



Field-Evolved Insect Resistance to Transgenic Bt Crops

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Genetically engineered crops that produce *Bacillus thuringiensis* (Bt) toxins kill some key insect pests and can help to reduce reliance on insecticide sprays. Bt crops have grown on more than 200 million ha worldwide since their commercial introduction in 1996¹. This widespread use has raised two pressing questions: “How quickly will insects evolve resistance to Bt toxins?” and “Will resistance be delayed by planting refuges of non-Bt crops near Bt crops?” Here we summarize our recent paper that addresses these questions by analyzing global resistance monitoring data in conjunction with results from computer simulations of the refuge strategy².

In brief, most insect pests targeted by Bt crops in Australia, China, Spain, and the U.S. did not evolve resistance during the first decade the crops were grown. An exception is the bollworm *Helicoverpa zea*. Field-evolved resistance to Cry1Ac, the Bt toxin in first generation transgenic cotton, was initially documented in some *H. zea* populations in the southeastern U.S. during 2003 and 2004, after seven to eight years of exposure to Bt cotton. Overall, extensive monitoring data for *H. zea* and five other pests are consistent with expectations arising from the theory underlying the refuge strategy, suggesting that non-Bt crop refuges help delay insect resistance to Bt crops.

We focused on the first generation of Bt crops, which consists almost entirely of transgenic cotton producing Bt toxin Cry1Ac and transgenic corn producing Bt toxin Cry1Ab. In nature, both toxins are produced in crystalline form by Bt bacteria (hence their names start with “Cry”). Initial efficacy of first generation Bt crops against larvae of major lepidopteran pests was high (>99%) for European corn borer (*Ostrinia nubilalis*), tobacco budworm (*Heliothis virescens*), and pink bollworm (*Pectinophora gossypiella*); intermediate (<99%) for bollworm (*Helicoverpa zea*) and cotton bollworm (*Helicoverpa armigera*); and low (<20%) for some other species such as beet armyworm (*Spodoptera exigua*). These differences reflect inherent variation among species in their susceptibility to Cry1Ab and Cry1Ac rather than evolution of resistance, which is defined below.

Field-evolved resistance to Bt toxins in sprays and transgenic crops

Evolution of resistance to a Bt toxin reflects a genetically-based decrease in the susceptibility of an insect population to the toxin³. This results from an increase in the frequency of individuals that have alleles conferring resistance, which occurs over time when populations are exposed to the toxin. Many insects harbor genetic variation in their susceptibility to Bt toxins; more than a dozen species have been selected for Bt resistance in the laboratory. To document field-evolved resistance, a field population exposed to one or more Bt toxins must show less susceptibility than conspecific field populations or lab strains with less exposure to the toxins.

Susceptibility is usually measured with lab bioassays that expose larvae to Bt toxins in their food. The most common index of susceptibility is the LC₅₀, which is the toxin concentration that kills 50% of larvae. The resistance ratio, which is the LC₅₀ of a field-derived strain divided by the LC₅₀ of a standard susceptible strain, is often used to gauge resistance. The higher the resistance ratio, the greater the resistance. Resistance ratios >10 are most likely to reflect genetically-based decreases in susceptibility³.

Evolution of resistance to Bt toxins used in sprays has been documented for field populations of diamondback moth (*Plutella xylostella*) and greenhouse populations of cabbage looper (*Trichoplusia ni*). In these two vegetable pests, initial documentation of resistance was based on a maximum resistance ratio of 36 for diamondback moth in Hawaii and 160 for cabbage looper in British Columbia (**Table 1**).

Insect	Bt spray or toxin	Resistance ratio			
		>10	>100	>1000	Maximum
<i>P. xylostella</i> (diamondback moth) ⁷	Dipel	2	0	0	36
<i>T. ni</i> (cabbage looper) ⁸	Dipel	23	2	0	160
<i>H. zea</i> (bollworm) ^{5, 9-12}	Cry1Ac	54	14	2	>1000

Table 1. Field-evolved resistance to the Bt toxins in sprays and transgenic cotton. Resistance ratio is the LC₅₀ (concentration killing 50%) of a field-derived strain divided by the LC₅₀ of a susceptible strain. Dipel is a spray formulation of Bt subsp. *kurstaki* that contains Cry1A and Cry2A toxins. Superscripts indicate references.



Evidence of field-evolved resistance to Cry1Ac in Bt cotton by *H. zea* includes data from 2002 to 2006 showing 14 field populations from Arkansas, Georgia, and Mississippi with resistance ratios >100, including two populations with resistance ratios >1000 (Tables 1, 2). Baseline data from 1992 and 1993 show that, before Bt cotton was commercialized, resistance was not detected in field populations of *H. zea* (Table 2).

Several experiments with *H. zea* show that the increased LC_{50} s of Cry1Ac in lab bioassays are linked with higher survival on plant tissues of Bt cotton producing Cry1Ac. In one set of experiments by Jackson et al.⁴, survival on Bt cotton relative to non-Bt cotton was 10% for a susceptible strain vs. 40% for a lab-selected strain with a resistance ratio of 100. In independent experiments with field-selected resistant strains, Luttrell and colleagues report similar results and conclude that reduced susceptibility to Cry1Ac in bioassays was “associated with a measurable increase in survival on Bt plant tissue,” and “Colonies collected as surviving larvae on Bt cotton tended to have reduced susceptibility suggesting that some component of observed field control problems may be associated with the presence of resistance genes.”⁵ In contrast to the resistance documented for some field populations of *H. zea*, similar monitoring efforts have not detected resistance in five other major pests targeted by Bt crops: *H. armigera*, *H. virescens*, *O. nubilalis*, *P. gossypiella*, and *Sesamia nonagrioides*².

Table 2. Field-derived strains of *H. zea* with resistance ratios >100 for Bt toxin Cry1Ac. LC_{50} is the concentration (μ g Cry1Ac per ml diet) killing 50% of larvae. Resistance ratio is the LC_{50} of a field-derived strain divided by the LC_{50} of a susceptible strain. Data summarized are from references 5 and 9 – 12.

Year	Strain	Collection site	Source	Resistance ratio
1992	None (maximum LC_{50} = 0.93)			
1993	None (maximum LC_{50} = 5.97)			
2002	None (maximum LC_{50} = 91.65)			
2003	F3603	Morgan City, MS	Bt cotton	515
	F3703	Morgan City, MS	Bt cotton	184
	F3803	Morgan City, MS	Bt cotton	354
2004	F3704	Pickens, AR	Non-Bt cotton	578
2005	F6605	Pickens, AR	Bt corn	102
	F6705	Pickens, AR	Bt corn	157
	F12105	Miller Co., GA	Bt cotton	153
	F13305	Foreman, AR	Light trap	319
	F0105	Texarkana, AR	Clover	>1000
	F5705	Foreman, AR	Non-Bt corn	>1000
2006*	F7605	Fayetteville, AR	Chickpea	710
	F9206	Fayetteville, AR	Chickpea	681
	F4106	Early Co., GA	Non-Bt corn	186
	F8306	Calhoun Co., GA	Light trap	254

AR, Arkansas; GA, Georgia; MS, Mississippi. Larvae were collected from the plants listed. Gravid female moths were collected from light traps. *A total of seven field-derived strains tested in 2006 had less than 50% mortality at a diagnostic concentration of 250 μ g Cry1Ac per ml diet¹².

Field monitoring data vs. predictions from the refuge theory

To determine if the field outcomes documented by monitoring data are consistent with the theory underlying the refuge strategy, we modeled resistance evolution in each of the six major pests listed above². The refuge strategy is



based on population genetics theory positing that refuges of non-Bt host plants delay evolution of resistance by allowing survival of susceptible insects. The refuge strategy is mandated in the U.S. and many other countries where Bt crops are grown. This strategy is expected to be especially effective when resistance is inherited as a recessive trait and most resistant adults surviving on Bt crops mate with susceptible adults from refuges. Under these conditions, the frequency of resistance is not expected to increase rapidly because Bt crops kill the hybrid progeny produced by matings between resistant and susceptible adults.

Consistent with the field data, modeling results projected that *H. zea* would evolve resistance faster than other pests, primarily because its resistance to Cry1Ac is dominant rather than recessive. Modeling results also showed that resistance is expected to evolve faster as refuges constitute a smaller percentage of the pests' host plants. This projection is consistent with field data showing that *H. zea* resistance to Cry1Ac evolved faster in states with lower refuge percentages.

Implications and conclusions

Even though large, genetically-based decreases in susceptibility to Cry1Ac are well documented for some field populations of *H. zea* and increased control problems have been noted anecdotally⁶, widespread control failures have not been reported. We think that several factors contribute to this pattern. First, even in the few states with documented cases of resistance, most populations are not resistant. Second, data from greenhouse experiments suggest that Cry1Ac in Bt cotton kills some resistant larvae, e.g., 60% of larvae in a strain with a resistance ratio of 100⁴. Third, insecticide sprays have been used extensively to control *H. zea* since the introduction of Bt cotton. Control achieved with insecticides would mask problems resulting from resistance to Cry1Ac. Finally, "pyramided" Bt cotton producing Bt toxins Cry1Ac and Cry2Ab was registered in December 2002 and planted on >1 million ha in the U.S. in 2006 and 2007. Control of Cry1Ac-resistant *H. zea* larvae by Cry2Ab also limits control problems associated with resistance to Cry1Ac.

The negative effects of resistance to Cry1Ac should decline further as the acreage of cotton producing only Cry1Ac decreases. This acreage decreased from 2.5 million ha in 2006 to only 1.3 million ha in 2007. In addition, Monsanto's registration for Bt cotton with only Cry1Ac is scheduled to expire in 2009. Cotton producing both Cry1Ac and Cry2Ab toxins could substantially delay evolution of resistance for pests like *H. virescens* that remain susceptible to both toxins. For Cry1Ac-resistant populations of *H. zea*, however, the resistance-delaying benefits of pyramiding these two toxins may not be fully realized.

In general, the second generation of crops genetically engineered for protection against insects offers a greatly increased diversity of toxins. The first generation of Bt crops was dominated by plants producing either Cry1Ab or Cry1Ac, two closely related toxins that kill only caterpillars. The U.S. EPA website of registered plant-incorporated protectants now lists commercially available varieties of Bt corn and Bt cotton with 12 different combinations of one to three Cry toxins that kill caterpillars, beetles, or both. In the near future, more extensive pyramiding is likely, with plans for up to six different Bt Cry proteins in single corn plants. Registration is also expected for transgenic varieties producing another type of Bt toxin called Vip (vegetative insecticidal protein). Other options for genetically engineered insect protection include modified Bt toxins specifically designed to kill insects resistant to native Bt toxins and gene-silencing technology based on RNA interference.

Looking back on the first generation of Bt crops, we think that their sustained efficacy against nearly all targeted pest populations exceeds the expectations of many scientists. An exceptional case involving field-evolved resistance to Cry1Ac in some populations of *H. zea* 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 Cry1Ac-susceptible and -resistant adults. In reference to this concept, the refuge strategy is sometimes called the "high-dose refuge strategy." Before Bt cotton was commercialized, scientists at the U.S. EPA and elsewhere reported that the high-dose criterion of the refuge strategy was not met for Bt cotton with Cry1Ac vs. *H. zea*. Therefore, the relatively rapid resistance that occurred in this pest is no surprise. As the second generation of Bt crops proceeds, we can use systematic analyses of monitoring data from the first decade to maximize benefits and minimize risks. The results summarized here suggest that refuges can delay pest resistance to Bt crops, especially when resistance is recessive and refuges are abundant.

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