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Insect resistance to Bt crops: lessons from the first billion acres

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Evolution of resistance in pests can reduce the effectiveness of insecticidal proteins from *Bacillus thuringiensis* (Bt) produced by transgenic crops. We analyzed results of 77 studies from five continents reporting field monitoring data for resistance to Bt crops, empirical evaluation of factors affecting resistance or both. Although most pest populations remained susceptible, reduced efficacy of Bt crops caused by field-evolved resistance has been reported now for some populations of 5 of 13 major pest species examined, compared with resistant populations of only one pest species in 2005. Field outcomes support theoretical predictions that factors delaying resistance include recessive inheritance of resistance, low initial frequency of resistance alleles, abundant refuges of non-Bt host plants and two-toxin Bt crops deployed separately from one-toxin Bt crops. The results imply that proactive evaluation of the inheritance and initial frequency of resistance are useful for predicting the risk of resistance and improving strategies to sustain the effectiveness of Bt crops.

Transgenic crops are one of the most widespread and controversial applications of biotechnology[1–4]. To reduce reliance on insecticide sprays, scientists have genetically engineered corn and cotton plants to make insecticidal proteins encoded by genes from the common bacterium *Bacillus thuringiensis* (Bt)[5]. These Bt proteins kill some devastating insect pests, but cause little or no harm to most other organisms, including people[4,5]. Benefits of Bt crops include reduced insecticide use, pest suppression, conservation of beneficial natural enemies, increased yield and higher farmer profits[6–12]. The area planted with Bt crops worldwide increased from 1.1 million hectares in 1996 to 66 million hectares in 2011, with a cumulative total of more than 420 million hectares (>1 billion acres) (**Fig. 1**). Bt corn accounted for 67% of corn planted in the United States during 2012 (http://www.ers.usda.gov/Data/BiotechCrops/) and Bt cotton accounted for 79–95% of cotton planted in Australia, China, India and the United States during 2010 to 2012 (**Fig. 2**).

The remarkable ability of insects to adapt to insecticides and other control tactics supports the conclusion that evolution of resistance by pests is the main threat to the continued success of Bt crops[13–23]. Many previous reviews have addressed pest resistance to Bt crops[13–23], including a 2011 mini-review emphasizing four successful cases of the high-dose/refuge resistance management strategy in North America[22] and our 2009 review of 17 cases involving 11 species of lepidopteran pests and four Bt toxins (B.E.T., Van Renburg, J.B.J. & Y.C.)[21]. Several papers have compared field outcomes for resistance to Bt crops with predictions from theory, but the rigor of these previous comparisons has been limited by small sample sizes for both the field outcomes and the factors predicted to affect resistance[19–22].

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Here we summarize the theory for managing pest resistance to Bt crops, outline new criteria for categorizing evidence of field-evolved resistance, review the global status of resistance to Bt crops based on current field monitoring data, and test the correspondence between theoretical predictions and observed patterns of field-evolved resistance. The criteria for categorizing field-evolved resistance described and applied here explicitly acknowledge that resistance is not 'all or none', which facilitates objective classification of monitoring data and may help to gauge management actions appropriately, depending on the severity of resistance. Compared with previous reviews on this topic, the field monitoring data analyzed here are more recent and represent more cases (24 in all), as well as larger and more diverse sets of Bt toxins (six toxins from four Cry families) and pest species (13 species from two insect orders). Using data from 77 studies published as of 2012, we report the first statistical analyses of the association between observed global patterns of field-evolved resistance and predicted effects of two key biological parameters: dominance of resistance and initial resistance allele frequency. The results provide insights that can be used proactively to improve resistance management.

Theory for managing pest resistance to Bt crops

The refuge strategy has been the primary approach used worldwide to delay pest resistance to Bt crops and has been mandated in the United States, Australia and elsewhere[8,16,23]. Despite implementation of some resistance management practices for conventional insecticides, the mandates for the refuge strategy are part of an unprecedented proactive effort to slow resistance to Bt crops that recognizes both their value and the strong threat of resistance. The concept underlying the refuge strategy is that most of the rare resistant pests surviving on Bt crops will mate with the relatively abundant susceptible pests from nearby refuges of host plants without Bt toxins[24–27]. If inheritance of resistance is recessive, the progeny from such matings will die on Bt crops, substantially delaying the evolution of resistance. This approach is sometimes called the 'high-dose refuge strategy' because it works best if the dose of toxin for insects

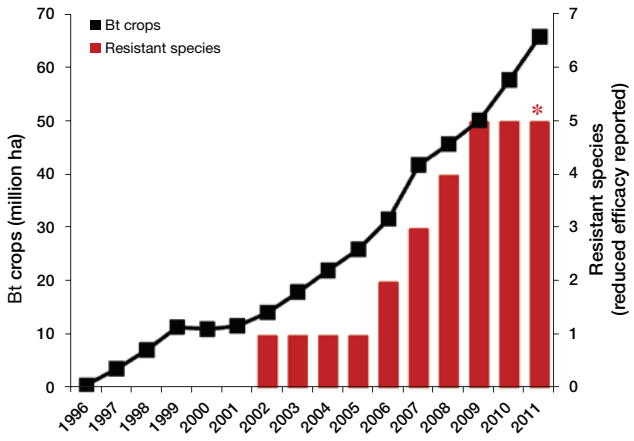

Figure 1 Planting of Bt crops globally and field-evolved resistance. Planting of Bt crops globally each year and cumulative number of insect species with field-evolved resistance and reduced efficacy reported. Planting of Bt crops increased from 1.1 million hectares (ha) in 1996 to 66 million ha in 2011 (ref. 2). Field-evolved resistance associated with reduced efficacy of Bt crops has been reported for five major target pests (year first detected): *H. zea* (2002), *S. frugiperda* (2006), *B. fusca* (2007), *P. gossypiella* (2008) and *D. v. virgifera* (2009) (**Tables 1** and **2**). *, For 2011, the number of species with resistant populations may be underestimated because reports of field-evolved resistance typically are published 2 or more years after resistance is first detected.

eating Bt plants is high enough to kill all (or almost all) of the offspring from matings between resistant and susceptible insects[16] (B.E.T., Y.C. *et al.*)[15,27]. Therefore, in theory, three key factors favor success of the refuge strategy: first, recessive inheritance of resistance; second, low resistance allele frequency; and third, abundant refuges of non-Bt host plants near Bt crops[16] (B.E.T., Y.C. *et al.*)[15,21].

Two additional factors predicted to delay resistance are fitness costs and incomplete resistance[16] (B.E.T., Y.C. *et al.*)[19,28,29]. Fitness costs occur when fitness on non-Bt host plants is lower for resistant insects than susceptible insects, so that refuges select against resistance[28,29]. 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[19,28].

The dominance of resistance on a Bt crop plant can be measured in terms of the parameter *h*, which varies from 0 for completely recessive to 1 for completely dominant[16] (Liu, Y.B. & B.E.T.)[30]. When such direct data are not available, dominance can be assessed indirectly by measuring survival of susceptible insects on Bt plants[31]. This indirect assessment relies on the idea that if Bt plants do not kill all or nearly all homozygous susceptible insects, they probably will not kill nearly all individuals heterozygous for resistance. If so, survival is likely to be higher for the heterozygotes than for the homozygous susceptible insects, which yields nonrecessive inheritance of resistance that accelerates adaptation[16,27]. Thus, the US Environmental Protection Agency guidelines specify that high-dose Bt plants should kill at least 99.99% of susceptible insects in the field[31].

Mathematical modeling consistently predicts that resistance will evolve more slowly if the initial resistance allele frequency is low[15,24,25,32,33]. Although several papers[22,34–38] propose (without theoretical or empirical evidence) that the success of the refuge strategy requires an initial resistance allele frequency ≤0.001, modeling results imply that the refuge strategy can be useful with much higher resistance allele frequencies, particularly if fitness costs are associated with resistance[24,28,33,39,40]. For example, with recessive resistance and fitness costs, refuges delayed resistance substantially in a model with an initial resistance allele frequency of 0.3 (Y.C. & B.E.T.)[28].

Refuge abundance can be measured for each pest in terms of the percentage of its host plants that are non-Bt plants. If more than one species of non-Bt host plant is available, the 'effective' refuge percentage can be estimated by adjusting for the relative abundance of susceptible pests produced on different host plant species[41–45]. The effective refuge percentage can also be adjusted downward for the effects of treating refuges with insecticides, because such treatments reduce the ability of refuges to delay resistance[41]. Because pest movement and mating patterns interact with the distribution and abundance of refuges and Bt crop fields to affect evolution of resistance, spatially explicit approaches are useful for assessing refuge effectiveness[46].

Modeling results suggest that when inheritance of resistance is not recessive, increasing refuge abundance can still substantially delay resistance. For example, 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 years with ≥5% refuges if resistance is completely recessive ($h = 0$) and with >50% refuges if resistance is partially dominant ($h \geq 0.4$) (ref. 20).

First-generation Bt crops each produce a single Bt toxin, but many second-generation Bt crops, named pyramids, produce two or more

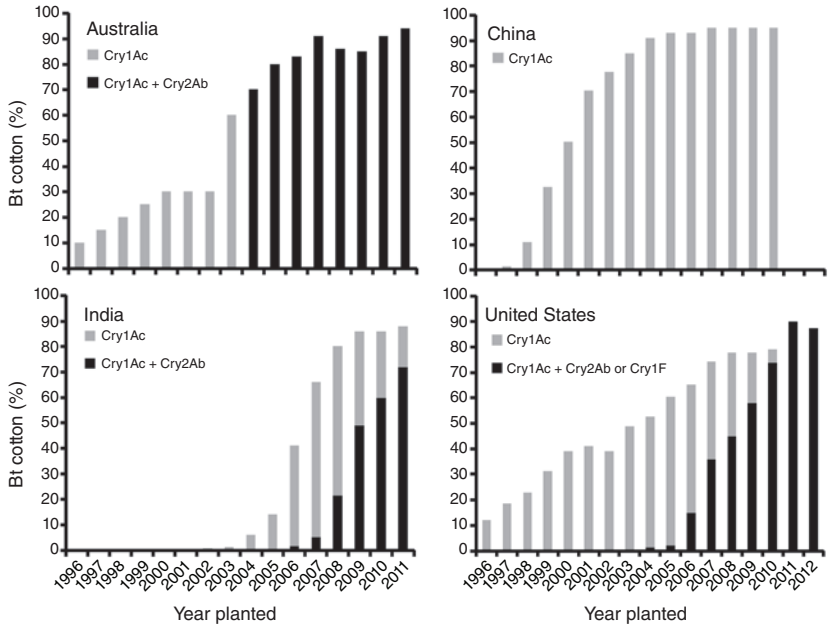

Figure 2 Percentage of cotton hectares planted with Bt cotton producing one toxin (gray) or two toxins (black) in four countries. All Bt cotton produced Cry1Ac. In Australia and India, all two-toxin cotton produced Cry1Ac and Cry2Ab. In the United States from 2004 to 2012, 86% of two-toxin cotton produced Cry1Ac and Cry2Ab and 14% produced Cry1Ac and Cry1F. The ranking of each country in terms of 2012 cotton production (percentage of world production) was 1 for China (27%), 2 for India (22%), 3 for the United States (15%) and 7 for Australia (3.4%) (see **Supplementary Methods** for details).

Box 1 An alternative definition of resistance

The Insecticide Resistance Action Committee (IRAC), composed of members from more than a dozen major agrochemical and biotech companies, aims to promote resistance management strategies for insecticides and Bt crops to support sustainable agriculture and improve public health (http://www.irac-online.org/about/irac/). IRAC defines resistance as "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species" (http://www.irac-online.org/about/resistance/). The first part of the IRAC definition, "a heritable change in the sensitivity of a pest population" and the definition of field-evolved resistance (see main text) both emphasize a genetically based decrease in susceptibility. The remainder of the IRAC definition sets additional conditions that are problematic for identifying resistance objectively and for proactive detection and responses to resistance. First, by the time a product has failed repeatedly, it is usually too late to respond most effectively to resistance. Second, the "expected level of control" is not specified, which allows variation in interpretation, including changes over time in expectations. Third, because the definition depends on the label recommendation, resistance cannot occur in any species that is not on the label, which excludes evolution of resistance in non-target pests and non-pest species[96,104]. By contrast, the term "field-evolved resistance" as defined here explicitly recognizes that resistance results from evolution, enables objective identification of resistance, facilitates proactive detection and management of resistance, and applies to resistance in pest and beneficial organisms. Various other definitions of resistance have been proposed and discussed in depth elsewhere[105,106].

distinct Bt toxins that are active against the same pest[21,47]. The assumption underlying this approach (which is not always true) is that selection for resistance to one toxin does not cause cross-resistance to the other toxins in the pyramid, so that insects resistant to all toxins in the pyramid are extremely rare[47,48]. Other factors favoring success of pyramids match 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[21,39,47,48]. Modeling results and small-scale experiments with noncommercial Bt broccoli plants indicate that resistance to pyramids evolves faster if one-toxin plants are grown concurrently with two-toxin plants[47]. This occurs because the one-toxin plants select for resistance to each toxin separately, which reduces the advantage of the two-toxin plants[47].

Field-evolved resistance: criteria and categories

Field-evolved (or field-selected) resistance is a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field[14,21,49]. A *Web of Science* search with 'topic = field-evolved resistance' identified 54 publications, starting with two 1996 papers about resistance to Bt toxins produced by two independent research teams[50,51] and including 31 papers published from 2010 to 2012. These 54 publications were authored by >150 academic, government and industry scientists from five continents and have been cited >900 times, including 300 citations in 2012. Despite this widespread and increasing use of the term 'field-evolved resistance', some scientists favor alternative terms and definitions for resistance (**Box 1**).

Natural genetic variation affecting responses to Bt toxins usually occurs in insect populations, with some alleles conferring susceptibility and others conferring resistance. Before an insect population is exposed to a Bt toxin, alleles conferring resistance are typically rare[16,19]. Field-evolved resistance occurs when exposure of a field population to a toxin increases the frequency of alleles conferring resistance in subsequent generations[21]. Thus, detecting resistance alleles without demonstrating that their frequency has increased in field populations does not constitute evidence of field-evolved resistance[21].

The primary goal of monitoring resistance to Bt crops is to detect field-evolved resistance early enough to enable proactive management before field failures occur[21]. Resistance monitoring includes sampling and testing of insects that survive on Bt crops as well as insects from other sources, including non-Bt host plants. Failure to sample insects from Bt crops favors underestimation of the frequency of resistance, which can postpone detection of resistance[21].

Pest control problems associated with field-evolved resistance vary from none to severe, depending on the frequency of resistant individuals, the extent to which resistance increases survival in the field, the geographical distribution of resistant populations, the insect's population density and the availability of alternative controls[21]. We define four categories of field-evolved resistance: 1) >50% resistant individuals and reduced efficacy of the Bt crop in the field has been reported; 2) >50% resistant individuals and reduced efficacy is expected, but has not been reported; 3) 1–6% resistant individuals; and 4) <1% resistant individuals. For categories 3 and 4, the percentage of resistant individuals is low enough that reduced efficacy of the Bt crop in the field is not expected. We adopt terms used previously and refer to cases in category 3 as an 'early warning' of resistance[52] and cases in category 4 as 'incipient resistance'[53]. In principle, an additional category could be 6–50% resistant individuals, but none of the cases reviewed here was in that range. The fifth category is cases in which monitoring data show no statistically significant decrease in susceptibility.

Each case reviewed here involves evaluation of field-evolved resistance to one Bt toxin in populations of one pest species from one country. Although initial detection of resistance can be based on data from a single field population, all of the cases of field-evolved resistance reviewed here entail evidence of genetically based, decreased susceptibility from several field populations. To classify each case of field-evolved resistance into one of the four categories we define above, we estimated the percentage of individuals resistant to a toxin based on survival on a diet treated with a 'diagnostic concentration' of the toxin that kills all or nearly all susceptible individuals, or survival on intact Bt plants or parts of Bt plants containing that toxin. For many cases, we also report the resistance ratio, which is the concentration of toxin killing 50% of insects tested (LC_{50}) for a field-derived strain divided by the LC_{50} for a conspecific susceptible strain. Large increases in LC_{50} in field-selected populations yield resistance ratios >10 and indicate that >50% of the population is resistant[21].

Resistance monitoring data

Here we review 24 cases for which resistance monitoring data are published in peer-reviewed journals for 13 species of major lepidopteran and coleopteran pests that are targeted by six Bt toxins in transgenic corn and cotton in eight countries (**Tables 1** and **2** and **Supplementary Tables 1–4**).

Reduced efficacy reported or expected. The cumulative number of major pest species with field-evolved resistance to Bt toxins in crops

Table 1 Evaluation of field-evolved resistance in 24 cases involving 13 species of major pests targeted by Bt crops[a]

Pest[a]	Bt crop	Toxin	Country	Years[b]	High dose[c]	Low initial freq.[d]
>50% resistant individuals and reduced efficacy reported						
B. fusca	Corn	Cry1Ab	South Africa	8	No	?[e]
D. v. virgifera	Corn	Cry3Bb	USA	7	No	No
H. zea	Cotton	Cry1Ac	USA	6	No	No
P. gossypiella	Cotton	Cry1Ac	India	6[f]	No	?
S. frugiperda	Corn	Cry1F	USA	3	No	?
>50% resistant individuals and reduced efficacy expected						
H. zea	Cotton	Cry2Ab	USA	2[g]	No	No
1–6% resistant individuals						
D. saccharalis	Corn	Cry1Ab	USA	10	No	No
H. armigera	Cotton	Cry1Ac	China	13	No	No
O. furnacalis	Corn	Cry1Ab	The Philippines	5	No	?
P. gossypiella	Cotton	Cry1Ac	China	13	No	?
<1% resistant individuals						
H. armigera	Cotton	Cry1Ac	Australia	15	No	Yes
H. armigera	Cotton	Cry2Ab	Australia	8	Yes	No
H. punctigera	Cotton	Cry2Ab	Australia	8	Yes	No
No decrease in susceptibility						
D. grandiosella	Corn	Cry1Ab	USA	6	?	Yes
D. v. virgifera	Corn	Cry34/35Ab	USA	4	No	No
H. punctigera	Cotton	Cry1Ac	Australia	10	?	Yes
H. virescens	Cotton	Cry1Ac	USA	11	Yes	No
H. virescens	Cotton	Cry1Ac	Mexico	11	?	?
H. virescens	Cotton	Cry2Ab	USAfa	2	Yes	?
O. nubilalis	Corn	Cry1Ab	USA	15	No	Yes
O. nubilalis	Corn	Cry1Ab	Spain	4	?	?
P. gossypiella	Cotton	Cry1Ac	USA	13	Yes	No
P. gossypiella	Cotton	Cry2Ab	USA	5	Yes	Yes
S. nonagroides	Corn	Cry1Ab	Spain	7	?	Yes

[a]See **Table 2** and **Supplementary Tables 1–6** for details. *D. v. virgifera* is a coleopteran; the other 12 pests are lepidopterans. [b]Years elapsed between the first year of commercialization in the region studied and: (i) for the six cases with >50% resistant individuals and reduced efficacy reported or expected (red and orange), the first year of field sampling that yielded evidence of resistance, or (ii) for all other cases, the most recent year of monitoring data reviewed here (see **Table 2** and **Supplementary Tables 1–4** for details). [c]Based on direct evaluation of recessive inheritance of resistance for 12 cases with relevant data and on survival of susceptible individuals on Bt plants for 7 cases without such direct data (**Supplementary Table 5**). [d]Based on an initial resistance allele frequency below the detection threshold; yes indicates initial screening did not detect any major resistance alleles (**Supplementary Table 6**). [e]"?" indicates answer could not be determined with available data. [f]Excludes years when Bt cotton was grown illegally in India before it was commercialized in 2002 (refs. 81,91,92). Resistance was first detected in samples collected in 2008, 6 years after commercialization. If illegal planting started in 2000, the total years elapsed would be 8. [g]May reflect some cross-resistance caused by selection with Cry1Ac[21,93,94].

and reduced transgenic crop efficacy increased from one in 2005 to five in 2010 (**Figs. 1**, **3** and **4**). These five cases include resistance to Bt corn in three pests (*Busseola fusca*, *Diabrotica virgifera virgifera* and *Spodoptera frugiperda*) and resistance to Bt cotton in two pests (*Helicoverpa zea* and *Pectinophora gossypiella*; **Tables 1** and **2** and **Boxes 2** and **3**). In each of these five cases, field-evolved resistance was detected fewer than 10 years after the Bt crop was commercialized. In a sixth case of field-evolved resistance of *H. zea* to Cry2Ab in Bt cotton, the percentage of resistant individuals exceeded 50% for several populations and reduced efficacy is expected, but has not yet been reported (**Box 4**).

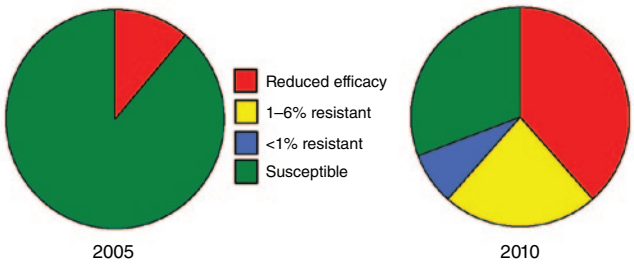

Figure 3 Resistance of major pest species to Bt crops in 2005 and 2010. For each pest species, the color indicates the status of the most resistant population. In 2005, the only pest with resistant field populations was *H. zea*; the other eight pests evaluated were susceptible. Data for 2005 (n = 9 species) are from reference 21. Data for 2010 (n = 13 species) are from **Table 1**.

Early warning: 1–6% resistant individuals. In four cases, the percentage of resistant individuals increased significantly to reach 1–6%, which is not expected to reduce efficacy in the field (**Table 1** and **Supplementary Table 2**). For field populations exposed to Cry1Ac cotton in China for 13 years, maximum survival at a diagnostic concentration of Cry1Ac was 2.6% for *Helicoverpa armigera*[52] and 5.6% for *P. gossypiella*[54], with 0% survival for susceptible control populations. For *Ostrinia furnicalis* exposed to Cry1Ab corn in the Philippines, the maximum survival at a diagnostic concentration of Cry1Ab increased 14-fold from 0.4% in 2007 to 5.5% in 2009 (ref. 55). For *Diatraea saccharalis* in Louisiana, data from F_2 screens show the frequency of alleles conferring resistance to Cry1Ab corn increased eightfold from 0.0023 in 2004 to 0.018 in 2009 (ref. 56). We estimate that the percentage of resistant individuals in the populations sampled in Louisiana in 2009 was 1.0–2.4%, based on the partial dominance of resistance (**Supplementary Table 5**) and an estimated frequency of heterozygous individuals of 0.031 (**Supplementary Table 2**).

Table 2 Bioassay data indicating field-evolved resistance to the toxins in Bt crops for five pests with >50% resistant individuals and reduced efficacy reported[a]

				Bioassay data						
Pest	Cry toxin	Country	Year comm.[b]	Strains tested[c]	Initial year[d]	Final year[e]	Parameter	Control value[e]	Test value[f]	References
B. fusca	1Ab	S. Africa	1998	2	2006	2006	Max. surv.[g]	0.0%	64%	21,95
B. fusca	1Ab	S. Africa	1998	8	2007	2007	Max. surv.[h]	0.0%	88%	96
H. zea	1Ac	USA	1996	2	2002	2002	Max. surv.[i]	0.0%	52%	97,98
H. zea	1Ac	USA	1996	64	1992	2004	Max. RR[j]	1.2	580	59,60
H. zea	1Ac	USA	1996	197	2002	2006	Max. RR[j]	40[k]	>1,000	59,97,99
P. gossypiella	1Ac	India	2002[l]	6	2007	2009	Survival[m]	2.0%	72%	100
P. gossypiella	1Ac	India	2002[l]	2	2007	2009	Max. RR[j]	1.0	47	100
S. frugiperda	1F	USA	2003	8	1990	2008	Max. RR[n]	1.0	>356	75
S. frugiperda	1F	USA	2003	13	2010	2011	Max. RR[o]	1.0	>970	76
S. frugiperda	1F	USA	2003	13	2010	2011	Survival[p]	0.8%	90%	76
D. v. virgifera	3Bb	USA	2003	9	2009	2009	Survival[q]	17%[r]	52%[s]	101
D. v. virgifera	3Bb	USA	2003	13	2010	2010	Survival[q]	5%[t]	74%[u]	102

[a]Cry1Ab, Cry1F and Cry3Bb produced by Bt corn; Cry1Ac produced by Bt cotton. [b]First year Bt crop was grown commercially in the location monitored. [c]Total number of field-derived strains tested in bioassays. [d]Initial and final years during which field populations were sampled. [e]Value for parameter from one or more susceptible strains, based on initial year unless noted otherwise. [f]Value for parameter from one or more field-selected resistant strains, based on final year unless noted otherwise. [g]Maximum survival in the field at 18 days on Bt corn plants relative to non-Bt corn plants for a susceptible field population (control value) and a resistant field population (test value). [h]Survival in the greenhouse at 35 days on Bt corn plants relative to non-Bt corn plants for the most susceptible field population (control value) and the most resistant field population (test value). [i]Survival at 4 days on Bt cotton leaves relative to non-Bt cotton leaves for a susceptible lab strain (control value) and the most resistant field population (test value); in addition, four strains derived from the field in 2004 had >50% survival at a diagnostic concentration of Cry1Ac in diet[59]. [j]Maximum resistance ratio, the highest LC_{50} for a field-derived strain divided by the LC_{50} for one or more susceptible strains. [k]The maximum resistance ratio of 40 in 2002 reflects field-evolved resistance that was detected in that year. [l]Bt cotton was grown illegally in Gujarat, India, for at least 2 years before it was commercialized[81,91,92]. [m]Mean survival at a concentration of 1 microgram Cry1Ac per ml diet in lab bioassays; resistance detected in a population from Amreli, Gujarat, sampled in 2008. [n]Field-selected resistant strains were derived from four populations sampled in Puerto Rico during 2007 to 2008 (test value); the control value is based on a strain derived from a field population sampled from Georgia in 2005. The most resistant field populations had <50% mortality and growth inhibition at the highest concentration tested, yielding a maximum resistance ratio >35 based on LC_{50} values and >356 based on the concentration causing 50% growth inhibition (IC_{50}). [o]Field-selected resistant strains were derived from four populations sampled in Puerto Rico during 2010 and 2011 (test value); the control value is the mean for nine strains derived from US mainland populations sampled during 2010 and 2011. [p]Mean survival at 7 days on Bt corn leaves relative to non-Bt corn leaves for the strains derived in 2010 and 2011 from Puerto Rico (test value) and the US mainland (control value). [q]Mean survival on Cry3Bb corn plants relative to non-Bt corn plants in lab bioassays. [r]Mean for five strains derived in 2010 from "control" fields in Iowa where severe corn rootworm damage was not seen. [s]Mean for four strains derived in 2009 from four "problem" fields in Iowa where growers reported severe corn rootworm injury to Bt corn fields planted with Cry3Bb corn (3 fields) or a combination of Cry3Bb corn and Cry34/35Ab corn (1 field). [t]Mean for six control strains derived before Cry3Bb corn was commercialized (1995–2001) from fields in four states. [u]Mean for seven strains derived in 2010 from problem fields in Iowa.

Incipient resistance: <1% resistant individuals. For three cases that entail *H. armigera* and *Helicoverpa punctigera* exposed to Bt cotton in Australia, F_1 and F_2 screens detected statistically significant increases over time in the frequency of alleles conferring resistance to Cry1Ac or Cry2Ab, yet the highest estimated percentage of resistant individuals was <1% (**Table 1** and **Supplementary Table 3**). Because of the low percentage of resistant individuals after many years of extensive exposure to Bt cotton, these three cases exemplify successful resistance management.

Among these three cases, the maximum resistance allele frequency detected is 0.048, based on results of the F_1 screen for *H. punctigera* resistance to Cry2Ab in Australia in 2008–2009 (refs. 53,57,58). Results from F_1 screens conducted in 2008–2009 also showed that the frequency of alleles conferring resistance to Cry2Ab was eight times higher in areas where Bt cotton was grown relative to non-cropping areas ($P < 0.0001$, ref. 53). Based on this difference between areas in the same season and a significantly increased frequency of resistance to Cry2Ab over time in Bt cotton growing areas, Downes *et al.*[53] termed this "incipient resistance." Based on recessive inheritance and Hardy-Weinberg equilibrium, they estimated 0.2% (0.048^2) of *H. punctigera* larvae were resistant to Cry2Ab in 2008–2009, which is too low to reduce the efficacy of Bt cotton in the field. Moreover, the frequency of resistance to Cry2Ab did not increase from 2008–2009 to 2010–2011 (ref. 57). These results show that the statistically significant yet small rises in resistance allele frequency characteristic of incipient resistance do not necessarily indicate that further increases in resistance are imminent.

No decrease in susceptibility. Monitoring data provide strong evidence of no decrease in susceptibility to toxins produced by Bt crops in 11 cases (**Table 1** and **Supplementary Table 4**). These cases include evidence of no decreased susceptibility to each Bt toxin tested against all populations examined for four species: *Diatraea grandiosella*, *Heliothis virescens*, *Ostrinia nubilalis* and *Sesamia nonagrioides*. In addition, the

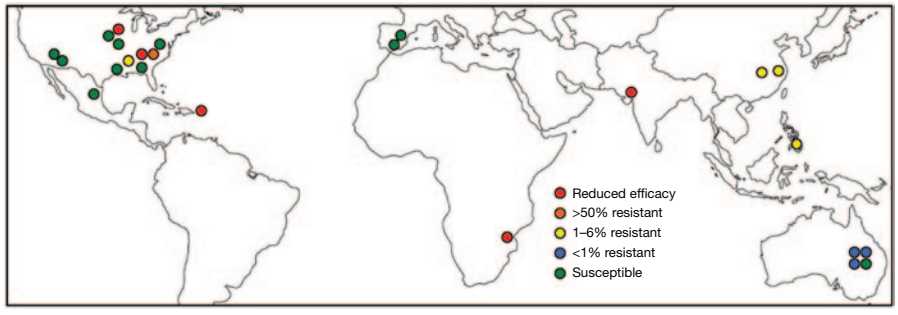

Figure 4 Global status of field-evolved resistance to Bt crops. Each circle represents 1 of 24 cases involving evaluation of field-evolved resistance to one toxin in Bt corn or Bt cotton in populations of one pest species from one country (**Tables 1** and **2** and **Supplementary Tables 1–4**).

Box 2 Field-evolved resistance to Bt corn with reduced efficacy reported

Field-evolved resistance of *S. frugiperda* (fall armyworm) to Bt corn producing Cry1F occurred in 3 years in the United States territory of Puerto Rico[75,76] (**Tables 1** and **2**). This is the fastest documented case of field-evolved resistance to a Bt crop with reduced efficacy reported and is consistent with worst-case scenarios envisioned in 1997 by some experts[32,68]. It is also the first case of resistance leading to withdrawal of a Bt crop from the marketplace. High levels of resistance persisted in Puerto Rico in 2011, 4 years after Cry1F corn had been voluntarily withdrawn from sales[76].

Field-evolved resistance to Bt corn producing Cry1Ab occurred in *B. fusca* (maize stem borer) in South Africa in 8 years[21,101] (**Tables 1** and **2**) and has some striking parallels with *S. frugiperda* resistance to Cry1F corn. In both cases, proactive resistance monitoring was not conducted and anecdotal evidence of reduced efficacy in the field preceded documentation of resistance with bioassays[73–76,101,102,107].

Bt corn producing Cry3Bb to kill beetles, particularly *D. v. virgifera* (western corn rootworm), was first registered in the United States in 2003 (ref. 108). By 2009, farmers planted Cry3Bb corn on 13 million ha, which was 36% of all corn in the United States[100,109]. Field and laboratory data show that control problems in the field during 2009 and 2010 were associated with resistance to Cry3Bb in some Iowa populations of *D. v. virgifera*[110–112]. In 'problem' fields, which had severe damage to Cry3Bb corn caused by rootworms, Cry3Bb corn had been planted for 3–7 years[110,111]. A 2011 field study of two of the problem fields identified in 2009 found that *D. v. virgifera* emergence did not differ significantly between Cry3Bb corn and non-Bt corn[112].

data show no decreased susceptibility of *D. v. virgifera* to Cry34/35Ab and of *P. gossypiella* to Cry1Ac and Cry2Ab in Arizona. Three cases in the United States show no decrease in susceptibility after ≥10 years of exposure to a toxin produced by a Bt crop: *O. nubilalis* to Cry1Ab in Bt corn, and *H. virescens* and *P. gossypiella* to Cry1Ac in Bt cotton. Overall, 5 of the 24 cases show no decrease in susceptibility after ≥10 years of exposure to the *Bt* crop; and 14 of the 24 cases (58%) show either no decrease in susceptibility (11 cases) or <1% resistant individuals (3 cases) after 2 to 15 years (mean = 9 years) (**Table 1**).

Testing theory with data

The data from resistance monitoring studies reviewed here generally confirm the main predictions from the evolutionary theory underlying the refuge and pyramid strategies for managing pest resistance to Bt crops. As detailed below, resistance was less likely to evolve rapidly if the high-dose standard was met (indicating recessive inheritance of resistance), the initial resistance allele frequency was low, refuges were abundant and Bt plants with two-toxin pyramids were grown separately from one-toxin Bt plants.

Box 3 Field-evolved resistance to Bt cotton with reduced efficacy reported

Both cases of field-evolved resistance to Bt cotton with reduced efficacy reported (**Tables 1** and **2**) have been controversial.

In India, Bt cotton hybrids generated by crossing a Bt cotton cultivar with local non-Bt cotton cultivars were commercialized in 2002, but illegal planting of Bt cotton hybrids began sooner in the western state of Gujarat[91,92]. Resistance of *P. gossypiella* (pink bollworm) to Bt cotton producing Cry1Ac (Bollgard) was first detected with laboratory bioassays of the offspring of insects collected from the field in 2008 in Gujarat[97] (**Table 2**). Monsanto (St. Louis) reported that their 2009 field monitoring confirmed *P. gossypiella* resistance to Cry1Ac in four districts of Gujarat[113]. This resistance seen in laboratory bioassays was associated with unusually high abundance of larvae on Cry1Ac cotton and moths caught in pheromone traps[98,113,114].

A prominent Indian entomologist challenged the conclusion of field-evolved resistance, claiming that resistance monitoring should be based only on insects collected from non-Bt cotton, yet Monsanto had collected larvae from Bt cotton plants[98,114]. Monsanto aptly countered this criticism by stating that their resistance monitoring based on insects collected from Bt cotton in India is "standard practice"[98]. Indeed, testing insects collected from Bt plants is an essential component of resistance monitoring[21,75,101,107,110–112,115].

Ironically, some of the dubious arguments disputing Monsanto's report of *P. gossypiella* resistance to Cry1Ac cotton in India mirror those offered by Monsanto and others[99] to challenge documentation of *H. zea* (bollworm) resistance to Cry1Ac cotton in the United States[116]. Researchers discovered the initial evidence of field-evolved resistance of *H. zea* to Cry1Ac cotton in the southeastern United States in 2002, 6 years after its commercialization in that region[59,117,118]. The extensive evidence confirming this case of resistance includes >50% survival at a diagnostic concentration for four strains derived from the field in 2003 (refs. 59,115).

One of the primary arguments disputing the conclusion of field-evolved resistance in this case was that "larval samples should not be collected from Bt crops" for resistance monitoring[99]. As noted above, testing insects sampled from Bt crops is critical for monitoring resistance. Moreover, the evidence in this case documents resistance in samples from non-Bt crops as well as from Bt crops, including a strain derived from non-Bt cotton in 2004 that had a resistance ratio >500 (refs. 59,115,119). Another challenge was that the evidence of field-evolved resistance came entirely from the laboratory[99]. However, "unacceptable levels of boll damage" observed in problem fields were associated with decreased susceptibility to Cry1Ac in laboratory bioassays[115,117], similar to the evidence from India[97,98,113].

In 2012, Luttrell and Jackson[118] asserted that selection of *H. zea* resistance to Cry1Ac in the laboratory before Bt cotton was commercialized "argues against conclusions of field-evolved resistance." Yet, the selection experiment they cite[60] demonstrates that resistance alleles were present, but not common, before Bt cotton was commercialized. This is reflected in the low LC_{50} of Cry1Ac before laboratory selection for the strain derived from the field in 1992, and the >100-fold increase in LC_{50} of this strain caused by seven generations of selection with Cry1Ac[60].

In the United States, the registration for Cry1Ac cotton expired in September 2009 (ref. 21) and this product was replaced progressively from 2003 to 2011 by cotton that produces two Bt toxins, either Cry1Ac and Cry2Ab or Cry1Ac and Cry1F (**Fig. 2**).

Box 4 Field-evolved resistance to Bt cotton with reduced efficacy likely

Like both cases of field-evolved resistance to *Bt* cotton producing Cry1Ac (**Box 3**), the case of *H. zea* resistance to Cry2Ab in the southeastern United States has been controversial. The initial data documenting resistance in this case show a significant increase in the proportion of populations screened that had an LC_{50} value greater than the diagnostic concentration of toxin (150 μg Cry2Ab per ml diet), which indicates >50% survival at the diagnostic concentration[21,93] (**Supplementary Table 1**). Based on this criterion, the percentage of *H. zea* populations tested that were resistant to Cry2Ab rose from 0% in 2002 to 50% in 2005, only 2 years after commercialization of Bt cotton producing Cry2Ab and Cry1Ac[21,93]. The percentage of populations with a resistance ratio >10 also increased from 0% in 2002 to 50% in 2005 (refs. 21,93). Three populations sampled from non-Bt plants in Arkansas in 2005 had such low mortality in bioassays that LC_{50} values could not be calculated, but were estimated to be >400 μg Cry2Ab per ml diet[93]. The decreased susceptibility to Cry2Ab detected in 2005, when cotton producing this toxin was not common (**Fig. 2**), suggests that resistance to Cry1Ac caused some cross-resistance to Cry2Ab[93], which is consistent with data showing a genetic correlation between resistance to these two toxins[94].

In addition, data from Arkansas show that mortality caused by a diagnostic concentration of Cry2Ab decreased substantially in 2010 compared with the previous 4 years for field populations relative to a susceptible laboratory strain[120]. This evidence of resistance to Cry2Ab coincided with higher abundance of *H. zea* in the field and increased insecticide sprays targeting *H. zea* on Bt cotton in 2010 (ref. 120). For the entire United States, the mean number of insecticide sprays per hectare of Bt cotton directed primarily at *H. zea* nearly doubled in 2009–2011 (0.88, s.e.m. = 0.1) compared with 1999–2008 (0.48, s.e.m. = 0.03) (data from ref. 121; *t*-test, $t = 4.9$, df = 11, $P < 0.001$). In the United States from 1999 to 2011, the percentage of Bt cotton producing two toxins increased from 0% to 90% (**Fig. 2**), whereas the number of sprayings against *H. zea* on Bt cotton tripled[121].

In the five states of the midsouth region, sprays for *H. zea* per hectare of Bt cotton were relatively low from 2004 to 2007 (mean = 0.75, s.e.m. = 0.04), compared with 2000–2003 and 2008–2010 (data from ref. 118; mean = 1.2, s.e.m. = 0.09; $t = 3.9$, df = 9, $P < 0.01$). One explanation for this pattern is that fewer sprays were needed during 2004 to 2007 because two-toxin plants producing Cry1Ac and Cry2Ab initially had relatively high efficacy against *H. zea*, but their efficacy declined because of resistance to Cry2Ab. An alternative hypothesis is that sprays increased because of increased planting of corn, which is a preferred host for *H. zea*[118]. However, we found no association between the area planted to corn and sprays for *H. zea* on Bt cotton in the midsouth from 1999 to 2010 (data from ref. 118; $r^2 = 0.01$, df = 10, $P = 0.76$). We also found no association between the area planted to corn and sprays for *H. zea* on all cotton in Arkansas, Georgia and Mississippi from 2000 to 2011 (**Supplementary Tables 7–9** and **Supplementary Fig. 1**).

Because some susceptible individuals can complete development in the field on cotton producing Cry1Ac and Cry2Ab[122] and resistance to Cry1Ac in diet tests is associated with increased survival on cotton leaves containing both toxins, the increased survival of field-selected strains on diet treated with diagnostic concentrations of Cry1Ac and Cry2Ab is probably associated with increased survival in the field on cotton plants producing both of these toxins[21,119]. Although Luttrell and Jackson[118] state that they did not find strong evidence of "sustained loss of field control or increased resistance levels over time," they conclude, "From a practical farm-level perspective, effective control of bollworm [*H. zea*] requires supplemental insecticides, even on dual-gene Bt cottons."

High dose. Field outcomes show that resistance was less likely to evolve quickly if plants met the high-dose standard indicating that resistance was inherited as a recessive trait (**Table 1** and **Fig. 5**). Available data enabled evaluation of this factor for 19 cases, based on direct assessment of dominance (*h*) (12 cases) or indirect assessment derived from survival of susceptible pests on Bt plants in the field (7 cases) (**Supplementary Table 5**). Bt plants met the high-dose standard in six of nine (67%) cases with either no decrease in susceptibility or <1% resistant individuals, but not in any of the ten cases with ≥1% resistant individuals (Fisher's exact test, $P = 0.003$; **Table 1**).

A compelling contrast confirming the importance of the high-dose criterion is seen between the rapid evolution of resistance to Cry1Ac in Bt cotton by *H. zea*, but not by the closely related pest *H. virescens* (**Table 1**). Cry1Ac cotton met the high-dose standard against *H. virescens*, but not *H. zea* (**Table 1**). These two polyphagous pests that attack cotton were sampled from the same region and tested side-by-side in some studies[59,60]. Moreover, evolution of resistance to insecticides other than Bt toxins has been faster in *H. virescens* than *H.* zea[61,62], which refutes the alternative hypothesis that resistance generally evolves faster in *H. zea* than *H. virescens*.

One of the three exceptional cases in which the high-dose standard was not met and the percentage of resistant individuals was <1% involves *D. v. virgifiera* and Bt corn producing Cry34/35Ab (**Table 1**). In this case, the monitoring data cover only 4 years since commercialization, during which adoption of this product has been limited[63].

The other two exceptions involve cases with <1% resistant individuals after 15 years of exposure to Bt crops: *O. nubilalis* and Cry1Ab corn in the United States and *H. armigera* and Bt cotton in Australia (**Table 1**). In both cases, inheritance of resistance was completely recessive on young plants ($h = 0$), but not on older plants ($h = 0.31$ for *O. nubilalis* and 0.63 for *H. armigera*)[64–66]. These direct estimates of dominance

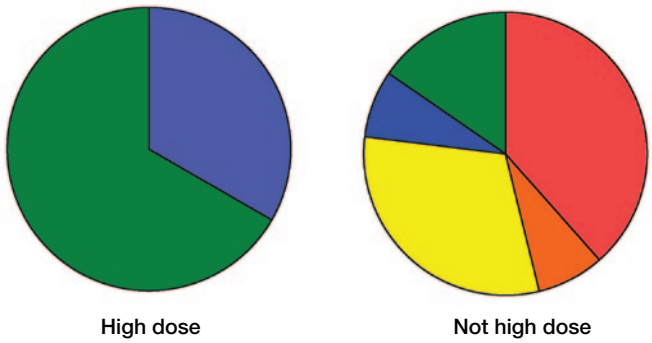

Figure 5 Resistance to Bt crops and dose criterion. Resistance evolved more slowly when the high-dose criterion was met (left, $n = 6$ cases) than when it was not met (right, $n = 13$ cases). Red, >50% resistance and reduced efficacy reported; orange, >50% resistance and reduced efficacy expected; yellow, 1–6% resistant individuals; blue, <1% resistant individuals; green, no decrease in susceptibility (see **Table 1** and text for details).

are based on survival of F_1 progeny on Bt plants to the adult stage for *H. armigera*[65,66], but only for 15 days for *O. nubilalis*, which might overestimate *h* for this pest[64]. In the experiments with *H. armigera* and Bt cotton, the concentration of Cry1Ac was 75% lower in the old plants compared with the young plants[66].

In both of these cases, non-Bt crop refuges were abundant. For *H. armigera* in Australia, the mean percentage of non-Bt cotton was 73% from 1996 to 2003 (range, 40–90%) when Cry1Ac cotton was planted, and 15% (range, 6–30%) from 2004 to 2011, when two-toxin Bt cotton producing Cry1Ac and Cry2Ab replaced Cry1Ac cotton[57] (**Fig. 2**). For *O. nubilalis* and Bt corn in the United States, the minimum percentage of corn planted with non-Bt corn for any state for a given year was 24%, which occurred in Iowa in 2010 and 2012 (ref. 67). From 1996 to 2012, the mean was 53% non-Bt corn in Iowa[67]. Overall, the results show that rapid evolution of resistance is less likely when the high-dose standard is met, and in some cases when this criterion is not met throughout the growing season, resistance can be delayed for more than a decade with abundant refuges.

Low initial resistance allele frequency. The monitoring data show that rapid resistance evolution was less likely when the initial resistance allele frequency was low (**Table 1**). The initial resistance allele frequency was below the detection threshold (no major resistance alleles detected, estimated frequency = 0) in 6 of 11 cases (55%) with either no decrease in susceptibility or <1% resistant individuals, compared with 0 of 5 cases with >1% resistant individuals (Fisher's exact test, one-tailed $P = 0.058$; **Table 1** and **Supplementary Table 6**).

With a criterion of an initial resistance allele frequency <0.001, however, the association with resistance was not significant. The initial resistance allele frequency was <0.001 in 7 of 11 cases (64%) with no decrease in susceptibility or <1% resistant individuals versus 2 of 5 cases (40%) with ≥1% resistant individuals (Fisher's exact test, one-tailed $P = 0.37$). Moreover, in two of the three cases from the United States with no decrease in susceptibility for more than a decade, the estimated initial resistance allele frequency was not <0.001; instead it was 0.0015 for *H. virescens* in four southern states and 0.16 for *P. gossypiella* in Arizona[68–71] (**Table 1** and **Supplementary Tables 4** and **6**). In laboratory-selected strains of these pests, resistance to Cry1Ac is recessive[68,71] (**Supplementary Table 5**). In addition, refuges were abundant for the first decade in both of these cases. The mean statewide percentage of cotton planted to non-Bt cotton from 1996 to 2005 was 42% in Arizona[9] and 50% in Arkansas, which had one of the highest adoption rates of Bt cotton of any state where *H. virescens* was monitored[59,69,72]. These results support the prediction from modeling studies that even when the initial resistance allele frequency exceeds 0.001, resistance can be delayed substantially, particularly if inheritance of resistance is recessive and refuges are abundant[28]. For *P. gossypiella* in Arizona, substantial fitness costs and incomplete resistance probably also helped to delay resistance[28,71].

Refuges. Consistent with previous reviews based on relatively limited data[20–22], the more extensive monitoring data reviewed here support the prediction that abundant refuges can delay resistance. Results from grower surveys in South Africa imply that the low abundance of refuges of non-Bt corn hastened evolution of *B. fusca* resistance to Bt corn producing Cry1Ab[73,74]. On average, from 1998 to 2004, fewer than 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[73]. In addition, pre-commercialization field data showing 2– 3% survival of susceptible larvae on Cry1Ab corn relative to non-Bt corn indicate that this Bt corn does not meet the high-dose standard against *B. fusca* (**Supplementary Table 5**). Thus, available evidence suggests that low refuge abundance and non-recessive inheritance of resistance accelerated evolution of resistance by *B. fusca* to Bt corn.

As with *B. fusca*, low refuge abundance and failure to meet the high-dose criterion apparently accelerated evolution of *S. frugiperda* resistance to Cry1F corn in Puerto Rico[75,76]. Based on mortality at the highest concentration of Cry1F tested, resistance was partially recessive[76] ($h = 0.14$), which does not meet the high-dose standard of at least 95% mortality of heterozygotes[31] ($h \leq 0.05$). Although the levels of Cry1F in Bt corn are "close to high dose" against this pest[76], modeling results suggest that such moderate doses can cause faster resistance evolution than either higher doses that kill all or nearly all heterozygotes, or lower doses that allow substantial survival of susceptibles[16] (B.E.T. & Croft, B.A.)[25]. Cross-resistance to Cry1F caused by exposure to Cry1A toxins in sprays and Bt corn might have also promoted resistance to Cry1F-producing corn[76]. Evolution of resistance in this case was probably also accelerated by continuous exposure to Bt corn during as many as 10 generations per year, which translates to 30 generations of selection in 3 years[76].

A scarcity of refuges in India and China may have promoted faster evolution of *P. gossypiella* resistance to Cry1Ac cotton in these two countries compared with the United States (**Table 1**), where refuges have been relatively abundant and high compliance with the refuge strategy was documented by our team in Arizona (Y.C., B.E.T *et al.*)[77,78]. Regulations in India mandate refuges of non-Bt cotton, but apparently compliance has been low[79,80]. China has not required non-Bt cotton refuges, and the non-Bt cotton percentage decreased to 8% in 2008 and 6% in 2009 and 2010 in six provinces of the Yangtze River Valley[54].

Another factor accelerating *P. gossypiella* resistance in India and China might be a lower concentration of Cry1Ac in the types of Bt cotton grown there compared with the varieties grown in the United States. In side-by-side field trials conducted in China, the abundance of *P. gossypiella* larvae was about five times higher on the predominant Bt cotton variety grown in China compared with a Bt cotton variety grown on a limited basis in the United States[54]. Although the efficacy and toxin concentrations have not been compared directly among the most popular types of Bt cotton grown in these countries, survival of susceptible *P. gossypiella* in the field was higher in both India and China than the United States, and the high-dose standard was met in the United States, but not in the two Asian countries (**Table 1** and **Supplementary Table 5**).

It is unclear why *P. gossypiella* resistance to Cry1Ac is a much more serious problem in India than in China (**Tables 1** and **2** and **Supplementary Table 2**). However, unlike the true-breeding varieties of Bt cotton planted in China, the United States, and elsewhere, hybrids account for nearly all Bt cotton planted in India[80]. In 2009, >500 Bt cotton hybrids were approved for planting in India[80]. Some of these diverse Bt cotton hybrids and the unapproved Bt cotton grown in India[81] may have lower toxin concentrations than the Bt cotton varieties grown in China.

Comparing field outcomes and refuge abundance in Australia, China and the United States for three congeneric pests (*H. armigera*, *H. punctigera* and *H. zea*) provides useful lessons for managing resistance when the high-dose standard is not met (**Table 1** and **Fig. 2**). Cotton plants producing Cry1Ac or both Cry1Ac and Cry2Ab do not meet the high-dose standard for any of these three pests (**Table 1** and **Supplementary Table 5**). After more than a decade of exposure to Bt cotton, the frequency of resistant individuals remained <1% for *H. armigera* and *H. punctigera* in Australia for Cry1Ac and Cry2Ab, increased to between 1% and 5% for *H. armigera* in China for Cry1Ac, and exceeded 50% for some populations of *H. zea* in the southeastern United States for both Cry1Ac and Cry2Ab (**Table 1**).

Of the three countries, Australia has applied the most stringent refuge requirements, which may have substantially delayed resistance. For cotton

producing only Cry1Ac, the minimum percentage of non-Bt cotton required on each farm in Australia was 70% from 1996 to 2003 (ref. 57) versus 4% in the United States[26,82]. For two-toxin cotton, Australia requires 10% non-Bt cotton or the equivalent in terms of other non-Bt crop host plants on each farm[83], whereas the United States has eliminated refuge requirements in most regions[84].

In China, however, virtually all Bt cotton planted produces only Cry1Ac (**Fig. 2**) and refuges of non-Bt cotton have not been required[52]. Nonetheless, non-Bt host plants other than cotton accounted for >92% of the cropping area planted to *H. armigera* host plants from 1997–2006 (ref. 7), which probably slowed resistance. Although *H. zea* in the United States also uses non-Bt host plants other than cotton, one of its major alternative hosts is Bt corn producing Cry1Ab, which is expected to select for cross-resistance to Cry1Ac[41,43]. Taking this and other factors including insecticide sprays into account, the 'effective refuge' for *H. zea* during the three generations in which it feeds on cotton was meticulously estimated as 39% in Arkansas for 2001–2005 (ref. 41).

Pyramids. Field outcomes are consistent with the prediction that resistance to pyramids will evolve faster if two-toxin plants are grown at the same time as plants producing only one of the toxins in the pyramid[47]. In the United States, farmers planted one-toxin cotton producing Cry1Ac concurrently with two-toxin cotton producing Cry1Ac and Cry2Ab from 2004 to 2010, whereas Australian growers completely replaced Cry1Ac cotton with two-toxin cotton during 2004 (**Fig. 2**). As noted above, the frequency of resistance to both toxins has exceeded 50% for some populations of *H. zea* in the United States, whereas it has remained <1% for *H. armigera* and *H. punctigera* in Australia (**Table 1**).

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*[21]. The available data suggest that initial resistance allele frequencies for Cry1Ac and Cry2Ab were not significantly higher for *H. zea* than for *H. armigera* (**Supplementary Table 6**), but resistance to both toxins appears to be more dominant in *H. zea* (**Supplementary Table 5**).

Conclusions

From 2005 to 2010, the data available to assess the effectiveness of resistance management tactics for Bt crops increased dramatically and the number of major target pests with some populations resistant to Bt crops and reduced efficacy reported surged from one to five (**Tables 1** and **2** and **Figs. 1**, **3** and **4**). The increase in documented cases of resistance likely reflects increases in the area planted to Bt crops (**Fig. 1**), the cumulative duration of pest exposure to Bt crops, the number of pest populations exposed and improved monitoring efforts.

Our review of field-evolved resistance to Bt crops based on monitoring data for up to two decades from 24 cases in eight countries generally confirms the principles of resistance management based on evolutionary theory. As predicted, factors associated with sustained susceptibility to the Bt toxins in transgenic crops are a toxin concentration that meets the high-dose standard and thus renders inheritance of resistance recessive (see Theory section above), a low initial frequency of resistance alleles, and abundant refuges of non-Bt host plants *near Bt* crops that promote survival of susceptible insects.

Before commercialization, scientists can evaluate insect responses to Bt crops to determine if the high-dose standard is met and if the initial frequency of resistance is low, using the techniques described in the studies reviewed here (**Supplementary Tables 5** and **6**). Because resistant strains are often not available before commercialization, the high-dose standard can be assessed proactively by measuring survival of susceptible insects on Bt crops[31]. In parallel, estimates of the frequency of individuals with a genetically based decrease in susceptibility relative to conspecific individuals can be made proactively with bioassays of field-derived strains using Bt plants, Bt plant parts, or diagnostic concentrations of toxin in diet. Although F_2 screens have been especially useful for detecting rare recessive resistance alleles, the modeling and empirical results reviewed here do not support the idea that it is critical to determine if the initial resistance allele frequency is <0.001.

The relevant theory and data suggest that if the criteria for high dose and low initial frequency are met, resistance can be delayed with limited refuges. Conversely, if these criteria are not met, resistance is likely to evolve rapidly unless refuges are abundant. Therefore, systematic assessment of these criteria can be used proactively to enhance resistance management. Moreover, if reporting the assessment of these criteria becomes standard practice, the data available for testing predictions will increase steadily, thereby facilitating refinements in resistance management strategies.

In the past decade, farmers in the United States, India and Australia have shifted largely from planting first-generation transgenic plants producing one Bt toxin to using 'pyramids' that produce two or more distinct Bt toxins active against a particular pest (**Fig. 2**). The limited field data available for pyramids confirm predictions from theory and small-scale experiments with a model system indicating that pyramids work best when implemented proactively[47], as has been done in Australia[57]. Conversely, when a pyramid of two toxins is adopted after resistance is no longer rare to one of the toxins, the benefits of this approach seem to

Table 3 Bt toxin pyramids used proactively and separately from one-toxin plants or remedially and concurrent with one-toxin plants

Pest	Crop	Country	Toxins in pyramid[21,57,63]	Resistance detected[a]
Proactive and separate from one-toxin plants				
H. armigera	Cotton	Australia	Cry1Ac, Cry2Ab	None
H. punctigera	Cotton	Australia	Cry1Ac, Cry2Ab	None
Remedial and concurrent with one-toxin plants				
D. v. virgifera	Corn	USA	Cry3Bb, Cry34/35Ab	Cry3Bb
H. zea	Cotton	USA	Cry1Ac, Cry2Ab	Cry1Ac
H. zea	Cotton	USA	Cry1Ac, Cry1F	Cry1Ac
P. gossypiella	Cotton	India	Cry1Ac, Cry2Ab	Cry1Ac
S. frugiperda	Corn	USA	Cry1F, Cry1A.105[b], Cry2Ab	Cry1F

[a]Resistance detected to one of the toxins in a pyramid before the pyramid completely replaced single-toxin Bt crops producing one of the toxins in the pyramid. Monitoring data and references are provided in **Supplementary Tables 3** and **4** for *H. armigera* and *H. punctigera*, and in **Table 2** for the four other pests. [b]Cry1A.105 is a chimeric Bt toxin with its amino acid sequence 99% identical to Cry1F for domain III, identical to Cry1Ab for domain I, and identical to Cry1Ac for domain II and C terminus[95]. Although data evaluating *S. frugiperda* responses to Cry1A.105 have not been reported, cross-resistance to Cry1A.105 is expected in Puerto Rico because populations there have been selected for resistance to each of its three parent toxins: Cry1F in Bt corn, and Cry1Ab and Cry1Ac in sprays[75,76]. For *S. frugiperda* populations and families from Puerto Rico resistant to Cry1F, resistance ratios for Cry1Ab and Cry1Ac ranged from 12 to 89 (refs. 75,103).

be greatly reduced, as exemplified by resistance to Cry2Ab in Bt cotton for *H. zea* in the United States (**Table 1**). In several other cases, pyramids are also being used as a remedial tactic following documented resistance to Bt crops producing only one of the toxins in the pyramid (**Table 3**).

Although all of the data reviewed here involve crystalline (Cry) Bt toxins, transgenic crops producing vegetative insecticidal proteins (Vips) from Bt are registered in the United States[21] and their use is expected in Australia[85]. In addition, genetically engineered Cry toxins that were more effective than native Bt toxins against resistant strains of several pests in laboratory tests might eventually broaden options for managing insects with transgenic plants[18] (B.E.T. *et al.*)[86]. Scientists are also developing transgenic plants that control insects by means of RNA interference and fusion proteins[87–90].

Even with a wider range of approaches used to engineer plants for protection against insects, resistance management will continue to be essential. Based on the 24 cases reviewed here, pests can evolve resistance to toxins in Bt crops in as few as 2 years under the worst circumstances; under the best circumstances, however, efficacy can be sustained for 15 years or more. Although regulations in the United States and elsewhere mandate refuges of non-Bt host plants for some Bt crops, farmer compliance is not uniformly high and the required refuge percentages may not always be large enough to achieve the desired delays in evolution of resistance[3,63,73–76,79–81]. Both in theory and practice, using Bt crops in combination with other tactics as part of integrated pest management may be especially effective for delaying pest resistance[9]. We hope that the lessons learned from the first billion acres of Bt crops will improve resistance management strategies in the future.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.C., T.B. and B.E.T. analyzed data. B.E.T. wrote the paper. All authors discussed the results and commented on the manuscript.

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The authors declare competing financial interests: details are available in the online version of the paper.

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