

# Modeling the Evolution of Resistance in Cotton Bollworm to Concurrently Planted Bt Cotton and Bt Maize in China

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## Research article

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

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30 **Abstract**

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

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

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

49  
50 **Keywords:** Bt maize, Bt cotton, Cotton bollworm, Time to resistance, Model prediction

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## 58 **Background**

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

63 One of the main threats to the long-term use of Bt crops is that target pests may evolve  
64 resistance to Bt toxin. To date, field resistance has been observed in the target pests of both Bt  
65 cotton and Bt maize [6-11]. Resistance can be affected by various ecological and genetic factors  
66 [12]. Among them, the most important factor is excessive planting of Bt plants [13], but other  
67 factors such as insect susceptibility to the Bt toxin, dominance of resistance, and strategy of  
68 resistance management are also very important [14-17].

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

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

86 Because maize has been one of the main categories of natural refuges for CBW in northern

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

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

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

## 111 **Results**

### 112 **The temporal patterns in the frequency of resistance alleles**

113 In the case of Bt cotton-1 & Bt maize-1 where resistance is governed by a single locus, the only  
114 resistance allele  $R_1$  increases in frequency rapidly over time (Fig. 1A). Because of fitness cost, the

115 frequency of resistance allele goes to a level less than 100%. The rapid increase in the frequency  
116 of resistance alleles is caused by a combination of dominant inheritance, high initial frequency and  
117 lack of natural refuge.

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

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

128

### 129 **The impact of fitness cost**

130 To see how the fitness cost at Locus 1 affects resistance evolution, the time to resistance (TTR) is  
131 derived when the fitness cost at Locus 1 varies from 0 to 0.5 (Fig. 2). In all four cases, i.e. Bt  
132 cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt  
133 maize-2, the TTRs increase as the fitness cost increases. This is expected because fitness cost acts  
134 against resistance. However, in all four cases, the effect of the fitness cost at Locus 1 appears very  
135 limited. For example, in the case of Bt cotton-2 & Bt maize-2, the TTR just increases from 31 to  
136 32 generations when the fitness cost at Locus 1 varies from 0 to 0.5. Among all four cases, for a  
137 fixed fitness cost the TTRs are much longer in the case of Bt cotton-2 & Bt maize-2 than in the  
138 cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2 and Bt cotton-2 & Bt maize-1.

139

### 140 **The impact of dominance of resistance**

141 To see how the dominance at Locus 1 affects resistance evolution, the time to resistance (TTR) is  
142 derived when the dominance at Locus 1 varies from 0 to 0.8 (Fig. 3). In all four cases, i.e. Bt  
143 cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt  
144 maize-2, the TTRs decrease as the dominance increases. This is expected because a dominant

145 resistance generally favors the spread of resistance alleles. In the first three cases, i.e. Bt cotton-1  
146 & Bt maize-1, Bt cotton-1 & Bt maize-2, and Bt cotton-2 & Bt maize-1, where there is either a  
147 one-toxin Bt cotton or a one-toxin Bt maize, the impact of dominance on TTR is relatively weaker.  
148 In the case of Bt cotton-2 & Bt maize-2, i.e. the case of two-toxin Bt cotton and two-toxin Bt  
149 maize, the effect of dominance is much stronger than in the first three cases. Among all four cases,  
150 for a fixed dominance the TTRs are much longer in the case of Bt cotton-2 & Bt maize-2 than in  
151 the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2 and Bt cotton-2 & Bt maize-1.

152

### 153 **The impact of natural refuge**

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

163

### 164 **Discussion**

165 Bt cotton is one of the earliest Bt crops that have been commercially planted in the world since  
166 1996 [5]. While most of countries have replaced the first generation Bt cotton with newer  
167 generations [13,33], China has been planting the first generation Bt cotton expressing *cry1Ac*  
168 [4,23]. Our modeling results here showed that planting such a first generation Bt cotton together  
169 with Bt maize expressing similar Bt toxins could significantly increase the risk of cotton  
170 bollworm resistance to the two Bt crops as a whole.

171 One of the main reasons that planting the first generation Bt cotton together with Bt maize  
172 could increase the risk of cotton bollworm resistance was that cotton bollworm in China had  
173 evolved a dominant resistance to Bt cotton. A literature had shown that the degree of dominance  
174 for the resistance allele was as high as 0.79 [23]. Our simulation results here showed that such a

175 highly dominant resistance could spread very fast as compared with a recessive or additive  
176 resistance.

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

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

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

200 Literatures have shown that compared to block refuge, seed mixtures of Bt and non-Bt plants  
201 can generally accelerate resistance, by reducing the effective refuge size or increasing the  
202 effective dominance of resistance [37, 39]. Our model simulations showed that this was also the  
203 case for CBW with cotton and maize seed mixture. However, the extent of acceleration in  
204 resistance depended on the rate of larval movement between plants. So far, researches about CBW

205 larval movement between maize plants have been very limited. Future studies on this aspect are  
206 needed in order to more accurately predict the risk of resistance associated with seed mixture.

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

215 Because our main aims here were to give a qualitative evaluation rather than a quantitative  
216 prediction for the risk of resistance in cotton bollworm when Bt cotton and Bt maize planted  
217 concurrently, only a simple model was used. In order to give a more accurate, quantitative  
218 prediction, the model needs to extend to include other complex factors. Those factors include the  
219 population dynamics [22], spatial structure [27], and the composition and distribution of refuges  
220 [31,35,38]. In addition to including those factors, model parameters must also be accurately  
221 estimated [32]. While our model here did not include all of those factors, it provided a basis for  
222 including them and giving a quantitative prediction for the risk of resistance in cotton bollworm in  
223 the future.

224

## 225 **Conclusions**

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

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234

## 235 **Methods**

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

243

### 244 **The effective proportions of host crops**

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

251 We assumed that the proportions of Bt and non-Bt plants in the cotton seed mixture are  $P_{Bt_1}$   
252 and  $1 - P_{Bt_1}$ , respectively, while the proportions of Bt and non-Bt plants in the maize seed mixture  
253 are  $P_{Bt_2}$  and  $1 - P_{Bt_2}$ , respectively.

254

### 255 **The Bt cotton and Bt maize products**

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

264

265 **The population genetic equations**

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

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

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

286 
$$W_{M1}(g) = P_{1,TT} \cdot W_{B1}(G) + P_{1,NN} \cdot W_N(G) + P_{1,TN} \cdot [W_{B1}(G)]^q \cdot [W_N(G)]^{1-q} + P_{1,NT} \cdot$$
  
287 
$$[W_N(G)]^q \cdot [W_{B1}(G)]^{1-q} \quad (1)$$

288 
$$W_{M2}(g) = P_{2,TT} \cdot W_{B2}(G) + P_{2,NN} \cdot W_N(G) + P_{2,TN} \cdot [W_{B2}(G)]^q \cdot [W_N(G)]^{1-q} + P_{2,NT} \cdot$$
  
289 
$$[W_N(G)]^q \cdot [W_{B2}(G)]^{1-q} \quad (2)$$

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

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

295 calculated as follows.

$$296 \quad P_{1,TT} = PBt_1 \cdot (m \cdot PBt_1 + 1 - m), \quad P_{1,NN} = (1 - PBt_1) \cdot [m \cdot (1 - PBt_1) + 1 - m]$$

$$297 \quad P_{1,TN} = PBt_1 \cdot m \cdot (1 - PBt_1), \quad P_{1,NT} = P_{1,TN} \quad (3)$$

298 Where  $PBt_1$  is the proportion of Bt in the cotton seed mixture.

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

$$301 \quad P_{2,TT} = PBt_2 \cdot (m \cdot PBt_2 + 1 - m), \quad P_{2,NN} = (1 - PBt_2) \cdot [m \cdot (1 - PBt_2) + 1 - m]$$

$$302 \quad P_{2,TN} = PBt_2 \cdot m \cdot (1 - PBt_2), \quad P_{2,NT} = P_{2,TN} \quad (4)$$

303 Where  $PBt_2$  is the proportion of Bt in the maize seed mixture.

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

$$309 \quad W(G) = P_1 \times W_{M1}(G) + P_2 \times W_{M2}(G) + (1 - P_1 - P_2) \times W_N(G) \quad (5)$$

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

$$312 \quad f'(G) = [f(G) \times W(G)] / [\sum_X f(X) \times W(X)], \text{ for any } G \quad (6)$$

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

318

### 319 **The model parameters**

#### 320 ***Host crop parameters***

321 We assumed that the ratio of the effective proportion of cotton to that of maize is fixed at  $P_1:P_2 =$   
322 0.64:0.36 and that the effective proportion of natural refuge ( $P_{nat}$ ) varies from 0 to 0.6. This  
323 ratio of 0.64:0.36 was obtained based on the effective proportions of cotton, maize and natural  
324 refuge in the study area in 2016, which are 0.27, 0.15, and 0.58, respectively [23]. The proportion

325 of Bt in cotton was fixed at 0.75, i.e.  $PBt_1=0.75$ . This value was used because the transgenic  
326 cotton plants in China were  $F_2$  hybrids between Bt and conventional cotton varieties, in which  
327 about 75% of the seeds expresses *cry1Ac* [4]. The proportion of Bt in maize was fixed at 0.8, i.e.  
328  $PBt_2=0.8$ . This value was recommended for insects that are intrinsically less susceptible to Bt  
329 proteins in the United States [40].

### 330 ***Fitness parameters***

331 We assumed the fitness of a two-locus genotype is multiplicative with respect to the two loci.  
332 With this assumption, we only needed to specify the fitnesses of one-locus genotypes, that is those  
333 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  
334 genotypes can be further computed based on fitness cost ( $c_j$ ), dominance of fitness cost ( $d_j$ ),  
335 Bt-caused mortality to susceptible CBW ( $\mu_{ij}$ ), dominance of resistance ( $h_{ij}$ ), and incomplete  
336 resistance ( $\sigma_{ij}$ ) (Table 1). Because the Bt proteins in Bt cotton and Bt maize are similar, we  
337 assumed that the Bt-caused mortality, dominance of resistance and incomplete resistance are the  
338 same between Bt cotton and Bt maize.

339 The first category of fitness parameters that we need to specify is the fitness cost ( $c_j$ ) and  
340 dominance of fitness cost ( $d_j$ ) for  $j=1,2$ . Among them,  $c_1$  is the fitness cost of resistance to  
341 *cry1Ac* or *cry1Ab*, while  $c_2$  is the fitness cost of resistance to *cry2Ab* or *cry2Aj*. Experimental  
342 results have showed that fitness cost of resistance to *cry1Ac* might be as large as 0.54 [23]. To be  
343 conservative, here we used a smaller value of 0.36 as the default but also studied the cases when  
344 this parameter varies between 0 and 0.5. Namely, we considered  $0 \leq c_1 \leq 0.5$ . Experimental  
345 studies have shown that there is no fitness cost to resistance to *cry2Ab* [33,41]. Therefore, we set  
346  $c_2 = 0$ . For the dominance of fitness cost, we followed Jin et al. [23] and Mahon and Young [33]  
347 and set  $d_1 = d_2 = 0$ . This means that there is no fitness cost to the heterozygous genotypes.

348 The second category of fitness parameters that we need to specify is the Bt-caused mortality to  
349 susceptible genotypes ( $\mu_{ij}$ ). For mortality caused by Bt cotton expressing *cry1Ac*, both a lower  
350 (0.95; [14]) and higher value (1; [20,23]) were used in literature. To be conservative, we used the  
351 lower value 0.95 as the mortality to susceptible genotype caused by Bt plants expressing *cry1Ac*  
352 or *cry1Ab*, i.e.  $\mu_{11} = \mu_{21} = 0.95$ . For mortality caused by Bt plants expressing *cry2Ab* or *cry2Aj*,  
353 we do not have specific experimental data. However, we have experimental data for the mortality  
354 caused by Bt maize expressing *cry1Ab+cry2Aj*, which is roughly 0.99 [42]. Based on this

355 experimental result and the mortality associated with *cry1Ab* alone, we derived the mortality  
356 associated with *cry2Aj* to be 0.8. Therefore, we assumed that the mortality associated with *cry2Ab*  
357 or *cry2Aj* is 0.8, i.e.  $\mu_{21} = \mu_{22} = 0.8$ .

358 For the dominance of resistance, Jin et al. found that resistance to Bt cotton expressing *cry1Ac*  
359 was dominant and that the degree of dominance was as high as 0.79 [23]. We followed this result  
360 and assumed that the dominance of resistance to *cry1Ac* or *cry1Ab* is 0.79, i.e.  $h_{11} = h_{21} = 0.79$ .  
361 For the incomplete resistance, Jin et al. used a value of 0.505 for Bt cotton expressing *cry1Ac* [23].  
362 Based on this value, we assumed that the incomplete resistance to *cry1Ac* or *cry1Ab* is 0.505, i.e.  
363  $\sigma_{11} = \sigma_{21} = 0.505$ . Because there was no experimental data to determine the dominance of  
364 resistance to *cry2Ab* or *cry2Aj* in China, we followed Edwards et al. [30] and used the value of  
365 0.25, i.e.  $h_{12} = h_{22} = 0.25$ . Also because there was no experimental data to determine the value  
366 of incomplete resistance to *cry2Ab* or *cry2Aj*, we adopted a conservative method ([32]) and  
367 assumed that the resistance is complete, i.e.  $\sigma_{12} = \sigma_{22} = 0$ . All fitness parameters were  
368 summarized in Table 2.

#### 369 *The larval movement parameter*

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

#### 377 *Initial frequencies of resistance alleles*

378 A study showed that the frequency of resistance allele to Bt cotton in northern China was 0.1 in  
379 2016 [23]. We used this value as the initial frequency of resistance allele associated with Locus 1.  
380 Because Bt maize has not yet planted in China, there is no way to determine the initial frequency  
381 of resistant allele empirically. Therefore, we used a theoretical value of 0.001 as the initial  
382 frequency of resistance allele associated with Locus 2 (see e.g. [27]).

383

#### 384 **Time to resistance (TTR)**

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

391

### 392 **Computer programing**

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

394

### 395 **Declarations**

### 396 **Abbreviations**

397 CBW: Cotton bollworm

398 TTR: Time to resistance

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

400 Bt maize-2: Bt maize containing two Bt toxins

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

402 Bt cotton-2: Bt cotton containing two Bt toxins

403

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406

### 407 **Authors' contributions**

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

409 and P.W. wrote the paper.

410

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414 data, and writing of the manuscript.

415

#### 416 **Availability of data and materials**

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

419

#### 420 **Ethics approval and consent to participate**

421 Not applicable.

422

#### 423 **Consent for publication**

424 Not applicable.

425

#### 426 **Competing interests**

427 The authors declare that they have no competing interests.

428

429

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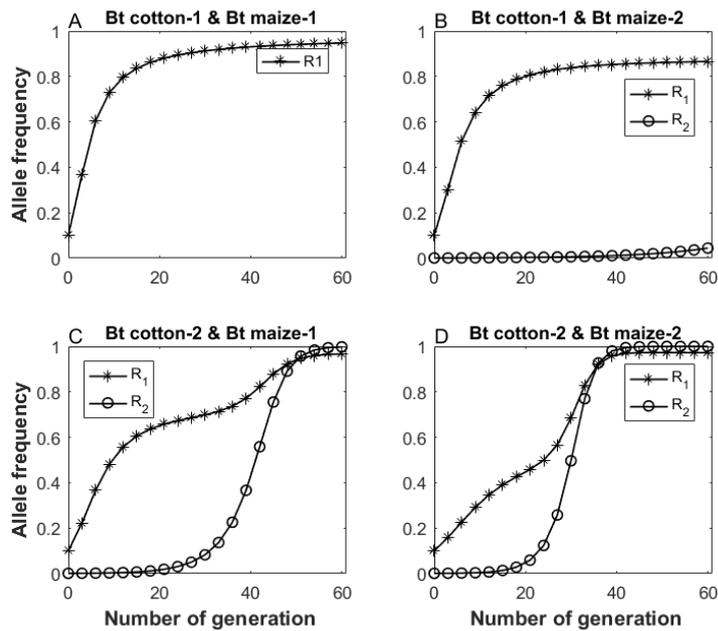
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563 **Figure legends**

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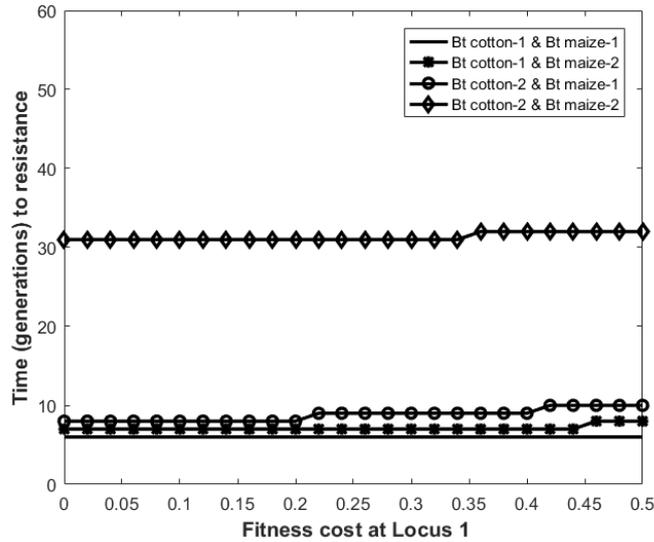


565

566 **Figure 1** Temporal patterns in the frequency of resistance alleles. **A.** one-locus model for Bt  
567 cotton-1 & Bt maize-1. **B.** two-locus model for Bt cotton-1 & Bt maize-2. **C.** two-locus model for  
568 Bt cotton-2 & Bt maize-1. **D.** two-locus model for Bt cotton-2 & Bt maize-2.  $R_1$  and  $R_2$  represent  
569 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  
570 of parameters are set at default.

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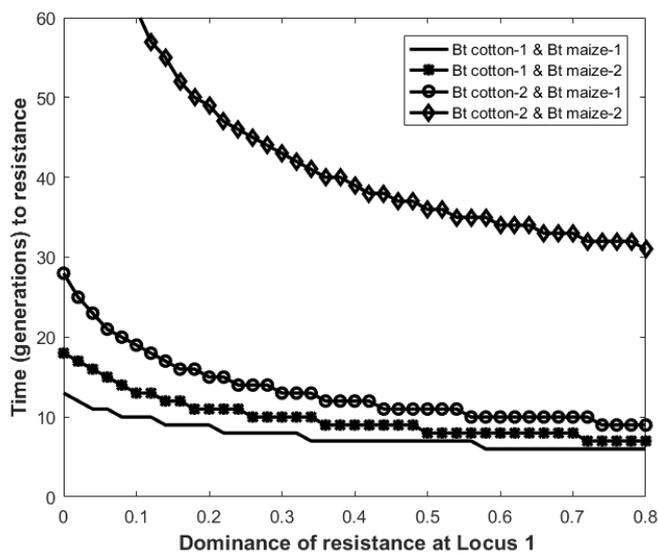
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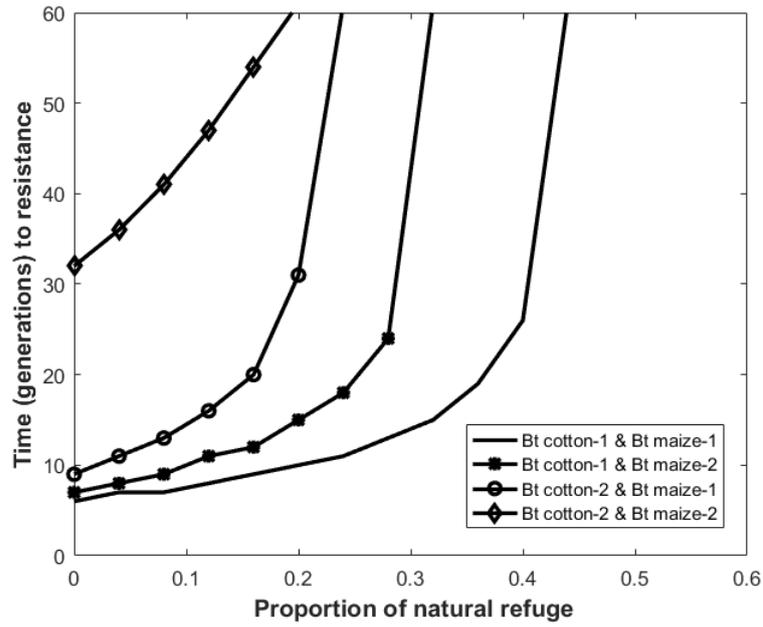
574 **Figure 2** Time to resistance versus fitness cost at locus 1. The four lines with none, star, circle and  
 575 diamond marks correspond to the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt maize-2,  
 576 Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, respectively. The horizontal axis is the  
 577 fitness cost at Locus 1 ( $c_1$ ) and the vertical axis is the time to resistance (TTR). In this figure,  
 578  $P_1=0.64$ ,  $P_2=0.36$ ,  $P_{nat}=0$ . The rest of parameters are set at default.

579



580

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



586

587 **Figure 4** Time to resistance versus proportion of natural refuge. The four lines with none, star,  
 588 circle and diamond marks correspond to the cases of Bt cotton-1 & Bt maize-1, Bt cotton-1 & Bt  
 589 maize-2, Bt cotton-2 & Bt maize-1 and Bt cotton-2 & Bt maize-2, respectively. The horizontal  
 590 axis is the proportion of natural refuge ( $P_{nat}$ ) and the vertical axis is the time to resistance (TTR).  
 591 The rest of parameters are set at default.

592

593 **Table 1** The formulae for fitness computation.

Notation	Meaning	formula
$W_N(S_j S_j)$	Fitness of $S_j S_j$ on non-Bt plants ( $j=1,2$ )	1
$W_N(S_j R_j)$	Fitness of $S_j R_j$ on non-Bt plants ( $j=1,2$ )	$1 - d_j \cdot c_j$
$W_N(R_j R_j)$	Fitness of $R_j R_j$ on non-Bt plants ( $j=1,2$ )	$1 - c_j$
$W_{B1}(S_j S_j)$	Fitness of $S_j S_j$ on Bt cotton ( $j=1,2$ )	$1 - \mu_{1j}$
$W_{B1}(S_j R_j)$	Fitness of $S_j R_j$ on Bt cotton ( $j=1,2$ )	$(1 - \mu_{1j}) + h_{1j} \cdot (\mu_{1j} - \sigma_{1j})$
$W_{B1}(R_j R_j)$	Fitness of $R_j R_j$ on Bt cotton ( $j=1,2$ )	$1 - \sigma_{1j}$
$W_{B2}(S_j S_j)$	Fitness of $S_j S_j$ on Bt maize ( $j=1,2$ )	$1 - \mu_{2j}$
$W_{B2}(S_j R_j)$	Fitness of $S_j R_j$ on Bt maize ( $j=1,2$ )	$(1 - \mu_{2j}) + h_{2j} \cdot (\mu_{2j} - \sigma_{2j})$
$W_{B2}(R_j R_j)$	Fitness of $R_j R_j$ on Bt maize ( $j=1,2$ )	$1 - \sigma_{2j}$

594

595

596 **Table 2** The notation, meaning and default value or range of parameters used in model  
 597 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 <i>cry1Ac</i> or <i>cry1Ab</i>	0.95	[14,20,23]
$\mu_{12}$	Mortality of $S_2S_2$ caused by Bt cotton containing <i>cry2Ab</i> or <i>cry2Aj</i>	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]

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

# Figures

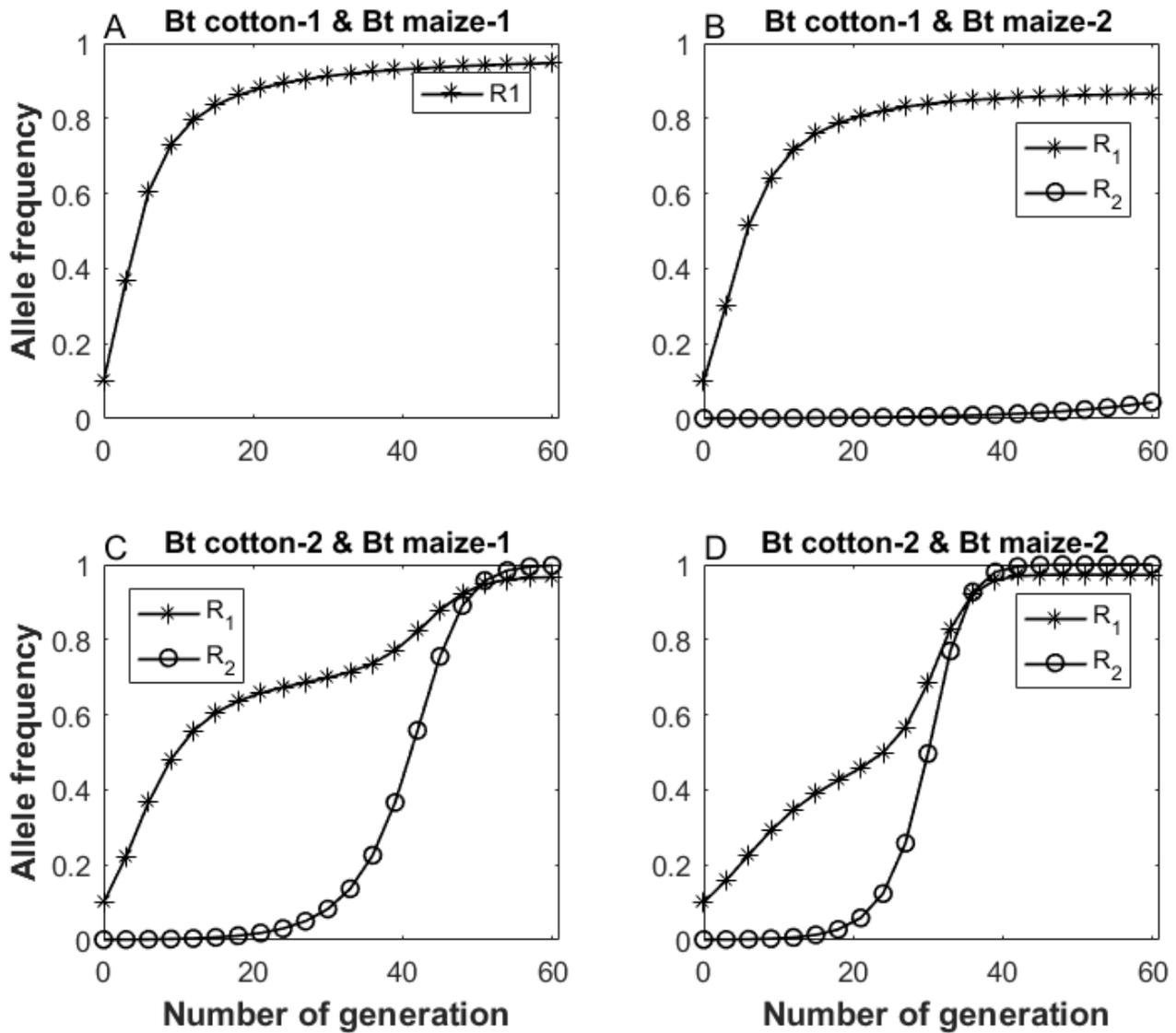
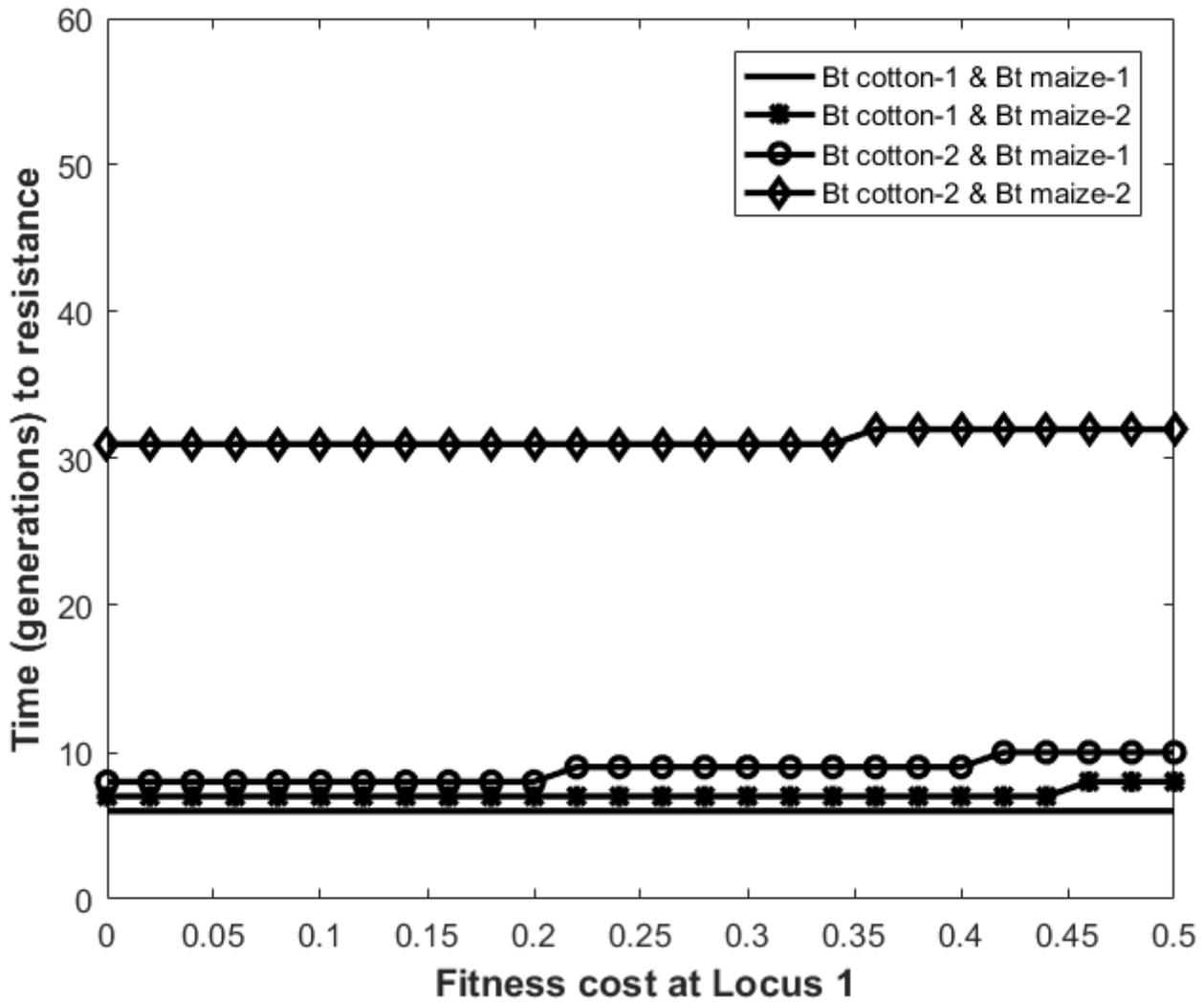


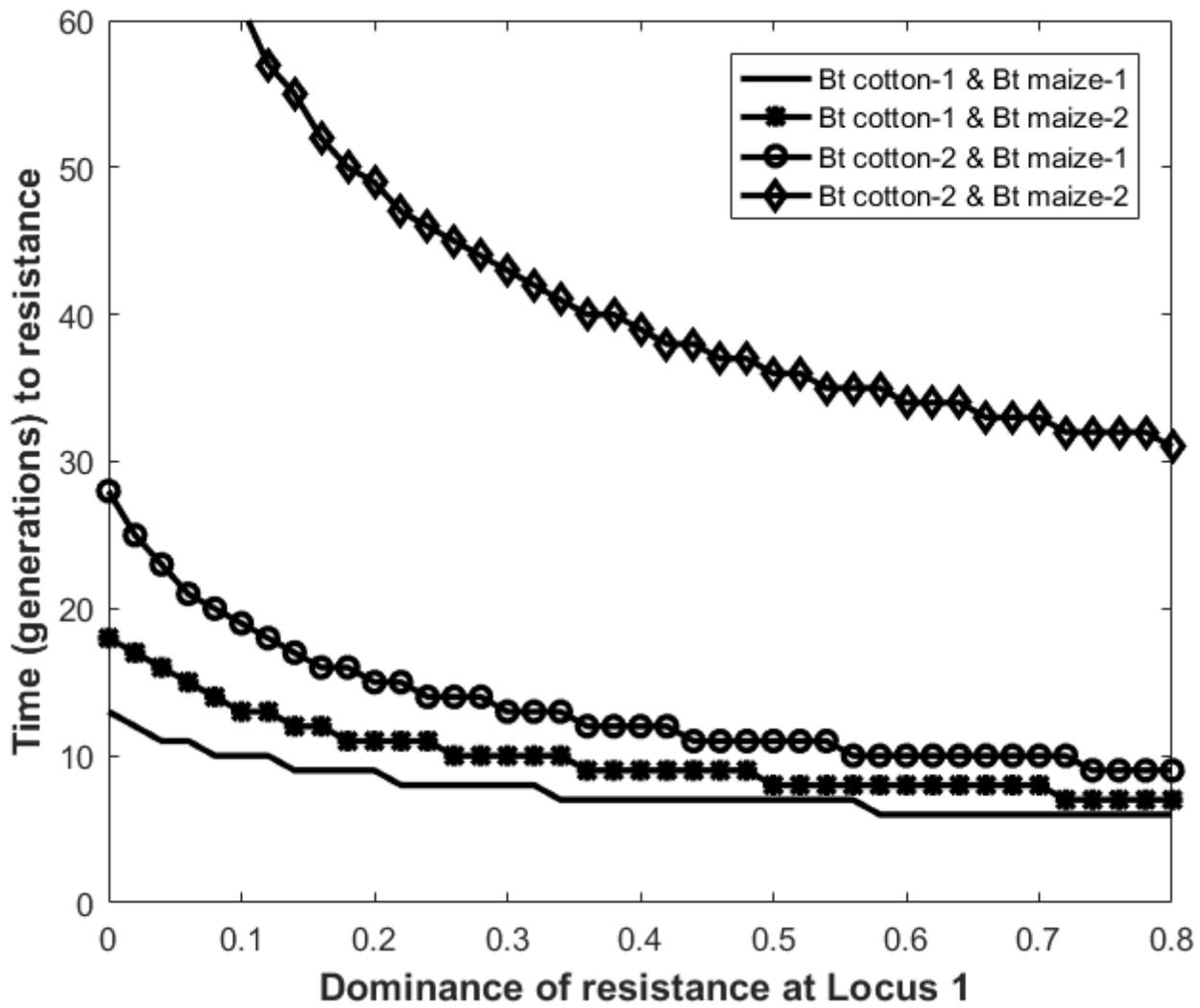
Figure 1

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. R1 and R2 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.



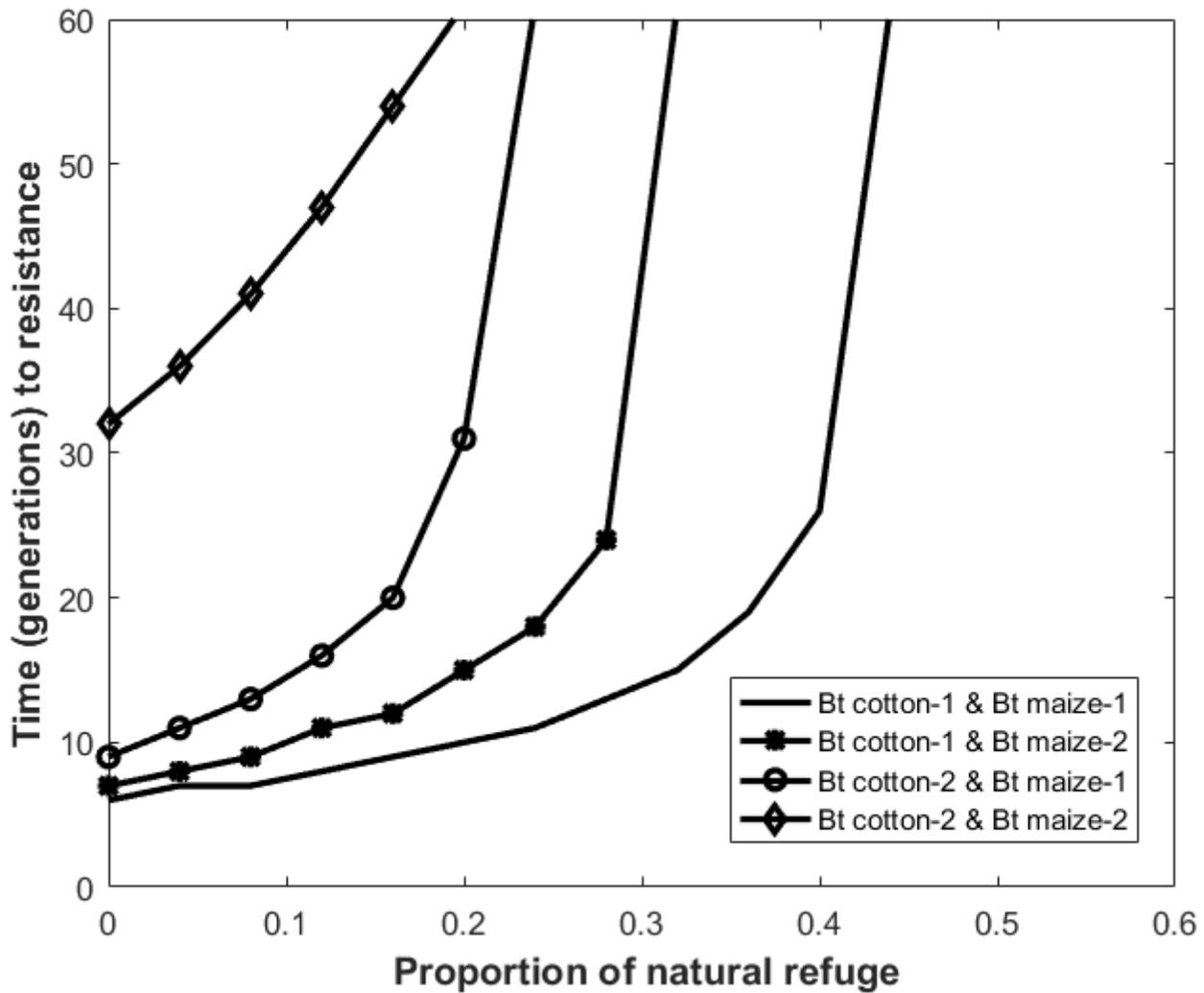
**Figure 2**

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.