doi:10.1111/j.1420-9101.2004.00730.x

SHORT COMMUNICATION

High dose refuge strategies and genetically modified crops – reply to Tabashnik *et al.*

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Keywords

Bacillus thuringiensis;
cotton;
dominance;
fitness cost;
Heliothis virescens;
resistance;
transgenic crops.

Abstract

Cultivating non-toxic conventional crops (refuges) in the proximity to transgenic crops that produce *Bacillus thuringienesis* (*Bt*) toxins is widely recommended to delay pest adaptation to these toxins. Using a spatially structured model of resistance evolution, Vacher and co-workers (Vacher, C., Bourguet, D., Rousset, F., Chevillon, C. & Hochberg, M.E. 2003. *J. Evol. Biol.* **16**: 378–387.) show that the percentage of refuge fields required for the sustainable control of pests can be reduced through intermediate levels of refuge field aggregation and by lowering the toxin dose produced by *Bt* plants. Tabashnik, B.E., Gould, F. & Carrière, Y. (2004 *J. Evol. Biol* doi: 10.1111/j1420–9101.2004.00695.x) call into question the results of Vacher *et al.* (2003) concerning the effect of toxin dose. They argue that these results arise from invalid assumptions about larval concentration–mortality responses for the insect considered, the cotton pest *Heliothis virescens*. We show here that the models presented by Vacher *et al.* (2003) and Tabashnik *et al.* (2004) both show inaccuracies in their definitions of genotypic fitness. The level of dominance estimated by Tabashnik *et al.* (2004) from larval mortality rates data is irrelevant to resistance evolution, and the fitness cost of resistance evolution, and the fitness cost of resistance is inaccurately integrated into their framework. Neverthless, the comments of Tabashnik *et al.* (2004) are very helpful in elucidating the definitions of genotypic fitness used in Vacher *et al.* (2003) and in pointing out the essential factors in predicting the evolution of insect resistance to *Bt* transgenic crops, namely, accurate estimations of the fitness cost of resistance, of the dominance level of this cost, and of the variations in the dominance level of the advantage conferred by the resistance with *Bt* toxin dose.

Introduction

Cultivating nontoxic conventional crops (refuges) in the proximity to transgenic crops that produce *Bacillus thuringiensis* (*Bt*) toxins is widely recommended to delay pest adaptation to these toxins. Using a spatially-structured model of resistance evolution, Vacher *et al.* (2003) argue that the spatial configuration of such refuge fields can be optimized to minimize pest densities over the cultivated region whilst preventing resistance rather than just slowing its evolution. Implementing refuge fields in such a way could promote the sustainable control of pests. Vacher *et al.* (2003) show that the percentage of refuge fields required for this sustainable control can be reduced through intermediate levels of refuge field aggregation and by lowering the toxin dose produced by *Bt* plants. They additionally show that the spatial

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configuration of fields optimal for sustainable pest control is also that which produces the longest delay in resistance evolution if the fitness cost associated with resistance decreases, due to for example the selection of modifier alleles. They conclude that the two principles underlying the 'high-dose refuge' strategy – refuge fields in close proximity to *Bt* plants and high toxin dose – are suboptimal in the context of sustainable pest control.

Tabashnik *et al.* (2004) call into question the results of Vacher *et al.* (2003) concerning the effect of toxin dose. They argue that these results arise from invalid assumptions about larval concentration-mortality responses. Tabashnik *et al.* (2004) present larval mortality data from two major cotton pests (*Heliothis virescens* and *Pectinophora gossypiella*) as a function of *Bt* toxin dose. With these bioassay data, they first estimate the effective dominance of resistance, *h*, defined as: (survival of RS − survival of SS)/(survival of RR − survival of SS). In a previous paper, Bourguet *et al.* (2000) referred to this dominance level based on larval mortality rates data as D_{ML}. Tabashnik *et al.* (2004) show that the effective dominance of resistance to *Bt* toxins decreases with toxin dose for *H. virescens* and *P. gossypiella* (Table 1 in Tabashnik *et al.*, 2004). They then introduce these bioassay data into a simple population genetic model that does not include the spatial distribution of *Bt*-crops and refuges, and hence, the selection/migration history of the pests. As increasing toxin dose delays resistance more effectively under the set of parameters they chose (Table 1 in Tabashnik *et al.*, 2004), they conclude that 'reduced (effective) dominance of resistance (…) delay(s) evolution of resistance to *Bt* crops'. Finally, and without having evaluated Vacher *et al.*'s spatial model, Tabashnik *et al.* (2004) claim that lowering *Bt* toxin dose delays resistance evolution in Vacher *et al.*'s (2003) model because of invalid changes in dominance levels with toxin dose (Table 1 in Tabashnik *et al.*, 2004).

Although model limitations are discussed on pages 385–386 in Vacher *et al.* (2003), the robustness of predictions to variations in the dominance level of

Table 1 Effects of resistance cost to *Bt* toxin on the dominance of the fitness advantage conferred by the resistance (R) allele in toxic fields, and change in R allele frequency in the first generation.

Bt toxin concentration (μg mL^{-1})	Genotypic fitnesses in *Bt* fields			Effective dominance of resistance (D_{ML})	Dominance of the fitness advantage conferred by the R allele (D_{WT})	Change in R allele frequency in the first generation*
	SS	RS	RR			
No cost of resistance†						
0.32	0.370	0.930	1.000	0.889	0.889	1.4×10^{-3}
1.6	0.040	0.520	1.000	0.500	0.500	2.5×10^{-3}
8.0	0.000	0.080	1.000	0.080	0.080	4.9×10^{-4}
40	0.000	0.005	1.000	0.005	0.005	3.9×10^{-5}
Recessive fitness cost of resistance of 15%‡						
0.32	0.370	0.930	0.850	0.889	1.167	1.4×10^{-3}
1.6	0.040	0.520	0.850	0.500	0.592	2.5×10^{-3}
8.0	0.000	0.080	0.850	0.080	0.094	4.9×10^{-4}
40	0.000	0.005	0.850	0.005	0.006	3.7×10^{-5}
Dominant fitness cost of resistance of 60%§						
0.32	0.370	0.372	0.400	0.889	0.067	-3.6×10^{-4}
1.6	0.040	0.208	0.400	0.500	0.467	9.5×10^{-5}
8.0	0.000	0.032	0.400	0.080	0.080	-7.1×10^{-4}
40	0.000	0.002	0.400	0.005	0.005	-8.9×10^{-4}

*In all cases we assumed that the initial frequency of the resistance (R) allele was 0.0015 as in Vacher *et al.* (2003) and Tabashnik *et al.* (2004). Changes in R allele frequency were calculated with Tabashnik *et al*'s model. As in Tabashnik *et al.* (2004), we assumed that the habitat had 80% *Bt* fields and 20% refuges.

†In the absence of any fitness cost of resistance, we assumed that genotypic fitnesses equal genotypic larval survival rates. Survival rates are those observed by Tabashnik *et al.* (2004) for *P. gossypiella* on *Bt* cotton. Fitnesses in refuges were 1 for SS, RS and RR. In this case, the dominance of the fitness advantage conferred by the R allele in toxic Bt fields (D_{WT}) equals the effective dominance of resistance (D_{ML} in Bourguet *et al.*, 2000; *h* in Tabashnik *et al.*, 2004).

‡With a recessive fitness cost of resistance of 15%, fitnesses in refuges were 1 for SS and RS, and 0.850 for RR. Contrary to Tabashnik *et al.* (2004), the cost of resistance decreased the fitness of RR individuals both in refuges and *Bt* fields. Fitness of RR individuals in *Bt* fields was defined as $W = (1 - c)S$, where *c* is the cost of resistance and *S* the larval survival rate.

§With a dominant fitness cost of resistance of 60%, fitnesses in refuges were 1 for SS, and 0.400 for RS and RR. Contrary to Tabashnik *et al.* (2004), cost of resistance decreased the fitness of RR and RS individuals both in refuges and *Bt* fields. Fitness of RR and RS individuals in *Bt* fields was defined as $W = (1 - c)S$, where *c* is the cost of resistance and *S* the larval survival rate.

resistance to *Bt* toxins are not explicitly studied. The comments of Tabashnik *et al.* (2004) are therefore welcome and our objective here is to begin to fill this gap. After having made a brief summary of the relationship between dominance levels and resistance evolution, we present new simulation results based on the bioassay data of Tabashnik *et al.* (2004).

Effective dominance of resistance – an index estimated from larval survival rates in the presence of *Bt* toxin – is not the relevant level of dominance to address evolutionary issues

Bourguet *et al.* (2000) have shown that the relevant level of dominance to address evolutionary issues is not the effective dominance of resistance (D_{ML}) estimated from larval survival rates, but rather the dominance of the fitness advantage conferred by the resistance allele in the toxic area (D_{WT}) estimated from genotypic fitnesses. There is no obvious correlation between these two dominance levels (Bourguet *et al.*, 2000). D_{WT} is defined as (fitness of RS − fitness of SS)/(fitness of RR − fitness of SS) (Bourguet *et al.*, 2000). Contrary to D_{ML}, D_{WT} accounts for the fitness costs of resistance that may arise on fitness components other than insect survival (Groeters *et al.*, 1994; Alyokhin & Ferro, 1999), and on the sublethal effects of larval exposure to the *Bt* toxin (Gould *et al.*, 1995; Liu *et al.*, 1999). The high dose/refuge strategy requires D_{WT} not D_{ML} be close to zero (Bourguet *et al.*, 2000). Below we demonstrate that Tabashnik *et al.*'s (2004) major point – i.e. reduced effective dominance of resistance delays resistance evolution – is not necessarily true, as it depends on the fitness cost of resistance.

Dominance of the fitness advantage conferred by the resistance allele – the relevant measure for evolutionary issues – does not necessarily decrease when toxin dose increases

Tabashnik *et al.* (2004) assume that the fitness cost of resistance to *Bt* toxin is *not* paid by resistant insects in toxic fields. Under this assumption, the fitness of RR insects is allowed to be lower in conventional crops ($RefW_{RR}$) than in toxic *Bt* crops (BtW_{RR}). For instance, taking the hypothetical value of 15% given to the fitness cost in Vacher *et al.* (2003), and assuming a toxin concentration of 8.0 μg mL^{-1} in *Bt* crops, they estimate the fitnesses of resistant homozygotes *H. virescens* as $RefW_{RR} = 0.85$ and $BtW_{RR} = 0.97$. Assuming a *Bt* toxin concentration of 1.6 μg mL^{-1}, these fitnesses become $RefW_{RR} = 0.85$ and $BtW_{RR} = 1$. Similarly, fitnesses of homozygous resistant *P. gossypiella* are computed as $RefW_{RR} = 0.85$ and $BtW_{RR} = 1$, whatever the toxin concentration encountered on *Bt* plants. These obviously unrealistic assumptions have important implications for their conclusions. Using the larval survival rates observed in *P. gossypiella*, we show in Table 1 that a fitness cost paid by resistant insects after the larval stage in conventional *and* in *Bt* fields can modify dominance levels, and their variations with toxin dose. For instance, a high cost of resistance can bring D_{WT} close to zero at a low toxin dose, and cause a decline in the resistance allele frequency (Table 1). It is noteworthy that in this case, resistance evolution is effectively delayed despite the fact that the effective level of dominance D_{ML} is very high (Table 1). Thus, correlations between changes in allele frequency and the effective level of dominance shown by Tabashnik *et al.* (2004) (their Table 1) are valid for their particular assumptions on the fitness cost of resistance but do not appear to be a general case.

Contrary to the conclusion of Tabashnik *et al.* (2004), the predictions of Vacher *et al.* (2003) are not due to the assumed pattern of dominance as a function of toxin dose

In Vacher *et al.* (2003), and contrary to the assumptions of Tabashnik *et al.* (2004), fitness cost of resistance is experienced by insects both in refuges *and* in *Bt* fields. Accordingly, in contrast to the assumptions of Tabashnik *et al.* (2004), Vacher *et al.* (2003) determine the fitness of RS insects in *Bt* fields not only by their sensitivity to the toxin at the larval stage, but also by the potential fitness cost associated with their single resistance allele. Vacher *et al.* (2003) define relative genotypic fitnesses following Lenormand & Raymond (1998) as:

$$W_{RR} = 1 - c$$

$$W_{RS} = 1 - h_c\, c - g\, h_s\, s$$

$$W_{SS} = 1 - g\, s$$

Parameter g is equal to one in toxic fields; otherwise it equals zero. Thus, in our formulation and as in Tabashnik *et al.* (2004), the maximal fitness is always that of SS insects in refuges (i.e. it equals one). Parameter s is the selection coefficient in the presence of *Bt* toxin that is the proportion of susceptible insects killed by the toxin. It increases with toxin dose. Parameter h_s is the dominance level associated with toxin selection. In the absence of toxin sublethal effects, h_s equals $(1 - h)$, where h is the effective dominance level estimated by Tabashnik *et al.* (2004). Parameter c is the fitness cost of resistance and h_c is the dominance level of the fitness cost. As the cost of resistance could act on other fitness components than larval survival, values given in Table 1 in Vacher *et al.* (2003) are not necessarily equal to genotypic survival rates. They correspond to relative genotypic fitness (i.e. the relative contribution of each genotype to the subsequent generation). The legend in Table 1 of Vacher *et al.*

(2003) is therefore unintentionally misleading and should be corrected. Based on the published information in Table 1 of Vacher *et al.* (2003), Tabashnik *et al.* (2004) interpret that the dominance levels calculated on the genotypic fitness assumed by Vacher *et al.* (2003) and the dominance levels calculated using the larval mortality rates they estimated from bioassays, are comparable. They are not: the former defines D_{WT} while the latter defines D_{ML}.

As mentioned above, the dominance of the fitness advantage conferred by the resistance allele (D_{WT}) does not necessarily decrease when toxin dose increases. Computation of D_{WT} from the above genotypic fitness equations gives:

$$D_{WT} = \frac{h_c\, c - (1 - h_s)s}{(c - s)}$$

A simple derivation of D_{WT} with respect to s shows that D_{WT} increases with s under the following condition:

$$h_c > 1 - h_s.$$

As the condition $h_c > 1 - h_s$ is verified in the set of parameters employed by Vacher *et al.* (2003), D_{WT} increases with s. Lowering the dominance of the fitness cost h_c leads to a decrease of D_{WT} with s (Table 2). Interestingly, whether D_{WT} increases or decreases when s increases, lowering the toxin dose (i.e. lowering s) *always reduces* the percentage of refuge required for a sustainable control of pests and *always slows* the initial progression of the *R* allele (Table 2). Hence, contrary to the corrections of Tabashnik *et al* (2004), our published results were not due to the assumed pattern of dominance as a function of toxin dose: our conclusion remains valid as long as h_s and h_c do not vary with toxin dose (see below for other situations). Moreover, the increase in dominance level (h) calculated by Tabashnik *et al.* (2004) with our parameters (their Table 1) is neither surprising nor invalid because it does not correspond to the effective level of dominance D_{ML}, but to the dominance of the fitness advantage conferred by the resistance allele D_{WT}. Finally, when h_c and h_s are fixed parameters, it is noteworthy that the conditions $h_c > 1 - h_s$ and $h_c < 1 - h_s$ have drastic consequences on the percentage of refuge required to prevent resistance evolution: reasonable refuge sizes require $h_c > 1 - h_s$ (Table 2). Hence, resistance evolution might be easier to prevent when the fitness cost of resistance is dominant.

Table 2 Effects of dominance of the fitness advantage conferred by the resistance (R) allele in *Bt* fields on change in R allele frequency in the first generation and on the percentage of refuge fields required to prevent resistance evolution.

Selection coefficient in the presence of *Bt* toxin (s)	Genotypic fitnesses in *Bt* fields			Dominance of the fitness advantage conferred by the R allele (D_{WT})	Change in R allele frequency in the first generation*	Percentage of refuge fields required to prevent resistance evolution† (%)
	SS	RS	RR			
Dominance D_{WT} increases with the selection coefficient ($h_c > 1 - h_s$)‡						
$h_c = 0.3$						
0.6	0.400	0.385	0.850	−0.033	-5.9×10^{-5}	0
0.8	0.200	0.195	0.850	−0.008	-5.1×10^{-5}	0
1	0.000	0.005	0.850	0.005	-3.0×10^{-5}	9
$h_c = 0.2$						
0.6	0.400	0.400	0.850	0.000	-1.6×10^{-5}	3
0.8	0.200	0.210	0.850	0.015	1.1×10^{-5}	21
1	0.000	0.020	0.850	0.024	8.3×10^{-5}	26
Dominance D_{WT} decreases with the selection coefficient ($h_c < 1 - h_s$)‡						
$h_c = 0.03$						
0.6	0.400	0.4255	0.850	0.057	4.3×10^{-5}	85
0.8	0.200	0.2355	0.850	0.055	9.7×10^{-5}	88
1	0.000	0.0455	0.850	0.053	2.4×10^{-4}	90
$h_c = 0.0$						
0.6	0.400	0.430	0.850	0.067	7.1×10^{-5}	100
0.8	0.200	0.240	0.850	0.061	1.4×10^{-4}	100
1	0.000	0.050	0.850	0.059	3.1×10^{-4}	100

*In all cases we assumed that the initial frequency of the resistance (R) allele was 0.0015 as in Vacher *et al.* (2003) and Tabashnik *et al.* (2004). Changes in R allele frequency were calculated with the model developed in Tabashnik *et al.* (2004). As in Tabashnik *et al.* (2004), we assumed that the habitat had 80% *Bt* fields and 20% refuges.

†In all cases we assumed that the initial R allele frequency was 0.0015 as in Vacher *et al.* (2003) and Tabashnik *et al.* (2004). Percentage refuge fields required to prevent resistance evolution were calculated with the model developed in Vacher *et al.* (2003). Refuge fields were aggregated into two strips.

‡Genotypic fitnesses were expressed as in Lenormand & Raymond (1998) and Vacher *et al.* (2003) with $c = 0.15$, $h_s = 0.95$.

Contrary to the previous conclusion of Vacher *et al.* (2003), increasing the toxin dose produced by *Bt* plants can decrease the percentage of refuge required for the sustainable control of *H. virescens*

With regard to the bioassay data on *H. virescens* presented by Tabashnik *et al.* (2004), we acknowledge that the genotypic fitness equations employed in Vacher *et al.* (2003) require modification. First, the selection coefficient in the presence of the *Bt* toxin (s) and the associated dominance level (h_s) should not be independent variables: h_s should be a decreasing function of s. Secondly, if the fitness cost of resistance is completely recessive, as is assumed in Tabashnik *et al.* (2004), h_c should be equal to zero. Thirdly, if RR larvae are not completely resistant to the toxin, then their fitness should not be a function of the cost of resistance only, but also of the toxin dose s. These important points will require further study.

Another approach is to use the larval mortality rates measured in bioassay data, and to properly integrate the fitness cost for estimating the genotypic fitnesses of these particular insect strains. This has the advantage of taking the three modifications simultaneously into account, but the disadvantage of being parameterized for a specific biological system. Using the bioassay data for *H. virescens* (Tabashnik *et al.*, 2004), we present the percentage refuge that would both prevent pest adaptation to *Bt* toxins and minimize their density, for three different fitness costs of resistance (Fig. 1). Contrary to our previous conclusion, the optimal percentage of refuge predicted with this new model decreases with toxin dose. Further investigations on the relative role of each of the three modifications introduced are required to determine why the case of *H. virescens* presented by Tabashnik *et al.* (2004) contradicts the predictions of our previous model. Interestingly though, it is worth noting that in this case the refuge size becomes very sensitive to the fitness cost of resistance and its level of dominance when *Bt* crops express a high dose of toxin. Similar to the predictions of our original model (Table 2), reasonable refuge sizes require the fitness cost of resistance to be dominant. Therefore, the accurate estimation of the cost of resistance and its associated level of dominance are needed before determining the optimal percentage refuge that would be sufficient to prevent the evolution of resistance to *Bt* toxins in this pest.

Further investigation on the fitness cost of resistance and its dominance are required to develop realistic models of resistance evolution to *Bt* toxins in agricultural pests

In conclusion, the models presented by Vacher *et al.* (2003) and Tabashnik *et al.* (2004) both show inaccuracies in their definitions of genotypic fitnesses. The level of dominance estimated by Tabashnik *et al.* (2004) from larval mortality rates data is irrelevant to resistance evolution, and the fitness cost of resistance is inaccurately integrated into their framework. Moreover, contrary to the conclusion of Tabashnik *et al.* (2004), the predictions of Vacher *et al.* (2003) concerning the effect of toxin dose on the optimal percentage of refuge are not due to the assumed pattern of dominance. The predictions based on the model of Vacher *et al.* (2003) are indeed robust to various patterns of dominance of the fitness advantage conferred by the resistance allele D_{WT} as a function of toxin concentration: as long as dominance levels associated with toxin selection and fitness cost of resistance (h_s and h_c, respectively) do not vary

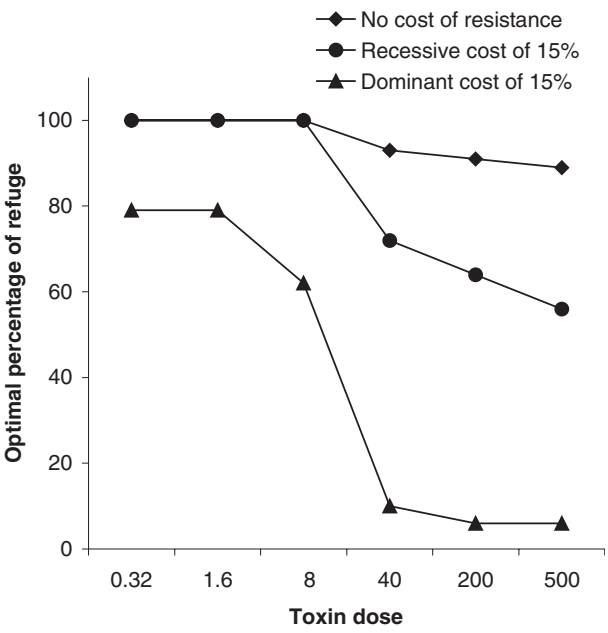

Fig. 1 Effects of *Bt* toxin dose and fitness cost of resistance to *Bt* toxin on the percentage of refuge required to prevent resistance evolution to *Bt* toxins in *Heliothis virescens*. In all cases the optimal percentage of refuge was calculated with the model published by Vacher *et al.* (2003). Refuges were aggregated into two strips. In the absence of any fitness cost of resistance, we assumed that genotypic fitnesses are equal to genotypic larval survival rates. Survival rates are those observed by Tabashnik *et al.* (2004) for *H. virescens* on *Bt* cotton. Fitnesses in refuges were 1 for SS, RS and RR. Contrary to the assumptions of Tabashnik *et al.* (2004), when assuming the occurrence of non-null fitness cost of resistance, such a cost occurring both in refuges and in *Bt* fields. With a recessive fitness cost of resistance of 15%, fitnesses in refuges were 1 for SS and RS, and 0.850 for RR. Fitness of RR individuals in *Bt* fields was defined as $W = (1 - c)S$, where c is the cost of resistance and S the larval survival rate. With a dominant fitness cost of resistance of 15%, fitnesses in refuges were 1 for SS, and 0.850 for RS and RR. Fitness of RR and RS individuals in *Bt* fields was defined as $W = (1 - c)S$, where c is the cost of resistance and S the larval survival rate.

with toxin dose s, and regardless of whether D_{WT} increases or decreases with toxin dose, lowering the toxin dose *reduces* the percentage of refuge required for sustainable pest control.

Nevertheless, the comments of Tabashnik *et al.* (2004) are very helpful in elucidating the definitions of genotypic fitness used in Vacher *et al.* (2003) and in pointing out that h_s is not independent of s. Based on the modified model presented here, and contrary to our previous predictions, increasing the toxin dose produced by *Bt* plants decreases the percentage of refuge required for the sustainable control of *H. virescens*. Further investigations are required to determine which of the three modifications made to our genotypic fitness estimation leads to this qualitative change in our previous predictions. However, it is noteworthy that this conclusion is not necessarily true for all pest species targeted by *Bt* crops. Indeed, while D_{ML} decreases when the toxin dose increases, fitness costs may induce concomitant increases in D_{WT}. In such cases, increasing the toxin dose produced by *Bt* plants could increase the percentage of refuge required for sustainable pest control.

Overall, this debate pinpoints the essential factors in predicting the evolution of insect resistance to *Bt* transgenic crops, namely, accurate estimations of the fitness cost of resistance, of the dominance level of this cost, and of the variations in the dominance level of the advantage conferred by resistance with *Bt* toxin dose.

References

Alyokhin, A.V. & Ferro, D.N. 1999. Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to the *Bacillus thuringiensis* Cry3A toxin. *J. Econ. Entomol.* **92**: 512–515.

Bourguet, D., Génissel, A. & Raymond, M. 2000. Insecticide resistance and dominance levels. *J. Econ. Entomol.* **93**: 1588–1595.

Gould, F., Anderson, A., Reynolds, A., Bumgarner, L. & Moar W. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *J. Econ. Entomol.* **88**: 1545–1559.

Groeters, F.R., Tabashnik, B.E., Finson, N. & Johnson, M.W. 1994. Fitness costs of resistance to *Bacillus thuringiensis* in the diamondback moth (*Plutella xylostella*). *Evolution* **48**: 197–201.

Lenormand, T. & Raymond M. 1998. Resistance management: the stable zone strategy. *Proc. R. Soc. Lond. B Biol. Sci.* **265**: 1985–1990.

Liu, Y.B., Tabashnik, B.E., Dennehy, T.J., Patin, A.L. & Barlett, A.C. 1999. Development time and resistance to Bt crops. *Nature* **400**: 519.

Tabashnik, B.E., Gould, F. & Carrière Y. 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. *J. Evol. Biol.*, doi: 10.1111/j.1420-9101.2004.00695.x

Vacher, C., Bourguet, D., Rousset F., Chevillon, C. & Hochberg M.E. 2003. Modelling the spatial configuration of refuges for a sustainable control of pests: a case study of *Bt* cotton. *J. Evol. Biol.* **16**: 378–387.

Received 23 December 2003; revised 23 January 2004; accepted 30 January 2004