

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

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

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23 **Abstract**

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

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

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

42
43 **Keywords:** Bt maize, Bt cotton, Cotton bollworm, Regional resistance, Model prediction

44

45 **Background**

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

50 One of the main threats to the long-term use of Bt crops is that target pests may evolve
51 resistance to Bt toxin. To date, field resistance has been observed in the target pests of both Bt
52 cotton and Bt maize [6-11]. Resistance can be affected by various ecological and genetic factors
53 [12]. Among them, the most important factor is excessive planting of Bt plants [13], but other
54 factors such as insect susceptibility to the Bt toxin, dominance of resistance, and strategy of
55 resistance management are also very important [14-17].

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

62 In China, Bt cotton is the only Bt crop that has been commercially planted so far [21]. One of
63 the main targets of Bt cotton is cotton bollworm (CBW), *Helicoverpa armigera* (Lepidoptera,
64 Noctuidae), a highly polyphagous insect pest that can feed on a number of different agricultural
65 crops. In northern China, CBW has 4 generations per year. The host crops of CBW include cotton,
66 maize, wheat, soybean, peanut, vegetables, and the availability of host crops vary among different

67 generations [22]. For the first generation, wheat is the primary host crop when other major host
68 crops like cotton and maize are absent. For the second through fourth generations, most of major
69 host crops are available. An earlier study has shown that abundant non-cotton host crops in
70 northern China served as natural refuges for CBW and contributed to delaying resistance of CBW
71 to Bt cotton [20]. However, a more recent study has found that resistance to Bt cotton in CBW is
72 accelerated by a dominant resistance allele [23].

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

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

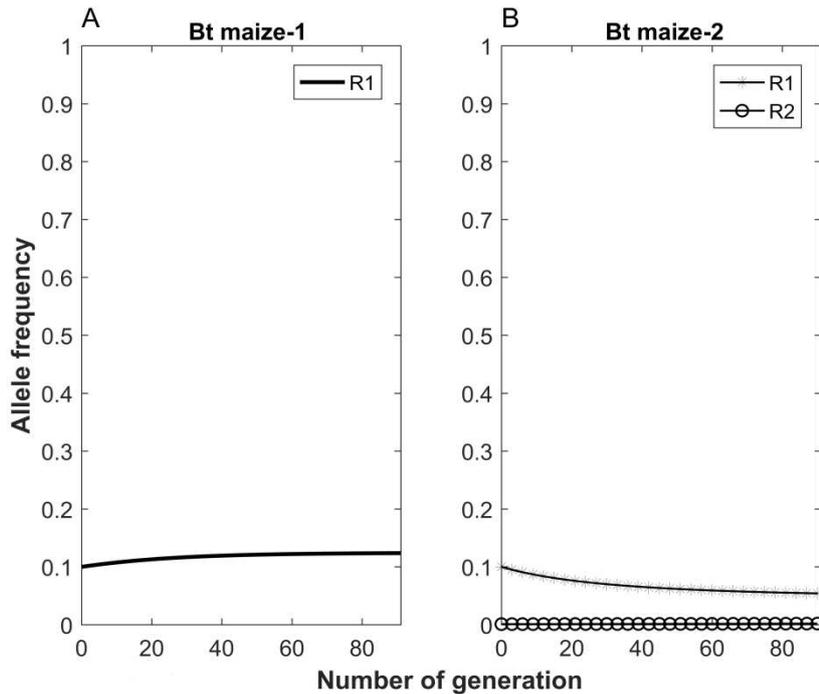
89 are very different from those in the United States.

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

98 **Results**

99 **The difference between Bt maize-1 and Bt maize-2**

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



107

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

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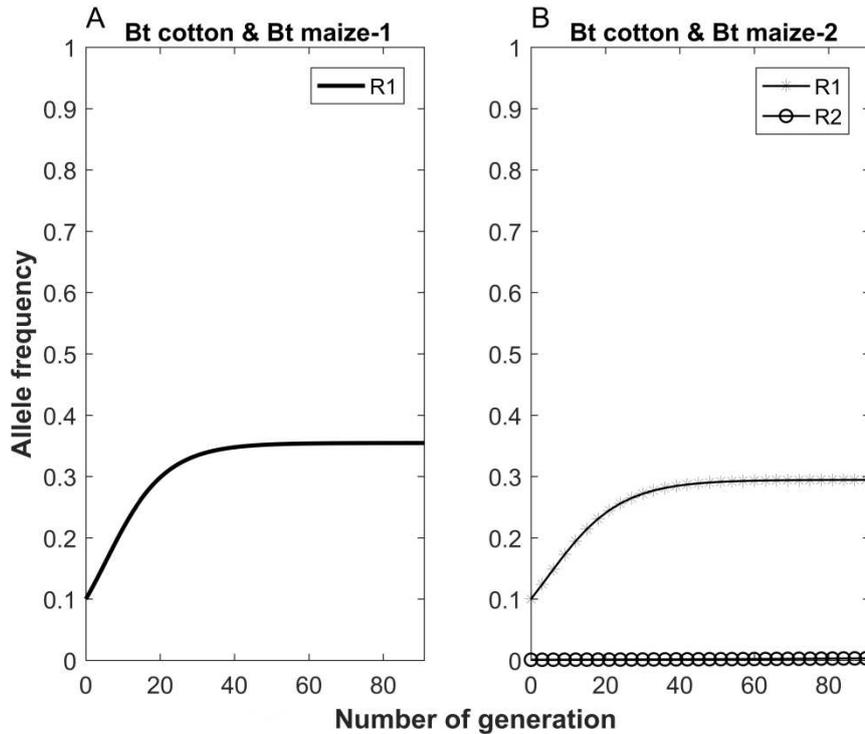
114

115 **The impact of Bt cotton**

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

121 Compared to without Bt cotton, the frequency of resistance allele R_1 evolves faster and higher

122 in both cases of Bt maize-1 and Bt maize-2, while the evolution in the frequency of resistance
123 allele R_2 does not increase over time. This is because resistance to Bt cotton is governed by Locus
124 1 only, so adding Bt cotton only affects the evolution of R_1 , not that of R_2 .



125
126 **Figure 2** Changes in the frequency of resistance alleles over time. **a** Bt cotton & Bt maize-1 where
127 Bt cotton and Bt maize contains a similar toxin and resistance is governed by one locus. **b** Bt
128 cotton & Bt maize-2 where Bt maize contains two toxins in which one is shared with Bt cotton
129 and resistance is governed by two loci. In this figure, R_1 and R_2 represent the resistance alleles at
130 loci 1 and 2, respectively. Parameters are all set at default.

131

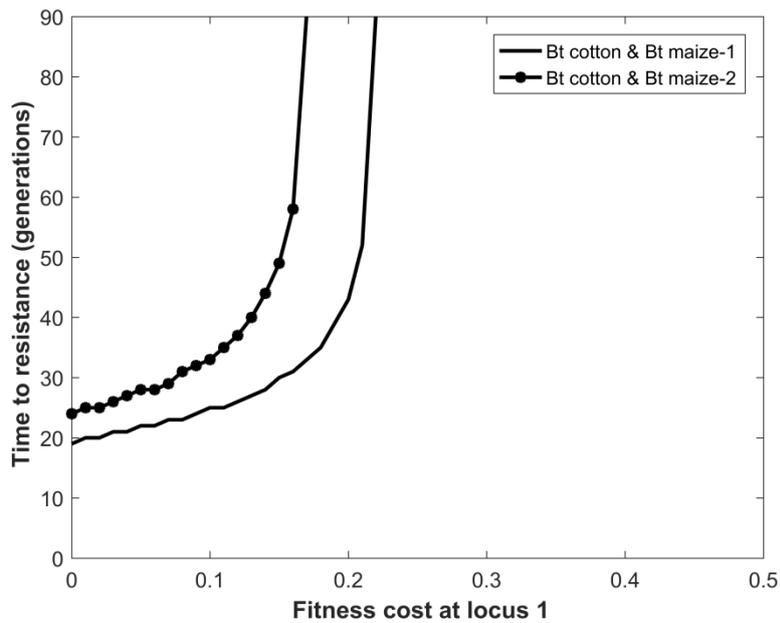
132

133 **The impact of fitness cost**

134 To investigate the impact of fitness cost at Locus 1 on resistance evolution, the time to resistance
135 (TTR) is derived when the fitness cost at Locus 1 varies from 0 to 0.5 (Fig. 3). In the case of Bt

136 cotton & Bt maize-1, when the fitness cost at Locus 1 varies from 0 to 0.22, the TTR increases
137 from 20 to >90 generations. In the case of Bt cotton & Bt maize-2, when the fitness cost at Locus
138 1 varies from 0 to 0.17, the TTR increases from 24 to >90 generations.

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



144
145 **Figure 3** Time to resistance versus fitness cost at locus 1. Bt maize-1: Bt maize contains only one
146 toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins and
147 resistance is governed by two loci. The rest of parameters are set at default.

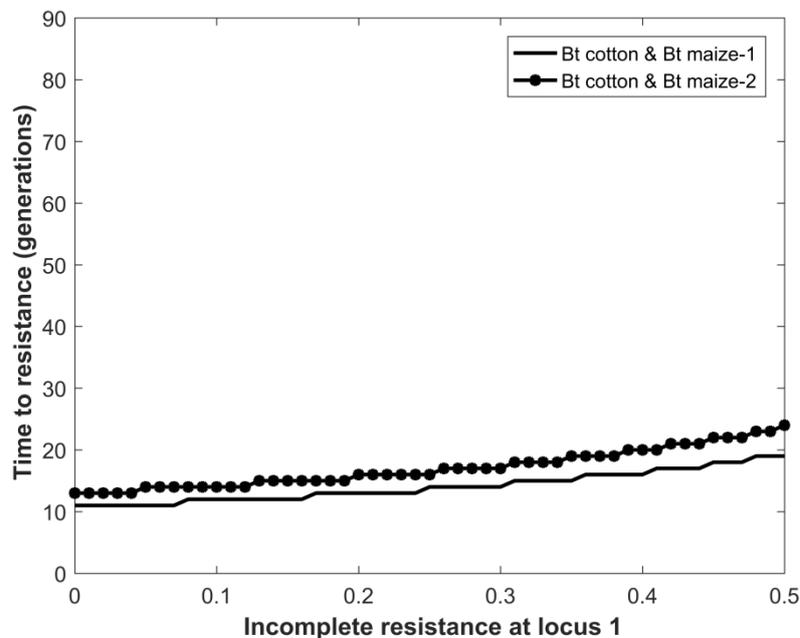
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149

150 **The impact of incomplete resistance**

151 To investigate the impact of incomplete resistance at Locus 1 on resistance evolution, the TTR is
152 derived by simulation when the incomplete resistance at Locus 1 varies from 0 to 0.5 (Fig. 4).
153 When the default fitness cost at Locus 1, i.e. 0.36, is used, the differences between the two cases
154 cannot be demonstrated because the TTRs are infinite in both cases of Bt cotton & Bt maize-1 and
155 Bt cotton & Bt maize-2. For this reason, a hypothetical value for the fitness cost at Locus 1, i.e. 0,
156 is used. In the case of Bt cotton & Bt maize-1, when the incomplete resistance at Locus 1 varies
157 from 0 to 0.5, the TTR increases from 11 to 20 generations. In the case of Bt cotton & Bt maize-2,
158 when the incomplete resistance at Locus 1 varies from 0 to 0.5, the TTR increases from 14 to 27
159 generations.

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



163
164 **Figure 4** Time to resistance versus incomplete resistance at locus 1. Bt maize-1: Bt maize contains
165 only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins

166 and resistance is governed by two loci. In this figure, the fitness cost at Locus 1 is zero, i.e. $c_1=0$.

167 The rest of parameters are set at default.

168

169

170 **The impact of dominance of resistance**

171 To investigate the impact of dominance of resistance at Locus 1 on resistance evolution, the TTR

172 is derived when the dominance of resistance at Locus 1 varies from 0 to 1 (Fig. 5). For the same

173 reason as described in the previous subsection, the fitness cost at Locus 1 is set at 0. In the case of

174 Bt cotton & Bt maize-1, when the dominance of resistance at Locus 1 varies from 0 to 1, the TTR

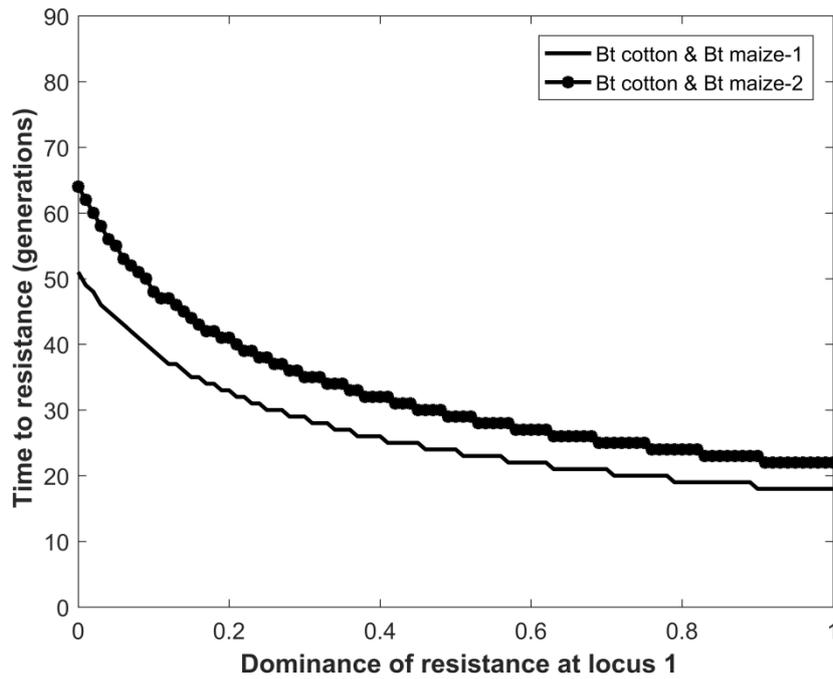
175 decreases from 51 to 18 generations. In the case of Bt cotton & Bt maize-2, when the dominance

176 of resistance at Locus 1 varies from 0 to 1, the TTR decreases from 64 to 22 generations.

177 With the same dominance of resistance at Locus 1, the TTR is always shorter in the case of Bt

178 cotton & Bt maize-1 than that in the case of Bt cotton & Bt maize-2. The higher the dominance of

179 resistance is, the smaller the difference.



180

181 **Figure 5** Time to resistance versus dominance of resistance at locus 1. Bt maize-1: Bt maize

182 contains only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains

183 two toxins and resistance is governed by two loci. In this figure, the fitness cost at Locus 1 is zero,

184 i.e. $c_1=0$. The rest of parameters are set at default.

185

186

187 **The impact of initial frequency of resistance allele**

188 To investigate the impact of the initial frequency of resistance allele at locus 1 on resistance

189 evolution, the TTR is derived when the initial frequency of R_1 varies from 0.001 to 0.1 (Fig. 6).

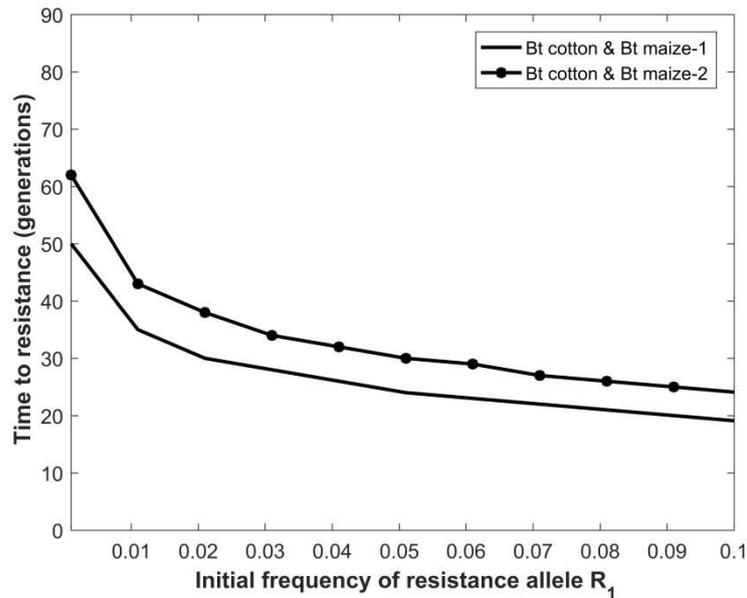
190 The fitness cost at Locus 1 is set at 0. In the case of Bt cotton & Bt maize-1, when the initial

191 frequency of resistance allele at Locus 1 varies from 0.001 to 0.1, the TTR decreases from 50 to

192 19 generations. In the case of Bt cotton & Bt maize-2, when the initial frequency of resistance

193 allele at Locus 1 varies from 0.001 to 0.1, the TTR decreases from 62 to 24 generations.

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



197
 198 **Figure 6** Time to resistance versus initial frequency of resistance allele at locus 1. Bt maize-1: Bt
 199 maize contains only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize
 200 contains two toxins and resistance is governed by two loci. In this figure, the fitness cost at Locus
 201 1 is zero, i.e. $c_1=0$. The rest of parameters are set at default.

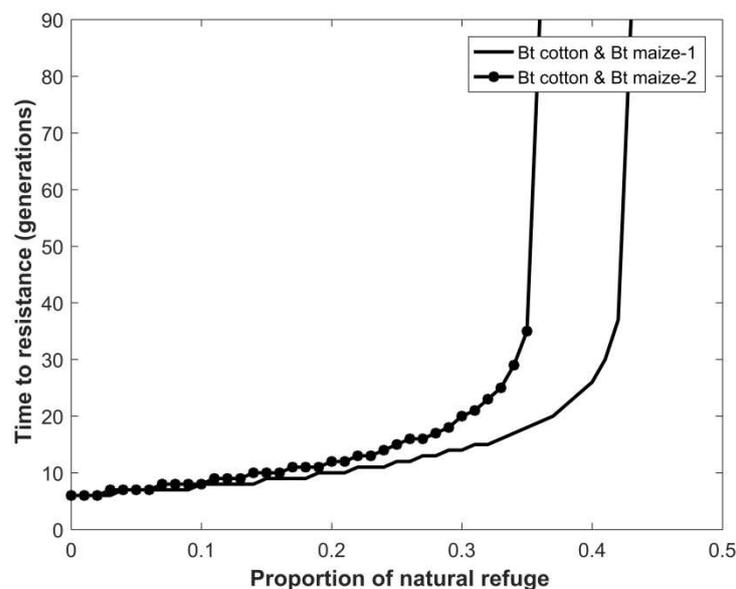
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 203

204 **The impact of proportion of natural refuge**

205 To investigate the impact of the proportion of natural refuge on resistance evolution, the TTR is
 206 derived when the proportion of natural refuge varies from 0 to 0.5 (Fig.7). When the proportion of
 207 natural refuge varies from 0 to 0.5, the proportion of cotton and maize as a whole varies from 0.5
 208 to 0. To be specific, the ratio of proportion of cotton to that of maize is fixed at 0.64 to 0.36 when
 209 proportion of natural refuge varies. In the case of Bt cotton & Bt maize-1, when the proportion of

210 natural refuge varies from 0 to 0.46, the TTR increases from 6 to >90 generations. In the case of
 211 Bt cotton & Bt maize-2, when the proportion of natural refuge varies from 0 to 0.37, the TTR
 212 increases from 6 to >90 generations.

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



218
 219 **Figure 7** Time to resistance versus proportion of natural refuge. Bt maize-1: Bt maize contains
 220 only one toxin and resistance is governed by one locus. Bt maize-2: Bt maize contains two toxins
 221 and resistance is governed by two loci. In this figure, the proportion of natural refuge (P_{nat}) varies
 222 from 0 to 0.5, while the proportion of cotton and that of maize are $P_1=0.64*(1-P_{nat})$ and
 223 $P_2=0.36*(1-P_{nat})$, respectively. The rest of parameters are set at default.

224
 225

226 **Discussion**

227 Bt cotton is one of the earliest Bt crops that have been commercially planted in the world since
228 1996 [5]. While most of countries have upgraded the first generation Bt cotton to the second and
229 third generations [13,33], China is still planting the first generation Bt cotton expressing *CryIAc*
230 [4,23]. Our modeling results here showed that planting Bt maize together with such a first
231 generation Bt cotton could significantly increase the risk of cotton bollworm resistance to the two
232 Bt crops as a whole.

233 One of the main reasons that planting Bt maize together with the first generation Bt cotton
234 could increase the risk of cotton bollworm resistance was that cotton bollworm in China had
235 evolved a dominant resistance to Bt cotton. A literature had shown that the degree of dominance
236 was as high as 0.79 [23]. Consistent with theoretical and empirical results elsewhere [18,23,34],
237 our simulation results here showed that such a dominant resistance could evolve much faster than
238 a recessive one.

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

247 While the preexisting dominant resistance could increase the risk of resistance when Bt

248 cotton and Bt maize planted together, our simulation results showed that fitness cost to the
249 resistance might dramatically decrease the risk. In particular, our results showed that when fitness
250 cost was sufficiently large, the frequency of resistance allele might not continue to increase from
251 its initial value and thus never reach the critical level of resistance risk. This is because when the
252 selection for resistance from planting Bt plants just matches the selection against resistance due to
253 fitness cost, an equilibrium may be reached at which the frequency of resistance remains constant
254 [12]. A previous study indicated that the fitness cost to the preexisting resistance in cotton
255 bollworm was as high as 0.54 [23]. Such a high fitness cost, if persists, should dramatically reduce
256 the risk of resistance when Bt maize and Bt cotton are planted together.

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

264 Literatures have shown that compared to block refuge, seed mixtures of Bt and non-Bt plants
265 can generally accelerate resistance, by reducing the effective refuge size or increasing the
266 dominance of resistance [38]. Our model simulations found that this was also the case for CBW
267 with seed mixture of Bt and non-Bt cotton and/or seed mixture of Bt and non-Bt maize. However,
268 the extent of acceleration in resistance depended on the rate of larval movement between plants.
269 So far, researches about CBW larval movement between maize plants have been very limited.

270 Future studies on this aspect are needed in order to more accurately predict the risk of resistance
271 associated with seed mixture.

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

280 Because our main aims here were to give a qualitative evaluation rather than a quantitative
281 prediction for the risk of resistance in cotton bollworm when Bt cotton and Bt maize planted
282 concurrently, only a simple model was used. In order to give a more accurate, quantitative
283 prediction, the model needs to extend to include other complex factors. Those factors include the
284 population dynamics [22], spatial structure [27], and the composition and distribution of refuges
285 [31,35,39]. In addition to including those factors, model parameters must also be accurately
286 estimated [32]. While our model here did not include all of those factors, it provided a basis for
287 including them and giving a quantitative prediction for the risk of resistance in cotton bollworm in
288 the future.

289

290 **Conclusions**

291 In northern China, planting the first generation Bt cotton together with Bt maize could

292 significantly increase the risk of CBW resistance to Bt toxins. The risk was higher in the case of
293 one-toxin Bt maize than in the case of pyramid two-toxin Bt maize. The risk of resistance could
294 be extremely high if natural refuges were absent. The approaches to reducing the risk of CBW
295 resistance include replacing the currently planted one-toxin Bt cotton with a pyramid two-toxin Bt
296 cotton, adopting a pyramid two-toxin Bt maize instead of one-toxin Bt maize, and maintaining a
297 sufficient proportion of natural refuges.

298

299

300 **Methods**

301 A population genetic model was developed to analyze the adaptation of CBW to Bt cotton and Bt
302 maize when the two types of Bt crops are planted together in northern China. In the study area,
303 CBW has 4 generations per year [20,22]. In the first generation, the primary host crop is wheat
304 when cotton and maize are absent. This means there is no selection for resistance to Bt in the first
305 generation. For this reason, we only modeled three generations per year. For simplicity, we did not
306 distinguish differences among generations and considered all model parameters as the averages
307 across the three generations. We modeled a maximum of 90 generations or 30 years.

308

309 **The effective proportions of Bt cotton, Bt maize and refuges**

310 We divided the host crops for CBW into three groups: cotton, maize and other non-Bt host crops
311 and assumed that the effective proportions of the three groups are given. Here the effective
312 proportion is the proportion of planting area weighted by the relative effectiveness in producing
313 susceptible insects [31]. We denoted the effective proportions of cotton, maize and other non-Bt

314 host crops by P_1 , P_2 , and P_{nat} , respectively, where $P_1+P_2+P_{nat}=1$. Throughout this article, we
315 referred to the “effective proportion” simply as “proportion” unless mentioned otherwise.

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

$$322 \quad P_{ref} = 1 - PBt_1 \times P_1 - PBt_2 \times P_2 \quad (1)$$

323 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).
324 Namely, the effective proportions of cotton, maize and natural refuge are 0.27, 0.15, and 0.58,
325 respectively. These two values were derived from real data in northern China in 2016 [23]. In
326 addition, we considered a theoretical case where the proportion of cotton and maize varies from
327 0.42 to 1, or that of natural refuges varies from 0 to 0.58. We assumed that the ratio of the
328 proportion of cotton to that of maize is fixed, which is approximately 0.64 to 0.36.

329 The proportion of Bt in cotton was fixed at 0.75, i.e. $PBt_1=0.75$ (Table 1). This value was used
330 because the transgenic cotton plants in China were F_2 hybrids between Bt and conventional cotton
331 varieties, in which about 75% of the seeds expresses *cryIAC* [4]. The proportion of Bt in maize
332 was fixed at 0.8, i.e. $PBt_2=0.8$. This value was recommended for insects that are intrinsically less
333 susceptible to Bt proteins in the United States [40].

334

335 **The population genetics**

336 In the studying area, the Bt cotton variety that has been planted is an one-toxin product expressing
337 *cryIAc* [20,23]. Therefore, we developed our model based on this specific one-toxin Bt cotton. Bt
338 maize has not been planted commercially when this article is written. The Bt maize varieties that
339 may potentially be used include an one-toxin product expressing *cryIAb* [41] and a two-toxin
340 product expressing *cryIAb/cry2Aj* [42]. In this article, we considered two cases for the Bt maize
341 varieties that might be potentially used. In Case 1, the Bt maize expresses *cryIAb* or a similar Bt
342 protein, which is denoted by “Bt maize-1”. In Case 2, the Bt maize expresses *cryIAb/cry2Aj* or
343 similar Bt proteins, which is denoted by “Bt maize-2”.

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

350 The two-locus population genetic model used here is a discrete-time, frequency-dependent
351 one in which the frequencies of genotypes are tracked from generation to generation. In the
352 two-locus model, there are a total of nine genotypes. These genotypes can be generally put in the
353 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
354 the subscript i indicating Locus 1 or 2. The key components of the model are the fitness functions
355 on cotton seed mixture, maize seed mixture, and natural refuge, which, for convenience of
356 expression, are denoted by W_1 , W_2 and W_3 , respectively.

357 We assumed that the fitness is multiplicative with respect to the two loci [27]. Namely, the

358 fitness of a two-locus genotype $X_1Y_1X_2Y_2$ is the product of the fitnesses of the two one-locus
 359 genotypes:

$$360 \quad W_j(X_1Y_1X_2Y_2) = W_j(X_1Y_1) \times W_j(X_2Y_2) \quad (2)$$

361 Where W_j ($j=1, 2, 3$) is the fitness over the entire larval period. Because W_1 and W_2 are the
 362 fitnesses on seed mixture, they depend on the ratio of Bt to non-Bt plants in the seed mixture and
 363 larval movement between plants. Following [43], we divided the entire period of CBW larvae into
 364 two stages and assumed that movement between plants occurs only when a larva completes the
 365 development of the first stage. Suppose that the movement from a Bt plant to a non-Bt plant or
 366 from a non-Bt plant to a Bt plant is completely random and that the probability of larval
 367 movement between plants is the same on cotton and maize plants (M), then for any single locus
 368 genotype g , we have

$$369 \quad W_1(g) = P_{1,TT} \cdot W_{B1}(g) + P_{1,NN} \cdot W_N(g) + P_{1,TN} \cdot [W_{B1}(g)]^q \cdot [W_N(g)]^{1-q} + P_{1,NT} \cdot$$

$$370 \quad [W_N(g)]^q \cdot [W_{B1}(g)]^{1-q} \quad (3)$$

$$371 \quad W_2(g) = P_{2,TT} \cdot W_{B2}(g) + P_{2,NN} \cdot W_N(g) + P_{2,TN} \cdot [W_{B2}(g)]^q \cdot [W_N(g)]^{1-q} + P_{2,NT} \cdot$$

$$372 \quad [W_N(g)]^q \cdot [W_{B2}(g)]^{1-q} \quad (4)$$

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

376 $P_{1,TT}$, $P_{1,NN}$, $P_{1,TN}$, and $P_{1,NT}$ are the probabilities that a larva moves from Bt to Bt, Non-Bt
 377 to Non-Bt, Bt to Non-Bt and Non-Bt to Bt cotton plants, respectively. These probabilities could be
 378 explicitly calculated as follows.

$$379 \quad P_{1,TT} = PBt_1 \cdot (M \cdot PBt_1 + 1 - M), \quad P_{1,NN} = (1 - PBt_1) \cdot [M \cdot (1 - PBt_1) + 1 - M]$$

380 $P_{1,TN} = PBt_1 \cdot M \cdot (1 - PBt_1), P_{1,NT} = P_{1,TN} \quad (5)$

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

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

384 $P_{2,TT} = PBt_2 \cdot (M \cdot PBt_2 + 1 - M), P_{2,NN} = (1 - PBt_2) \cdot [M \cdot (1 - PBt_2) + 1 - M]$

385 $P_{2,TN} = PBt_2 \cdot M \cdot (1 - PBt_2), P_{2,NT} = P_{2,TN} \quad (6)$

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

387 The fitnesses of single-locus genotypes on non-Bt, Bt cotton and Bt maize plants, that is
388 $W_N(g)$, $W_{B1}(g)$ and $W_{B2}(g)$, can be further expressed as functions of Bt-caused mortality (μ_i),
389 dominance of resistance (h_i), incomplete resistance (σ_i), fitness costs (c_i) and dominance of
390 fitness cost (d_i), where the subscript i stands for Loci 1 or 2 (Table 1). We assumed that there is no
391 cross-resistance among different loci. The detailed expressions of fitness functions are given as
392 follows.

393 On non-Bt plants:

394 $W_N(S_iS_i) = 1, W_N(R_iR_i) = 1 - c_i, W_N(S_iR_i) = 1 - d_i \times c_i, i = 1, 2. \quad (7)$

395 On Bt cotton plants:

396 $W_{B1}(S_1S_1) = 1 - \mu_1, W_{B1}(R_1R_1) = 1 - \sigma_1,$

397 $W_{B1}(S_1R_1) = W_{B1}(S_1S_1) + h_1 \times [W_{B1}(R_1R_1) - W_{B1}(S_1S_1)] \quad (8)$

398 $W_{B1}(S_2S_2) = W_N(S_2S_2), W_{B1}(S_2R_2) = W_N(S_2R_2), W_{B1}(R_2R_2) = W_N(R_2R_2) \quad (9)$

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

400 $W_{B2}(S_1S_1) = 1 - \mu_1, W_{B2}(R_1R_1) = 1 - \sigma_1,$

401 $W_{B2}(S_1R_1) = W_{B2}(S_1S_1) + h_1 \times [W_{B2}(R_1R_1) - W_{B2}(S_1S_1)] \quad (10)$

402 $W_{B2}(S_2S_2) = W_N(S_2S_2), W_{B2}(S_2R_2) = W_N(S_2R_2), W_{B2}(R_2R_2) = W_N(R_2R_2)$ (11)

403 On Bt maize plants, in the case of Bt maize-2:

404 $W_{B2}(S_iS_i) = 1 - \mu_i, W_{B2}(R_iR_2) = 1 - \sigma_i,$

405 $W_{B2}(S_iR_i) = W_{B2}(S_iS_i) + h_i \times [W_{B2}(R_iR_i) - W_{B2}(S_iS_i)], i = 1, 2.$ (12)

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

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

413 $W(G) = P_1 \times W_1(G) + P_2 \times W_2(G) + (1 - P_1 - P_2) \times W_3(G)$ (13)

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

416 $f'(G) = [f(G) \times W(G)] / [\sum_X f(X) \times W(X)],$ for any G (14)

417 Where $f(X)$ and $W(X)$ are the frequency and fitness of the two-locus genotype X in the
418 present generation, respectively. Based on equation (1)-(14), the frequencies of all genotypes can
419 be tracked from generation to generation. Once the frequencies of genotypes are available, the
420 frequency of a resistance allele is obtained by summing up the frequencies of all genotypes having
421 the resistance allele.

422

423 **The fitness parameters**

424 The fitness parameters on non-Bt host plants that we needed to specify are the fitness cost per
425 locus (c_1 and c_2) and the dominance of fitness cost (d_1 and d_2). Among them, c_1 is the fitness
426 cost of resistance to *CryIAc* or a similar Bt protein, while c_2 is the fitness cost of resistance to
427 *Cry2Ab* or a similar Bt protein. Experimental results have showed that fitness cost of resistance to
428 *CryIAc* might be as large as 0.54 [23]. To be conservative, here we used a smaller value of 0.36 as
429 the default while conducted sensitivity analysis by allowing this parameter varying between 0 and
430 0.5. Namely, we considered $0 \leq c_1 \leq 0.5$. Experimental studies have shown that there is no
431 fitness cost to resistance to *Cry2Ab* [33,44]. Therefore, we set $c_2 = 0$. For the dominance of
432 fitness cost, we followed Jin et al. [23] and Mahon and Young [33] and set $d_1 = d_2 = 0$. This
433 means that there is no fitness cost to the heterozygous genotypes.

434 On Bt cotton, the main parameters we need to specify are those associated with Locus 1,
435 which include the Bt-caused mortality to susceptible CBW (μ_1), the dominance of resistance (h_1),
436 and the incomplete resistance (σ_1). About Bt-caused mortality to susceptible CBW associated
437 with *CryIAc*, both a lower (0.953; [14]) and higher value (1; [20,23]) were used in literatures. To
438 be conservative, we used the lower value as the mortality of susceptible insect on Bt cotton, i.e.
439 $\mu_1 \approx 0.95$. For the dominance of resistance, Jin et al. found a dominant resistance gene for which
440 the degree of dominance was as high as 0.79 [23]. We followed this result and set $h_1 = 0.79$. For
441 the incomplete resistance, Jin et al. used a value of 0.505 [23]. Based on this value, we set $\sigma_1 =$
442 0.505.

443 On Bt maize, we assumed that fitness parameters associated with Locus 1 are the same as
444 those associated with Locus 2. Therefore, the main parameters that we need to specify are those
445 associated with Locus 2 in the case of two-toxin Bt maize, which include μ_2 , h_2 , and σ_2 . To

446 specify μ_2 , we studied the experimental results concerning the Bt-caused mortality of susceptible
447 CBWs associated with Bt maize expressing *cry1Ab/cry2Aj* [42]. In that experiment, the
448 mortalities of susceptible CBWs on Bt maize and a control were 0.9889 and 0.1278, or the
449 survivals were 0.0111 and 0.8722, respectively. Based on these two survival values, the corrected
450 survival of susceptible CBWs on Bt maize then is calculated as $1 - (0.8722 - 0.0111)/$
451 $0.8722 \approx 0.01$. Based on this calculation, we set $W_{B2}(S_1S_1S_2S_2) = (1 - \mu_1)(1 - \mu_2) \approx 0.01$.
452 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
453 for μ_2 . Because there was no Bt-plant-based data to determine the dominance of resistance
454 associated with Cry2Ab or a similar protein in China, we followed Edwards et al. [30] and set
455 $h_2=0.25$ as the default value. Also because there was no Bt-plant-based data to determine the value
456 of incomplete resistance, we adopted a conservative method (see e.g. [32]) and set the incomplete
457 resistance associated with Cry2Ab at $\sigma_2 = 0$.

458

459 **The larval movement parameter**

460 The probability of larval movement between plants depends on several factors, such as the
461 insect's tendency and ability to move, the distance between plants and the growing stage of the
462 plants. So far there is no evidence of significant CBW larval movement among plants. However,
463 because larval movement generally increases the risk of resistance evolution [43], we adopted a
464 conservative approach and considered a 10% larval movement between plants. Namely, we
465 assumed that the probability that a CBW larva moves from one plant to another during the entire
466 larvae stage is 0.1, i.e. $M=0.1$ (Table 1).

467

468 **Initial frequencies of resistance alleles**

469 A study showed that the frequency of resistance allele to Bt cotton in northern China was 0.1 in
470 2016 [23]. We used this value as the initial frequency of resistance allele associated with Locus 1.
471 Because Bt maize has not yet planted in China, there is no way to determine the initial frequency
472 of resistant allele empirically. Therefore, we used a theoretical value of 0.001 as the initial
473 frequency of resistance allele associated with Locus 2 (see e.g. [27]).

474

475 **Time to resistance (TTR)**

476 We regarded the threshold of resistance risk as the frequency of a resistance allele reaches 50%.
477 When resistance is governed by a single locus, we referred to the time it takes for the frequency of
478 the resistance allele to reach 50% as the “time to resistance” (TTR). When resistance is governed
479 by two loci, we referred to the time it takes for both of the frequencies of resistance alleles to
480 reach 50% as the TTR.

481

482 **Computer programming**

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

484

485 **Table 1** The notation, meaning and default value or range of parameters used in model
486 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]

P_{Bt_2}	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
μ_1	Mortality of S_1S_1 on Bt cotton and Bt maize with Cry1Ac or similar protein	0.95	[14,20,23]
μ_2	Mortality of S_2S_2 on Bt maize with Cry2Ab or similar protein	0.8	[44]
c_1	Fitness cost of R_1R_1	0.36	[23]
c_2	Fitness cost of R_2R_2	0	[33,43]
d_i	Dominance of fitness cost for R_i ($i=1,2$)	0	[23,33]
σ_1	Incomplete resistance of R_1R_1	0.505	[23]
σ_2	Incomplete resistance of R_2R_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]

487

488

489

490 **Declarations**

491 **Abbreviations**

492 CBW: Cotton bollworm

493 TTR: Time to resistance

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

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

496

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499

500 **Authors' contributions**

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

502 and P.W. wrote the paper.

503

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508 and interpretation of the data, and writing of the manuscript.

509

510 **Availability of data and materials**

511 All data generated or analyzed during this study are included in this published article.

512

513 **Ethics approval and consent to participate**

514 Not applicable.

515

516 **Consent for publication**

517 Not applicable.

518

519 **Competing interests**

520 The authors declare that they have no competing interests.

521

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Figures

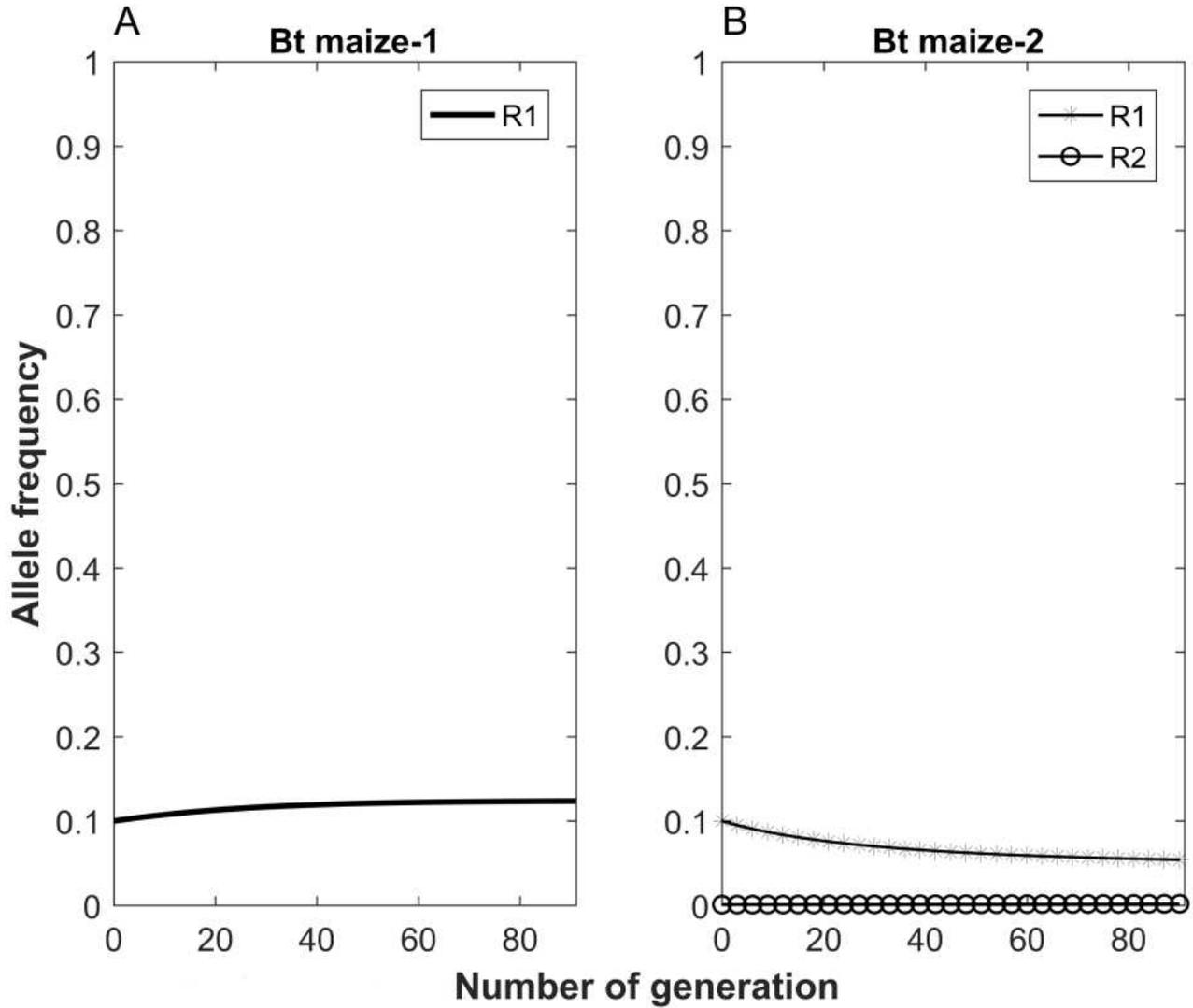


Figure 1

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. R1 and R2 represent the resistance alleles at loci 1 and 2, respectively. In this figure, $P1=0$, which means that cotton plants are absent. The rest of parameters are set at default.

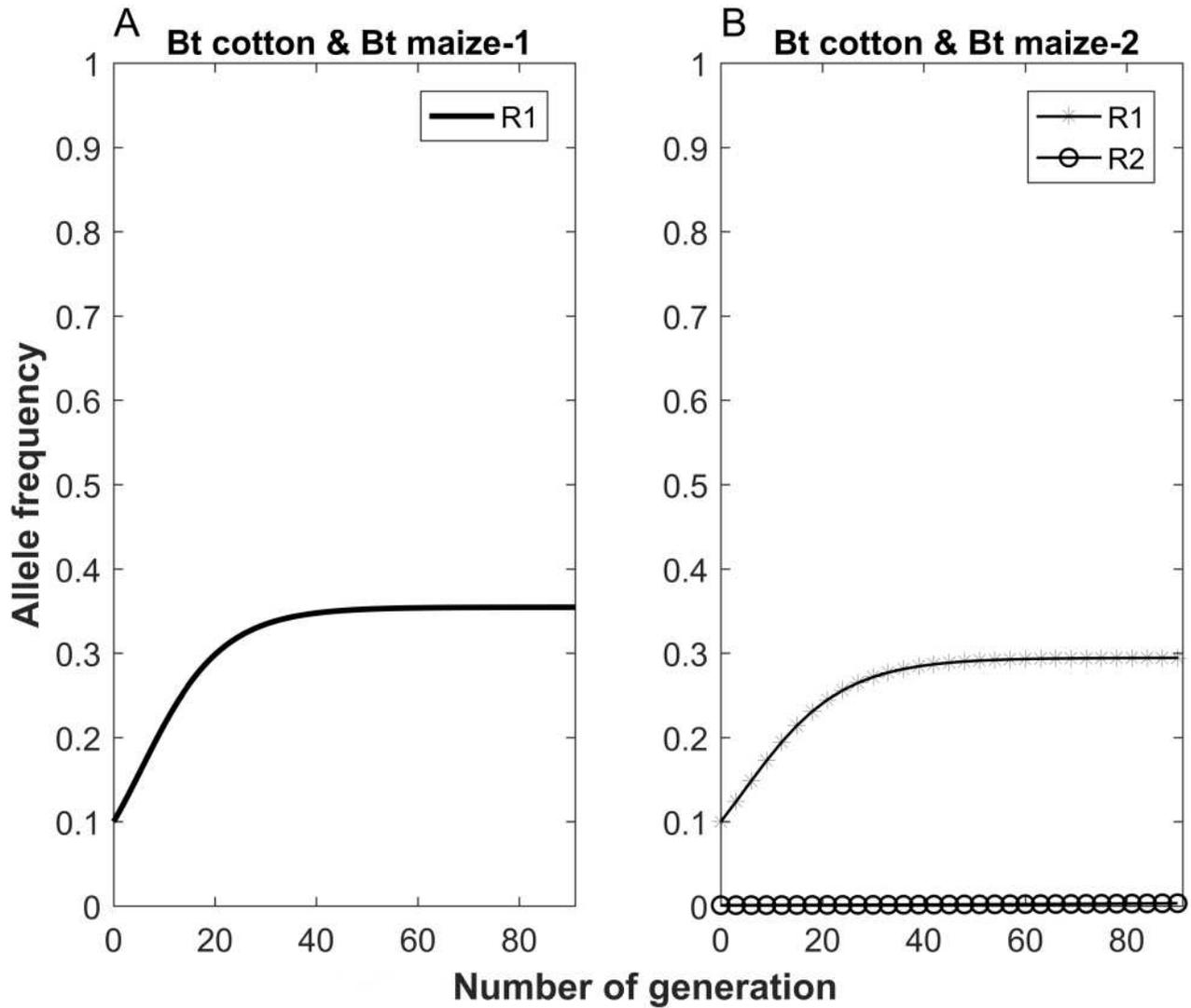


Figure 2

Changes in the frequency of resistance alleles over time. a Bt cotton & Bt maize-1 where Bt cotton and Bt maize contains 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, R1 and R2 represent the resistance alleles at loci 1 and 2, respectively. Parameters are all set at default.

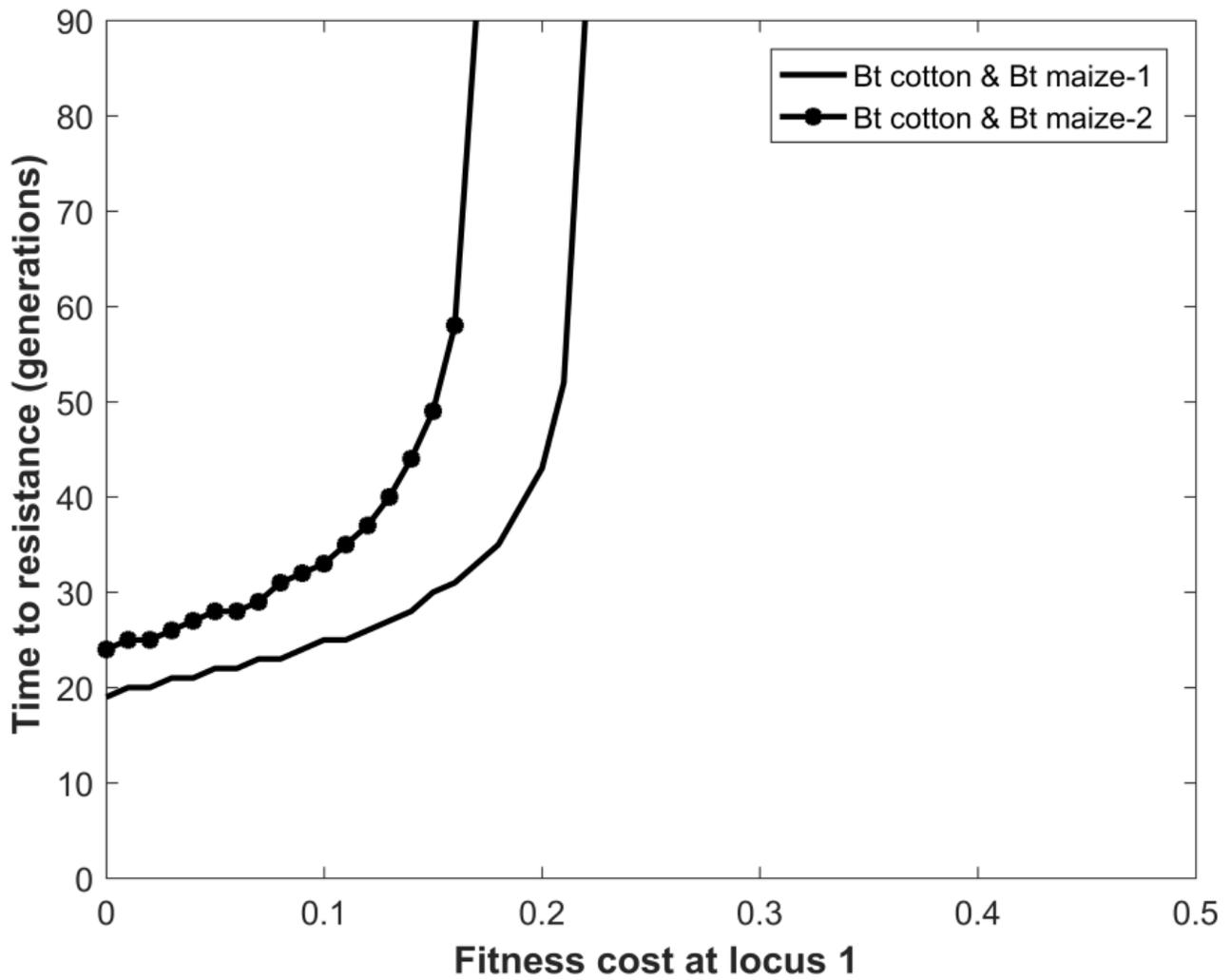


Figure 3

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.

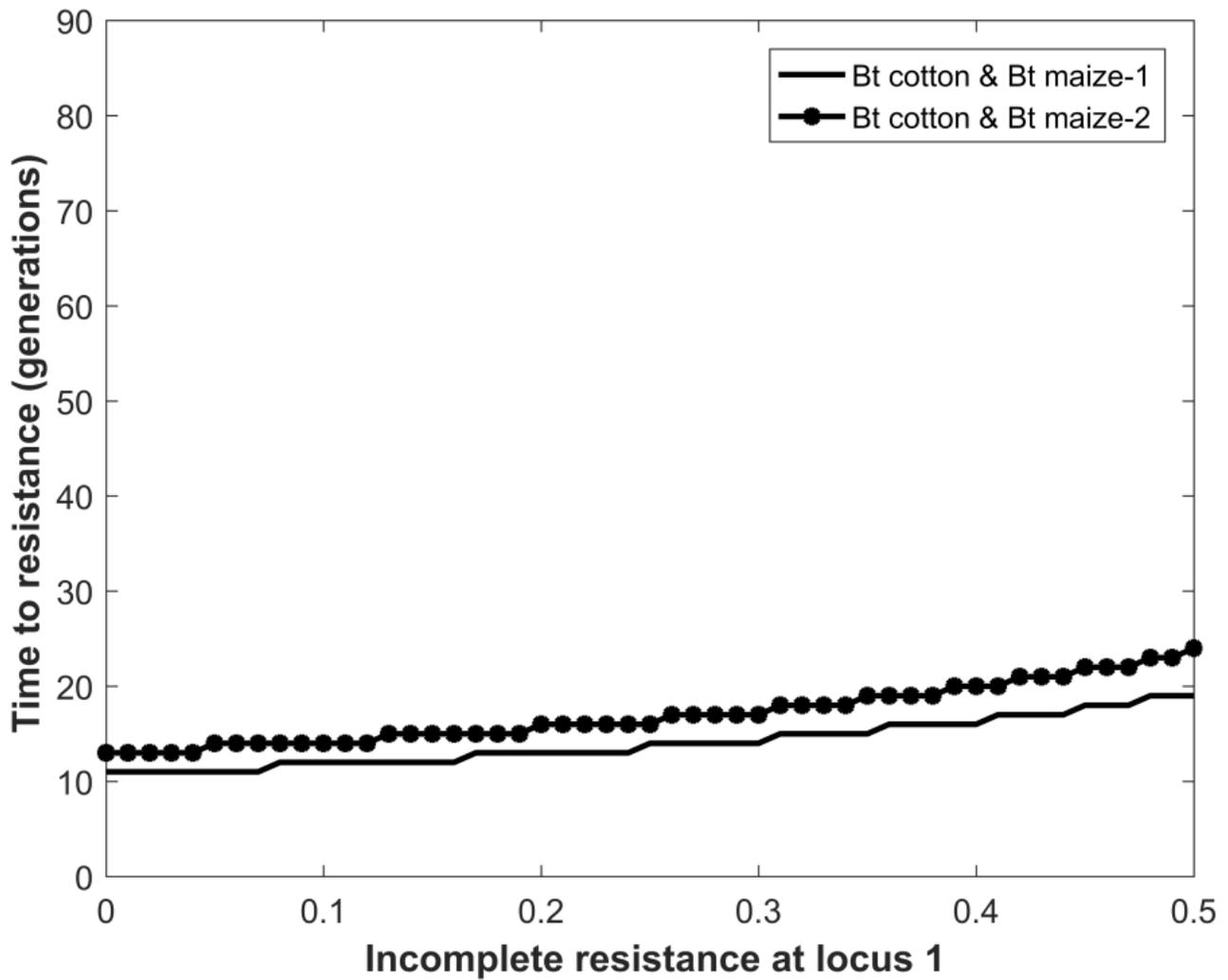


Figure 4

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.

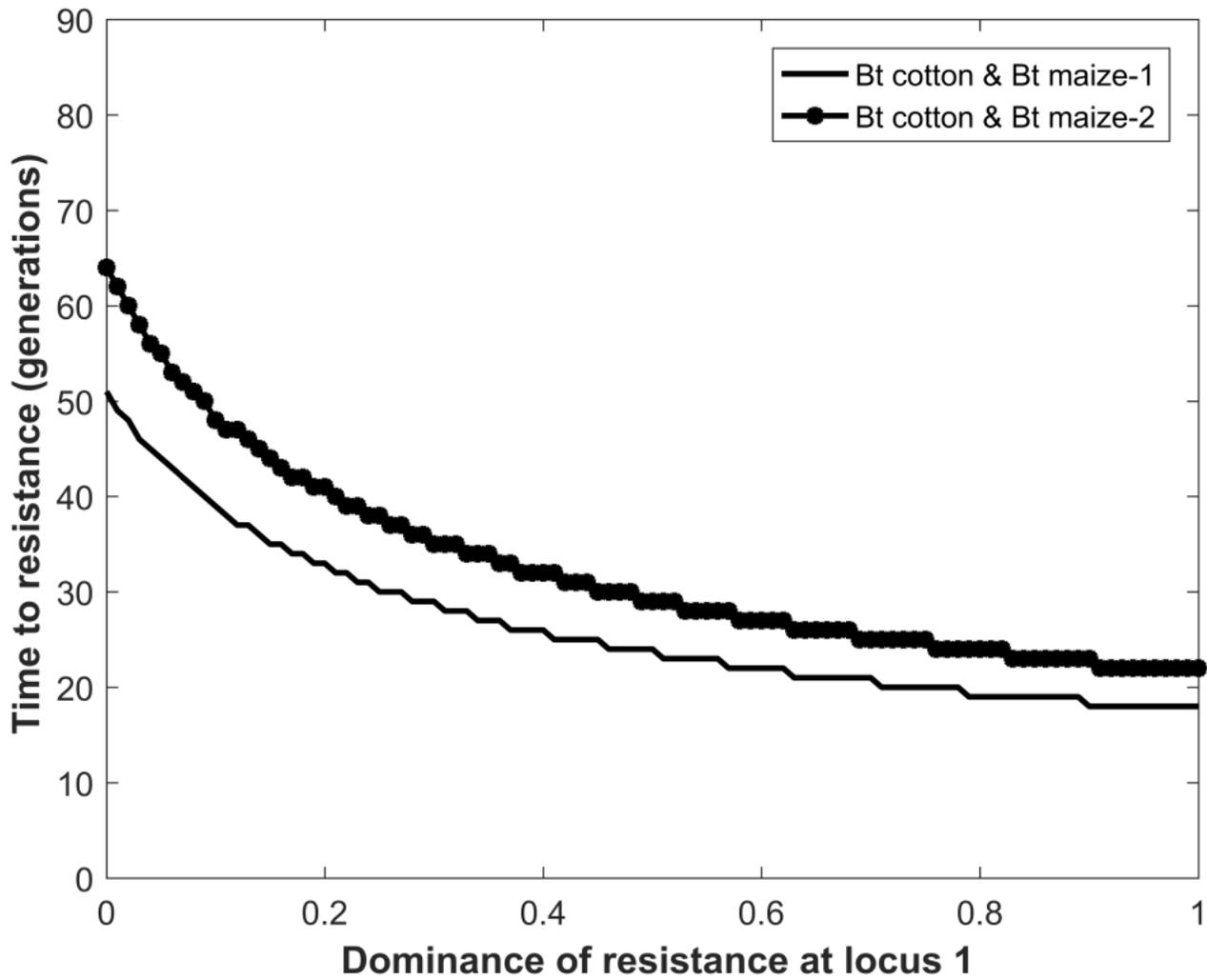


Figure 5

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.

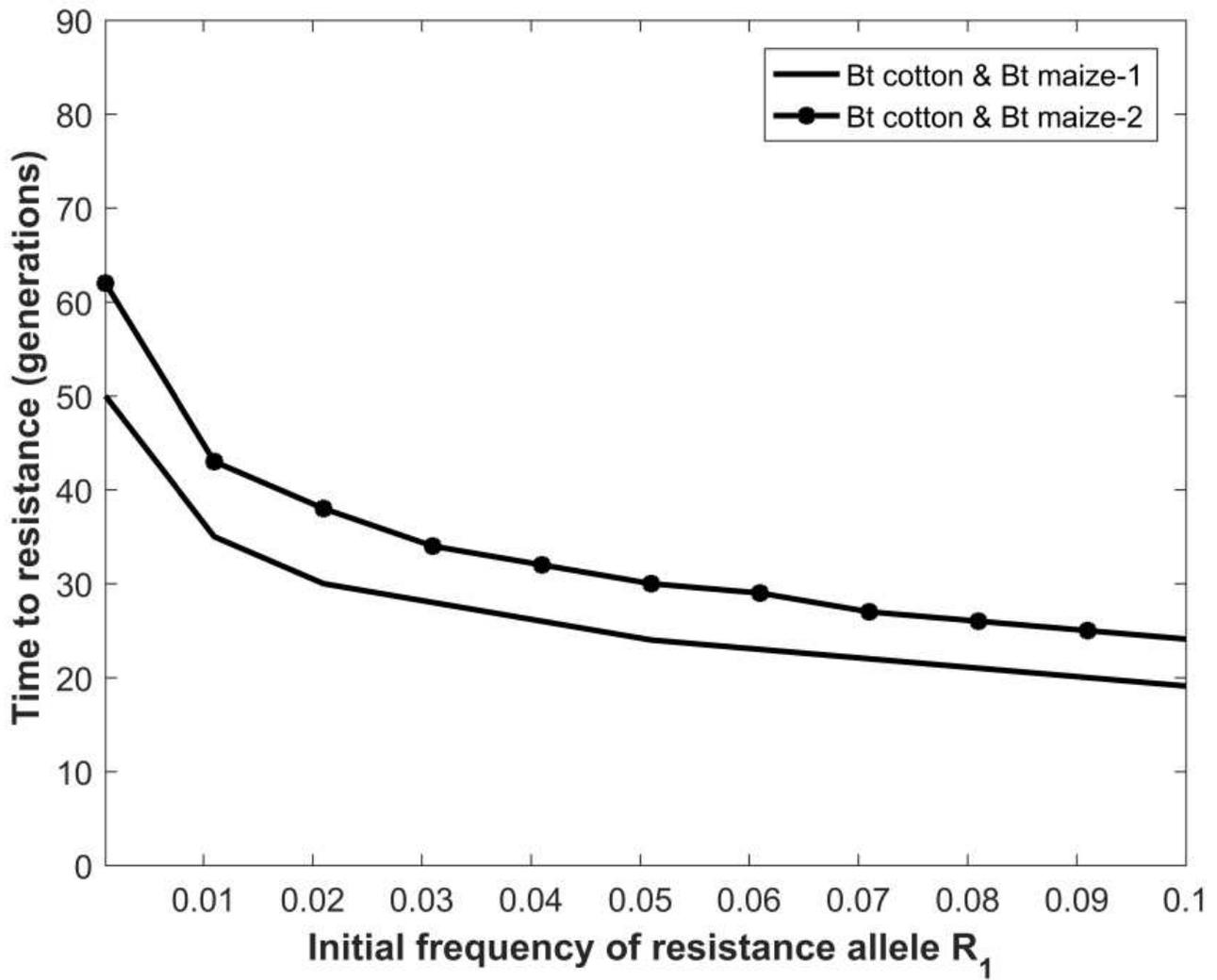


Figure 6

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.

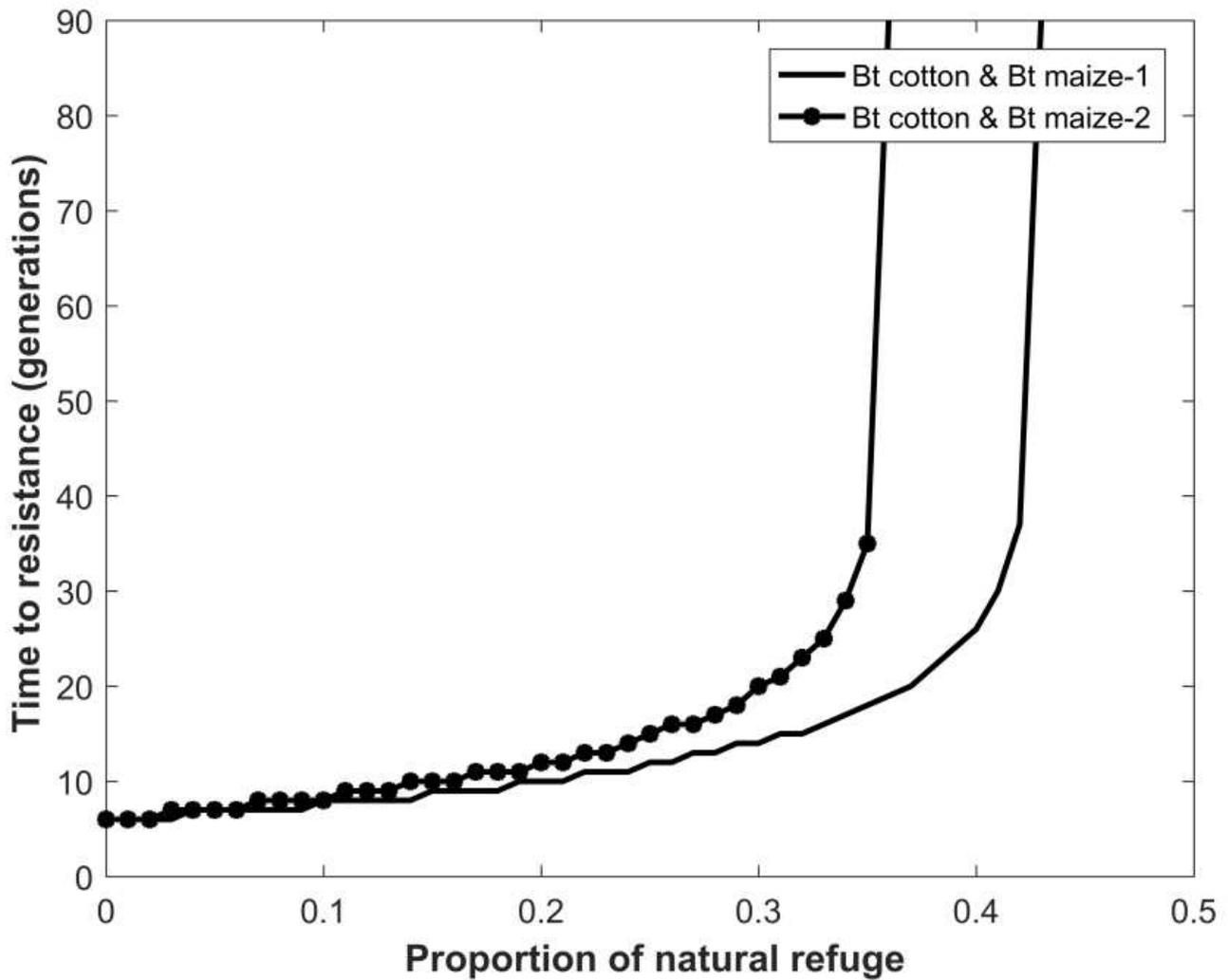


Figure 7

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 $P_1 = 0.64 \cdot (1 - P_{nat})$ and $P_2 = 0.36 \cdot (1 - P_{nat})$, respectively. The rest of parameters are set at default.