

Field-Evolved Insect Resistance to *Bt* Crops: Definition, Theory, and Data

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ABSTRACT Transgenic crops producing *Bacillus thuringiensis* (*Bt*) toxins for insect pest control have been successful, but their efficacy is reduced when pests evolve resistance. Here we review the definition of field-evolved resistance, the relationship between resistance and field control problems, the theory underlying strategies for delaying resistance, and resistance monitoring methods. We also analyze resistance monitoring data from five continents reported in 41 studies that evaluate responses of field populations of 11 lepidopteran pests to four *Bt* toxins produced by *Bt* corn and cotton. After more than a decade since initial commercialization of *Bt* crops, most target pest populations remain susceptible, whereas field-evolved resistance has been documented in some populations of three noctuid moth species: *Spodoptera frugiperda* (J. E. Smith) to Cry1F in *Bt* corn in Puerto Rico, *Busseola fusca* (Fuller) to Cry1Ab in *Bt* corn in South Africa, and *Helicoverpa zea* (Boddie) to Cry1Ac and Cry2Ab in *Bt* cotton in the southeastern United States. Field outcomes are consistent with predictions from theory, suggesting that factors delaying resistance include recessive inheritance of resistance, abundant refuges of non-*Bt* host plants, and two-toxin *Bt* crops deployed separately from one-toxin *Bt* crops. The insights gained from systematic analyses of resistance monitoring data may help to enhance the durability of transgenic insecticidal crops. We recommend continued use of the long-standing definition of resistance cited here and encourage discussions about which regulatory actions, if any, should be triggered by specific data on the magnitude, distribution, and impact of field-evolved resistance.

KEY WORDS resistance, genetically engineered crops, transgenic crops, *Bacillus thuringiensis*, evolution

Crop plants genetically engineered to produce toxins from *Bacillus thuringiensis* (*Bt*) for insect control have been planted on >200 million ha since 1996 (James 2009). The first generation of *Bt* crops was dominated by plants producing single toxins to kill key caterpillar pests: corn producing *Bt* toxin Cry1Ab and cotton producing the closely related toxin Cry1Ac. Although *Bt* corn and *Bt* cotton still dominate, varieties of these crops currently registered in the United States collectively produce 18 different combinations of 11 *Bt* toxins (Table 1). Each variety produces 1–6 *Bt* toxins that kill caterpillars, beetles, or both (Table 1).

The primary threat to the continued success of *Bt* crops is evolution of resistance by pests (Tabashnik 1994, Gould 1998, Tabashnik et al. 2003, Griffiths and Aroian 2005, Bravo and Soberón 2008, Onstad 2008). The literature on this topic has grown rapidly, with hundreds of papers published in the past 5 yr and confusion arising from differences in definitions of

resistance, resistance monitoring methods, and interpretation of data. This paper aims to help clarify the current status of field-evolved resistance to *Bt* crops. Here we define field-evolved resistance, describe its relationship to field control problems, and explain how it is measured. We summarize the theory underlying the refuge and pyramid strategies for delaying resistance. We analyze resistance monitoring data for *Bt* crops that produce Cry1 and Cry2 toxins targeting caterpillar pests, including many studies published since we last reviewed this topic (Tabashnik et al. 2008a). We compare the observed outcomes in the field with the expectations based on theory and conclude by considering the implications of current knowledge and prospects for the future of insect control with transgenic crops.

Field-Evolved Resistance: Definition and Relationship to Field Control

We define field-evolved (or field-selected) resistance as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (National Research

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Table 1. *Bt* crops registered for commercial use in the United States (USEPA 2009^a)

<i>Bt</i> toxin(s)	Target pests ^b	First registered
Corn		
Cry1Ab	L	1995
Cry1F	L	2001
Cry3Bb1	C	2003
Cry1Ab + Cry3Bb1	L, C	2003
Cry34Ab1 + Cry35Ab1	C	2005
Cry1F + Cry34Ab1 + Cry35Ab1	L, C	2005
Modified Cry3A ^c	C	2006
Cry1Ab + modified Cry3A ^c	L, C	2007
Cry1A.105 ^d + Cry2Ab2	L	2008
Cry1A.105 + Cry2Ab2 + Cry3Bb1	L, C	2008
Vip3Aa20	L	2008
Cry1Ab + Vip3Aa20	L	2009
Cry1Ab + Vip3Aa20 + modified Cry3A ^c	L, C	2009
Cry1A.105 ^d + Cry2Ab2 + Cry1F + Cry3Bb1 + Cry34Ab1 + Cry35Ab1	L, C	2009
Cotton		
Cry1Ac	L	1995 ^e
Cry1Ac + Cry2Ab2	L	2002
Cry1Ac + Cry1F	L	2004
Modified Cry1Ab ^f + Vip3Aa19	L	2008

^a Website accessed 29 October 2009.

^b L, lepidopteran larvae; C, coleopteran larvae. The specific target pests depend on the toxins produced and where the crop is grown. Tables 2–5 provide species names and monitoring data for key lepidopteran pests targeted by *Bt* crops. In the United States, the major coleopteran pests targeted by modified Cry3A, Cry3Bb1, Cry34Ab1, and Cry35Ab1 in *Bt* corn are the western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*D. barberi*) and Mexican corn rootworm (*D. virgifera zeae*).

^c Modified Cry3A has 46 fewer amino acids at the N-terminus and three amino acid substitutions internally compared with Cry3A (USEPA 2006).

^d Cry1A.105 is a chimeric protein containing domain I of Cry1Ab, domain II of Cry1Ac, most of domain III from Cry1F, and the C-terminus from Cry1Ac (Biosafety Clearing-House 2008).

^e Expired 30 September 2009.

^f Modified Cry1Ab has 26 extra amino acids at the C-terminus (USEPA 2008).

Council 1986, Tabashnik 1994). In contrast, laboratory-selected resistance occurs when exposure to a toxin in the laboratory causes a heritable decrease in susceptibility of a laboratory strain. Because both field-evolved and laboratory-selected resistance entail changes in gene frequency across generations, they exemplify evolution.

Our definition of field-evolved resistance is based on the definition provided by a group of resistance experts convened by the United States National Academy of Sciences (National Research Council 1986) that was later paraphrased and applied to *Bt* toxins and *Bt* crops (Tabashnik 1994, Tabashnik et al. 2008a). Like the National Research Council (1986) definition, our definition of field-evolved resistance does not necessarily imply loss of economic efficacy in the field. Nonetheless, field-evolved resistance to toxins in *Bt* crops is expected to confer decreased susceptibility to *Bt* crops in the field, whereas laboratory-selected resistance achieved by feeding insects on toxin-treated diet does not always increase survival on *Bt* crops (Tabashnik et al. 2003). Although the terms “field-evolved resistance” and “evolution of resistance” refer

to populations, the word “resistance” can be used to indicate heritable, lower susceptibility of an individual relative to conspecific individuals.

Insect populations often have natural genetic variation affecting response to a toxin, with some alleles conferring susceptibility and others conferring resistance. Alleles conferring resistance are typically rare in insect populations before the populations are exposed to a *Bt* toxin, with empirical estimates often close to one in a thousand (Tabashnik 1994, Gould et al. 1997, Burd et al. 2003, Tabashnik et al. 2008a, Downes et al. 2009, Huang et al. 2009). Field-evolved resistance occurs when exposure of a field population to a toxin increases the frequency of alleles conferring resistance in subsequent generations. Hence, inherently low susceptibility of a species to a toxin does not signify field-evolved resistance. Likewise, merely detecting resistance-conferring alleles without demonstrating that their frequency has increased does not constitute evidence of field-evolved resistance.

The main goal of monitoring resistance to *Bt* crops is to detect field-evolved resistance early enough to enable proactive management before control failures occur (United States Environmental Protection Agency [USEPA] 2001, Siegfried et al. 2007, Tabashnik et al. 2008b). Therefore, as noted above, documentation of field-evolved resistance does not necessarily imply that control problems have occurred in the field (National Research Council 1986, Tabashnik et al. 2008a). This means that regulatory decisions about continued use of *Bt* crops should incorporate information about the relationship between field-evolved resistance and field control problems.

The relationship between field-evolved resistance and field-control problems depends on many factors including the frequency of resistance alleles, the magnitude of resistance, the extent to which resistance increases survival in the field, the number and spatial distribution of resistant populations, the insect's population density, the availability of alternative control tactics, and the extent to which the insect is a pest. For example, field-evolved resistance to insecticides in species such as *Drosophila melanogaster* (L.) (Pedra et al. 2004) may have virtually no implications for control, whereas field-evolved resistance in natural enemies can enhance pest control (Tabashnik and Johnson 1999). Even for major target pests, field-evolved resistance to a *Bt* crop may not cause widespread problems if alternative control methods are effective (Tabashnik et al. 2008a). Furthermore, if a *Bt* crop targets several species of pests, its efficacy may be fully maintained against species that remain susceptible, even though field-evolved resistance reduces its efficacy against other species. Accordingly, regulatory decisions should be based on the net benefits and drawbacks of a particular *Bt* crop in a particular location, so that detection of field-evolved resistance in one or more pest populations does not automatically trigger large-scale removal of valuable varieties from the marketplace.

Resistance Monitoring Methods

Accurate resistance monitoring requires evaluation of insect field populations on *Bt* crops as well as from other sources including non-*Bt* host plants. Sampling and testing of target pest insects surviving on or near *Bt* crops is essential for early detection of field-evolved resistance (Tabashnik et al. 2008b). Failure to sample such insects favors underestimation of the frequency of resistance, which can postpone detection of resistance and is contrary to the primary purpose of resistance monitoring. Although most research on *Bt* toxins focuses on physiologically based resistance, behavioral changes can also cause resistance by reducing exposure to a toxin (Onstad 2008).

To measure susceptibility, insects are exposed to toxins in bioassays. Susceptibility of a field population is usually measured by sampling insects from the field, rearing their progeny in the laboratory, and determining how the progeny respond to diet treated with toxin or to parts of *Bt* plants such as leaves. With rigorous control of environmental conditions, this approach allows one to infer that any differences in susceptibility are heritable.

In some cases, field-collected insects are pooled in large groups for mating in the laboratory to generate field-derived strains for bioassays (Tabashnik et al. 2000). Alternatively, families derived from single wild gravid females or from single-pair crosses done in the laboratory can be reared and tested separately using F_1 or F_2 screening procedures (Gould et al. 1997, Andow and Alstad 1998, Blanco et al. 2009). Whereas bioassays with plants allow more direct inferences about survival on *Bt* crops in the field, diet tests enable determination of responses to specific toxin concentrations. Both approaches are valuable; they are most powerful when used in concert (Tabashnik et al. 2000).

Results of diet or plant bioassays document field-evolved resistance if they show that exposure to a toxin in the field has caused a genetically based decrease in the susceptibility of one or more populations. Field-evolved resistance can be demonstrated directly by showing decreases in susceptibility over time for one or more field populations exposed to toxin. More commonly, field-evolved resistance is documented indirectly by showing that one or more field populations with a history of exposure to toxin are less susceptible than conspecific field populations or laboratory strains that have had little or no such exposure (Tabashnik 1994). Thus, susceptible strains used for comparison should be representative of susceptible field populations; they should not be contaminated with resistance alleles from resistant laboratory strains or infused with resistance alleles from field populations exposed to toxin.

The most common and definitive measure of susceptibility is based on mortality of insects exposed to toxin. Many resistance monitoring studies have used diet bioassays to compare the concentration of toxin causing 50% mortality (LC_{50}) in strains derived from field populations exposed to *Bt* crops to susceptible

laboratory or field-derived strains (Tabashnik et al. 2008a). A statistically significant difference between strains is typically demonstrated by no overlap of the 95% fiducial limits of their LC_{50} values, which is a conservative criterion (Tabashnik et al. 1987). LC_{50} data also enable calculation of the resistance ratio, which is the LC_{50} value of a field-derived strain divided by the LC_{50} value of a conspecific susceptible strain, with both strains tested under the same conditions. Higher resistance ratios provide stronger evidence of resistance. Resistance ratios >10 are more likely to reflect genetically based decreases in susceptibility (Tabashnik 1994).

An alternative to comparing LC_{50} values is comparing responses to diagnostic toxin concentrations that kill all or nearly all susceptible individuals, but few or no resistant individuals. Exposure to diagnostic concentrations can be achieved with diet or plant bioassays. This approach is especially efficient for detecting 1–10% of resistant individuals in a population (Roush and Miller 1986). If the genetic basis of resistance is known, survival at a diagnostic concentration can be used to estimate the resistance allele frequency (Andow and Alstad 1998, Tabashnik et al. 2000). Field-evolved resistance can be documented by showing a statistically significant increase in the percentage survival at a diagnostic concentration or in the estimated resistance allele frequency.

Estimating LC_{50} values requires data from several concentrations, usually including at least one diagnostic concentration. Large increases in LC_{50} indicate that $>50\%$ of the individuals in a population are resistant. Thus, in the early stages of resistance evolution, the diagnostic concentration approach is more likely to detect resistance (Roush and Miller 1986). LC_{50} values and survival at a diagnostic concentration are typically correlated when some populations are highly resistant (Tabashnik et al. 1993). When a population is extremely resistant, the highest concentrations tested may kill $<50\%$ of the insects tested, making it difficult to precisely estimate LC_{50} values. Although most resistance monitoring has focused on mortality, growth inhibition caused by toxin can also be a useful indicator of susceptibility. Whether evaluated as the toxin concentration causing 50% inhibition (IC_{50}) or the extent of inhibition caused by a diagnostic concentration, measures of growth inhibition and mortality are often correlated (Ali et al. 2007).

Although the commonly used methods described above focus on bioassays conducted under controlled conditions, resistance can also be monitored in the field by comparing pest population density in paired fields of *Bt* and non-*Bt* crops (Tabashnik et al. 2000, Venette et al. 2000). A significant increase over time in the pest population density in the *Bt* crop relative to the non-*Bt* crop provides suggestive, but not definitive, evidence of resistance. Because this relative population density can be affected by variation in toxin concentration of *Bt* plants and other environmental and ecological factors, tests conducted under controlled conditions are needed to demonstrate that

survivors on *Bt* plants in the field have genetically based resistance.

Whereas nearly all monitoring for resistance to *Bt* toxins has been based on bioassays, DNA screening for cadherin alleles linked with resistance has been done in a few cases (Tabashnik et al. 2006, Gahan et al. 2007, Yang et al. 2007). Advantages of this approach compared with bioassays include the ability to detect single resistance alleles in heterozygotes, in pools of many individuals, in different life stages, and in preserved, dead individuals (Morin et al. 2004). However, while bioassays can detect resistance caused by any mechanism, DNA screening detects only resistance alleles associated with previously identified mechanisms of resistance and can underestimate the frequency of all alleles that confer resistance (Morin et al. 2004, Gahan et al. 2007). The combination of DNA screening and bioassays can be especially effective for monitoring resistance (Tabashnik et al. 2005, 2006).

Resistance Management Theory: Refuges, High Dose, and Pyramids

The refuge strategy has been the chief approach used worldwide to delay pest resistance to *Bt* crops (Tabashnik 2008). This strategy, which has been mandated in the United States and elsewhere, is based on the idea that most of the rare resistant pests surviving on *Bt* crops will mate with abundant susceptible pests from nearby refuges of host plants without *Bt* toxins (Tabashnik and Croft 1982, Gould 1998, USEPA 1998a). If inheritance of resistance is recessive, the hybrid progeny from such matings will die on *Bt* crops, substantially slowing the evolution of resistance. This approach is sometimes called the "high-dose refuge strategy" because it works best if the dose of toxin ingested by insects on *Bt* plants is high enough to kill all or nearly all of the aforementioned hybrid progeny (Gould 1998, Tabashnik et al. 2004).

The most direct way to test the high-dose hypothesis is to let resistant and susceptible adults mate in the laboratory and measure survival of their hybrid progeny on *Bt* plants. Because suitable resistant strains for direct tests may not be available, indirect tests are used. One indirect test relies on the reasonable assumption that if *Bt* plants do not kill close to 100% of susceptible individuals, they probably will not kill nearly all hybrid individuals. Furthermore, in such cases, survival is likely to be higher for the hybrid individuals than for their susceptible counterparts, which yields nonrecessive inheritance of resistance that accelerates adaptation (Tabashnik et al. 2004). Thus, the USEPA (1998b) guidelines for a high dose specify that *Bt* plants should kill at least 99.99% of susceptible insects in the field.

In principle, if a high dose is not achieved, resistance can be delayed by increasing refuge abundance to compensate for survival of hybrid progeny on *Bt* plants (Gould 1998, Tabashnik et al. 2004). Factors favoring success of the refuge strategy are abundant refuges of non-*Bt* host plants near *Bt* crops, recessive inheritance of resistance, low initial resistance allele frequency,

fitness costs, and incomplete resistance (Gould 1998, Carrière and Tabashnik 2001, Tabashnik et al. 2003, 2004, Crowder and Carrière 2009). Fitness costs occur when fitness on non-*Bt* host plants is lower for resistant insects than susceptible insects (Gassmann et al. 2009). Incomplete resistance occurs when resistant insects can complete development on *Bt* plants, but they are at a disadvantage compared with resistant insects that develop on non-*Bt* plants (Carrière and Tabashnik 2001, Carrière et al. 2006).

The dominance of resistance on a *Bt* crop plant can be measured in terms of the parameter h , which varies from zero for completely recessive to one for completely dominant (Liu and Tabashnik 1997). Refuge abundance can be measured for each pest in terms of the percentage of host plants that is accounted for by non-*Bt* plants adjusted for the relative production of pests by different types of host plants (Carrière et al. 2004, Gustafson et al. 2006, Baker et al. 2008). Results from a single-locus, two-allele model of a generic pest with an initial resistance allele frequency of 0.001 suggest that resistance can be delayed for >20 yr with refuges of $\geq 5\%$ if resistance is completely recessive ($h = 0$) and with refuges of >50% if resistance is partially dominant ($h \geq 0.4$) (Tabashnik et al. 2008a).

Although first-generation *Bt* crops each produce a single *Bt* toxin, some second-generation *Bt* crops produce two distinct *Bt* toxins that are active against the same pest (Table 1). This approach, which is called a "pyramid," is expected to delay pest resistance most effectively when selection for resistance to one of the toxins does not cause cross-resistance to the other toxin (Zhao et al. 2005). Other factors favoring success of pyramided *Bt* crops parallel those listed above for the refuge strategy, including abundant refuges and the following conditions for each toxin in the pyramid: recessive inheritance of resistance, low initial resistance allele frequency, fitness costs, and incomplete resistance (Gould 1998, Zhao et al. 2005, Gould et al. 2006, Tabashnik et al. 2009). Results from population genetic models and small-scale experiments with *Plutella xylostella* (L.) indicate that resistance to pyramids will evolve faster if two-toxin plants are grown concurrently with single-toxin plants (Zhao et al. 2005). This occurs because the single-toxin plants select for resistance to each toxin separately, which diminishes the ability of the two-toxin plants to delay resistance (Zhao et al. 2005).

Resistance Monitoring Data

Here we review the status of field-evolved pest resistance to *Bt* crops, including monitoring data from seven countries testing responses of field populations of 11 species of lepidopteran pests to four toxins produced by *Bt* corn and cotton (Cry1Ab, Cry1Ac, Cry1F, and Cry2Ab) (Tables 2–5). We use Cry2Ab to refer to Cry2Ab2 because these toxins have the same amino acid sequence (Tabashnik et al. 2009). As in our previous reviews (Tabashnik et al. 2003, 2008a), we focus on studies published in refereed journals, using references to other sources primarily to supplement in-

Table 2. Strong evidence of field-evolved resistance to the toxins in *Bt* crops for three lepidopteran pests in the family Noctuidae

Location	Crop	Toxin	Year comm. ^a	Strains ^b	Initial year ^c	Final year ^c	Parameter	Initial value	Final value	Reference
<i>Busseola fusca</i> (Fuller)										
South Africa	Corn	Cry1Ab	1998	2	NA ^d	2006	Max survival ^e	NA	63.6%	Van Rensburg 2007
<i>Helicoverpa zea</i> (Boddie)										
AR, MS	Cotton	Cry1Ac	1996	64	1992	2004	Max RR ^f	1.2	578	Luttrell et al. 1999, Ali et al. 2006
AR, GA, MS	Cotton	Cry2Ab	2003	67	2002	2005	Max RR	7.7	>27	Ali and Luttrell 2007
AR, GA, MS	Cotton	Cry2Ab	2003	67	2002	2005	R strains ^g	0%	50%	Ali and Luttrell 2007
<i>Spodoptera frugiperda</i> (J. E. Smith)										
Puerto Rico	Corn	Cry1F	2003	2	NA	2006	Max RR	NA	>100	Matten et al. 2008

^a First year *Bt* crop was grown commercially in the location monitored.

^b No. field-derived strains tested in bioassays.

^c Initial and final years during which field populations were sampled.

^d Not available.

^e Maximum survival in the field at 18 d on *Bt* corn plants relative to non-*Bt* corn plants (see Fig. 1).

^f Maximum resistance ratio (RR), the highest LC₅₀ value of a field-derived strain divided by the LC₅₀ of one or more susceptible laboratory strains.

^g % field-derived strains with a resistance ratio >10 and an LC₅₀ value greater than the diagnostic concentration of 150 µg Cry2Ab/ml diet (see Fig. 2).

formation about cases described in refereed journal articles. One exceptional case is field-evolved resistance to Cry1F in *Spodoptera frugiperda*, for which the original data have not been reported in a refereed journal article as far as we know. In this case, the data have been summarized by the USEPA (Matten 2007, Matten et al. 2008) and were obtained via the United States Freedom of Information Act. As detailed below, the global monitoring data provide strong evidence of field-evolved resistance for three target pests, ambiguous evidence of field-evolved resistance to Cry1Ac in *Helicoverpa armigera* in China and India, and strong evidence of sustained susceptibility in some or all populations examined for nine target pests (Tables 2–5).

Strong Evidence of Field-Evolved Resistance. Strong evidence of field-evolved resistance to the *Bt* toxins in transgenic crops has been reported for some

populations of three targeted noctuid moths: *Busseola fusca*, *Helicoverpa zea*, and *S. frugiperda* (Table 2). Field-evolved resistance of *S. frugiperda* to *Bt* corn producing Cry1F occurred in 4 yr in the United States territory of Puerto Rico (Matten et al. 2008), making this the fastest documented case of field-evolved resistance to a *Bt* crop. This is also the first case of resistance leading to withdrawal of a *Bt* crop from the marketplace.

In Puerto Rico, where *S. frugiperda* is a primary corn pest, Cry1F corn was first commercially available in 2003. Based on review of data submitted by Dow AgroSciences and Pioneer Hi-Bred International, the USEPA concluded that “unexpected” damage to Cry1F corn observed in the field in 2006 was caused by Cry1F-resistant *S. frugiperda* (Matten 2007, Matten et al. 2008). From fields of Cry1F corn showing unexpected damage, live *S. frugiperda* were collected and their progeny were tested using laboratory diet bioassays. The highest toxin concentration tested (10 µg Cry1F/cm² diet) did not cause significant mortality of these larvae (Matten 2007). In contrast, larvae from a concurrently tested susceptible strain of *S. frugiperda* had an LC₅₀ (µg Cry1F/cm² diet with 95% fiducial limits) of 0.06 (0.03–0.25) (Matten 2007) and another, independently tested susceptible strain had an LC₅₀ of 0.11 (0.03–0.17) (Luo et al. 1999). Because of the extremely high resistance of the Puerto Rican larvae, the resistance ratio cannot be calculated precisely. However, the data show that the LC₅₀ of the Cry1F-resistant larvae was much greater than 10 µg Cry1F/cm² diet, which indicates that their resistance ratio relative to both of the susceptible strains was much higher than 100.

After the reports of unusually high damage were received, growers were advised to treat affected Cry1F corn fields with insecticides to kill *S. frugiperda* and steps were taken voluntarily by Dow AgroSciences and Pioneer Hi-Bred International to stop commercial sales of Cry1F corn in Puerto Rico. Bioassay data from Puerto Rico for *S. frugiperda* versus

Table 3. Field-derived strains of *Helicoverpa zea* with an LC₅₀ value > 150 µg Cry2Ab/ml and RR > 10 (data from Ali and Luttrell 2007)

Year	Strain	Collection site	Source	LC ₅₀ ^a	RR ^b
2002	None ^c	NA ^d	NA	NA	NA
2003	F3803	Morgan City, MS	Cotton (Cry1Ac)	207	25
2004	F1804	Pickens, AR	Soybean	172	29
2005	F2205	Foreman, AR	Non- <i>Bt</i> corn	162	11
	F12205	Taylor Co., GA	Cotton (Cry1Ac + Cry2Ab)	185	12
	F0705	Dumus, AR	Clover	>400 ^e	>27
	F2405	Foreman, AR	Non- <i>Bt</i> corn	>400 ^e	>27
	F15105	Fayetteville, AR	Chickpea	>400 ^e	>27

^a Concentration that killed 50% of larvae tested, in micrograms Cry2Ab per milliliter diet.

^b Resistance ratio (RR), the LC₅₀ value of a field-derived strain divided by the LC₅₀ value of the UALab strain of *H. zea* tested in the same year (UALab LC₅₀ for 2002 = 6.71, 2003 = 8.41, 2004 = 5.98, 2005 = 14.87).

^c In 2002, the max LC₅₀ value was 51.6 µg Cry2Ab/ml diet and the max resistance ratio was 51.6/6.71 = 7.7.

^d Not applicable.

^e LC₅₀ value could not be estimated accurately because of high survival at the highest concn tested.

Table 4. Ambiguous evidence of field-evolved resistance to Cry1Ac in *Bt* cotton for *Helicoverpa armigera* (Hübner) (Noctuidae) in China and India

Location	Field sample	Initial year ^a	Final year ^a	Parameter	Initial value	Final value	Reference
China, Qiuxian Co.	406 families	1999	2005	r frequency ^b	0.0058	0.0146	He et al. 2001, Xu et al. 2009
China, Qiuxian Co.	399 families	2006	2007	r frequency	0.094	0.107	Liu et al. 2008, 2009
India	20 strains	2000	2006	Max RR ^c	NA	120 ^d	Gujar et al. 2007

Large-scale planting of Cry1Ac cotton in the locations monitored first occurred in 1998 in China and in 2002 in India.

^a Initial and final years during which field populations were sampled.

^b Estimated resistance allele frequency based on bioassays.

^c Maximum resistance ratio (RR), the highest ratio of an LC₅₀ (μg Cry1Ac/g diet) of a field-derived strain divided by the LC₅₀ of a field-derived strain tested previously from the same region (from the Bhatinda region, LC₅₀ of 5.1 for 2004 divided by LC₅₀ of 0.04 for 2000).

^d Although the max resistance ratio reported was 120, the significance of this is unclear as concurrent testing of a standard susceptible strain was not reported (see text).

Cry1F have not been reported for populations derived from non-*Bt* crops or for baseline susceptibility before *Bt* corn was introduced. Nonetheless, based on previous efficacy of Cry1F corn in Puerto Rico, the damage observed in some fields in 2006 was considered unusual and unexpected (Matten 2007, Matten et al. 2008). This case provides strong evidence of field-evolved resistance because bioassay results show that the observed field damage was associated with resistance ratios >100 for field-derived strains relative to two susceptible strains (Luo et al. 1999, Matten 2007).

Monitoring data show that field-evolved resistance to *Bt* corn producing Cry1Ab occurred in a population of stem borer, *B. fusca*, in South Africa in 8 yr or less (Van Rensburg 2007). The area of Cry1Ab corn planted in South Africa increased from 50,000 ha (<3% of corn) in 1998, the first year of commercialization, to 943,000 ha (34.9% of corn) in 2006 (James 2007). During the 2004–2005 growing season, *B. fusca* caused severe damage to Cry1Ab corn at some locations in South Africa (Van Rensburg 2007). In 2006, Van Rensburg (2007) collected diapausing *B. fusca* larvae from stubble in a Cry1Ab corn field (R strain) near Christiana that had experienced damage and in a non-*Bt* corn field (S strain) near Ventersdorp, an area where *Bt* corn had not been adopted. The larvae from both fields were reared to adults in the laboratory and their neonate progeny were used to infest corn plants in the field. Van Rensburg (2007) reported that larval weight gain occurred significantly faster for the R strain than the S strain on *Bt* corn, but did not differ between strains on non-*Bt* corn. Analysis here of survival data described qualitatively by Van Rensburg (2007) shows the same pattern: survival after 18 d was significantly higher for the R strain than the S strain on *Bt* corn, but did not differ between strains on non-*Bt* corn (Fig. 1). Survival on *Bt* corn relative to non-*Bt* corn was 43–64% for the R strain versus 0% for the S strain (Fig. 1).

A second resistant population of *B. fusca* was found and farmers reported increased damage to *Bt* corn in the Vaalharts area of South Africa, 60 km from the site of the first resistant population (Kruger et al. 2009). The percentage of Vaalharts farmers reporting medium or severe damage to *Bt* corn from stem borers rose from 2.5% in the 2005–2006 growing season to 58.8% in the 2007–2008 growing season. In contrast to

previous years when insecticides were not used for stem borer control on *Bt* corn, 55% of farmers applied insecticides to both *Bt* corn and non-*Bt* corn for stem borer control during the 2007–2008 growing season. Furthermore, for early planted *Bt* corn, Monsanto covered spaying costs when stem borer density exceeded the economic threshold (Kruger et al. 2009). The data provide strong evidence that field-evolved resistance increased larval survival on Cry1Ab corn in the field.

Luttrell and colleagues published a series of five papers revealing that field-evolved resistance to Cry1Ac, the first *Bt* toxin produced by transgenic cotton, occurred in as little as 7–8 yr in some populations of *H. zea* in the southeastern United States (Luttrell et al. 1999, 2004; Ali et al. 2006, 2007; Luttrell and Ali 2007). Although an attempt to challenge the documentation of resistance in this case considered only a small subset of the relevant data (Moar et al. 2008), more comprehensive analyses reveal strong evidence of resistance (Tabashnik et al. 2008a, 2008b). The extensive resistance monitoring data from 1992–2006 document significant, genetically based decreases in susceptibility to Cry1Ac. Decreased susceptibility in laboratory diet bioassays was associated with increased larval survival on leaves of Cry1Ac cotton plants and control problems in the field (Luttrell et al. 1999, 2004; Ali et al. 2006, 2007; Luttrell and Ali 2007). For *H. zea* strains derived from the field in 1992–1993, before Cry1Ac cotton was commercialized, the maximum resistance ratio was 1.2 and the maximum LC₅₀ value among field-derived strains was 5.97 μg Cry1Ac/ml diet (Luttrell et al. 1999, Tabashnik et al. 2008a). For strains derived from the field from 2003–2006, 14 strains of *H. zea* had resistance ratios >100, including two strains with resistance ratios >1,000 (Luttrell and Ali 2007). Eight of these 14 strains originated from *Bt* crops, the other six from either non-*Bt* crops or light traps (Luttrell and Ali 2007). In 2003 and 2004, a diagnostic concentration of 150 μg Cry1Ac/ml diet killed <50% of larvae in 4 of 46 (9%) field-derived strains (Ali et al. 2006). In 2006, mortality at 250 μg Cry1Ac/ml was <50% in 7 of 39 (18%) field-derived strains (Ali et al. 2007).

Based on related work using similar methods, Ali and Luttrell (2007) and Ali et al. (2007) published data showing field-evolved resistance to Cry2Ab in some of

Table 5. Strong evidence of sustained susceptibility to the toxins in *Bt* crops for nine lepidopteran pests

Location	Crop	Toxin	Year comm. ^a	Field sample	Years ^b		Parameter	Initial value	Final value	Reference
					Initial	Final				
Gelechiidae										
<i>Pectinophora gossypiella</i> (Saunders)										
AZ	Cotton	Cry1Ac	1996	106 strains	1997	2004	r frequency ^c	0.16	0.004	Tabashnik et al. 2005
AZ, CA, TX	Cotton	Cry1Ac	1996	5,571 insects	2001	2005	r frequency ^d	0.0	0.0	Tabashnik et al. 2006
Noctuidae										
<i>Helicoverpa armigera</i> (Hübner)										
Australia	Cotton	Cry1Ac	1996	17 strains	2001/2	2002/3	Max RR ^e	1.2	1.5	Bird and Akhurst 2007
Australia	Cotton	Cry1Ac	1996	826 families	2002/3	2005/6	r frequency	0.0	0.0	Mahon et al. 2007a
Australia	Cotton	Cry2Ab	2004/5	827 families	2002/3	2005/6	r frequency	0.0089	0.0049	Mahon et al. 2007a
China	Cotton	Cry1Ac	1998	94 strains	1998	2004	Survival ^f	0.0095	0.0017	Wu et al. 2006
China, Henan	Cotton	Cry1Ac	1997	9,984 insects	NA ^g	2005	r frequency	NA	0.00035	Yang et al. 2007
China, Anci	Cotton	Cry1Ac	1998	2,036 families	2002	2008	r frequency	0.0011	0.00	Li et al. 2007, Gao et al. 2009
China, Xijian	Cotton	Cry1Ac	1998	3,857 families	2002	2008	r frequency	0.0006	0.0003	Li et al. 2007, Gao et al. 2009
<i>Helicoverpa punctigera</i> (Wallengren)										
Australia	Cotton	Cry1Ac	1996	545 families	2002/3	2006/7	r frequency	0.0	0.0	Downes et al. 2009
Australia	Cotton	Cry2Ab	2004/5	548 families	2002/3	2006/7	r frequency	0.0	0.0030	Downes et al. 2009
<i>Helicoverpa zea</i> (Boddie)										
NC	Cotton	Cry1Ac	1996	1,835 families	2000	2002	r frequency	0.0004	0.0	Burd et al. 2003, Jackson et al. 2006
NC	Cotton	Cry2Ab	2003	1,252 families	2001	2002	r frequency	0.0	0.0	Jackson et al. 2006
<i>Heliothis virescens</i> (F.)										
United States	Cotton	Cry1Ac	1996	21 strains	1992/3	2002/4	Max RR	1.1	3.2	Ali et al. 2006
LA, TX	Cotton	Cry1Ac	1996	7,050 males	1996	2002	r frequency ^d	0.0	0.0	Gahan et al. 2007
United States, Mexico	Cotton	Cry1Ac	1996	1,001 families	2006	2007	r frequency	NA	0.0009	Blanco et al. 2009
United States	Cotton	Cry2Ab	2003	26 strains	2002	2005	Max RR	7.6	8.5	Ali and Luttrell 2007
<i>Sesamia nonagrioides</i> Lefebvre										
Spain	Corn	Cry1Ab	1998	12 strains	1999	2002	Max RR	3.0	2.9	Farinós et al. 2004
Spain	Corn	Cry1Ab	1998	85 families	2004	2005	r frequency	0.0	0.0	Andreadis et al. 2007
Pyralidae										
<i>Diatraea grandiosella</i> (Dyar)										
LA	Corn	Cry1Ab	1999	210 families	NA	2005	r frequency	NA	0.0	Huang et al. 2007a
<i>Diatraea saccharalis</i> (F.)										
LA	Corn	Cry1Ab	1999	824 families	2004	2006	r frequency	0.0023	0.0030	Huang et al. 2007b, 2008; Yue et al. 2008
TX	Corn	Cry1Ab	1999	494 families	2006	2007	r frequency	0.0	0.0	Huang et al. 2009
<i>Ostrinia nubilalis</i> (Hübner)										
United States	Corn	Cry1Ab	1996	933 families	1996	2003	r frequency	0.0	0.0	Stodola et al. 2006
United States	Corn	Cry1Ab	1996	32 strains	1995	2005	Mean LC ₅₀ ^h	4.5	2.2	Siegfried et al. 2007
Spain	Corn	Cry1Ab	1998	5 strains	1999	2002	Max RR	1.2	2.7	Farinós et al. 2004

^a First year *Bt* crop was grown commercially in the location monitored.

^b Initial and final years during which field populations were sampled.

^c Estimated resistance allele frequency based on bioassays unless noted otherwise.

^d Estimated frequency of cadherin alleles conferring resistance to Cry1Ac based on DNA screening.

^e Maximum resistance ratio, the highest LC₅₀ of a field-derived strain divided by the LC₅₀ of one or more susceptible laboratory strains.

^f Survival to third instar on diet with 1 microliter of Cry1Ac per ml diet.

^g Not available.

^h Mean LC₅₀ in ng Cry1Ab per cm² diet.

the same populations of *H. zea* screened for resistance to Cry1Ac. The area in the United States planted to cotton making *Bt* toxins Cry2Ab and Cry1Ac (called Bollgard II) increased quickly: from 40,000 ha in 2003 to 100,000 ha in 2004; 300,000 ha in 2005; and >1 million ha in 2006 (Monsanto 2008). To analyze the extensive Cry2Ab monitoring data for 2002–2005 of Ali and Luttrell (2007), we calculated the resistance ratio as the LC₅₀ of a field-derived strain divided by the LC₅₀ for the susceptible University of Arkansas lab strain of *H. zea* (UALab) tested in the same year. These comparisons between field-derived strains and a susceptible strain tested in the same year minimize

any potential effects of changes in bioassay conditions across years, including changes in the potency of toxin.

We used the LC₅₀ value of the UALab strain of *H. zea* in each year to calculate resistance ratios because it was the only lab strain tested in all 4 yr of the study. Furthermore, UALab was not unusually susceptible to Cry2Ab. From 2002–2005, the mean LC₅₀ of Cry2Ab (in µg Cry2Ab/ml diet) was slightly higher for UALab (mean = 9.0, range = 6.7–15) than for the most susceptible field-derived strain tested in each year (mean = 8.5, range = 4.4–18). Of the three lab strains tested, USDALab was most susceptible (LC₅₀ = 5.3), UALab was intermediate (mean LC₅₀ = 9.0), and the

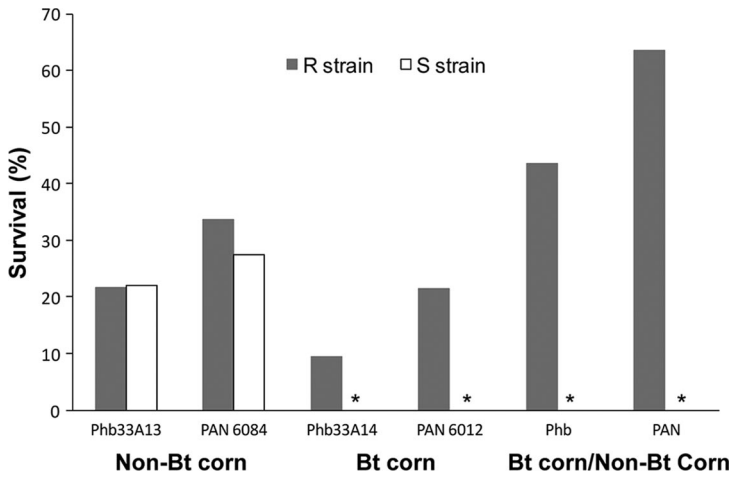


Fig. 1. Survival of resistant (R) and susceptible (S) strains of *Busseola fusca* on Bt corn (cultivars Phb33A14 and PAN 6012) and non-Bt corn (cultivars Phb33A13 and PAN 6084) plants in the field ($n = 84$ –106 larvae per strain for each corn cultivar). Asterisks show 0% survival of the S strain on Bt corn. Bt corn/non-Bt corn shows survival on Bt corn divided by survival on non-Bt corn. Survival on non-Bt corn did not differ between strains ($P > 0.4$ in each comparison, Fisher's exact test). Survival on Bt corn was significantly greater for the R strain than the S strain ($P = 0.0016$ on Phb33A14 and $P < 0.0001$ on PAN6012, Fisher's exact test). See Van Rensburg (2007) for details of the methods.

Monsanto strain was least susceptible (mean $LC_{50} = 84$, which is 16 times higher than USDALab). The Monsanto strain was infused yearly with field-collected insects, which probably increased its frequency of resistance alleles and contributed to its high LC_{50} values for Cry2Ab and Cry1Ac relative to susceptible lab and field-derived strains of *H. zea* (Ali and Luttrell 2007, Anilkumar et al. 2008, Tabashnik et al. 2008b).

The percentage of field-derived strains of *H. zea* with a resistance ratio >10 and an LC_{50} value greater than a diagnostic concentration ($150 \mu\text{g}$ Cry2Ab/ml diet) rose from 0% in 2002 to 50% in 2005 (Fig. 2; Table 3). Of the five Cry2Ab-resistant strains derived from the field in 2005, only one was from cotton producing

Cry2Ab and Cry1Ac; the other four were from non-Bt crops (Fig. 2; Table 5). Of 31 field-derived strains tested at the diagnostic concentration in 2006, 26 (84%) and 12 (39%) strains had higher survival than the laboratory strains from the University of Arkansas and Monsanto, respectively (Ali et al. 2007). For 2006, positive correlations occurred between survival at the diagnostic concentration and two other key resistance parameters: LC_{50} value ($r = 0.705$, $df = 29$, $P < 0.0001$) and MIC_{50} value (the toxin concentration causing inhibition of molting to second instar or stunting in 50% of larvae; $r = 0.684$, $df = 29$, $P < 0.0001$) (Ali et al. 2007).

For 61 strains of *H. zea* tested from 2002–2004 against both Cry1Ac and Cry2Ab, the LC_{50} values for Cry2Ab were positively correlated with those for Cry1Ac ($r = 0.32$, $df = 59$, $P = 0.01$) (Ali and Luttrell 2007). Despite the predicted lack of cross-resistance between these two toxins based on differences in their structures and binding sites (Hernández-Rodríguez et al. 2008), cross-resistance to Cry2Ab caused by resistance to Cry1Ac is a plausible explanation for this observed correlation (Ali and Luttrell 2007, Tabashnik et al. 2009). Selection first for resistance to Cry1Ac followed by selection for resistance to Cry2Ab could also cause or contribute to this pattern. In particular, survival on cotton with both toxins would be more likely for individuals with Cry2Ab resistance alleles if they were already resistant to Cry1Ac (Zhao et al. 2005). However, responses to Cry1Ac and Cry2Ab were genetically correlated in field populations of *H. zea* sampled during 2001 and 2002 (Jackson et al. 2006), before cotton with both toxins was registered in December 2002 (USEPA 2009). Furthermore, because cotton that produces Cry2Ab was still relatively rare from 2002–2004, cross-resistance probably contrib-

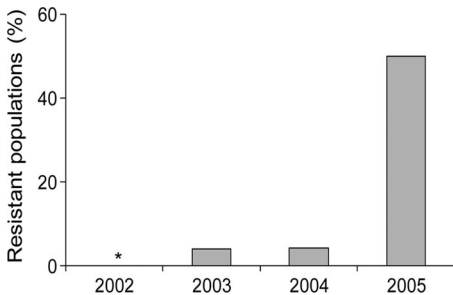


Fig. 2. Field populations of *Helicoverpa zea* with resistance to Cry2Ab in laboratory diet bioassays. A population was considered resistant if it had an LC_{50} value $>150 \mu\text{g}$ Cry2Ab/ml diet and its LC_{50} value was >10 times higher than the LC_{50} value of the susceptible UALab strain tested in the same year (i.e., resistance ratio was >10). The number of resistant populations of the total populations tested was 0 of 8 in 2002 (indicated by asterisk), 1 of 25 in 2003, 1 of 24 in 2004, and 5 of 10 in 2005. The proportion of resistant populations was significantly higher in 2005 (0.5, 5 of 10) than in 2002–2004 (2 of 57, 0.035) ($P = 0.00047$, Fisher's exact test).

uted to the positive correlation detected during this period.

Similar to data showing that *H. zea* resistance to Cry1Ac increases survival on leaves of *Bt* cotton producing Cry1Ac (Luttrell et al. 2004, Jackson et al. 2004b, Ali et al. 2006, Luttrell and Ali 2007, Tabashnik et al. 2008b), survival on *Bt* cotton leaves producing Cry1Ac and Cry2Ab was positively associated with the LC_{50} for Cry1Ac (Luttrell and Ali 2007). Relative to the susceptible UALab strain, two resistant *H. zea* strains derived from the field in 2006 had fivefold and sevenfold higher LC_{50} s for Cry1Ac, as well as fourfold and sixfold higher survival on cotton leaves producing Cry1Ac and Cry2Ab (Luttrell and Ali 2007). Furthermore, for strains started in 2002–2005 from larvae surviving on plants in the field, the mean LC_{50} (in micrograms Cry2Ab per milliliter diet) was more than double for strains from Bollgard II cotton (124) compared with strains from non-*Bt* crops (49.6) (*t*-test, *t* = 2.65, *df* = 32, *P* = 0.01) (Ali and Luttrell 2007).

In addition, field data from seven sites in North Carolina during 2000–2002 show that mean production of *H. zea* adults was 1,697/ha (SE = 682) on cotton producing Cry1Ac and Cry2Ab, which was 3.6% relative to non-*Bt* cotton (Jackson et al. 2004a). This 3.6% survival likely reflects survival of susceptible individuals, because no genes conferring substantial resistance to Cry1Ac or Cry2Ab were detected in screening of 1,252 families of *H. zea* from North Carolina in 2001 and 2002 (Jackson et al. 2006). Given that some susceptible individuals can complete development in the field on cotton with both toxins, and that resistance to Cry1Ac in diet tests was associated with increased survival on cotton leaves containing Cry1Ac and Cry2Ab (Luttrell and Ali 2007), we suspect that the observed increased survival of field-selected strains on diet treated with diagnostic concentrations of Cry1Ac and Cry2Ab is linked with increased survival in the field on cotton plants producing these toxins. However, we are not aware of published data that directly test this hypothesis.

Ambiguous Evidence. Five studies from China and India report ambiguous evidence about resistance of *H. armigera* to Cry1Ac in *Bt* cotton (Table 4). Results from Qiuxian County of Hebei Province in northern China show that the estimated Cry1Ac resistance allele frequency increased from 1999 to 2007 (Table 4). Results in 1999 and 2003–2005 were based on F2 screening that involved collecting wild gravid females, sib-mating of F₁ adults, and testing of second generation (F2) progeny on *Bt* cotton plants (He et al. 2001, Xu et al. 2009). The estimated resistance allele frequency is 0.0146 for 2003–2005 pooled (Xu et al. 2009), which is 2.5 times higher than the estimate of 0.0058 in 1999 (He et al. 2001). However, in the screening bioassays from 2003–2005, the survival rate on *Bt* cotton plants after 5 d was only 0.39% in the 15 of 278 families scored as carrying resistance alleles, which is considerably lower than the expected 6.25% (Xu et al. 2009). These results imply deviation from one or more of the assumptions of the one-locus model that was used to estimate the resistance allele frequency.

In 2006 and 2007 bioassays, F₁ and F₂ screening was done on *Bt* cotton leaves (Liu et al. 2008, 2009) rather than on *Bt* cotton plants as in previous years (He et al. 2001, Xu et al. 2009). Results from an F₁ screen in which field-caught *H. armigera* males were mated singly with females from a resistant lab-selected strain yielded Cry1Ac resistance allele frequency estimates of 0.094 for 2006 and 0.107 for 2007 (Liu et al. 2008). The estimate from F2 screen results in 2007 is 0.075 (Liu et al. 2009). As far as we know, these are the highest estimates of Cry1Ac resistance allele frequency for *H. armigera*. The mean estimated resistance allele frequency for 2006 and 2007 is 0.092, which is 16 times higher than the estimate for 1999. However, the change in screening methods from intact *Bt* cotton plants (1999 and 2003–2005) to *Bt* cotton leaves (2006 and 2007) cannot be excluded as a factor contributing to the increase in estimated resistance allele frequency. In addition, the data show no significant increase in resistance allele frequency from 2006 to 2007, which is not consistent with the rapid increase expected given the estimated resistance allele frequency of 0.094 in 2006, 100% adoption of *Bt* cotton in Qiuxian County since 2001, limited availability of non-*Bt* host plants other than cotton, and the finding that Cry1Ac cotton does not provide a high dose against *H. armigera* (Liu et al. 2008, 2009; Xu et al. 2009).

Concurrent with the eightfold increase in the estimated resistance allele frequency from 2003 to 2007, the density of *H. armigera* eggs on *Bt* cotton plants in Qiuxian County increased dramatically (Liu et al. 2009). However, the changes in these two parameters are not consistently correlated over shorter intervals. For example, from 2003 to 2005, the egg density in the fourth generation of *H. armigera* increased fivefold, but the estimated resistance allele frequency did not increase significantly (Liu et al. 2009). Conversely, from 2005 to 2007, the egg density in the fourth generation declined slightly, whereas the estimated resistance allele frequency increased sixfold. These results may reflect the fact that egg density on *Bt* cotton is affected by many factors other than susceptibility to Cry1Ac, such as weather (Liu et al. 2009), insecticides, and natural enemies.

Based on bioassays with Cry1Ac in diet, Liu et al. (2009) reported a resistance ratio of 11 for a strain of *H. armigera* derived from the field in Qiuxian County in 2007 compared with data for a susceptible lab strain they cited from a separate, unpublished study by Z. Xiaomei. This resistance ratio does not provide strong evidence of field-evolved resistance because the two strains were tested in different studies and one cannot judge if the lab strain was unusually susceptible to Cry1Ac.

Monitoring data summarized by Gujar et al. (2007) for *H. armigera* from India during 2000–2006 are difficult to interpret because the paper does not include data from a concurrently tested susceptible strain. Increases in LC_{50} of Cry1Ac occurred over time in each of the four regions of India studied, but without concurrent comparative data from a susceptible strain we cannot exclude the hypothesis that the increases in

LC₅₀ were caused partly or entirely by decreasing toxin potency over time (Tabashnik et al. 2008a).

Strong Evidence of Sustained Susceptibility. Strong evidence of no increase in the frequency of resistance to the *Bt* toxins in transgenic crops has been reported for all populations monitored of seven target pests (*Diatraea grandiosella*, *D. saccharalis*, *Helicoverpa punctigera*, *Heliothis virescens*, *Ostrinia nubilalis*, *Pectinophora gossypiella*, and *Sesamia nonagrioides*) as well as for populations of *H. armigera* and *H. zea* from some regions (Table 5).

In contrast with the results summarized above showing field-evolved resistance of *H. zea* to Cry1Ac, data from the same studies show sustained susceptibility of *H. virescens* to Cry1Ac (Table 5). For example, Ali et al. (2006) reported that for *H. virescens* in 2004, the maximum Cry1Ac resistance ratio was 3.2 and the maximum LC₅₀ value was 3.25 μ g Cry1Ac/ml diet. Using the same methods and sampling from the same region in the same year, their results for *H. zea* show a maximum Cry1Ac resistance ratio of 578 and a maximum LC₅₀ value of 1,746 μ g Cry1Ac/ml diet (Ali et al. 2006). Consistent with the data reported by Ali et al. (2006), Hardee et al. (2002) reported evidence showing decreased susceptibility to Cry1Ac in *H. zea*, but not in *H. virescens*.

In parallel with Ali et al. (2006) and our analysis above of the Cry2Ab data for *H. zea*, we used the Cry2Ab LC₅₀ of the UALab strain of *H. virescens* tested in the same year as the divisor to calculate resistance ratios for field-derived strains of *H. virescens* tested with Cry2Ab (Ali and Luttrell 2007). As with the UALab *H. zea* strain, the UALab *H. virescens* strain was the only lab strain of *H. virescens* tested in all 4 yr of the study (2002–2005) and it was not unusually susceptible (Ali and Luttrell 2007). Among the 26 field-derived strains of *H. virescens* tested, the maximum Cry2Ab resistance ratio was 8.5 and the maximum LC₅₀ value was 24.9 μ g Cry2Ab/ml diet (Ali and Luttrell 2007). Again, the same methods revealed a different result for *H. zea*: three strains from 2005 were so resistant that their LC₅₀ values could not be calculated accurately, but were estimated to be >400 μ g Cry2Ab/ml diet (Ali and Luttrell 2007), which yields resistance ratios >27 (Table 3).

Monitoring data of Siegfried et al. (2007) for *O. nubilalis* in the United States show no increase in the mean LC₅₀ of Cry1Ab for field populations sampled in 2005 compared with 1995 (Table 5). In addition, the ratio of the highest to lowest LC₅₀ among field-derived strains was between 3 and 5 in all years. Of >150 field-derived strains tested at a putative diagnostic concentration, all but one had <1% survival. The exceptional strain was derived in 2001 from Kandiyohi County, MN, and had 1.6% survival at the diagnostic concentration. After two rounds of laboratory selection, larvae from this strain showed no feeding or survival on corn plants producing Cry1Ab. Strains derived from the same area in subsequent years had <1% survival at the diagnostic concentration (Siegfried et al. 2007). However, Siegfried et al. (2007) collected insects only “some distance (>1.5 mile if

possible) from the nearest *Bt* cornfield,” which would have delayed detection of resistance if any insects were surviving on *Bt* corn.

Monitoring data of Li et al. (2007) and Gao et al. (2009) for *H. armigera* from two counties in China (Anci County, Hebei Province and Xiajin County, Shandong Province) show no significant change in the frequency of major resistance alleles from 2002 to 2008 (Table 5). Although Li et al. (2007) reported a significant increase in “tolerance” from 2002 to 2005 based on larval developmental rate on toxin-treated diet relative to untreated diet, these experiments did not include a concurrently tested susceptible strain. Accordingly, Li et al. (2007) state: “the possibility that the results were because of testing conditions being less stringent in each successive year cannot be ruled out.” Subsequent studies by Gao et al. (2009) using the same methods showed that tolerance decreased from 2006 to 2008 in both counties, with a statistically significant decline in Anci but not Xiajin. The combination of data on the frequency of major resistance alleles and tolerance provide strong evidence of continued susceptibility to Cry1Ac in Anci and Xiajin.

Correspondence Between Data and Theory

The data from resistance monitoring studies reviewed here generally confirm the main predictions from the population genetics theory underlying the refuge and pyramid strategies for managing pest resistance to *Bt* crops. Observed field outcomes are consistent with predictions that resistance evolves slower as (1) the inheritance of resistance is more recessive and (2) the abundance of non-*Bt* host plant refuges increases.

As summarized previously, the observed rapid evolution of resistance to Cry1Ac in *H. zea* is consistent with theoretical predictions (Tabashnik et al. 2008a). In particular, *H. zea* shows nonrecessive inheritance of resistance to Cry1Ac, which is expected to accelerate evolution of resistance. Before *Bt* cotton was commercialized, results showing substantial survival of *H. zea* on *Bt* cotton producing only Cry1Ac indicated that these plants did not meet the high dose criterion for *H. zea* (USEPA 1998a, 1998b; Gould 1998). Subsequent experiments with *H. zea* confirmed that the hybrid progeny produced by the matings between a laboratory-selected resistant strain and a susceptible strain were resistant to Cry1Ac, with almost completely dominant resistance ($h = 0.83$) (Burd et al. 2000). In contrast, recessive inheritance of resistance to Cry1Ac occurs in *H. virescens* and *P. gossypiella* (Gould et al. 1997, Liu et al. 1999), which have remained susceptible to the Cry1Ac in *Bt* cotton after more than a decade of field exposure (Tabashnik et al. 2005, 2006; Ali et al. 2006, Blanco et al. 2009). The comparison between *H. zea* and *H. virescens* is especially compelling because these two species are closely related taxonomically, they are both polyphagous pests that attack cotton, and they were sampled from the same region and tested side-by-side in some studies (Luttrell et al. 1999, Ali et al. 2006).

Results from monitoring *H. zea* resistance to Cry1Ac are also consistent with prediction (2) above that resistance evolves slower as the abundance of refuges increases. Guftafson et al. (2006) meticulously estimated the "effective refuge" percentage for *H. zea*, which takes into account moth production on various *Bt* and non-*Bt* host plants. During each of the three generations when *H. zea* feeds on cotton, the effective refuge was 82% in North Carolina versus 39% in Arkansas and Mississippi. Consistent with expectations, the monitoring data show that resistance was detected in Arkansas and Mississippi, but not in North Carolina (Tabashnik et al. 2008a). Furthermore, lower resistance to Cry1Ac in southwestern versus southeastern Arkansas was associated with the lower abundance of *Bt* crops in southwestern Arkansas (Allen and Luttrell 2008).

Results from grower surveys in South Africa suggest that the low abundance of refuges of non-*Bt* corn contributed to rapid evolution of *B. fusca* resistance to Cry1Ab in *Bt* corn (Kruger et al. 2009). On average, from 1998 to 2004, <30% of the farmers planting *Bt* corn in the Vaalharts area of South Africa complied with contracts requiring them to plant non-*Bt* corn refuges (Kruger et al. 2009). Although *Bt* corn accounted for only 34.9% of South Africa's corn in 2006 (James 2007), the adoption rate was much higher in some regions, including the Vaalharts area where >90% of farmers planted *Bt* corn (Kruger et al. 2009). Although data have not been reported on dominance of *B. fusca* resistance to Cry1Ab, precommercialization field data imply that the Cry1Ab corn in South Africa does not kill 99.99% of larvae (Van Rensburg 1999) and does not meet the USEPA (1998b) standard for a high dose. In field trials during 1996–1997, larval mortality on *Bt* corn was 99.2–99.6%, and mortality on *Bt* corn relative to non-*Bt* corn was 97–98% (Van Rensburg 1999). Therefore, available evidence suggests that nonrecessive inheritance of resistance as well as low refuge abundance might have hastened evolution of resistance by *B. fusca* to Cry1Ab in *Bt* corn.

As with *B. fusca*, data on dominance of *S. frugiperda* resistance to Cry1F have not been reported, but available evidence suggests that the high-dose criterion is not met. When registering Cry1F corn, the USEPA (2005) concluded that a high dose was achieved against *O. nubilalis*, but only "a high level of efficacy" was achieved against *S. frugiperda*. This conclusion was confirmed in field trials conducted in Georgia during 2006 and 2007 (Buntin 2008). Infestation by *S. frugiperda* of whorls of Cry1F corn ranged from 0 to 7.6% and the average *S. frugiperda* infestation of whorls of Cry1F corn relative to non-*Bt* corn was 9.6% (range, = 0–15.8%, Buntin 2008), implying average mortality of 90.4%. These results suggest that nonrecessive inheritance of Cry1F may have contributed to rapid resistance evolution by *S. frugiperda* in Puerto Rico. In addition, it has been suggested that the multiple yearly generations of *S. frugiperda* in Puerto Rico accelerated resistance evolution there (Matten et al. 2008).

Although some *H. zea* populations in the southeastern United States have evolved resistance to Cry1Ac and Cry2Ab, Australian populations of the closely related congener *H. armigera* have remained susceptible to both toxins (Table 5). This pattern is consistent with prediction (2), because refuge requirements for *Bt* cotton have been more stringent in Australia than in the United States. For cotton producing only Cry1Ac, the minimum refuge percentage for non-*Bt* cotton was 70% in Australia (Mahon et al. 2007a) versus 4–5% in the United States (USEPA 1998a, 2001). For two-toxin cotton, Australia has detailed requirements for 10% non-*Bt* cotton or the equivalent in terms of other non-*Bt* crops on each farm (Farrell 2008), while the USEPA (2007) has eliminated refuge requirements in much of the United States for two-toxin cotton with Cry1Ac and Cry2Ab. The field outcomes are also consistent with the prediction from population genetic models and small-scale experiments with *P. xylostella* that resistance to pyramids will evolve faster if two-toxin plants are grown concurrently with single-toxin plants (Zhao et al. 2005). In the southeastern United States, Cry1Ac cotton has been grown concurrently with two-toxin cotton since 2003 (Table 1). In contrast, Australian growers completely replaced Cry1Ac cotton with two-toxin cotton during the 2004–2005 growing season (Downes et al. 2009). In principle, faster evolution of resistance in *H. zea* than in *H. armigera* could also reflect higher initial resistance allele frequencies or more dominant inheritance of resistance in *H. zea*. The available data suggest initial resistance allele frequency was not substantially higher in *H. zea* than in *H. armigera* for Cry1Ac or Cry2Ab, and that resistance to both toxins may be more dominant in *H. zea* (Akhurst et al. 2003, Burd et al. 2003, Jackson et al. 2006, Mahon et al. 2007a, 2007b; and see supplementary Table 1 in Tabashnik et al. 2008a).

Conclusions and Implications

Bt cotton and corn have been remarkably successful since their commercial introduction more than 12 yr ago. Despite a few documented cases of field-evolved resistance to the *Bt* toxins in these transgenic crops, most pest populations remain susceptible. The evidence from field monitoring is generally consistent with the principles of resistance management underlying the refuge and pyramid strategies. To more rigorously test the correspondence between evidence and theory, scientists must thoroughly document and systematically analyze current and future examples of field-evolved resistance to transgenic crops, as well as cases in which pest susceptibility is sustained. For example, if additional data confirm trends in China, it would be useful to know why *H. armigera* resistance to Cry1Ac in *Bt* cotton is evolving faster in Qiuxian County than in neighboring areas. The results of such analyses can bolster the scientific basis for improving resistance management strategies.

We favor continued use of the long-standing definition of field-evolved resistance cited here (National

Research Council 1986, Tabashnik 1994) because it promotes early detection and proactive management of resistance. In contrast, definitions that incorporate criteria about field control problems are likely to delay recognition of resistance and postpone management actions that can limit the negative consequences of resistance. Rather than debating the definition of resistance, we think it will be more productive to focus discussions on which regulatory actions, if any, are appropriate in response to specific data on the magnitude, distribution, and impact of field-evolved resistance.

As use of transgenic insecticidal crops increases, resistance management will be increasingly important. Expanded use of transgenic crops for insect control will likely include more varieties with combinations of two or more *Bt* toxins, novel *Bt* toxins such as vegetative insecticidal proteins (Table 1), and modified *Bt* toxins that have been genetically engineered to kill insects resistant to standard *Bt* toxins (Soberón et al. 2007). Transgenic plants that control insects via RNA interference are also under development (Baum et al. 2007, Mao et al. 2007). Increasing use of transgenic crops in developing nations is likely, with a broadening range of genetically modified crops and target insect pests (Showalter et al. 2009). Incorporating enhanced understanding of observed patterns of field-evolved resistance into future resistance management strategies can help to minimize the drawbacks and maximize the benefits of current and future generations of transgenic crops.

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