

# Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability

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*Pectinophora gossypiella*;  
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## Abstract

The refuge strategy is used widely for delaying evolution of insect resistance to transgenic crops that produce *Bacillus thuringiensis* (Bt) toxins. Farmers grow refuges of host plants that do not produce Bt toxins to promote survival of susceptible pests. Many modelling studies predict that refuges will delay resistance longest if alleles conferring resistance are rare, most resistant adults mate with susceptible adults, and Bt plants have sufficiently high toxin concentration to kill heterozygous progeny from such matings. In contrast, based on their model of the cotton pest *Heliothis virescens*, Vacher *et al.* (Journal of Evolutionary Biology, 16, 2003, 378) concluded that low rather than high toxin doses would delay resistance most effectively. We demonstrate here that their conclusion arises from invalid assumptions about larval concentration-mortality responses and dominance of resistance. Incorporation of bioassay data from *H. virescens* and another key cotton pest (*Pectinophora gossypiella*) into a population genetic model shows that toxin concentrations high enough to kill all or nearly all heterozygotes should delay resistance longer than lower concentrations.

## Introduction

'If Darwin were alive today the insect world would delight and astound him with its impressive verification of his theories of survival of the fittest. Under the stress of intensive chemical spraying the weaker members of the insect populations are being weeded out' (Carson, 1962). Evolution of insecticide resistance, which has been documented in more than 500 species of insects and mites (Georghiou & Lagunes-Tejeda, 1991), threatens agriculture and human health worldwide. The myriad examples of insecticide resistance offer rich opportunities for examining hypotheses about how response to selection is affected by various factors, including dominance, gene flow, fitness tradeoffs, genetic constraints, major and minor genes, haplodiploidy, founder events, life-history traits and multitrophic interactions (Roush & McKenzie, 1987; Rosenheim & Tabashnik, 1991, 1993;

Carrière & Roff, 1995; Rosenheim *et al.*, 1996; French-Constant *et al.*, 1998; Bourguet & Raymond, 1998; Groeters & Tabashnik, 2000; Carrière *et al.*, 2001a; Tabashnik, 2002; Carrière, 2003). Whereas study of resistance can provide fundamental insights about evolution, efforts to manage resistance enable application and testing of evolutionary theory. One of the most urgent challenges is to determine if strategies based on evolutionary principles can delay pest resistance to transgenic crops.

Transgenic corn and cotton that produce insecticidal proteins derived from *Bacillus thuringiensis* (Bt) grew on 14 million ha during 2002, with a cumulative total of more than 62 million ha since 1996 (James, 2002; Tabashnik *et al.*, 2003). Such Bt crops can reduce reliance on insecticide sprays, thereby providing economic, health, and environmental benefits (Shelton *et al.*, 2002; Carrière *et al.*, 2003). Evolution of resistance by pests, however, would cut short the efficacy of Bt crops and the associated benefits. Pest resistance to Bt crops in the field has not been documented yet (Tabashnik *et al.*, 2003), but *Plutella xylostella* (diamondback moth) has

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evolved resistance to Bt sprays in the field, and many key pests have evolved resistance to Bt toxins in the laboratory (Tabashnik, 1994; Ferré & Van Rie, 2002).

To delay pest resistance to Bt crops, the refuge strategy has been widely adopted. This strategy is based on evolutionary theory developed in dozens of papers (e.g. Georghiou & Taylor, 1977; Curtis *et al.*, 1978; Tabashnik & Croft, 1982; Roush, 1994; Gould, 1998; Peck *et al.*, 1999; Caprio, 2001; Carrière & Tabashnik, 2001; Onstad *et al.*, 2002) and on small-scale experiments with *P. xylostella* (Liu & Tabashnik, 1997a; Shelton *et al.*, 2000; Tang *et al.*, 2001). The theory underlying the strategy is to reduce heritability of resistance by providing refuges of non-Bt host plants that produce susceptible adults, by promoting mating between resistant and susceptible adults, and by decreasing the dominance of resistance. The strategy relies on the principle that dominance can be altered by the environment (Roush & Daly, 1990; Bourguet *et al.*, 1996, 2000). In particular, bioassay results from some key pests show that the dominance of their resistance to Bt toxins decreases as toxin concentration increases (Tabashnik *et al.*, 1992, 2002; Gould *et al.*, 1995; Liu & Tabashnik, 1997b; Tang *et al.*, 1997; Zhao *et al.*, 2000; Liu *et al.*, 2001b).

To implement the refuge strategy, farmers grow refuges of non-Bt host plants near Bt crops to promote survival of susceptible pests. This strategy is expected to work best if resistance is conferred by rare, recessive alleles and if most of the extremely rare resistant adults emerging from Bt crops mate with susceptible adults from refuges. The theory predicts that such conditions will greatly delay evolution of resistance.

The refuge strategy is sometimes called the 'high-dose refuge' (HDR) strategy because many modelling studies show that refuges are most effective if the dose of toxin ingested by insects eating Bt plants is high enough to kill all or nearly all individuals heterozygous for resistance (e.g. Curtis *et al.*, 1978; Tabashnik & Croft, 1982; Roush, 1997; Gould, 1998). In other words, refuges are predicted to work best if the toxin concentration in Bt plants is high enough to make resistance functionally recessive.

In contrast to the aforementioned results, Vacher *et al.* (2003) concluded that the HDR strategy is suboptimal, in part, because sustainable control of pests is achievable with 'transgenic plants producing lower doses of toxin'. They based this conclusion on simulations of a diallelic (R for resistance, S for susceptibility) single-locus model of the interaction between Bt cotton and the major pest *Heliothis virescens* (tobacco budworm). They noted, however, that their model relies on unverified assumptions. In particular, they state, 'first and foremost, toxin dose-mortality curves must be accurately estimated for insect larvae'. Modelling results reported here, which incorporate mortality data from bioassays instead of hypothetical mortality assumed by Vacher *et al.* (2003), indicate that high rather than low toxin concentration should delay pest resistance longest. We focus on *H. virescens*, because

it was modelled by Vacher *et al.* (2003), and on *Pectinophora gossypiella* (pink bollworm), because it is another key lepidopteran pest targeted by Bt cotton for which relevant bioassay data are available.

## Methods

### Sources of survival data

We used published data on the relationship between Bt toxin concentration, larval mortality, and functional dominance from bioassays with Bt toxin Cry1Ab against *H. virescens* (Gould *et al.*, 1995) and Bt toxin Cry1Ac against *P. gossypiella* (Tabashnik *et al.*, 2002). Cry1Ab is closely related to Cry1Ac (Tabashnik *et al.*, 1996), the toxin in commercially grown Bt cotton. The data for *H. virescens* were obtained from the 14-day bioassay summarized in the upper left corner of Figure 7 of Gould *et al.* (1995). The data for *P. gossypiella* were obtained from the 21-day bioassay summarized in Figure 1 and Table 3 of Tabashnik *et al.* (2002).

In the bioassays, larvae ate artificial diet into which various concentrations of Bt toxin had been incorporated. Each set of bioassays tested three groups of larvae representing putative RR, SS and RS genotypes. These three groups are labelled in the figures, respectively, as selected (YHD2), control (YDK) and F<sub>1</sub> (YHD2 × YDK) for *H. virescens*; and as resistant (AZP-R), susceptible (APHIS-S), and F<sub>1</sub> (AZP-R × APHIS-S) for *P. gossypiella*. In addition to the 10 concentration-mortality data points for *H. virescens* plotted in the upper left corner of Figure 7 (Gould *et al.*, 1995), the bioassay results showed survival of 0% for SS at 40 µg toxin mL<sup>-1</sup> diet; 0% for SS and RS at 200 µg toxin mL<sup>-1</sup> diet; and 100 and 97%, respectively, for RR at 1.6 and 8.0 µg toxin mL<sup>-1</sup> diet (F. Gould, unpublished data).

Both resistant strains (YHD2 and AZP-R) show 'mode 1' resistance, which is the most common type of resistance to Bt toxins in Lepidoptera (Tabashnik *et al.*, 1998, 2003). Mode 1 is characterized by >500-fold resistance to at least one Cry1A toxin, recessive inheritance, little or no cross-resistance to Cry1C, and reduced binding of at least one Cry1A toxin. In both the YHD2 and AZP-R strains, resistance is tightly linked to a cadherin locus that encodes a receptor for Cry1A toxins (Gahan *et al.*, 2001; Morin *et al.*, 2003).

### Estimation of dominance

For each Bt toxin concentration, dominance (*h*) was estimated as (Liu & Tabashnik, 1997b):

$$h = \frac{\text{Survival of RS} - \text{Survival of SS}}{\text{Survival of RR} - \text{Survival of SS}}$$

Values of *h* range from 0 (completely recessive resistance) to 1 (completely dominant resistance). The parameter *h* is a standard measure of dominance (Hartl

& Clark, 1989) that is not the same as  $h_s$  of Vacher *et al.* (2003). Bourguet *et al.* (2000) refer to  $h$  as  $D_{ML}$ .

### Population genetic model

We used a standard population genetic model (Hartl & Clark, 1989) to predict the change in resistance (R) allele frequency in the first generation:

$$\Delta p = pq(p[W_{RR} - W_{RS}] + q[W_{RS} - W_{SS}])/W_M$$

where  $p$  is the R allele frequency,  $q$  is the S allele frequency,  $W_M$  is mean fitness, and  $W_{RR}$ ,  $W_{RS}$  and  $W_{SS}$  are the fitnesses of RR, RS, and SS, respectively. Although this equation can be iterated to project changes over many generations, we focus here on relative rates of resistance evolution, which are indicated by the change in R allele frequency in the first generation. Fitness for each genotype was calculated as (Carrière & Tabashnik, 2001):

$$W_{RR} = BtW_{RR} \cdot P_{Bt} + RefW_{RR} \cdot P_{Ref}$$

$$W_{RS} = BtW_{RS} \cdot P_{Bt} + RefW_{RS} \cdot P_{Ref}$$

$$W_{SS} = BtW_{SS} \cdot P_{Bt} + RefW_{SS} \cdot P_{Ref}$$

where  $P_{Bt}$  is the proportion of Bt plants and  $P_{Ref}$  is the proportion of non-Bt plants (refuge), and fitnesses for RR, RS, and SS, respectively, are  $BtW_{RR}$ ,  $BtW_{RS}$ , and  $BtW_{SS}$  on Bt plants, and  $RefW_{RR}$ ,  $RefW_{RS}$ , and  $RefW_{SS}$  in refuges. Mean fitness was calculated with the standard equation (Hartl & Clark, 1989):

$$W_M = p^2W_{RR} + 2pqW_{RS} + q^2W_{SS}$$

As in Vacher *et al.* (2003), the initial R allele frequency was 0.0015, which is the empirical estimate for field populations of *H. virescens* from Gould *et al.* (1997). For simplicity, we also used this assumption for *P. gossypiella*, for which mean estimated R allele frequency ranged from 0 to 0.16 in Arizona during a 5-year period (Tabashnik *et al.*, 2003). Fitnesses on Bt plants ( $BtW_{RR}$ ,  $BtW_{RS}$ , and  $BtW_{SS}$ ) at a series of Bt toxin concentrations were based either on the assumptions of Vacher *et al.* (2003) for low, middle and high doses or on mortality data from bioassays as described above (Gould *et al.*, 1995; Tabashnik *et al.*, 2002). We evaluated the effects of hypothetical vs. observed mortality on resistance evolution by holding all other assumptions constant and changing assumptions about the effect of Bt toxin concentration on mortality.

### Fitness costs, refuge size and sublethal effects

We compared effects of hypothetical vs. observed mortality with two sets of assumptions about fitnesses in refuges. In the first set, we used the assumptions of Vacher *et al.* (2003), which incorporate an additive fitness cost associated with resistance:  $RefW_{RR} = 0.85$ ,

$RefW_{RS} = 0.97$ , and  $RefW_{SS} = 1$ . In the second set, we used the more realistic assumption that the fitness cost is recessive (Carrière *et al.*, 2001a,b):  $RefW_{RR} = 0.85$ ,  $RefW_{RS} = 1$ , and  $RefW_{SS} = 1$ . Analysis of these two sets, which differ only in that RS have reduced fitness in refuges in set 1 (0.97) but not in set 2 (1), enabled direct examination of the effect of dominance of fitness cost.

To evaluate the effects of refuge size and toxin concentration on resistance evolution, we varied refuge size (5, 20 or 50%) for each of six concentrations tested against *H. virescens* larvae in bioassays (Gould *et al.*, 1995). In these calculations, we used observed survival from the bioassays and assumed a recessive fitness cost (see above).

In all of the calculations described above, we assumed that larval survival corresponds to genotypic fitness, as in Vacher *et al.* (2003). However, for larvae exposed to Bt toxin, larval survival provides a good estimate of overall fitness when all larvae die, but not necessarily when some survive (Bourguet *et al.*, 2000). Indeed, larval exposure to Bt toxin has sublethal effects on survivors of *H. virescens* and *P. gossypiella*, including reduced developmental rate and fecundity (Gould *et al.*, 1995; Liu *et al.*, 1999, 2001a). To evaluate the impact of sublethal effects and toxin concentration, we examined resistance evolution for each of six concentrations tested in bioassays against *H. virescens* larvae under three sets of assumptions about sublethal effects: (a) none, (b) fixed and (c) genotype-specific. Under (a), we assumed that sublethal effects were negligible, such that larval survival was a good estimate of overall fitness. Under (b), we assumed that the proportional reduction in fitness caused by sublethal effects of Bt toxin was the same for each genotype (0.50 × survival). Under (c), we assumed that the proportional reduction in fitness caused by sublethal effects of Bt toxin was greatest for SS (0.25 × survival), intermediate for RS (0.50 × survival), and weakest for RR (0.75 × survival). For example, survival of *H. virescens* larvae exposed to 0.32 µg toxin mL<sup>-1</sup> diet was 0.47 for SS, 1 for RS, and 1 for RR. For individuals exposed to this concentration, fitness values for SS, RS, and RR were, respectively, 0.47, 1, and 1 under (a); 0.235, 0.5, and 0.5 under (b); and 0.1175, 0.5 and 0.75 under (c). The genotype-specific trend in (c) is consistent with data on developmental rate for *H. virescens* and *P. gossypiella* (Gould *et al.*, 1995; Liu *et al.*, 2001a). In these calculations, we assumed a recessive fitness cost (see above).

## Results

### Observed vs. hypothetical effects of Bt toxin concentration on survival and functional dominance

Variation in larval survival as a function of Bt toxin concentration in bioassays with *H. virescens* and *P. gossypiella* differed markedly from the assumptions of Vacher *et al.* (2003) (Table 1). Bioassays with *H. virescens*

**Table 1** Effects of hypothetical vs. observed survival of larvae exposed to *Bacillus thuringiensis* (Bt) toxin on functional dominance of resistance and change in resistance (R) allele frequency in the first generation\*.

Bt toxin concentration ( $\mu\text{g mL}^{-1}$ )	Survival of larvae exposed to Bt (%)				Dominance ( $h$ )	Change in R allele frequency in the first generation	
	SS	RS	RR	RS – SS		Additive cost†	Recessive cost‡
Hypothetical: <i>Heliiothis virescens</i> (Vacher <i>et al.</i> , 2003)							
Low	40	40	85	0	0.000	$-1.6 \times 10^{-5}$	$1.4 \times 10^{-6}$
Middle	20	21	85	1	0.015	$1.1 \times 10^{-5}$	$3.6 \times 10^{-5}$
High	0	2	85	2	0.024	$8.2 \times 10^{-5}$	$1.3 \times 10^{-4}$
Observed: <i>Heliiothis virescens</i> (Gould <i>et al.</i> , 1995)							
0.32	47	100	ND§	53	1.000	$1.1 \times 10^{-3}$	$1.1 \times 10^{-3}$
1.6	16	75	100	59	0.702	$2.1 \times 10^{-3}$	$2.1 \times 10^{-3}$
8.0	0	31	97	31	0.320	$1.8 \times 10^{-3}$	$1.9 \times 10^{-3}$
40	0	0	97	0	0.000	$-3.6 \times 10^{-5}$	$8.4 \times 10^{-6}$
200	0	0	75	0	0.000	$-3.8 \times 10^{-5}$	$6.4 \times 10^{-6}$
500	ND	ND	59	ND	0.000	$-4.0 \times 10^{-5}$	$5.0 \times 10^{-6}$
Observed: <i>Pectinophora gossypiella</i> (Tabashnik <i>et al.</i> , 2002)							
0.32	37	93	ND	56	0.889	$1.3 \times 10^{-3}$	$1.3 \times 10^{-3}$
1.0	4	52	ND	48	0.500	$2.4 \times 10^{-3}$	$2.5 \times 10^{-3}$
3.2	0	8	ND	8	0.080	$4.4 \times 10^{-4}$	$4.9 \times 10^{-4}$
10	0	0.5	100	0.5	0.005	$-2.6 \times 10^{-6}$	$3.9 \times 10^{-5}$

\*In all cases, we assumed that the initial R allele frequency was 0.0015 as in Vacher *et al.* (2003) and that the habitat had 80% Bt cotton and 20% refuges.

†With additive fitness costs, fitnesses in refuges were 1 for SS, 0.97 for RS and 0.85 for RR, as in Vacher *et al.* (2003).

‡With recessive fitness costs, fitnesses in refuges were 1 for SS, 1 for RS and 0.85 for RR.

§ND, no data available; if concentration higher than concentration causing 0% survival, assumed to be 0% survival; if concentration lower than concentration causing 100% survival, assumed to be 100% survival for calculation of  $h$  and change in R allele frequency in the first generation.

larvae show the following: (1) survival at the lowest concentration tested ( $0.32 \mu\text{g toxin mL}^{-1}$  diet) was substantially lower for SS (47%) than for RS (100%), (2) survival of RS was 0% at high toxin concentrations ( $\geq 40 \mu\text{g toxin mL}^{-1}$  diet), and (3) survival of RR decreased from 100% at a low concentration ( $1.6 \mu\text{g toxin mL}^{-1}$  diet) to 59% at the highest concentration tested ( $500 \mu\text{g toxin mL}^{-1}$  diet).

In contrast, Vacher *et al.* (2003) made the following three assumptions about *H. virescens* larvae exposed to Bt toxin: (1) survival at a low dose is equal for SS (40%) and RS (40%), (2) survival of RS is 2% at a high dose, and (3) survival of RR does not vary as a function of dose (85% survival at low, middle, and high doses). The bioassay data for *H. virescens* show that the assumptions of Vacher *et al.* (2003) underestimate survival of RS at low toxin concentration and overestimate survival of RS and RR at high toxin concentration.

The differences between observed and hypothetical survival for *H. virescens* larvae yield opposite patterns of dominance ( $h$ ) as a function of toxin concentration (Table 1). In bioassays, resistance was completely dominant ( $h = 1$ ) at the lowest concentration tested and completely recessive ( $h = 0$ ) at the highest concentrations. In contrast, the assumptions of Vacher *et al.* (2003) yield complete recessiveness ( $h = 0$ ) at the low dose and the highest degree of dominance ( $h = 0.024$ ) at the high

dose. Thus, bioassays showed that dominance decreased as toxin concentration increased, but Vacher *et al.* (2003) assumed the reverse.

Observed patterns of survival and functional dominance for *P. gossypiella* were similar to those observed for *H. virescens* (Table 1). In bioassays of *P. gossypiella* larvae, survival at the lowest concentration tested ( $0.32 \mu\text{g toxin mL}^{-1}$  diet) was substantially lower for SS (37%) than for RS (93%). At the highest concentration at which RS were tested ( $10 \mu\text{g toxin mL}^{-1}$  diet), their survival was 0.5%. Functional dominance ( $h$ ) was 0.889 at the low concentration and 0.005 at the high concentration. Thus, similar to results with *H. virescens*, dominance decreased as toxin concentration increased.

### Effects of observed vs. hypothetical survival on resistance evolution

As expected from the differences described above, the predicted effects of toxin concentration on resistance evolution differ markedly between observed and hypothetical values for survival of *H. virescens* larvae (Table 1). When survival data for *H. virescens* were incorporated in the model, resistance evolved faster at the three lowest concentrations than at the three highest concentrations. Resistance evolved fastest at a concentration of  $1.6 \mu\text{g toxin mL}^{-1}$  diet, which allowed survival of 16% for SS,

75% for RS and 100% for RR. Resistance evolved slowest at the highest concentration tested ( $500 \mu\text{g}$  toxin  $\text{mL}^{-1}$  diet), which allowed survival of 0% for SS and RS, and 59% for RR. In contrast to these findings, the hypothetical survival values for *H. virescens* larvae assumed by Vacher *et al.* (2003) make resistance evolve faster as dose increases.

With bioassay data for *P. gossypiella* larvae incorporated in the model, the predicted effects of toxin concentration on resistance evolution were similar to those based on bioassay data for *H. virescens* larvae (Table 1). For *P. gossypiella*, resistance evolved faster at the three lowest concentrations than at the highest concentration. Resistance evolved fastest at  $1.0 \mu\text{g}$  toxin  $\text{mL}^{-1}$  diet, which allowed survival of 4% for SS and 52% for RS. Resistance evolved slowest at the highest concentration tested against RS ( $10 \mu\text{g}$  toxin  $\text{mL}^{-1}$  diet), which allowed 0% survival for SS, 0.5% for RS and 100% for RR.

### Effects of recessive vs. additive fitness costs on resistance evolution

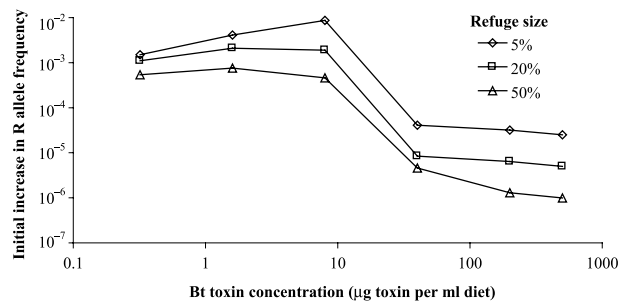
Consistent with previous modelling results (Carrière & Tabashnik, 2001; Carrière *et al.*, 2002), the additive fitness cost assumed by Vacher *et al.* (2003) delayed resistance more than a recessive fitness cost (Table 1). When resistance was completely recessive ( $h = 0$ ) or almost completely recessive ( $h = 0.005$ ), an additive fitness cost caused decreases in R allele frequency in five cases, but no such decreases occurred when the fitness cost was recessive. As the dominance of resistance increased, the effect of the dominance of the fitness cost diminished. For example, the difference between recessive and additive fitness cost was negligible when  $h$  was 1 or 0.889 (observed data for  $0.32 \mu\text{g}$  toxin  $\text{mL}^{-1}$  diet for *H. virescens* and *P. gossypiella*, respectively).

### Effects of Bt toxin concentration and refuge size on resistance evolution

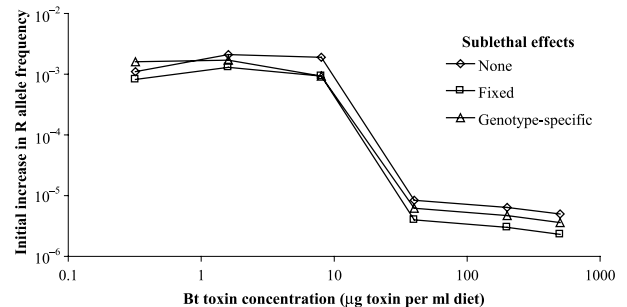
With refuges of 5, 20 or 50% and survival of *H. virescens* larvae exposed to Bt toxins based on bioassay data, resistance evolved faster at the three lowest concentrations than at the three highest concentrations (Fig. 1). As expected, at each concentration examined, resistance evolved slower as refuge size increased (Fig. 1).

### Effects of Bt toxin concentration and sublethal effects on resistance evolution

With no sublethal effects, sublethal effects fixed across genotypes, or genotype-specific sublethal effects (see Methods) and survival of *H. virescens* larvae exposed to Bt toxins based on bioassay data, resistance evolved faster at the three lowest concentrations than at the three highest concentrations (Fig. 2). Sublethal effects that reduced the fitness of individuals surviving exposure to Bt toxin



**Fig. 1** Effects of refuge size and *Bacillus thuringiensis* (Bt) toxin concentration on increase in resistance (R) allele frequency in the first generation. Survival of *Heliothis virescens* larvae exposed to Bt toxin was based on bioassays (Gould *et al.*, 1995, see Table 1). A recessive fitness cost was assumed; fitnesses in refuges were 1 for SS, 1 for RS and 0.85 for RR. We assumed that the initial R allele frequency was 0.0015 as in Vacher *et al.* (2003).



**Fig. 2** Effects of *Bacillus thuringiensis* (Bt) toxin concentration and sublethal effects on increase in resistance (R) allele frequency in the first generation. Survival of *Heliothis virescens* larvae exposed to Bt toxin was based on bioassays (Gould *et al.*, 1995, see Table 1). We assumed that the initial R allele frequency was 0.0015 as in Vacher *et al.* (2003) and that the habitat had 80% Bt cotton and 20% refuges. Fitness of larvae exposed to Bt toxin depended on survival and sublethal effects. For no sublethal effects, larval survival values were used as fitness values. For fixed sublethal effects, fitness of each genotype was larval survival  $\times$  0.50. For genotype-specific sublethal effects, fitness of SS was survival  $\times$  0.25, fitness of RS was survival  $\times$  0.50 and fitness of RR was survival  $\times$  0.75.

generally slowed resistance evolution (Fig. 2). This is not surprising, as such sublethal effects reduce the fitness of larvae exposed to Bt toxin relative to larvae in refuges. An exception to this general trend occurred at the lowest concentration tested in bioassays ( $0.32 \mu\text{g}$  toxin  $\text{mL}^{-1}$  diet). At this concentration, resistance evolved faster with genotype-specific sublethal effects than without sublethal effects (Fig. 2). This occurred because the acceleration in resistance caused by the substantial reduction in fitness of SS associated with strong sublethal effects (from 0.47 to 0.1175) outweighed the delaying effect of proportionately smaller reductions in fitness of RS (from 1 to 0.5) and RR (from 1 to 0.75).

## Discussion

The results here illustrate the principle that dominance can be altered by the environment. In particular, the dominance of resistance to Bt toxins of some key pests can be reduced by increasing toxin concentration. If most of the rare resistant adults emerging from Bt crops mate with susceptible adults from refuges, survival of their heterozygous progeny largely determines the response to selection. Under such conditions, reduced dominance of resistance decreases the heritability of resistance, thereby delaying evolution of resistance to Bt crops. When heritability is constant, increasing selection intensity increases the response to selection (Falconer, 1989). However, incorporation of bioassay data into a simple population genetic model indicates that increasing toxin concentration from moderate to high levels can decrease the heritability of resistance sufficiently to decrease the response to selection, despite the higher intensity of selection.

The effects of Bt toxin concentration on mortality of *H. virescens* larvae observed in bioassays (Gould *et al.*, 1995) differ markedly from the effects assumed by Vacher *et al.* (2003). Results from a population genetic model reported here demonstrate that the differences between the observed and assumed patterns of mortality yield opposite predictions about the effect of toxin concentration on resistance evolution. Confirming the results of many earlier studies and refuting Vacher *et al.* (2003), incorporation of bioassay data for *H. virescens* and *P. gossypiella* larvae into a model led to the prediction that concentrations high enough to kill all or nearly all RS larvae will delay resistance most effectively. Also confirming patterns described from earlier models (e. g. Tabashnik & Croft, 1982), results reported here based on incorporation of bioassay data from *H. virescens* and *P. gossypiella* show that resistance evolved fastest at intermediate concentrations causing <20% survival of SS and >50% survival of RS. These conclusions were robust across various assumptions about fitness costs (Table 1), refuge size (Fig. 1) and sublethal effects (Fig. 2).

We emphasized one bioassay data set for each pest species (Table 1), but additional data for these two pests and data for other pests suggest that typical concentration-mortality responses favour high doses for delaying pest resistance to Bt toxins. Although we focused on data for *H. virescens* from one set of bioassays with the resistant YHD2 strain, the susceptible YDK strain, and their F<sub>1</sub> progeny (Gould *et al.*, 1995, Fig. 7 upper left), data from a second set of bioassays with these three groups of larvae show the same trends (Gould *et al.*, 1995, Fig. 7 lower left). At a low concentration (1.6 µg toxin mL<sup>-1</sup> diet) survival was much lower for SS (45%) than for RS (93%), whereas at a high concentration (200 µg toxin mL<sup>-1</sup> diet) survival was 0% for SS and RS. So, as with the data for *H. virescens* summarized in Table 1, resistance was more dominant at a low concentration ( $h = 0.87$ )

than at a high concentration ( $h = 0$ ). Other bioassay data with Bt toxins showing a similar pattern include the APHIS-98R strain of *P. gossypiella* vs. Cry1Ac (Liu *et al.*, 2001b), *P. xylostella* vs. Cry1C (Liu & Tabashnik, 1997b; Zhao *et al.*, 2000), and *P. xylostella* vs. formulated Bt toxins (Tabashnik *et al.*, 1992; Tang *et al.*, 1997).

In some published bioassay studies with Bt toxins, the potential impact of high toxin concentrations cannot be assessed readily because survival of RS larvae is not reported for concentrations higher than those killing 95% of RS larvae (e. g. McGaughey, 1985; McGaughey & Beeman, 1988; Gould *et al.*, 1992; Huang *et al.*, 1999). In some cases, only concentrations killing 50% (LC<sub>50</sub>s) and slopes of concentration-mortality lines are reported, or estimated concentration-mortality lines are plotted in figures without individual data points. Further, some studies report estimates of dominance (D, Stone, 1968) based only on LC<sub>50</sub>s. These practices reflect an emphasis on probit analysis, in which concentrations causing 0% survival are problematic and estimation of LC<sub>50</sub>s is a primary focus. However, to facilitate evaluation of the refuge strategy, we encourage researchers to test SS, RS and RR larvae at concentrations killing <25 to >99% of RS larvae and to report estimates of dominance ( $h$ ) as a function of concentration across this range.

We are not aware of published bioassay results with Bt toxins showing the type of dose-mortality relationships assumed by Vacher *et al.* (2003). For example, we know of no bioassays where survival of 40% occurs for both SS and RS at the same Bt toxin concentration. Further, bioassay results show that if Bt toxin concentration is increased sufficiently, survival of RR declines. Because the assumptions of Vacher *et al.* (2003) do not correspond with published data for *H. virescens* or other pests, the relevance of their conclusions is unclear.

The results reported here confirm numerous other studies showing that, in theory, high concentrations of Bt toxins in transgenic plants will be most effective for delaying resistance. Experimental evidence shows that commercial cultivars of Bt cotton are sufficiently toxic to kill all or nearly all F<sub>1</sub> larvae from crosses of resistant and susceptible adults (putative RS) of *P. gossypiella* (Liu *et al.*, 1999, 2001b; Tabashnik *et al.*, 2000) and *H. virescens* (F. Gould, L. Carter and L. Seltmann unpublished data). Thus, for these two pests that are extremely susceptible to the toxin in Bt cotton, it appears that the theoretically envisioned high dose is achieved. This also appears to be true for *P. xylostella* tested on noncommercial cultivars of transgenic crucifers that produce high concentrations of Bt toxins (Metz *et al.*, 1995; Tang *et al.*, 1997; Zhao *et al.*, 2000). On the contrary, as noted by Vacher *et al.* (2003) and others, some pests of cotton such as *Helicoverpa armigera* and *Helicoverpa zea* are much less susceptible to the Cry1Ac toxin in Bt cotton. Their resistance to commercial cultivars of Bt cotton might not be functionally recessive (Akhurst *et al.*, 2003; Burd *et al.*, 2003).

In conclusion, models incorporating data from bioassays with Bt toxins in diet show that high rather than low toxin concentrations are expected to delay pest adaptation longest. Further, bioassays testing *H. virescens* and *P. gossypiella* larvae on transgenic plants indicate that the toxin concentration in Bt cotton is high enough to kill all or nearly all F<sub>1</sub> progeny of matings between resistant and susceptible adults. Thus, for these pests, the assumption of functionally recessive inheritance of resistance to Bt cotton appears valid. Nonetheless, the hypothesis that high doses are best for delaying pest resistance remains to be tested experimentally.

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