host crop when other major host crops like cotton and maize are absent. For the second through fourth generations, most of major host crops are available. An earlier study has shown that abundant non-cotton host crops in northern China served as natural refuges for CBW and contributed to delaying resistance of CBW to Bt cotton [20]. However, a more recent study has found that resistance to Bt cotton in CBW is accelerated by a dominant resistance allele [23].

Because maize has been one of the main categories of natural refuges for CBW in northern
China so far, a practical question is what if the conventional maize is replaced with Bt maize in the region? Planting Bt maize will increase the proportion of Bt plants while decrease the proportion of natural refuge, so one can expect the risk of resistance to Bt to increase if the current composition of host plants is not changed. However, it is unknown how such an increase in the risk of resistance to Bt is affected by important factors associated with the Bt cotton and Bt maize varieties that have been or will be planting. These factors include the number and types of toxins contained in Bt cotton and Bt maize, the fitness parameters associated with Bt cotton and Bt maize, the mode of action in resistance to Bt cotton and Bt maize.

Simulation models perhaps are the best approach to addressing the questions above. Compared to experimental studies, simulation models have the advantages of reducing complexity and thus are widely used to assess the risk of insect resistance to Bt plants [24-30]. Simulation models have been used to assess the risk of Helicoverpa zea resistance to Bt cotton and Bt maize in the United States, where H. zea is a closely related species to CBW [31,32]. Similar models can be used to assess the risk of resistance to Bt cotton and Bt maize for CBW in northern China, but must take into account the fact that the Bt cotton and Bt maize varieties and their planting history in China are very different from those in the United States.

In this paper, a two-locus population genetic model is developed to analyze the resistance evolution of CBW to Bt cotton and Bt maize when the two species of Bt crops are planted concurrently. The model takes into account the actual Bt cotton variety and the existing resistance to Bt cotton. The questions to be addressed include: (1) What are the differences in the risk of resistance between an one-toxin Bt maize (Bt maize-1) and two-toxin Bt maize (Bt maize-2)? (2) How does the preexisting resistance to Bt cotton affect the risk of resistance when Bt cotton and Bt maize are planted together? (3) What are the key parameters that will impact on the risk of resistance?

**Results**

The temporal patterns in the frequency of resistance alleles

In the case of Bt cotton-1 & Bt maize-1 where resistance is governed by a single locus, the only resistance allele $R_1$ increases in frequency rapidly over time (Fig. 1A). Because of fitness cost, the
The frequency of resistance allele goes to a level less than 100%. The rapid increase in the frequency of resistance alleles is caused by a combination of dominant inheritance, high initial frequency and lack of natural refuge.

In the case of Bt cotton-1 & Bt maize-2 where resistance is governed by two loci, the temporal pattern in the frequency of $R_1$ is very different from that in the frequency of $R_2$ (Fig. 1B). The frequency of $R_1$ increases rapidly, similar to that in the single-locus case. Compared to that in the frequency of $R_1$, the frequency of $R_2$ increases much slower. It is worthy to note that the frequency of $R_1$ increases slower than that in the single-locus case even if all parameters related to Locus 1 are the same. Namely, there is an interaction between $R_1$ and $R_2$, which results in a slower increase in the frequency of $R_1$.

In the case of Bt cotton-2 & Bt maize-1 and of Bt cotton-2 & Bt maize-2, where resistance is governed by two loci, similar interactions between $R_1$ and $R_2$ are observed (Fig. 1C&D). Compared to that in the single-locus case, the frequencies of $R_1$ in all three two-locus cases increase slower.

The impact of fitness cost

To see how the fitness cost at Locus 1 affects resistance evolution, the time to resistance (TTR) is derived when the fitness cost at Locus 1 varies from 0 to 0.5 (Fig. 2). In all four cases, i.e. Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, the TTRs increase as the fitness cost increases. This is expected because fitness cost acts against resistance. However, in all four cases, the effect of the fitness cost at Locus 1 appears very limited. For example, in the case of Bt cotton-2 & Bt maize-2, the TTR just increases from 31 to 32 generations when the fitness cost at Locus 1 varies from 0 to 0.5. Among all four cases, for a fixed fitness cost the TTRs are much longer in the case of Bt cotton-2 & Bt maize-2 than in the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2 and Bt cotton-2 & Bt maize-1.

The impact of dominance of resistance

To see how the dominance at Locus 1 affects resistance evolution, the time to resistance (TTR) is derived when the dominance at Locus 1 varies from 0 to 0.8 (Fig. 3). In all four cases, i.e. Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, the TTRs decrease as the dominance increases. This is expected because a dominant
resistance generally favors the spread of resistance alleles. In the first three cases, i.e. Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, and Bt cotton-2 & Bt maize-1, where there is either a one-toxin Bt cotton or a one-toxin Bt maize, the impact of dominance on TTR is relatively weaker. In the case of Bt cotton-2 & Bt maize-2, i.e. the case of two-toxin Bt cotton and two-toxin Bt maize, the effect of dominance is much stronger than in the first three cases. Among all four cases, for a fixed dominance the TTRs are much longer in the case of Bt cotton-2 & Bt maize-2 than in the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2 and Bt cotton-2 & Bt maize-1.

The impact of natural refuge

To examine the impact of natural refuge on resistance, the time to resistance (TTR) is derived when the proportion of natural refuge varies from 0 to 0.6 (Fig. 4). In all four cases, i.e. Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, the TTRs increase as the proportion of natural refuge increases. This is expected because refuges delays resistance. For a fixed proportion of natural refuge there are large differences in the TTRs among the four cases. The larger the proportion of natural refuge, the larger the differences in TTRs. Among all four cases, for a fixed proportion of refuge the TTRs are much longer in the case of Bt cotton-2 & Bt maize-2 than in the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2 and Bt cotton-2 & Bt maize-1.

Discussion

Bt cotton is one of the earliest Bt crops that have been commercially planted in the world since 1996 [5]. While most of countries have replaced the first generation Bt cotton with newer generations [13,33], China has been planting the first generation Bt cotton expressing cry1Ac [4,23]. Our modeling results here showed that planting such a first generation Bt cotton together with Bt maize expressing similar Bt toxins could significantly increase the risk of cotton bollworm resistance to the two Bt crops as a whole.

One of the main reasons that planting the first generation Bt cotton together with Bt maize could increase the risk of cotton bollworm resistance was that cotton bollworm in China had evolved a dominant resistance to Bt cotton. A literature had shown that the degree of dominance for the resistance allele was as high as 0.79 [23]. Our simulation results here showed that such a
highly dominant resistance could spread very fast as compared with a recessive or additive resistance.

Another reason that planting the first generation Bt cotton with Bt maize could increase the risk of cotton bollworm resistance was that the Bt maize and Bt cotton contain a similar Bt toxin. When the two Bt crops contain a similar Bt toxin, resistance is most likely governed by the same locus or loci [32]. In this case, the resistance to Bt maize is superimposed on that to Bt cotton and therefore evolves much faster than without the preexisting resistance to Bt cotton. Our simulation results confirmed the above scenario. In particular, our results showed that the high initial frequency of resistance could result in a rapid increase in the frequency of resistance when Bt maize is planted and the proportion of natural refuge is reduced.

Our results showed that planting a pyramid product of two-toxin Bt maize with the first generation Bt cotton could reduce the risk of resistance as compared with planting a single-toxin Bt maize. This is consistent with our previous work in a more general setting [34]. It is also generally consistent with the results in other literatures [35,36]. However, when the first generation Bt cotton is planted, the differences between the two-toxin Bt maize and one-toxin Bt maize are limited. This is because when the first generation Bt cotton is planted, the risk of resistance in the landscape is mainly determined by that in cotton fields, even when a two-toxin Bt maize reduces the risk of resistance in maize fields.

Our results showed that planting a two-toxin Bt maize with a two-toxin Bt cotton could substantially reduce the risk of resistance as compared with planting the first generation Bt cotton. When both two-toxin Bt cotton and Bt maize are planted, the risk of resistance in the landscape is determined by two resistance alleles and the risk of resistance in the landscape is low as long as one of the frequencies of the resistance alleles is low [41]. This is exactly the case when both two-toxin Bt maize and Bt cotton are planted. Therefore, planting a two-toxin Bt maize with a two-toxin Bt cotton could effectively counter the risk of resistance in CBW.

Literatures have shown that compared to block refuge, seed mixtures of Bt and non-Bt plants can generally accelerate resistance, by reducing the effective refuge size or increasing the effective dominance of resistance [37, 39]. Our model simulations showed that this was also the case for CBW with cotton and maize seed mixture. However, the extent of acceleration in resistance depended on the rate of larval movement between plants. So far, researches about CBW
larval movement between maize plants have been very limited. Future studies on this aspect are needed in order to more accurately predict the risk of resistance associated with seed mixture.

Our modeling results here have important implications for the strategy of resistance management in cotton bollworm when Bt cotton and Bt maize are planted together. Firstly, our results suggest that in order to reduce the risk of resistance, it is much better to plant a two-toxin Bt maize than to plant an one-toxin Bt maize. Secondly, if possible, the Bt maize should avoid having a similar Bt toxin to that in Bt cotton, which can reduce the effect of preexisting resistance on the evolution of resistance to Bt maize. Thirdly, for the very best, the Bt cotton should be replaced with a two-toxin one. A pyramid two-toxin Bt cotton not only reduces the risk of resistance to the Bt cotton itself but also that to Bt maize.

Because our main aims here were to give a qualitative evaluation rather than a quantitative prediction for the risk of resistance in cotton bollworm when Bt cotton and Bt maize planted concurrently, only a simple model was used. In order to give a more accurate, quantitative prediction, the model needs to extend to include other complex factors. Those factors include the population dynamics [22], spatial structure [27], and the composition and distribution of refuges [31,35,38]. In addition to including those factors, model parameters must also be accurately estimated [32]. While our model here did not include all of those factors, it provided a basis for including them and giving a quantitative prediction for the risk of resistance in cotton bollworm in the future.

Conclusions

In northern China, planting the first generation Bt cotton with Bt maize could significantly increase the risk of CBW resistance to Bt toxins as compared to planting a pyramid two-toxin Bt cotton. The risk is the highest when both one-toxin Bt cotton and one-toxin Bt maize are planted and lowest when both two-toxin Bt cotton and two-toxin Bt maize are planted. The strategies to reducing the risk of CBW resistance include replacing the currently planted one-toxin Bt cotton with a pyramid two-toxin Bt cotton, adopting a pyramid two-toxin Bt maize, and maintaining a sufficient proportion of natural refuges.
Methods

A population genetic model was developed to analyze the adaptation of CBW to Bt cotton and Bt maize when the two types of Bt crops are planted together in northern China. In the study area, CBW has 4 generations per year [20,22]. In the first generation, the primary host crop is wheat when cotton and maize are absent. This means there is no selection for resistance to Bt in the first generation. For this reason, we only modeled three generations per year. For simplicity, we did not distinguish differences among generations and considered all model parameters as the averages across the three generations. We modeled a maximum of 120 generations or 40 years.

The effective proportions of host crops

We divided the host crops for CBW into three groups: cotton seed mixture, maize seed mixture and natural refuge (i.e. non-Bt host crops other than cotton and maize). We assumed that the effective proportions of the three groups are given, which are denoted by $P_1$, $P_2$, and $P_{nat} = 1 - P_1 - P_2$, respectively. Here the effective proportion is the proportion of planting area weighted by the relative effectiveness in producing susceptible insects [31]. Throughout this article, we referred to the “effective proportion” simply as “proportion” unless mentioned otherwise.

We assumed that the proportions of Bt and non-Bt plants in the cotton seed mixture are $P_{Bt1}$ and 1- $P_{Bt1}$, respectively, while the proportions of Bt and non-Bt plants in the maize seed mixture are $P_{Bt2}$ and 1- $P_{Bt2}$, respectively.

The Bt cotton and Bt maize products

We considered two possible products for Bt cotton: a one-toxin product expressing cry1Ac or similar protein and a two-toxin product expressing cry1Ac/cry2Ab or similar proteins, and denoted them by Bt cotton-1 and Bt cotton-2, respectively. We also considered two possible products for Bt maize: a one-toxin product expressing cry1Ab or similar protein and a two-toxin product expressing cry1Ab/cry2Aj or similar proteins, and denoted them by Bt maize-1 and Bt maize-2, respectively. There are totally four combinations of Bt cotton and Bt maize: “Bt cotton-1 & Bt maize-1”, “Bt cotton-1 & Bt maize-2”, “Bt cotton-2 & Bt maize-1” and “Bt cotton-2 & Bt maize-2”.

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The population genetic equations

We developed a general two-Bt-crop two-locus population genetic model to cover the four combinations of Bt cotton and Bt maize mentioned above. In the case of Bt cotton-1 & Bt maize-1, we assumed that the Bt-resistance is governed by a single-locus with two alleles because Bt cotton-1 and Bt maize-2 express similar Bt proteins. In this case, a single-locus model is sufficient, which can be achieved by setting no selection at the second locus in the two-locus model. In the cases of “Bt cotton-1 & Bt maize-2”, “Bt cotton-2 & Bt maize-1” and “Bt cotton-2 & Bt maize-2”, we assumed that the Bt-resistance is governed by two independently segregated loci with two alleles at each locus because Bt cotton-2 and Bt maize-2 share similar Bt proteins. Because a single-locus model is a special case of the two-locus model, we only described the two-locus model as follows.

The two-locus model is a discrete-time, frequency-dependent one in which the frequencies of genotypes are tracked from generation to generation. In the model, there are a total of nine genotypes. The key components of the model are the fitness functions on cotton seed mixture, maize seed mixture, and natural refuge, which, for convenience of expression, are denoted by $W_{M1}$, $W_{M2}$ and $W_N$, respectively.

Following [39], we divided the entire period of CBW larvae into two stages and assumed that movement between plants occurs only when a larva completes the development of the first stage. Suppose that the movement from a Bt plant to a non-Bt plant or from a non-Bt plant to a Bt plant is completely random and that the probability of larval movement between plants is the same on cotton and maize plants ($m$), then for any genotype $G$, we have

$$W_{M1}(G) = P_{1,TT} \cdot W_{B1}(G) + P_{1,NN} \cdot W_{N}(G) + P_{1,TN} \cdot [W_{B1}(G)]^q \cdot [W_{N}(G)]^{1-q} + P_{1,NT} \cdot [W_{B1}(G)]^q \cdot [W_{N}(G)]^{1-q}$$

(1)

$$W_{M2}(G) = P_{2,TT} \cdot W_{B2}(G) + P_{2,NN} \cdot W_{N}(G) + P_{2,TN} \cdot [W_{B2}(G)]^q \cdot [W_{N}(G)]^{1-q} + P_{2,NT} \cdot [W_{B2}(G)]^q \cdot [W_{N}(G)]^{1-q}$$

(2)

Where $W_{B1}(G)$, $W_{B2}(G)$ and $W_N(G)$ represent the fitnesses of genotype $G$ on Bt cotton, Bt maize and any non-Bt plants, respectively. The parameter $0 < q < 1$ designates the distribution of fitness over the two larval stages. Throughout the paper, we set $q = 0.5$.

$P_{1,TT}$, $P_{1,NN}$, $P_{1,TN}$, and $P_{1,NT}$ are the probabilities that a larva moves from Bt to Bt, Non-Bt to Non-Bt, Bt to Non-Bt and Non-Bt to Bt cotton plants, respectively. These probabilities were...
calculated as follows.

\[ P_{1,TT} = \frac{PB_{1} \cdot (m \cdot PB_{1} + 1 - m)}{f(X) \times W(X)} \times W(G) \]

Where \( PB_{1} \) is the proportion of Bt in the cotton seed mixture.

Similarly, we can obtain the corresponding probabilities for larval movement between maize plants as follows.

\[ P_{2,TT} = \frac{PB_{2} \cdot (m \cdot PB_{2} + 1 - m)}{f(X) \times W(X)} \times W(G) \]

Where \( PB_{2} \) is the proportion of Bt in the maize seed mixture.

We assumed that moths emerged from different host crops mate randomly. This assumption is reasonable because in the study area, host crops are planted by small-holder farmers and it is very common that different host crops are planted side by side in small fields [1]. With the assumption of random mating, the overall fitness of any two-locus genotype \( G \) across cotton plants, maize plants and natural refuge is expressed by the following formula:

\[ W(G) = P_{1} \times W_{1}(G) + P_{2} \times W_{2}(G) + (1 - P_{1} - P_{2}) \times W_{3}(G) \]

With the fitness function given above, the frequency of any genotype \( G \) in the next generation, \( f'(G) \), is derived by the following recursive equation:

\[ f'(G) = \frac{f(G) \times W(G)}{\sum_{X} f(X) \times W(X)} \times W(G) \]

Where \( f(X) \) and \( W(X) \) are the frequency and fitness of the two-locus genotype \( X \) in the present generation, respectively. Based on equation (1)-(6), the frequencies of all genotypes can be tracked from generation to generation. Once the frequencies of genotypes are available, the frequency of a resistance allele is obtained by summing up the frequencies of all genotypes having the resistance allele.

The model parameters

Host crop parameters

We assumed that the ratio of the effective proportion of cotton to that of maize is fixed at \( P_{1}:P_{2} = 0.64:0.36 \) and that the effective proportion of natural refuge (\( P_{nat} \)) varies from 0 to 0.6. This ratio of 0.64:0.36 was obtained based on the effective proportions of cotton, maize and natural refuge in the study area in 2016, which are 0.27, 0.15, and 0.58, respectively [23]. The proportion
of Bt in cotton was fixed at 0.75, i.e. \( PBt_1 = 0.75 \). This value was used because the transgenic cotton plants in China were \( F_2 \) hybrids between Bt and conventional cotton varieties, in which about 75% of the seeds expresses \( cry1Ac \) [4]. The proportion of Bt in maize was fixed at 0.8, i.e. \( PBt_2 = 0.8 \). This value was recommended for insects that are intrinsically less susceptible to Bt proteins in the United States [40].

**Fitness parameters**

We assumed the fitness of a two-locus genotype is multiplicative with respect to the two loci. With this assumption, we only needed to specify the fitnesses of one-locus genotypes, that is those of \( S_jS_j, S_jR_j, R_jR_j \), where \( j = 1, 2 \) stands for loci 1 and 2, respectively. The fitnesses of one-locus genotypes can be further computed based on fitness cost \( (c_j) \), dominance of fitness cost \( (d_j) \), Bt-caused mortality to susceptible CBW \( (\mu_{ij}) \), dominance of resistance \( (h_{ij}) \), and incomplete resistance \( (\sigma_{ij}) \) (Table 1). Because the Bt proteins in Bt cotton and Bt maize are similar, we assumed that the Bt-caused mortality, dominance of resistance and incomplete resistance are the same between Bt cotton and Bt maize.

The first category of fitness parameters that we need to specify is the fitness cost \( (c_j) \) and dominance of fitness cost \( (d_j) \) for \( j = 1, 2 \). Among them, \( c_1 \) is the fitness cost of resistance to \( cry1Ac \) or \( cry1Ab \), while \( c_2 \) is the fitness cost of resistance to \( cry2A_b \) or \( cry2A_j \). Experimental results have showed that fitness cost of resistance to \( cry1Ac \) might be as large as 0.54 [23]. To be conservative, here we used a smaller value of 0.36 as the default but also studied the cases when this parameter varies between 0 and 0.5. Namely, we considered \( 0 \leq c_1 \leq 0.5 \). Experimental studies have shown that there is no fitness cost to resistance to \( cry2A_b \) [33,41]. Therefore, we set \( c_2 = 0 \). For the dominance of fitness cost, we followed Jin et al. [23] and Mahon and Young [33] and set \( d_1 = d_2 = 0 \). This means that there is no fitness cost to the heterozygous genotypes.

The second category of fitness parameters that we need to specify is the Bt-caused mortality to susceptible genotypes \( (\mu_{ij}) \). For mortality caused by Bt cotton expressing \( cry1Ac \), both a lower (0.95; [14]) and higher value (1; [20,23]) were used in literature. To be conservative, we used the lower value 0.95 as the mortality to susceptible genotype caused by Bt plants expressing \( cry1Ac \) or \( cry1Ab \), i.e. \( \mu_{11} = \mu_{21} = 0.95 \). For mortality caused by Bt plants expressing \( cry2A_b \) or \( cry2A_j \), we do not have specific experimental data. However, we have experimental data for the mortality caused by Bt maize expressing \( cry1Ab + cry2A_j \), which is roughly 0.99 [42]. Based on this
experimental result and the mortality associated with cry1Ab alone, we derived the mortality associated with cry2Aj to be 0.8. Therefore, we assumed that the mortality associated with cry2Ab or cry2Aj is 0.8, i.e. \( \mu_{21} = \mu_{22} = 0.8 \).

For the dominance of resistance, Jin et al. found that resistance to Bt cotton expressing cry1Ac was dominant and that the degree of dominance was as high as 0.79 [23]. We followed this result and assumed that the dominance of resistance to cry1Ac or cry1Ab is 0.79, i.e. \( h_{11} = h_{21} = 0.79 \).

For the incomplete resistance, Jin et al. used a value of 0.505 for Bt cotton expressing cry1Ac [23]. Based on this value, we assumed that the incomplete resistance to cry1Ac or cry1Ab is 0.505, i.e. \( \sigma_{11} = \sigma_{21} = 0.505 \). Because there was no experimental data to determine the dominance of resistance to cry2Ab or cry2Aj in China, we followed Edwards et al. [30] and used the value of 0.25, i.e. \( h_{12} = h_{22} = 0.25 \). Also because there was no experimental data to determine the value of incomplete resistance to cry2Ab or cry2Aj, we adopted a conservative method ([32]) and assumed that the resistance is complete, i.e. \( \sigma_{12} = \sigma_{22} = 0 \). All fitness parameters were summarized in Table 2.

The larval movement parameter

The probability of larval movement between plants depends on several factors, such as the insect’s tendency and ability to move, the distance between plants and the growing stage of the plants. So far there is no evidence of significant CBW larval movement among plants. However, because larval movement generally increases the risk of resistance evolution [39], we adopted a conservative approach and considered a 10% larval movement between plants. Namely, we assumed that the probability that a CBW larva moves from one plant to another during the entire larvae stage is 0.1, i.e. \( m=0.1 \) (Table 2).

Initial frequencies of resistance alleles

A study showed that the frequency of resistance allele to Bt cotton in northern China was 0.1 in 2016 [23]. We used this value as the initial frequency of resistance allele associated with Locus 1. Because Bt maize has not yet planted in China, there is no way to determine the initial frequency of resistant allele empirically. Therefore, we used a theoretical value of 0.001 as the initial frequency of resistance allele associated with Locus 2 (see e.g. [27]).

Time to resistance (TTR)
We regarded the threshold of resistance risk as the frequency of a resistance allele reaches 50%. In the case of one-toxin Bt cotton or Bt maize where resistance is governed by a single locus, we defined the “time to resistance” (TTR) as the time it takes for the frequency of the corresponding single resistance allele to reach 50%. In the case of two-toxin Bt cotton or Bt maize where resistance is governed by two loci, we defined the “time to resistance” (TTR) as the time it takes for the frequencies of both resistance alleles to reach 50%.

**Computer programing**

The model was programmed with C++ and ran at a personal computer.

**Declarations**

**Abbreviations**

CBW: Cotton bollworm

TTR: Time to resistance

Bt maize-1: Bt maize containing only one Bt toxin

Bt maize-2: Bt maize containing two Bt toxins

Bt cotton-1: Bt cotton containing only one Bt toxin

Bt cotton-2: Bt cotton containing two Bt toxins

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**Authors' contributions**

W.W. and F.X. conducted the model analysis; Y.H. designed the study and wrote the article; H.F.
and P.W. wrote the paper.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its additional information files.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Figure legends

**Figure 1** Temporal patterns in the frequency of resistance alleles. **A.** one-locus model for Bt cotton-1 & Bt maize-1. **B.** two-locus model for Bt cotton-1 & Bt maize-2. **C.** two-locus model for Bt cotton-2 & Bt maize-1. **D.** two-locus model for Bt cotton-2 & Bt maize-2. $R_1$ and $R_2$ represent the resistance alleles at loci 1 and 2, respectively. In this figure, $P_1=0.64$, $P_2=0.36$, $P_{nat}=0$. The rest of parameters are set at default.
Figure 2 Time to resistance versus fitness cost at locus 1. The four lines with none, star, circle and diamond marks correspond to the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, respectively. The horizontal axis is the fitness cost at Locus 1 ($c_1$) and the vertical axis is the time to resistance (TTR). In this figure, $P_1=0.64$, $P_2=0.36$, $P_{nat}=0$. The rest of parameters are set at default.

Figure 3 Time to resistance versus dominance of resistance at locus 1. The four lines with none, star, circle and diamond marks correspond to the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, respectively. The horizontal axis is the dominance of resistance at Locus 1 ($h_{11}$) and the vertical axis is the time to resistance (TTR). In this figure, $P_1=0.64$, $P_2=0.36$, $P_{nat}=0$. The rest of parameters are set at default.
Figure 4 Time to resistance versus proportion of natural refuge. The four lines with none, star, circle and diamond marks correspond to the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, respectively. The horizontal axis is the proportion of natural refuge ($P_{nat}$) and the vertical axis is the time to resistance (TTR). The rest of parameters are set at default.

Table 1 The formulae for fitness computation.

| Notation      | Meaning                                                                 | formula |
|---------------|-------------------------------------------------------------------------|---------|
| $W_N(S_jS_j)$ | Fitness of $S_jS_j$ on non-Bt plants (j=1,2)                            | 1       |
| $W_N(S_jR_j)$ | Fitness of $S_jR_j$ on non-Bt plants (j=1,2)                            | $1 - d_j \cdot c_j$ |
| $W_N(R_jR_j)$ | Fitness of $R_jR_j$ on non-Bt plants (j=1,2)                            | $1 - c_j$ |
| $W_{B1}(S_jS_j)$ | Fitness of $S_jS_j$ on Bt cotton (j=1,2)                        | $1 - \mu_{1j}$ |
| $W_{B1}(S_jR_j)$ | Fitness of $S_jR_j$ on Bt cotton (j=1,2)                        | $(1 - \mu_{1j}) + h_{1j} \cdot (\mu_{1j} - \sigma_{1j})$ |
| $W_{B1}(R_jR_j)$ | Fitness of $R_jR_j$ on Bt cotton (j=1,2)                        | $1 - \sigma_{1j}$ |
| $W_{B2}(S_jS_j)$ | Fitness of $S_jS_j$ on Bt maize (j=1,2)                            | $1 - \mu_{2j}$ |
| $W_{B2}(S_jR_j)$ | Fitness of $S_jR_j$ on Bt maize (j=1,2)                            | $(1 - \mu_{2j}) + h_{2j} \cdot (\mu_{2j} - \sigma_{2j})$ |
| $W_{B2}(R_jR_j)$ | Fitness of $R_jR_j$ on Bt maize (j=1,2)                            | $1 - \sigma_{2j}$ |
Table 2 The notation, meaning and default value or range of parameters used in model simulation.

| Notation | Meaning                                                                 | Default/range | Reference   |
|----------|------------------------------------------------------------------------|---------------|-------------|
| \(p_{10}\) | Initial frequency of resistance allele at Locus 1                       | 0.1           | [23]        |
| \(p_{20}\) | Initial frequency of resistance allele at Locus 2                       | 0.001         | [27]        |
| \(m\)    | Probability of larval movement between plants                           | 0.1           | here        |
| \(\mu_{11}\) | Mortality of \(S_1S_1\) caused by Bt cotton containing \(cry1Ac\) or \(cry1Ab\) | 0.95          | [14,20,23]  |
| \(\mu_{12}\) | Mortality of \(S_2S_2\) caused by Bt cotton containing \(cry2Ab\) or \(cry2Aj\) | 0.8           | [41]        |
| \(c_1\)  | Fitness cost of \(R_1R_1\)                                            | 0.36          | [23]        |
| \(c_2\)  | Fitness cost of \(R_2R_2\)                                            | 0             | [33,39]     |
| \(d_j\)  | Dominance of fitness cost for \(R_j\) \((j=1,2)\)                      | 0             | [23,33]     |
| \(\sigma_{11}\) | Incomplete resistance of \(R_1R_1\) on Bt cotton                       | 0.505         | [23]        |
| \(\sigma_{12}\) | Incomplete resistance of \(R_2R_2\) on Bt cotton                       | 0             | [32]        |
| \(h_{11}\) | Dominance of resistance for \(R_1\) on Bt cotton                       | 0.79          | [23]        |
| \(h_{12}\) | Dominance of resistance for \(R_2\) on Bt cotton                       | 0.25          | [30]        |

Note: The parameters associated with Bt maize \((\mu_{2j}, \sigma_{2j} \text{ and } h_{2j})\) are the same as those with Bt cotton: \(\mu_{2j} = \mu_{1j}, \sigma_{2j} = \sigma_{1j}, h_{2j} = h_{1j}, \text{ for } j=1,2.\)