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PERSPECTIVE

Insect resistance to *Bt* crops: evidence versus theory

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Evolution of insect resistance threatens the continued success of transgenic crops producing *Bacillus thuringiensis* (*Bt*) toxins that kill pests. The approach used most widely to delay insect resistance to *Bt* crops is the refuge strategy, which requires refuges of host plants without *Bt* toxins near *Bt* crops to promote survival of susceptible pests. However, large-scale tests of the refuge strategy have been problematic. Analysis of more than a decade of global monitoring data reveals that the frequency of resistance alleles has increased substantially in some field populations of *Helicoverpa zea*, but not in five other major pests in Australia, China, Spain and the United States. The resistance of *H. zea* to *Bt* toxin Cry1Ac in transgenic cotton has not caused widespread crop failures, in part because other tactics augment control of this pest. The field outcomes documented with monitoring data are consistent with the theory underlying the refuge strategy, suggesting that refuges have helped to delay resistance.

Rapid responses to laboratory selection show that many pests naturally harbor genetic variation in susceptibility to *Bt* toxins and thus have the potential to evolve resistance to *Bt* crops in the field[1–4]. Although some newer varieties of *Bt* crops produce two *Bt* toxins (http://www.epa.gov/pesticides/biopesticides/pips/pip_list.htm), we focus here on first-generation *Bt* crops that target lepidopteran pests and produce only one *Bt* toxin: cotton producing *Bt* toxin Cry1Ac (referred to hereafter as *Bt* cotton) and corn producing *Bt* toxin Cry1Ab (referred to hereafter as *Bt* corn). *Bt* cotton and *Bt* corn have been grown on more than 162 million ha worldwide[5], generating one of the largest selections for insect resistance ever known. The theory underlying the refuge strategy[1–4] for delaying insect resistance is that most of the rare resistant pests surviving on *Bt* crops will mate with abundant susceptible pests from refuges of host plants without *Bt* toxins. If inheritance of resistance is recessive, the hybrid offspring produced by such matings will be killed by *Bt* crops, markedly slowing the evolution of resistance. Results from models and small-scale experiments suggest that refuges can delay resistance, but comparisons between observed and predicted outcomes in commercial agriculture have been limited[1–4].

The major pests targeted by *Bt* crops have been monitored for the evolution of resistance, which is a heritable decrease in a population's susceptibility to a toxin[3,6]. Susceptibility is usually measured by sampling insects from a field population and determining how their progeny respond to the toxin in laboratory bioassays. Such bioassays document field-evolved resistance if one or more populations with a history of exposure to the toxin in the field are less susceptible than conspecific field populations or laboratory strains that have had less exposure[3]. Decreased susceptibility is typically demonstrated as a significant increase in the toxin concentration killing 50% (LC_{50}) of the insects tested or in the percentage of insects surviving exposure to a fixed amount of toxin[3,6]. Laboratory documentation of resistance, however, does not always indicate control problems in the field[6].

In the sections below, we analyze the results of studies from Australia, China, Spain and the United States monitoring the resistance to *Bt* crops in field populations of six major insect pests (*Helicoverpa armigera, H. zea, Heliothis virescens, Ostrinia nubilalis, Pectinophora gossypiella* and *Sesamia nonagrioides*). Next, for each of these six pests, we compare the evidence from monitoring studies to results from computer modeling of resistance evolution that is based on the theory underlying the refuge strategy. We conclude with a discussion of the implications of our findings for managing insect resistance to current and future transgenic crops.

Evidence from resistance monitoring studies

Analysis of the published monitoring data for six major lepidopteran pests targeted by *Bt* crops shows field-evolved resistance in *H. zea*, but not in *H. armigera, H. virescens, O. nubilalis, P. gossypiella* or *S. nonagrioides* (**Table 1** and **Fig. 1**). Evaluation of the large datasets from two landmark studies[7,8] reveals resistance to Cry1Ac in some field populations of *H. zea* from Arkansas and Mississippi (**Fig. 1**) but not in concurrently tested populations of *H. virescens* from the same region (**Table 1**). Field sampling of both pests was done from 1992 to 1993 in the first study and from 2002 to 2004 in the second study, enabling comparison of each pest's susceptibility before and after the commercialization of *Bt* cotton. Both studies used bioassays involving toxin incorporated in the diet to determine the LC_{50} of Cry1Ac for strains derived from field populations and for laboratory strains that were not exposed to *Bt* toxins. These data allow calculation of resistance ratios, which are LC_{50} values of field-derived strains divided by LC_{50} values of conspecific, susceptible laboratory strains. Resistance ratios >10 are most likely to reflect heritable decreases in susceptibility[3]. For the two pest species in which resistance to *Bt* sprays evolved outside of the laboratory, the initial documentation was based on resistance ratios up to 36 for *Plutella xylostella* from field populations and 160 for *Trichoplusia ni* from glasshouses[3,9].

Bioassay results for *H. zea* sampled during 2003 and 2004 in Arkansas and Mississippi show resistance ratios for Cry1Ac >50 for six field-derived strains, including resistance ratios >100 for four strains and >500 for two (**Fig. 1** and **Supplementary Methods** online). Data from field populations sampled in 2005 and 2006 also demonstrate *H. zea* resistance to Cry1Ac, yielding resistance ratios >100 for seven additional strains from Arkansas, including two strains with resistance ratios >1,000 (ref. 10). In contrast, field populations of *H. zea* sampled in 1992

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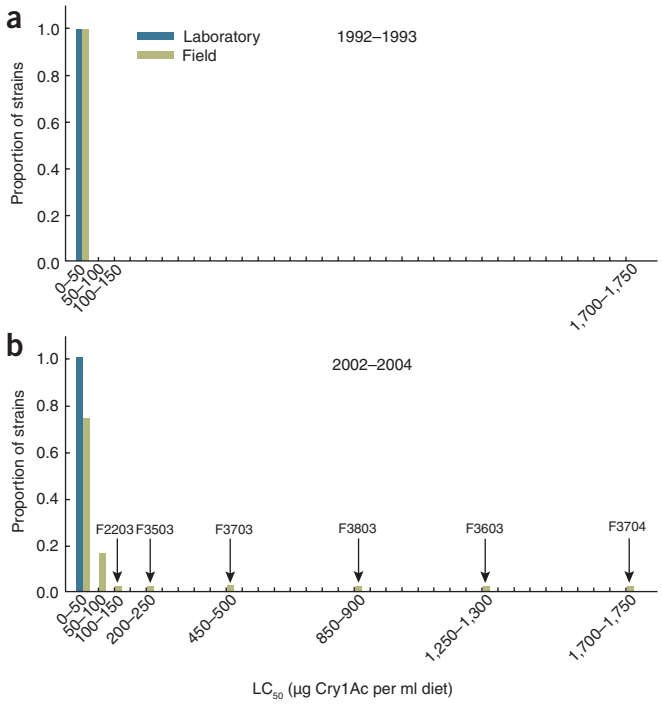

Figure 1 Field-evolved resistance of *Helicoverpa zea* to *Bt* cotton demonstrated by increases in the median lethal concentration (LC_{50}) of the *Bt* toxin Cry1Ac for field populations. (**a**) Before the commercialization of *Bt* cotton (1992–1993, ref. 7), no significant difference in LC_{50} values existed between field-derived strains (mean = 1.36, $n = 7$) and laboratory strains (mean = 2.53, $n = 4$) (Mann-Whitney *U*-test, $U = 15.5$, $P = 0.39$). (**b**) After the commercialization of *Bt* cotton (2002–2004, ref. 8), LC_{50} values were significantly higher for field-derived strains (mean = 111, $n = 57$) than for laboratory strains (mean = 9.29, $n = 7$) ($U = 340$, $P = 0.0013$). Arrows show the six field-derived strains with LC_{50} >100. For these six strains, the resistance ratios, from Table 4 of ref. 8, were: F2203, 53; F3503, 83; F3703, 184; F3803, 354; F3603, 515; F3704, 578 (**Supplementary Methods**).

and 1993, before commercialization of *Bt* cotton, were not resistant to Cry1Ac (**Table 1** and **Fig. 1**).

Although field-evolved resistance to Cry1Ac occurred in some Arkansas and Mississippi populations 7–8 years after commercialization of *Bt* cotton, two studies[11,12] show no decrease in susceptibility to Cry1Ac in *H. zea* populations from North Carolina (**Table 1**). Diet bioassays testing more than 80,000 first-generation progeny of 1,835 field-collected females show that the estimated frequency of major non-recessive resistance alleles remained low, declining from 0.00043 in 2000 to below detectable levels in 2001 and 2002.

Evidence versus theory

To determine whether field outcomes are consistent with the theory underlying the refuge strategy, we modeled resistance evolution in each of the six major pests listed in **Table 1**. We used the same basic population genetic model for all pests, incorporating realistic estimates of the key biological parameters for each species (**Supplementary Methods** and **Supplementary Table 1** online). For each pest, we used sensitivity analysis to assess how resistance evolution might be affected by variation in the relative abundance of refuges of non-*Bt* host plants.

The modeling results are in accord with the patterns documented by monitoring data, suggesting that the principles of the refuge strategy apply in the field for this set of pests and *Bt* crops (**Fig. 2a**). Consistent with the monitoring data, the modeling results indicate that *H. zea* is expected to evolve resistance faster than the other pests (**Fig. 2a**). With realistic estimates of refuge abundance incorporated, the modeling

Table 1 Global monitoring of resistance of six major pests to the *Bt* toxins in transgenic crops

Location	Field sample	Years	Parameter	Initial	Final
Helicoverpa armigera					
Australia[29]	17 strains	2001–2003	max RR	1.2	1.5
Australia[15]	404 families	2002–2003 to 2005–2006	r freq	0.0	0.0
China[30]	94 strains	1998–2004	survival	0.0095	0.0017
China: Anci[31]	766 families	2002–2005	r freq	0.00107	0.0
China: Xiajian[31]	1,551 families	2002–2005	r freq	0.00059	0.0023
Helicoverpa zea					
USA[8]	64 strains	1992–1993 to 2002–2004	max RR	1.2	578
NC[12]	1,835 families	2000–2002	r freq	0.00043	0.0
Heliothis virescens					
USA[8]	21 strains	1992–1993 to 2002–2004	max RR	1.1	4.3
LA and TX[32]*	7,050 males	1996–2002	r freq	0.0	0.0
Ostrinia nubilalis					
USA[33]	933 families	1996–2003	r freq	0.0	0.0
Spain[18]	5 strains	1999–2002	max RR	1.2	2.7
Pectinophora gossypiella					
AZ[28]	106 strains	1997–2004	r freq	0.16	0.004
AZ, CA,TX[34]*	5,571 insects	2001–2005	r freq	0.0	0.0
Sesamia nonagrioides					
Spain[18]	12 strains	1999–2002	max RR	3.0	2.9
Spain[35]	85 families	2004–2005	r freq	0.0	0.0

Data are from bioassays of *O. nubilalis* and *S. nonagrioides* versus Cry1Ab (*Bt* corn) or other pests versus Cry1Ac (*Bt* cotton), with two exceptions (*): Gahan *et al.*[32] and Tabashnik *et al.*[34] screened DNA of field-collected insects for cadherin mutations conferring resistance to Cry1Ac in *H. virescens* and *P. gossypiella*, respectively. 'Max RR' is the maximum resistance ratio, the highest LC_{50} among field-derived strains divided by the LC_{50} of one or more susceptible laboratory strains. 'r freq' is the estimated resistance allele frequency. 'Survival' is defined as survival to third instar on diet with 1 μg Cry1Ac per ml of diet. AZ, Arizona; CA, California; LA, Louisiana; NC, North Carolina; TX, Texas. See **Supplementary Methods** for details.

results are also consistent with the monitoring data showing that field-evolved resistance of *H. zea* to Cry1Ac occurred faster in Arkansas and Mississippi than in North Carolina (**Table 1**). Gustafson *et al.*[13] meticulously estimated that the effective refuge abundance during each of three generations when *H. zea* fed on cotton was 39% in Arkansas and Mississippi and 82% in North Carolina. With these refuge sizes, *H. zea* is projected to evolve resistance after 9 years in Arkansas and Mississippi. By contrast, in North Carolina, resistance evolution should take >20 years, with the expected resistance allele frequency still <0.005 after 10 years.

Although the results suggest that high refuge abundance in North Carolina delayed resistance in *H. zea*, an alternative hypothesis is that initial susceptibility to Cry1Ac was greater in North Carolina than in Arkansas and Mississippi. However, monitoring data from 1992 and 1993 show the opposite pattern: initial susceptibility to Cry1Ac was greater in Mississippi than in North Carolina[7]. In addition, the rapid response to laboratory selection in a strain started from 354 larvae collected from North Carolina in 1998 demonstrates the presence of genetic variation for resistance to Cry1Ac[14]. Selection with Cry1Ac in diet yielded 86-fold resistance in eight generations in the North Carolina strain[14], similar to the 120-fold resistance attained in seven generations of selection in a composite strain started in 1992 from 363 insects collected in Mississippi and Texas[7]. These comparable responses to selection suggest that genetic variation for resistance was similar in the two independently selected strains.

As seen for *H. zea* in North Carolina, relatively high refuge abundances are associated with the observed lack of field-evolved resistance to Cry1Ac for *H. armigera* in Australia and China, *P. gossypiella* in Arizona and *S. nonagrioides* in Spain. In Australia, *Bt* cotton producing Cry1Ac was limited to 30% of total cotton planted, providing a minimum 70% refuge[15]. In China, small fields of *Bt* cotton are close to fields of other, non-*Bt* crops, providing refuges that accounted for 87–95% of the available hosts for *H. armigera* in each province[16]. In Arizona, the mean refuge abundance was close to 50%, ranging from 14% to 78% per county[17]. In Spain, refuge abundance was about 95%[18]. Consistent with the monitoring data, *H. virescens* and *O. nubilalis* were not projected to evolve resistance in 10 years, even with the smallest refuge examined (5%).

Supporting the theory underlying the refuge strategy, dominant inheritance of resistance to Cry1Ac appears to have hastened the evolution of resistance in *H. zea*. The hybrid progeny produced by matings between a laboratory-selected resistant strain and a susceptible strain of *H. zea* were resistant to Cry1Ac[14], yielding a dominance value (h) of 0.826 (**Supplementary Table 1**), where 0 indicates completely recessive and 1 completely dominant inheritance. Of the five other major pests examined here, *H. armigera* is the only one with laboratory-selected resistance to *Bt* crop plants that is not completely recessive ($h = 0.300$, **Supplementary Table 1**).

Consistent with the monitoring data, modeling results for a generic pest of *Bt* crops show that, while all other factors are held constant, the dominance of resistance (h) and the refuge abundance greatly affect the rate of resistance evolution (**Fig. 2b**). With completely recessive inheritance of resistance to *Bt* crops ($h = 0$), refuges of ≥5% are expected to delay resistance >20 years in the generic pest. On the other hand, with $h \geq 0.4$, refuges of >50% are needed for delays of >20 years.

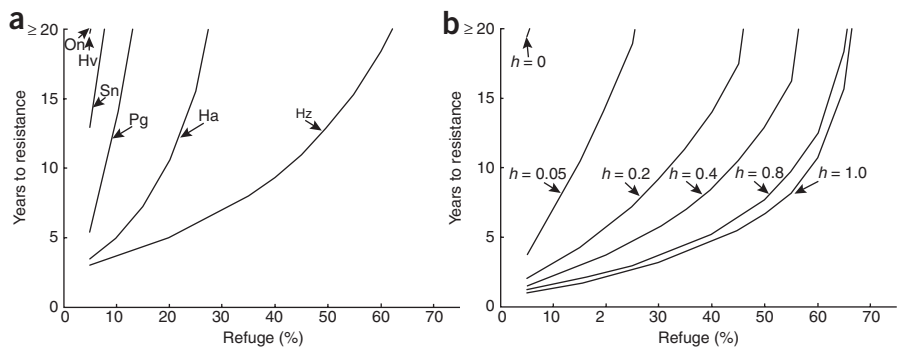

Figure 2 Simulated effect of refuge abundance (%) on pest resistance to *Bt* crops. The criterion for resistance was a resistance (r) allele frequency > 0.50. (**a**) Resistance evolution in six major pests modeled with realistic estimates for key biological parameters (**Supplementary Table 1**). Ha, *Helicoverpa armigera*; Hz, *Helicoverpa zea*; Hv, *Heliothis virescens*; On, *Ostrinia nubilalis*; Pg, *Pectinophora gossypiella*; Sn, *Sesamia nonagrioides*. For On with a 5% refuge, resistance evolution required >20 years and the r allele frequency was <0.001 after 10 years. For Hv with a 5% refuge, resistance evolved in 20 years. (**b**) Effect of dominance (h) on resistance evolution in a generic pest. With $h = 0$ and a 5% refuge, resistance evolution required >20 years and the r allele frequency increased from 0.001 to 0.0014 in 10 years.

Conclusions and implications

Our analysis shows that laboratory bioassays of *H. zea* document the first case of field-evolved resistance to a *Bt* toxin produced by a transgenic crop. Although tests of survival on *Bt* cotton plants from neonate to adult have not been reported for field-selected resistant strains of *H. zea*, the available evidence suggests that survival on *Bt* cotton in the field is higher for resistant populations than for susceptible populations. Susceptible strains of *H. zea* show some survival on *Bt* cotton[2,19,20], which means that any decrease in susceptibility to Cry1Ac could increase survival. Moreover, in greenhouse experiments with *Bt* cotton plants, survival was 40% for a laboratory-selected strain with 100-fold resistance to Cry1Ac compared with 10% for a feral strain[19]. Also, in bioassays with *Bt* cotton leaves, a field-derived strain of *H. zea* from Mississippi with 44-fold resistance to Cry1Ac had 52% survival versus 0% for a susceptible strain[8,21]. Accordingly, Luttrell and Ali[10] state that their results suggest the low susceptibility of some field-derived strains was "heritable" and "associated with a measurable increase in survival on *Bt* plant tissue." They also note that strains started by collecting larvae surviving on *Bt* cotton plants in the field "tended to have reduced susceptibility suggesting that some component of the observed field control problems may be associated with the presence of resistance genes"[10].

Nonetheless, resistance of *H. zea* to Cry1Ac has not caused widespread control failures for several reasons. First, even in the few states with documented resistance, most populations tested were not resistant to Cry1Ac (**Fig. 1**). Second, insecticides have been used from the outset to augment control of *H. zea* on *Bt* cotton because Cry1Ac alone is not sufficient to control high-density populations of the pest[2,20]. Insecticide sprays decrease any problems associated with reduced control of *H. zea* by *Bt* cotton. Third, against strains with 44- to 100-fold resistance to Cry1Ac, the Cry1Ac in *Bt* cotton still caused 48–60% larval mortality[8,19,21]. Finally, 'pyramided' transgenic cotton producing *Bt* toxins Cry2Ab and Cry1Ac was registered in December 2002 and planted on more than 1 million ha in the United States in 2006 and 2007 (ref. 22). Control of Cry1Ac-resistant *H. zea* by Cry2Ab also limits problems associated with resistance to Cry1Ac[19].

Negative effects of *H. zea* resistance to Cry1Ac should further diminish as use of cotton that produces only Cry1Ac decreases and use of two-toxin cotton with Cry2Ab and Cry1Ac increases. In the US, the area planted to cotton producing only Cry1Ac declined from 2.5 million ha in 2006

to 1.3 million ha in 2007 (ref. 22). Also, Monsanto's US registration of Cry1Ac cotton is scheduled to expire in September 2009 (http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm). For *H. virescens*, which has remained susceptible to Cry1Ac and is also susceptible to Cry2Ab, cotton producing both of these toxins may greatly delay resistance[23]. For Cry1Ac-resistant populations of *H. zea*, however, the two-toxin cotton may act like single-toxin cotton, with control exerted primarily by Cry2Ab. If so, the potential benefits of pyramiding these two toxins for delaying resistance will not be fully realized[23]. In May 2007, Syngenta applied for US registration of transgenic cotton with the pyramid of the *Bt* toxins Vip3Aa and Cry1Ab (http://www.epa.gov/fedrgstr/EPA-PEST/2007/May/Day-30/p10230.htm), which would provide another option for controlling key lepidopteran pests including *H. zea* and *H. virescens*. More generally, options for pest control with transgenic crops have been broadened by the development of corn with *Bt* toxins that kill corn rootworms and may be further expanded by gene-silencing technology and modified *Bt* toxins designed to kill resistant pests[24–27].

The sustained efficacy of the first generation of *Bt* crops for a decade against nearly all targeted pest populations has exceeded the expectations of many[4,28]. The exceptional case, *H. zea* resistance to *Bt* cotton producing Cry1Ac, is consistent with the theory underlying the refuge strategy because this resistance is not recessive. In other words, the concentration of Cry1Ac in *Bt* cotton is not high enough to kill the hybrid offspring produced by matings between susceptible and resistant *H. zea*. Thus, the so-called 'high dose' requirement is not met[1,2]. As the second decade of transgenic crop use begins, knowledge gained from systematic analyses of monitoring data from the first decade can help to minimize the risks and maximize the benefits. The results summarized here suggest that the refuge strategy can delay resistance to *Bt* crops, especially when resistance is recessive and refuges are abundant.

Note: Supplementary information is available on the Nature Biotechnology website.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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