Preexisting resistance in cotton bollworm increases the risk of resistance to the concurrently planted Bt cotton and Bt maize

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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 used a two-locus population genetic model to assess the risk of resistance in CBW when Bt cotton and Bt maize are planted concurrently. Results of model simulations showed that planting Bt maize together with Bt cotton significantly increases the risk of resistance if Bt cotton and Bt maize share a similar Bt toxin. The risk of resistance is higher in the case of one-toxin Bt maize than in the case of two-toxin Bt maize. Parameters associated with the preexisting resistance in CBW all could impact on the risk of resistance but with different extents. Among them, the most notable ones are the dominance of resistance and fitness cost, which can dramatically affect the risk of resistance, especially when the proportion of natural refuges is reduced.

Conclusions: We concluded that the preexisting resistance in CBW to Bt cotton can significantly increase the risk of resistance when Bt maize and Bt cotton are planted concurrently and that using two-toxin Bt cotton and maize instead of one-toxin ones are needed in order to reduce the risk of resistance.

Keywords: Bt maize, Bt cotton, Cotton bollworm, Regional resistance, Model prediction
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 difference between Bt maize-1 and Bt maize-2

In the absence of cotton plants, there are both qualitative and quantitative differences in the evolution of resistance between the case of Bt maize-1 and that of Bt maize-2 (Fig. 1). In the case of Bt maize-1, where resistance is governed by a single locus, the frequency of resistance allele $R_1$ increases from its initial value before tending to a stable level (Fig. 1a). However, in the case of Bt maize-2, where resistance is governed by two loci, the frequency of resistance allele $R_1$ decreases from its initial value (Fig. 1b). In the case of Bt maize-2, the frequency of $R_2$ does not increase from its initial value within 90 generations.
Figure 1 Changes in the frequency of resistance alleles over time.  

a Bt maize-1: Bt maize contains only one toxin and resistance is governed by one locus.  
b Bt maize-2: Bt maize contains two toxins and resistance is governed by two loci.  
$R_1$ and $R_2$ represent the resistance alleles at loci 1 and 2, respectively. In this figure, $P_1=0$, which means that cotton plants are absent. The rest of parameters are set at default.

The impact of Bt cotton

When Bt cotton is planted together with Bt maize, the same qualitative differences in the evolution of resistance between the case of Bt maize-1 and that of Bt maize-2 are observed (Fig. 2). In the case of Bt maize-1, the frequency of resistance allele $R_1$ increases from its initial value before tending to a stable level (Fig. 2a), while in the case of Bt maize-2, the frequency of resistance allele $R_1$ decreases from its initial value (Fig. 2b).

Compared to without Bt cotton, the frequency of resistance allele $R_1$ evolves faster and higher
in both cases of Bt maize-1 and Bt maize-2, while the evolution in the frequency of resistance allele $R_2$ does not increase over time. This is because resistance to Bt cotton is governed by Locus 1 only, so adding Bt cotton only affects the evolution of $R_1$, not that of $R_2$.

**Figure 2** Changes in the frequency of resistance alleles over time. **a** Bt cotton & Bt maize-1 where Bt cotton and Bt maize contain a similar toxin and resistance is governed by one locus. **b** Bt cotton & Bt maize-2 where Bt maize contains two toxins in which one is shared with Bt cotton and resistance is governed by two loci. In this figure, $R_1$ and $R_2$ represent the resistance alleles at loci 1 and 2, respectively. Parameters are all set at default.

**The impact of fitness cost**

To investigate the impact of fitness cost at Locus 1 on resistance evolution, the time to resistance (TTR) is derived when the fitness cost at Locus 1 varies from 0 to 0.5 (**Fig. 3**). In the case of Bt
cotton & Bt maize-1, when the fitness cost at Locus 1 varies from 0 to 0.22, the TTR increases from 20 to >90 generations. In the case of Bt cotton & Bt maize-2, when the fitness cost at Locus 1 varies from 0 to 0.17, the TTR increases from 24 to >90 generations.

With the same fitness cost at Locus 1, the TTR is always shorter in the case of Bt cotton & Bt maize-1 than that in the case of Bt cotton & Bt maize-2. The higher the fitness cost is, the larger the difference. For example, when the fitness cost is zero the difference in TTR between the two cases is just 4 generations. When the fitness cost is 0.2, the difference is greater than 45 generations.

Figure 3 Time to resistance versus fitness cost at locus 1. Bt maize-1: Bt maize contains only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins and resistance is governed by two loci. The rest of parameters are set at default.

The impact of incomplete resistance
To investigate the impact of incomplete resistance at Locus 1 on resistance evolution, the TTR is derived by simulation when the incomplete resistance at Locus 1 varies from 0 to 0.5 (Fig. 4).

When the default fitness cost at Locus 1, i.e. 0.36, is used, the differences between the two cases cannot be demonstrated because the TTRs are infinite in both cases of Bt cotton & Bt maize-1 and Bt cotton & Bt maize-2. For this reason, a hypothetical value for the fitness cost at Locus 1, i.e. 0, is used. In the case of Bt cotton & Bt maize-1, when the incomplete resistance at Locus 1 varies from 0 to 0.5, the TTR increases from 11 to 20 generations. In the case of Bt cotton & Bt maize-2, when the incomplete resistance at Locus 1 varies from 0 to 0.5, the TTR increases from 14 to 27 generations.

With the same incomplete resistance at Locus 1, the TTR is always shorter in the case of Bt cotton & Bt maize-1 than that in the case of Bt cotton & Bt maize-2. The higher the incomplete resistance is, the smaller the difference.

**Figure 4** Time to resistance versus incomplete resistance at locus 1. Bt maize-1: Bt maize contains only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins.
and resistance is governed by two loci. In this figure, the fitness cost at Locus 1 is zero, i.e. \( c_1 = 0 \).

The rest of parameters are set at default.

The impact of dominance of resistance

To investigate the impact of dominance of resistance at Locus 1 on resistance evolution, the TTR is derived when the dominance of resistance at Locus 1 varies from 0 to 1 (Fig. 5). For the same reason as described in the previous subsection, the fitness cost at Locus 1 is set at 0. In the case of Bt cotton & Bt maize-1, when the dominance of resistance at Locus 1 varies from 0 to 1, the TTR decreases from 51 to 18 generations. In the case of Bt cotton & Bt maize-2, when the dominance of resistance at Locus 1 varies from 0 to 1, the TTR decreases from 64 to 22 generations.

With the same dominance of resistance at Locus 1, the TTR is always shorter in the case of Bt cotton & Bt maize-1 than in the case of Bt cotton & Bt maize-2. The higher the dominance of resistance is, the smaller the difference.
Figure 5 Time to resistance versus dominance of resistance at locus 1. Bt maize-1: Bt maize contains only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins and resistance is governed by two loci. In this figure, the fitness cost at Locus 1 is zero, i.e. $c_1=0$. The rest of parameters are set at default.

The impact of initial frequency of resistance allele

To investigate the impact of the initial frequency of resistance allele at locus 1 on resistance evolution, the TTR is derived when the initial frequency of $R_1$ varies from 0.001 to 0.1 (Fig. 6).

The fitness cost at Locus 1 is set at 0. In the case of Bt cotton & Bt maize-1, when the initial frequency of resistance allele at Locus 1 varies from 0.001 to 0.1, the TTR decreases from 50 to 19 generations. In the case of Bt cotton & Bt maize-2, when the initial frequency of resistance allele at Locus 1 varies from 0.001 to 0.1, the TTR decreases from 62 to 24 generations.
With the same initial frequency of resistance allele at Locus 1, the TTR is always shorter in the case of Bt cotton & Bt maize-1 than that in the case of Bt cotton & Bt maize-2. The higher the initial frequency of resistance allele is, the smaller the difference.

**Figure 6** Time to resistance versus initial frequency of resistance allele at locus 1. Bt maize-1: Bt maize contains only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins and resistance is governed by two loci. In this figure, the fitness cost at Locus 1 is zero, i.e. $c_1=0$. The rest of parameters are set at default.

**The impact of proportion of natural refuge**

To investigate the impact of the proportion of natural refuge on resistance evolution, the TTR is derived when the proportion of natural refuge varies from 0 to 0.5 (Fig. 7). When the proportion of natural refuge varies from 0 to 0.5, the proportion of cotton and maize as a whole varies from 0.5 to 0. To be specific, the ratio of proportion of cotton to that of maize is fixed at 0.64 to 0.36 when proportion of natural refuge varies. In the case of Bt cotton & Bt maize-1, when the proportion of
natural refuge varies from 0 to 0.46, the TTR increases from 6 to >90 generations. In the case of Bt cotton & Bt maize-2, when the proportion of natural refuge varies from 0 to 0.37, the TTR increases from 6 to >90 generations.

With the same proportion of natural refuge, the TTR is always shorter in the case of Bt cotton & Bt maize-1 than that in the case of Bt cotton & Bt maize-2. The higher the proportion of natural refuge is, the larger the difference. For example, when the proportion of natural refuge is zero the difference in TTR between the two cases is just 2 generations. When the proportion of natural refuge is 0.37, the difference is greater than 75 generations.

**Figure 7** Time to resistance versus proportion of natural refuge. Bt maize-1: Bt maize contains only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins and resistance is governed by two loci. In this figure, the proportion of natural refuge ($P_{nat}$) varies from 0 to 0.5, while the proportion of cotton and that of maize are $P_1=0.64*(1-P_{nat})$ and $P_2=0.36*(1-P_{nat})$, respectively. The rest of parameters are set at default.
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 upgraded the first generation Bt cotton to the second and third generations [13,33], China is still planting the first generation Bt cotton expressing Cry1Ac [4,23]. Our modeling results here showed that planting Bt maize together with such a first generation Bt cotton could significantly increase the risk of cotton bollworm resistance to the two Bt crops as a whole.

One of the main reasons that planting Bt maize together with the first generation Bt cotton 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 was as high as 0.79 [23]. Consistent with theoretical and empirical results elsewhere [18,23,34], our simulation results here showed that such a dominant resistance could evolve much faster than a recessive one.

Another reason that planting Bt maize together with the first generation Bt cotton 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.

While the preexisting dominant resistance could increase the risk of resistance when Bt
cotton and Bt maize planted together, our simulation results showed that fitness cost to the resistance might dramatically decrease the risk. In particular, our results showed that when fitness cost was sufficiently large, the frequency of resistance allele might not continue to increase from its initial value and thus never reach the critical level of resistance risk. This is because when the selection for resistance from planting Bt plants just matches the selection against resistance due to fitness cost, an equilibrium may be reached at which the frequency of resistance remains constant [12]. A previous study indicated that the fitness cost to the preexisting resistance in cotton bollworm was as high as 0.54 [23]. Such a high fitness cost, if persists, should dramatically reduce the risk of resistance when Bt maize and Bt cotton are planted together.

Our results showed that planting a pyramid product of two-toxin Bt maize could substantially reduce the risk of resistance than a single-toxin Bt maize, either planted alone or planted together with Bt cotton. This is consistent with our previous work in a more general setting [35]. It is also generally consistent with the results in other literatures [36,37]. In particular, our results showed that when a two-toxin Bt maize variety is planted with an one-toxin Bt cotton that expresses a similar toxin to those in Bt maize, the risk of resistance to the two Bt crops is solely determined by the risk of resistance to Bt cotton.

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 dominance of resistance [38]. Our model simulations found that this was also the case for CBW with seed mixture of Bt and non-Bt cotton and/or seed mixture of Bt and non-Bt maize. 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 \[31,35,39\]. 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 together with Bt maize could
significantly increase the risk of CBW resistance to Bt toxins. The risk was higher in the case of one-toxin Bt maize than in the case of pyramid two-toxin Bt maize. The risk of resistance could be extremely high if natural refuges were absent. The approaches 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 instead of one-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 90 generations or 30 years.

The effective proportions of Bt cotton, Bt maize and refuges

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

We assumed that cotton plants consist of Bt and non-Bt plants in seed mixture, with the proportions of Bt and non-Bt plants being $PBt_1$ and 1-$PBt_1$, respectively. Similarly, we assumed that maize plants consist of Bt and non-Bt plants in seed mixture, with the proportions being $PBt_2$ and 1-$PBt_2$, respectively. We also assumed that the host crops other than cotton and maize are all non-Bt, which serve as the “natural refuge” for CBW. Based on the above notations, the total proportion of all types of non-Bt host plants or the total proportion of refuges is

$$P_{\text{ref}} = 1 - PBt_1 \times P_1 - PBt_2 \times P_2$$  \hspace{1cm} (1)

We adopted a result from Jin et al. [23] and set $P_1=0.27$ and $P_2=0.15$ as the default (Table 1). Namely, the effective proportions of cotton, maize and natural refuge are 0.27, 0.15, and 0.58, respectively. These two values were derived from real data in northern China in 2016 [23]. In addition, we considered a theoretical case where the proportion of cotton and maize varies from 0.42 to 1, or that of natural refuges varies from 0 to 0.58. We assumed that the ratio of the proportion of cotton to that of maize is fixed, which is approximately 0.64 to 0.36.

The proportion of Bt in cotton was fixed at 0.75, i.e. $PBt_1=0.75$ (Table 1). 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].

The population genetics
In the studying area, the Bt cotton variety that has been planted is an one-toxin product expressing \textit{cry}1\textit{Ac} \cite{20,23}. Therefore, we developed our model based on this specific one-toxin Bt cotton. Bt maize has not been planted commercially when this article is written. The Bt maize varieties that may potentially be used include an one-toxin product expressing \textit{cry1}\textit{Ab} \cite{41} and a two-toxin product expressing \textit{cry1}\textit{Ab}/\textit{cry2}\textit{Aj} \cite{42}. In this article, we considered two cases for the Bt maize varieties that might be potentially used. In Case 1, the Bt maize expresses \textit{cry1}Ab or a similar Bt protein, which is denoted by “Bt maize-1”. In Case 2, the Bt maize expresses \textit{cry1}Ab/\textit{cry2}Aj or similar Bt proteins, which is denoted by “Bt maize-2”.

In the case of Bt maize-1, we assumed that resistance to Bt cotton and Bt maize is governed by the same single locus and used a single-locus model to simulate the evolution of resistance. In the case of Bt maize-2, we assumed that resistance to Bt cotton and Bt maize is governed by two loci that are independently segregated and used a two-locus model to simulate the evolution of resistance. Because the single-locus model can be described as a special case of the two-locus model, here we only described the two-locus model.

The two-locus population genetic model used here is a discrete-time, frequency-dependent one in which the frequencies of genotypes are tracked from generation to generation. In the two-locus model, there are a total of nine genotypes. These genotypes can be generally put in the form of $X_1Y_1X_2Y_2$, where $X_i$ and $Y_i$ are either the resistance allele $R_i$ or susceptible allele $S_i$, with the subscript $i$ indicating Locus 1 or 2. 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 $W_1$, $W_2$ and $W_3$, respectively.

We assumed that the fitness is multiplicative with respect to the two loci \cite{27}. Namely, the
fitness of a two-locus genotype $X_1Y_1X_2Y_2$ is the product of the fitnesses of the two one-locus genotypes:

$$W_j(X_1Y_1X_2Y_2) = W_j(X_1Y_1) \times W_j(X_2Y_2) \quad (2)$$

Where $W_j$ ($j=1, 2, 3$) is the fitness over the entire larval period. Because $W_1$ and $W_2$ are the fitnesses on seed mixture, they depend on the ratio of Bt to non-Bt plants in the seed mixture and larval movement between plants. Following [43], 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 single locus genotype $g$, we have

$$W_1(g) = P_{1,TT} \cdot W_{B1}(g) + P_{1,NN} \cdot W_N(g) + P_{1,NT} \cdot [W_{B1}(g)]^q \cdot [W_N(g)]^{1-q} + P_{1,NT} \cdot [W_N(g)]^q \cdot [W_{B1}(g)]^{1-q} \quad (3)$$

$$W_2(g) = P_{2,TT} \cdot W_{B2}(g) + P_{2,NN} \cdot W_N(g) + P_{2,NT} \cdot [W_{B2}(g)]^q \cdot [W_N(g)]^{1-q} + P_{2,NT} \cdot [W_N(g)]^q \cdot [W_{B2}(g)]^{1-q} \quad (4)$$

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

$P_{1,TT}$, $P_{1,NN}$, $P_{1,NT}$, 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 could be explicitly calculated as follows.

$$P_{1,TT} = PBt_1 \cdot (M \cdot PBt_1 + 1 - M), \quad P_{1,NN} = (1 - PBt_1) \cdot [M \cdot (1 - PBt_1) + 1 - M]$$
\[ P_{1,TN} = P_{Bt_1} \cdot M \cdot (1 - P_{Bt_1}), \quad P_{1,NT} = P_{1,TN} \quad (5) \]

Where \( P_{Bt_1} \) 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.

\[ P_{2,TT} = P_{Bt_2} \cdot (M \cdot P_{Bt_2} + 1 - M), \quad P_{2,NN} = (1 - P_{Bt_2}) \cdot [M \cdot (1 - P_{Bt_2}) + 1 - M] \]

\[ P_{2,TN} = P_{Bt_2} \cdot M \cdot (1 - P_{Bt_2}), \quad P_{2,NT} = P_{2,TN} \quad (6) \]

Where \( P_{Bt_2} \) is the proportion of Bt in the maize seed mixture.

The fitnesses of single-locus genotypes on non-Bt, Bt cotton and Bt maize plants, that is \( W_N(g), W_{B1}(g) \) and \( W_{B2}(g) \), can be further expressed as functions of Bt-caused mortality \( (\mu_i) \), dominance of resistance \( (h_i) \), incomplete resistance \( (\sigma_i) \), fitness costs \( (c_i) \) and dominance of fitness cost \( (d_i) \), where the subscript \( i \) stands for Loci 1 or 2 (Table 1). We assumed that there is no cross-resistance among different loci. The detailed expressions of fitness functions are given as follows.

On non-Bt plants:

\[ W_N(S_iS_i) = 1, \quad W_N(R_iR_i) = 1 - c_i, \quad W_N(S_iR_i) = 1 - d_i \times c_i, \quad i = 1, 2. \quad (7) \]

On Bt cotton plants:

\[ W_{B1}(S_1S_1) = 1 - \mu_1, \quad W_{B1}(R_1R_1) = 1 - \sigma_1, \]

\[ W_{B1}(S_1R_1) = W_{B1}(S_1S_1) + h_1 \times [W_{B1}(R_1R_1) - W_{B1}(S_1S_1)] \quad (8) \]

\[ W_{B1}(S_2S_2) = W_N(S_2S_2), \quad W_{B1}(S_2R_2) = W_N(S_2R_2), \quad W_{B1}(R_2R_2) = W_N(R_2R_2) \quad (9) \]

On Bt maize plants, in the case of Bt maize-1:

\[ W_{B2}(S_1S_1) = 1 - \mu_1, \quad W_{B2}(R_1R_1) = 1 - \sigma_1, \]

\[ W_{B2}(S_1R_1) = W_{B2}(S_1S_1) + h_1 \times [W_{B2}(R_1R_1) - W_{B2}(S_1S_1)] \quad (10) \]
On Bt maize plants, in the case of Bt maize-2:

\[ W_{B2}(S_iS_i) = W_N(S_2S_2), \quad W_{B2}(S_iR_i) = W_N(S_2R_2) \quad (11) \]

\[ W_{B2}(S_iR_i) = 1 - \mu_i, \quad W_{B2}(R_iR_i) = 1 - \sigma_i, \]

\[ W_{B2}(S_iR_i) = W_{B2}(S_iS_i) + h_i \times [W_{B2}(R_iR_i) - W_{B2}(S_iS_i)], \quad i = 1, 2. \quad (12) \]

Note that in Equations (9) and (11), the fitnesses of genotypes associated with Locus 2 on Bt plants equal to those on non-Bt plants because the resistance is governed by only Locus 1.

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. 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:

\[ W(G) = P_1 \times W_1(G) + P_2 \times W_2(G) + (1 - P_1 - P_2) \times W_3(G) \quad (13) \]

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:

\[ f'(G) = [f(G) \times W(G)] / [\sum_X f(X) \times W(X)], \quad \text{for any } G \quad (14) \]

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)-(14), 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 fitness parameters
The fitness parameters on non-Bt host plants that we needed to specify are the fitness cost per locus ($c_1$ and $c_2$) and the dominance of fitness cost ($d_1$ and $d_2$). Among them, $c_1$ is the fitness cost of resistance to Cry1Ac or a similar Bt protein, while $c_2$ is the fitness cost of resistance to Cry2Ab or a similar Bt protein. 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 while conducted sensitivity analysis by allowing this parameter varying 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 Cry2Ab [33,44]. 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.

On Bt cotton, the main parameters we need to specify are those associated with Locus 1, which include the Bt-caused mortality to susceptible CBW ($\mu_1$), the dominance of resistance ($h_1$), and the incomplete resistance ($\sigma_1$). About Bt-caused mortality to susceptible CBW associated with Cry1Ac, both a lower (0.953; [14]) and higher value (1; [20,23]) were used in literatures. To be conservative, we used the lower value as the mortality of susceptible insect on Bt cotton, i.e. $\mu_1 \approx 0.95$. For the dominance of resistance, Jin et al. found a dominant resistance gene for which the degree of dominance was as high as 0.79 [23]. We followed this result and set $h_1 = 0.79$. For the incomplete resistance, Jin et al. used a value of 0.505 [23]. Based on this value, we set $\sigma_1 = 0.505$.

On Bt maize, we assumed that fitness parameters associated with Locus 1 are the same as those associated with Locus 2. Therefore, the main parameters that we need to specify are those associated with Locus 2 in the case of two-toxin Bt maize, which include $\mu_2$, $h_2$, and $\sigma_2$. To
specify $\mu_2$, we studied the experimental results concerning the Bt-caused mortality of susceptible
CBWs associated with Bt maize expressing $\text{cry1Ab/cry2Aj}$ [42]. In that experiment, the
mortalities of susceptible CBWs on Bt maize and a control were 0.9889 and 0.1278, or the
survivals were 0.0111 and 0.8722, respectively. Based on these two survival values, the corrected
survival of susceptible CBWs on Bt maize then is calculated as $1 - (0.8722 - 0.0111)/$
0.8722 ≈ 0.01. Based on this calculation, we set $W_{B2}(S_1S_1S_2S_2) = (1 - \mu_1)(1 - \mu_2) \approx 0.01$.
When $\mu_1 = 0.95$, it is easy to find that $\mu_2 = 0.8$. Therefore, we set the 0.8 as the default value
for $\mu_2$. Because there was no Bt-plant-based data to determine the dominance of resistance
associated with Cry2Ab or a similar protein in China, we followed Edwards et al. [30] and set
$h_2=0.25$ as the default value. Also because there was no Bt-plant-based data to determine the value
of incomplete resistance, we adopted a conservative method (see e.g. [32]) and set the incomplete
resistance associated with Cry2Ab at $\sigma_2 = 0$.

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 [43], 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 1).
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%. When resistance is governed by a single locus, we referred to the time it takes for the frequency of the resistance allele to reach 50% as the “time to resistance” (TTR). When resistance is governed by two loci, we referred to the time it takes for both of the frequencies of resistance alleles to reach 50% as the TTR.

Computer programing

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

Table 1 The notation, meaning and default value or range of parameters used in model simulation..

| Notation | Meaning                     | Default/range | Reference |
|----------|-----------------------------|---------------|-----------|
| P_1      | Effective proportion of cotton | 0.27          | [23]      |
| P_2      | Effective proportion of maize | 0.15          | [23]      |
| PBt_1    | Proportion of Bt in cotton  | 0.75          | [4]       |
| Parameter | Description                                                                 | Value | Reference |
|-----------|------------------------------------------------------------------------------|-------|-----------|
| PB_{t2}   | Proportion of Bt in maize                                                    | 0.8   | [40]      |
| P_{nat}   | Effective proportion of natural refuge (1 - P_{1} - P_{2})                   | 0.58  | -         |
| 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_{1}   | Mortality of S_{1}S_{1} on Bt cotton and Bt maize with Cry1Ac or similar protein | 0.95  | [14,20,23]|
| \mu_{2}   | Mortality of S_{2}S_{2} on Bt maize with Cry2Ab or similar protein           | 0.8   | [44]      |
| c_{1}     | Fitness cost of R_{1}R_{1}                                                   | 0.36  | [23]      |
| c_{2}     | Fitness cost of R_{2}R_{2}                                                   | 0     | [33,43]   |
| d_{i}     | Dominance of fitness cost for R_{i} (i=1,2)                                  | 0     | [23,33]   |
| \sigma_{1}| Incomplete resistance of R_{1}R_{1}                                         | 0.505 | [23]      |
| \sigma_{2}| Incomplete resistance of R_{2}R_{2}                                         | 0     | [32]      |
| h_{1}     | Dominance of resistance at Locus 1                                           | 0.79  | [23]      |
| h_{2}     | Dominance of resistance at Locus 2                                           | 0.25  | [30]      |

### 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
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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.

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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