

Surge in insect resistance to transgenic crops and prospects for sustainability

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Transgenic crops have revolutionized insect pest control, but their effectiveness has been reduced by evolution of resistance in pests. We analyzed global monitoring data reported during the first two decades of transgenic crops, with each case representing the responses of one pest species in one country to one insecticidal protein from *Bacillus thuringiensis* (Bt). The cases of pest resistance to Bt crystalline (Cry) proteins produced by transgenic crops increased from 3 in 2005 to 16 in 2016. By contrast, in 17 other cases there was no decrease in pest susceptibility to Bt crops, including the recently introduced transgenic corn that produces a Bt vegetative insecticidal protein (Vip). Recessive inheritance of pest resistance has favored sustained susceptibility, but even when inheritance is not recessive, abundant refuges of non-Bt host plants have substantially delayed resistance. These insights may inform resistance management strategies to increase the durability of current and future transgenic crops.

Genetically engineered crops have the potential to help meet the challenge of sustainably providing food and fiber for the world's growing population¹. In particular, transgenic crops producing insecticidal proteins from Bt have revolutionized pest control^{2–5}. Bt proteins kill some voracious insect pests, but cause little or no harm to most other organisms, including humans, wildlife, and most beneficial insects^{6–8}. The hectares (ha) planted with Bt crops worldwide increased from 1.1 million in 1996 to 98.5 million in 2016, with a cumulative total of more than 830 million⁴ (Fig. 1a). Bt corn, cotton, and soybean have accounted for >99% of this total. In addition to the crystalline (Cry) proteins from Bt produced by transgenic crops for the past two decades, some recently introduced types of Bt corn and cotton produce a vegetative insecticidal protein (Vip) from Bt^{9–13}. When produced naturally by the bacteria, Cry proteins are produced during sporulation and retained within the cell wall, whereas Vips are produced during the vegetative phase and secreted¹¹. Benefits of Bt crops include pest suppression, decreased use of conventional insecticides, conservation of beneficial natural enemies, increased yields, and higher farmer profits^{14–20}.

The benefits of Bt crops, however, are threatened by the evolution of pest resistance^{3,12,21–24}. The scientific literature on this topic has exploded; a Web of Science search for “*Bacillus thuringiensis* and resistance” identified >1,100 papers published from 2013 to April 2017. Here, we analyze the relevant literature from the past two decades to elucidate the current status of pest resistance to transgenic crops, to better understand how we got where we are, and to determine how we can move forward effectively. We simplify the criteria for classifying resistance to Bt crops, update the global status of resistance to Bt crops, briefly summarize theory and tactics for delaying resistance to Bt crops (Box 1), and test the theory with data from the field.

We end by assessing the future prospects for managing resistance to recently introduced Bt crops that produce Vip3Aa and transgenic crops in the pipeline that combine RNA interference (RNAi) with Bt proteins for pest control.

Compared with previous reviews on this topic^{24–26}, the field-monitoring data analyzed here represent a more diverse set of Bt toxins (one Vip and nine Cry toxins), crops (corn, cotton, and soy), pests (15 species from two insect orders), and countries (ten countries on six continents). Strikingly, the number of cases of resistance to Bt crops with practical consequences for pest control has more than tripled (Fig. 1b) since completion of our previous review based on monitoring data published as of 2012 (B.E.T., Y.C. *et al.*²⁴).

Field-evolved resistance

Previous publications provide detailed discussion of various definitions and criteria for resistance to Bt crops^{24,27,28}. In this Review, we define field-evolved resistance as a genetically based decrease in susceptibility of an insect population to a Bt toxin caused by selection in the field. This is similar to, but broader than, our previous definitions (B.E.T., Y.C. *et al.*)^{24,27} because it includes the possibility of selection in the field by one toxin that causes cross-resistance to another toxin. As in previous work, each case reviewed here represents responses of one pest species in one country to one Bt toxin²⁴.

Although we have previously used up to six categories of susceptibility and resistance to Bt crops²⁴, here we classify each case into one of three categories: category 1, practical resistance; category 2, no decrease in susceptibility; or category 3, early warning of resistance. Practical resistance to a Bt crop is field-evolved resistance that reduces the efficacy of the Bt crop and has practical consequences for pest control²⁷. The criteria for practical resistance are that >50% of individuals in a population are resistant and the efficacy of the Bt crop is reduced in the field²⁷. The percentage of resistant individuals can be estimated from survival of insects exposed to a concentration of Bt toxin that kills all, or nearly all, susceptible individuals^{24,27} (Supplementary Methods). Exposure can be mediated by allowing insects to eat Bt

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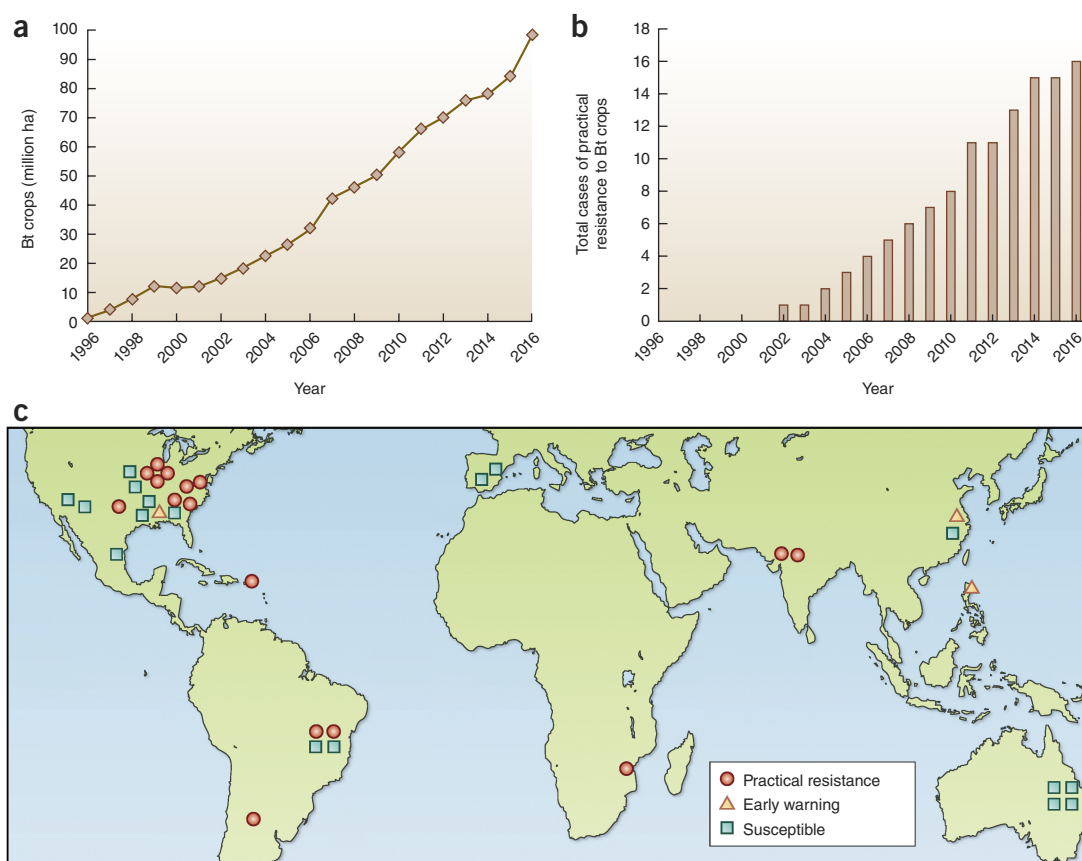


Figure 1 Global status of pest resistance to Bt crops. **(a)** Hectares planted to Bt crops each year. **(b)** Cumulative cases of field-evolved practical resistance to Bt crops. **(c)** Each symbol represents 1 of 36 cases indicating responses of one pest species in one country to one toxin in Bt corn, cotton, or soy (Tables 1 and 2).

plants, Bt plant tissues, or diet containing a 'diagnostic concentration' of Bt toxin^{24,27}. Large increases in the concentration of toxin killing 50% of the insects tested (LC_{50}) also indicate >50% of individuals in a population are resistant²⁷. Cases fit category 2 when the monitoring data show no statistically significant decrease in susceptibility after field populations have been exposed to a Bt crop²⁴. Whereas definitions for the first two categories are the same as before, for simplicity here, we broaden the intermediate category of 'early warning of resistance' to include all cases of field-evolved resistance where monitoring data show a statistically significant decrease in susceptibility, yet reduced efficacy of the Bt crop has not been reported.

Global status of insect resistance to Bt crops

The 36 cases reviewed here consist of 16 cases of practical resistance, 17 cases of no decrease in susceptibility, and 3 cases of early warning of resistance (Fig. 1, Tables 1 and 2, and Supplementary Notes 1 and 2). Of the 15 pest species monitored, 14 are lepidopterans and one is a coleopteran (*Diabrotica virgifera virgifera*, western corn rootworm).

Increasingly rapid evolution of practical resistance. The cumulative number of cases of practical resistance to the Bt toxins in transgenic crops surged from 3 in 2005 to 16 in 2016 (Fig. 1b and Table 1). These 16 cases represent resistance of some populations of seven major pests in five countries to each of the nine Cry toxins produced by widely grown Bt crops: Cry1Ab, Cry1Ac, Cry1A.105, Cry1Fa, Cry2Ab, Cry3Bb, mCry3A, eCry3.1Ab, and Cry34/35Ab (Table 1).

For these 16 cases of practical resistance, the average time from the first commercial planting of a Bt crop in a region to the first sampling of field populations in the region that provided evidence of resistance was 5.2 years (s.e.m. = 0.7, range: 0 to 10 years) (Table 1). Practical resistance to Bt corn has been documented for some populations of five pest species (*Busseola fusca*, *Diatraea saccharalis*, *D. v. virgifera*, *Spodoptera frugiperda*, and *Striacosta albicosta*), to Bt cotton for one species (*Pectinophora gossypiella*), and to both Bt corn and cotton for the remaining species (*Helicoverpa zea*). The rise in total reported cases of practical resistance from 5 in 2012 (ref. 24) to 16 in 2016 reflects 2 cases for species that had no practical resistance before (*D. saccharalis* and *S. albicosta*) and 9 additional cases for four species that had practical resistance to only one Bt toxin previously and now resist up to four toxins (Table 1). Practical resistance has reduced the number of Bt toxins in transgenic crops that are effective against some populations of major pests to two, one, or none (Table 3).

Evolution of practical resistance has accelerated over the past two decades, as shown by the significant negative association between the time for practical resistance to occur and the year when a Bt crop was first grown commercially (Fig. 2). Cross-resistance to one Bt toxin caused by selection with another Bt toxin is an important factor accelerating the evolution of practical resistance (B.E.T.)²⁹ (Fig. 2). For *D. v. virgifera*, cross-resistance caused by resistance to Cry3Bb is implicated in resistance to the closely related toxins mCry3A and eCry3.1Ab^{30,31}. Resistance to eCry3.1Ab was detected in the field before plants producing this toxin were commercialized, providing

Box 1 Resistance management from theory to practice

The most widely used strategy for delaying evolution of pest resistance to Bt crops is to grow 'refuges' of host plants that do not make Bt toxins and thereby boost survival of susceptible pests^{16,21,24,66,132,133}. The hope is that rare resistant pests that survive on Bt plants will mate with the relatively abundant susceptible pests that thrive in refuges. If inheritance of resistance is recessive, the resulting heterozygous offspring will die on Bt crops, greatly delaying the evolution of resistance. This is sometimes called the 'high-dose refuge strategy' because it works best if the dose of toxin ingested is high enough to kill all, or almost all, of the heterozygous insects that feed on Bt plants.

In principle, the high-dose standard can be assessed by measuring the survival of resistant insects, susceptible insects, and their F_1 progeny on Bt plants²⁴. This allows calculation of the dominance parameter h , which varies from 0 for completely recessive to 1 for completely dominant¹³⁴. Values of h less than 0.05 satisfy the high-dose criterion²⁴. Alternatively, several indirect tests measure survival of susceptible insects on Bt plants¹³⁵. The US Environmental Protection Agency (EPA) indicates that Bt plants meet the high-dose standard if they kill at least 99.99% of susceptible insects¹³⁵. This criterion reflects the concept that if Bt plants do not kill all or nearly all susceptible insects, they probably will not kill nearly all insects that are heterozygous for resistance. If survival of susceptible insects is $>0.01\%$, then survival is likely to be higher for the heterozygotes than for the homozygous susceptible insects, which yields nonrecessive inheritance of resistance that accelerates adaptation²⁴. If a high dose is not achieved, resistance can be delayed by increasing refuge abundance, which compensates for survival of heterozygous progeny on Bt plants by reducing the proportion of the population selected for resistance²⁴.

Extending the efficacy of Bt crops by increasing refuge abundance can impose a short-term cost of greater pest damage to the non-Bt crop refuge. However, in the United States, planting non-Bt corn refuges yielded growers \$4.3 billion in short-term benefits because of the lower cost of non-Bt seed and regional suppression of the primary pest *O. nubilalis* by Bt corn¹⁶. Also, in China, millions of farmers voluntarily increased planting of non-Bt cotton refuges, apparently to achieve short-term economic gains¹²⁴ (see below).

Overall, five factors favor success of the refuge strategy for delaying resistance: recessive inheritance of resistance (i.e., plants meet the high-dose standard), low resistance allele frequency, abundant refuges of non-Bt host plants near Bt plants, fitness costs, and incomplete resistance^{24,136}. When fitness costs occur, fitness on non-Bt host plants is higher for susceptible insects than resistant insects, so refuges select against resistance. Incomplete resistance occurs when homozygous resistant insects can survive on Bt plants, but they suffer a disadvantage relative to resistant insects on non-Bt plants. When a potentially heterogeneous mixture of resistant and susceptible individuals is tested, less than 100% survival on Bt plants is expected, so such results alone are not sufficient to infer incomplete resistance (**Supplementary Methods**).

Whereas each of the first-generation Bt plants makes a single Bt toxin, second-generation Bt plants each produce two or more Bt toxins to address one or more of the following goals: to delay or counter resistance, improve efficacy, and broaden the spectrum of pests killed⁶⁴. Bt crop 'pyramids' are designed to delay the evolution of resistance by producing two or more distinct toxins or other traits that kill the same pest^{34,64}. Based on modeling and experimental evidence, pyramids are considered more effective for delaying resistance than temporal alternations or spatial mosaics of crops with different Bt toxins¹³⁷. First commercialized in 2003, Bt crop pyramids have become prevalent globally, with the notable exception of transgenic cotton producing a single Bt toxin still grown throughout China²⁴. Although some cotton producing a cowpea trypsin inhibitor plus Bt toxin Cry1Ac has been planted in China, a three-year field study found that relative to cotton producing Cry1Ac alone, addition of the trypsin inhibitor did not significantly decrease the population density of *H. armigera*¹³⁸.

The five factors listed above favor durability of pyramids as well as single-toxin transgenic crops. In addition, the following three factors are especially important for delaying pest resistance to pyramids: the concentration of each toxin in the pyramid is high enough to kill all or nearly all susceptible insects, no cross-resistance occurs between toxins in the pyramid, and pyramids are not grown simultaneously with single-toxin plants that produce one of the toxins in the pyramid^{34,64,139}. In some cases, the efficacy and durability of Bt crop pyramids has been reduced by resistance to single-toxin crops producing the same toxins used in pyramids, as well as cross-resistance and antagonism between Bt toxins^{34,64}.

Although refuges are essential for durability of both pyramids and single-toxin transgenic crops, the optimal spatial configuration of refuges remains unresolved. Blocks of non-Bt plants called 'structured refuges' have been cultivated in separate fields or within fields of Bt crops to delay pest resistance since 1996 (refs. 34,45,64). In 2007, to delay pest resistance to Bt cotton pyramids, the US EPA approved 'natural refuges' consisting of non-Bt host plants other than cotton⁴⁶. This approach has been effective for prolonging the efficacy of Bt cotton against *H. virescens*, but not against *H. zea* (**Tables 1 and 2**). Although both of these pests use many non-Bt host plants other than cotton, the Cry1 and Cry2 toxins in Bt cotton meet the high-dose standard for *H. virescens*, but not for *H. zea* (**Tables 1 and 2**).

Natural refuges have helped to slow, but not stop, adaptation to Bt cotton by *H. armigera* in northern China⁷⁴ (**Table 2**). The effective refuge percentage including non-cotton host plants was estimated as 56% for *H. armigera* in northern China⁷⁴ versus 39% for *H. zea* in Arkansas and Mississippi of the southeastern United States¹⁴⁰. Cultivation of non-Bt host plants and Bt cotton in small fields that are close to each other may also boost the success of the natural refuge strategy in northern China⁷⁴.

Since 2010, seed mixtures (also called 'refuge-in-a-bag' or RIB) yielding a random mixture of Bt plants and non-Bt plants side-by-side within fields have been planted to delay pest resistance to Bt corn pyramids⁶⁴. Seed mixtures solve the problem of farmers not complying with block refuge requirements. However, results from modeling and small-scale experiments indicate that if larvae move between Bt and non-Bt plants, seed mixtures may accelerate evolution of resistance by reducing the survival of susceptible insects and the effective refuge size, or by increasing the survival of heterozygotes relative to susceptible homozygotes, thereby increasing the dominance of resistance in seed mixtures relative to blocks of Bt crops (Y.C., B.E.T. *et al.*)⁶⁴.

In the Yangtze River Valley of China, millions of growers serendipitously implemented a novel seed mixture strategy by planting second-generation seeds from crosses between Bt and non-Bt cotton, which yields a refuge of 25% non-Bt plants randomly interspersed within fields of Bt cotton¹²⁴. Analysis of 11 years of field monitoring data from six provinces implies that this approach delayed or even reversed *P. gossypiella* resistance to single-toxin Bt cotton while sustaining pest suppression¹²⁴.

Table 1 Practical resistance to Bt crops

Insect	Crop	Toxin	Country	Year marketed ^a	Years ^b	High dose ^c	References ^d
<i>B. fusca</i>	Corn	Cry1Ab	S. Africa	1998	8	No	87,88
<i>D. saccharalis</i>	Corn	Cry1A.105	Argentina	2010	4	?	51,89–91
<i>D. v. virgifera</i>	Corn	Cry3Bb	USA	2003	6	No	28,86
<i>D. v. virgifera</i>	Corn	Cry34/35Ab	USA	2006	7	No	28,92,93
<i>D. v. virgifera</i>	Corn	mCry3A	USA	2007	4 ^e	No	28,94
<i>D. v. virgifera</i>	Corn	eCry3.1Ab	USA	2014	0 ^e	No	28,30,31
<i>H. zea</i>	Corn	Cry1Ab	USA	1996	8	No	95,96
<i>H. zea</i>	Corn	Cry1A.105	USA	2010	6 ^e	No	95
<i>H. zea</i>	Cotton	Cry1Ac	USA	1996	6	No	24,97,98
<i>H. zea</i>	Cotton	Cry2Ab	USA	2003	2 ^e	No	24,32,99
<i>P. gossypiella</i>	Cotton	Cry1Ac	India	2002	6	No	50,100–102
<i>P. gossypiella</i>	Cotton	Cry2Ab	India	2006	8	?	75
<i>S. albicosta</i>	Corn	Cry1Fa	USA	2003	10	No	103–106
<i>S. frugiperda</i>	Corn	Cry1Ab	Brazil	2008	2 ^e	No	40
<i>S. frugiperda</i>	Corn	Cry1F	Brazil	2009	2	No	38,107
<i>S. frugiperda</i>	Corn	Cry1F	USA	2003	4	No	60,108

^aFirst year of commercial planting of a Bt crop in the region monitored. ^bYears from the first commercial planting of a Bt crop in the region to the first sampling of field populations in the region yielding evidence of resistance. ^cTest for the high-dose standard based on direct or indirect evidence (Box 1). If both types were available, the table reflects the direct evidence. ^d“?” indicates data not available. ^eEach reference provides evidence of practical resistance, data for evaluating the high-dose criterion, or both. ^cCross-resistance suspected or known as a factor contributing to resistance.

direct evidence of cross-resistance³¹. Several lines of evidence suggest that cross-resistance to Cry2Ab caused by resistance to Cry1Ac in Bt cotton hastened the markedly decreased susceptibility of *H. zea* field populations to Cry2Ab in the southern United States in 2005, only two years after commercial planting of Bt cotton producing Cry2Ab^{32,33}. Although strong cross-resistance generally does not occur between Cry1 and Cry2 toxins, statistically significant but weak cross-resistance is typical^{34–37}. Resistance to Cry1Ab in *H. zea* probably caused some cross-resistance to Cry1A.105^{36,95}. In Brazil, cross-resistance between Cry1Ab and Cry1Fa cannot be excluded as a factor accelerating evolution of practical resistance to these toxins in *S. frugiperda*, which occurred two years after Bt corn producing each toxin was grown commercially^{38–40}.

In addition to cross-resistance, factors favoring faster evolution of resistance to more recently commercialized Bt crops are increased adoption rates and concomitantly reduced percentages of host plants that are refuges of non-Bt crops. The increase in cases of practical resistance in the second decade of Bt crops is also associated with an increase in the total area planted with Bt crops (Fig. 1), exposure of pests to Bt crops in more countries, and longer cumulative exposure of pests to Bt crops. The number of new cases of practical resistance reported relative to the yearly mean area planted with Bt crops globally is similar for the first and second decades of Bt crops: 2.3 and 2.0 new cases per 10 million ha, respectively (3 cases/12.9 million ha for 1996–2005 and 13 cases/63.8 million ha for 2006–2016). Nonetheless, the 16 cases of practical resistance analyzed here underestimate the situation in the field, in part because of the delay between field sampling that yields evidence of resistance and publication of the data (mean = 3.4 years, s.e.m. = 0.7, $n = 16$ cases).

We tested the hypothesis that resistance evolved faster for pests that fed on Bt crops for more generations per year (GPY). The negative association between time to practical resistance and GPY was not significant when all 16 cases of practical resistance were considered ($n = 16$, $P = 0.19$), but it was significant when the five cases of cross-resistance were excluded ($n = 11$, $P = 0.022$) (Supplementary Table 1 and Supplementary Fig. 1). Multiple regression for all 16 cases also shows that the negative relationship between time to practical resistance and GPY was significant ($P = 0.049$) after accounting for the significant effect of cross-resistance ($P = 0.005$) (Supplementary Table 2). The mean GPY did not differ significantly between the 16 cases of practical resistance (4.2, s.e.m. = 0.9) and the 17 cases of sustained

susceptibility (4.1, s.e.m. = 0.5) (t -test, $t = 0.04$, $df = 31$, $P = 0.97$) (Supplementary Tables 1 and 3).

Sustained susceptibility. In contrast with the 16 cases of practical resistance described above, the global monitoring data reveal 17 cases where no significant decrease in susceptibility occurred after 1 to 19 years of exposure to Bt crops (mean = 10.6 years, s.e.m. = 1.4; Table 2). These 17 cases include data from six countries indicating susceptibility to five toxins in Bt crops for populations of nine species of lepidopteran pests: *Chrysodeixis includens*, *Diatraea grandiosella*, *Helicoverpa armigera*, *Helicoverpa punctigera*, *Heliothis virescens*, *Ostrinia nubilalis*, *P. gossypiella*, *S. frugiperda*, and *Sesamia nonagroides*. In 11 of these 17 cases, no decrease in susceptibility has been demonstrated for at least 10 years (Table 2). Moreover, in all of these cases, the currently available monitoring data underestimate the ultimate duration of sustained susceptibility, because this can be known only after resistance occurs.

Early warning of resistance. The three cases of early warning of resistance involve responses of *Diatraea saccharalis* in the United States and *Ostrinia furnacalis* in the Philippines to Cry1Ab, and *H. armigera* in China to Cry1Ac (Table 2 and Supplementary Note 2).

Testing theory with data

Consistent with previous results from smaller data sets^{24,25}, the data from the 36 cases reviewed here support the main predictions from the evolutionary theory underlying the refuge strategy (Box 1). When the high-dose standard is met, which indicates recessive inheritance of resistance, resistance is less likely to evolve rapidly. In the 30 cases where the available data enable evaluation of this factor, the high-dose standard was met for 69% (9 of 13) of the cases with no decrease in susceptibility and with none of the 17 cases showing either practical resistance or early warning of resistance (Tables 1 and 2). This pattern demonstrates a significant association between meeting the high-dose standard and a lower risk of rapid evolution of resistance (Fisher's exact test, $n = 30$, $P < 0.0001$).

In two of the four exceptional cases where the high-dose standard was not met and susceptibility did not decrease (Table 2), pest exposure to the relevant Bt toxins was limited: *O. nubilalis* exposure to Cry1F corn in the United States⁴¹ and *C. includens* exposure to Cry1Ac soy in Brazil⁴². In the third exceptional case, *O. nubi-*

Table 2 No decrease in susceptibility and early warning of resistance to Bt crops

Insect	Crop	Toxin	Country	Year marketed ^a	Years ^b	High dose ^c	Reference
No decrease in susceptibility							
<i>C. includens</i>	Soy ^d	Cry1Ac	Brazil	2013 ^d	1 ^d	No	42,109
<i>D. grandiosella</i>	Corn	Cry1Ab	USA	1999	6	?	110
<i>H. armigera</i>	Cotton	Cry1Ac	Australia	1996	19	No	111–113
<i>H. armigera</i>	Cotton	Cry2Ab	Australia	2004	11	Yes	12,114
<i>H. punctigera</i>	Cotton	Cry1Ac	Australia	1996	19	?	111
<i>H. punctigera</i>	Cotton	Cry2Ab	Australia	2004	11	Yes	12,114
<i>H. virescens</i>	Cotton	Cry1Ac	Mexico	1996	11	?	115
<i>H. virescens</i>	Cotton	Cry1Ac	USA	1996	11	Yes	98,115,116
<i>H. virescens</i>	Cotton	Cry2Ab	USA	2003	2	Yes	32,117–119
<i>O. nubilalis</i>	Corn	Cry1Ab	Spain	1998	15	?	120,121
<i>O. nubilalis</i>	Corn	Cry1Ab	USA	1996	15	No	43,122
<i>O. nubilalis</i>	Corn	Cry1Fa	USA	2003	8	No	41,123
<i>P. gossypiella</i>	Cotton	Cry1Ac	China	2000	15	Yes	124
<i>P. gossypiella</i>	Cotton	Cry1Ac	USA	1996	12	Yes	17
<i>P. gossypiella</i>	Cotton	Cry2Ab	USA	2003	5	Yes	17,125,126
<i>S. frugiperda</i>	Corn	Vip3Aa	Brazil	2010	5	Yes	10
<i>S. nonagroides</i>	Corn	Cry1Ab	Spain	1998	15	Yes	127
Early warning of resistance^e							
<i>D. saccharalis</i>	Corn	Cry1Ab	USA	1999	10	No	24,128,129
<i>H. armigera</i>	Cotton	Cry1Ac	China	1997	16	No	74
<i>O. furnacalis</i>	Corn	Cry1Ab	Philippines	2003	6	No	130,131

^aFirst year of commercial planting of a Bt crop in the region monitored. ^bFor cases with no decrease in susceptibility, this column shows years of documented susceptibility, calculated as the year of the most recent monitoring data cited minus the first year of commercialization in the region. For early warning of resistance, this column shows the years from the first year of commercial planting in the region to the most recent year of monitoring data reviewed here. ^cTest for the high-dose standard based on direct or indirect evidence (**Box 1**). If both types were available, the table reflects the direct evidence. '?' indicates data not available. ^dThe first season of commercial planting in Brazil for transgenic plants producing Cry1Ac was 2013–14 for soy and 2006–2007 for cotton, which is an occasional host of *C. includens*⁴². Based on monitoring data from the 2014–2015 season, documented susceptibility to Cry1Ac is 1 year since introduction of Bt soy and 8 years since introduction of Bt cotton. ^eThe highest percentage of resistant individuals reported for any field population screened (based on survival at a diagnostic concentration in diet bioassays) was 2.4% for *D. saccharalis*, 11.3% for *H. armigera*, and 5.5% for *O. furnacalis*.

lalis and Cry1Ab corn, larvae from the resistant strain tested did not survive on young vegetative-stage plants, but larvae from the resistant strain and the progeny from a cross between the resistant strain and a susceptible strain survived on older reproductive-stage plants, yielding partially recessive resistance that does not meet the high-dose standard ($h = 0.31$) (ref. 43). Larvae surviving on reproductive-stage corn were found feeding on reproductive tissues that have lower toxin concentration than the leaves eaten by larvae on vegetative-stage corn⁴³. However, this evaluation of the high-dose criterion is based on survival after only 15 days, which could overestimate dominance⁴³.

The fourth exceptional case, the sustained efficacy of Cry1Ac against *H. armigera* in Australia for two decades (**Table 2**), is particularly instructive. Recognizing that the high-dose standard was not fully satisfied, the Australian resistance management plan for cotton producing Cry1Ac proactively required a non-Bt cotton refuge of at least 70% from 1996 to 2003 (ref. 44). Susceptibility was

maintained to Cry1Ac cotton until it was replaced in 2004 by two-toxin cotton producing Cry1Ac and Cry2Ab. When the two-toxin cotton was introduced, the refuge requirement dropped to 10% unsprayed non-Bt cotton or its equivalent⁴⁴. This approach has yielded no net decrease in susceptibility of *H. armigera* to either of the two toxins. By contrast, practical resistance to both toxins has evolved in the closely related species *H. zea* in the United States (**Table 1**), where refuge requirements have been less stringent. In the United States, the minimum refuge required was 4% unsprayed non-Bt cotton for Cry1Ac cotton when it was first grown commercially in 1996 (ref. 45); and in most regions, no refuge has been required for two-toxin Bt cotton since 2007 (refs. 24,46).

The outcomes summarized above imply that when the high-dose standard is not met, proactive deployment of abundant refuges can substantially delay resistance. Conversely, along with failure to meet the high-dose standard, the scarcity of refuges seems to be a key factor contributing to the cases of practical resistance in Argentina, Brazil, India, South Africa, and the United States^{24,28,38,39,47–51} (**Table 1**).

Table 3 Limited availability of transgenic crops to control pests that have practical resistance to two or more Bt toxins

Insect	Crop	Country	Practical resistance reported ^a	Practical resistance not reported and toxin effective
<i>D. v. virgifera</i>	Corn	USA	Cry3Bb, eCry3.1Ab, mCry3A, Cry34/35Ab	None ^b
<i>P. gossypiella</i>	Cotton	India	Cry1Ac, Cry2Ab	None ^b
<i>H. zea</i>	Corn and cotton	USA	Cry1Ab, Cry1Ac, Cry1A.105, Cry2Ab	Vip3Aa
<i>S. frugiperda</i>	Corn	Brazil	Cry1Ab, Cry1F	Cry2Ab, Vip3Aa ^c

^aSee **Table 1**. ^bIn the country listed, no Bt toxins in currently commercialized transgenic crops remain effective against all populations of the pest, but some Bt toxins in currently commercialized transgenic crops do remain effective against some populations of the pest. Note: Vip3Aa is not effective against *D. v. virgifera* or *P. gossypiella*. ^cThe combination of Cry2Ab and Vip3Aa is not produced by any widely adopted transgenic corn hybrids, so each toxin acts alone against pest populations resistant to Cry1 toxins.

Managing resistance of lepidopteran pests to Vip3Aa

Compared with the extensive exposure of pests to Cry proteins during the past two decades, exposure to vegetative insecticidal proteins (Vips) from Bt has been limited and no field-evolved resistance has been reported. Bt produces both Cry and Vip toxins¹¹, which could be a 'natural pyramid strategy' (**Box 1**). Although four Vip families with a total of >100 toxins are known¹¹, Vip3Aa is the only Vip in commercialized transgenic crops. Because Vip3Aa19 in Bt corn and Vip3Aa20 in Bt cotton are 99.9% identical in their amino acid sequence (Y.C., B.E.T. *et al.*)³⁴, we refer to both as Vip3Aa. Vip3Aa kills some lepidopteran pests and is produced in combination with the lepidopteran-active proteins Cry1Ab, Cry1F, or both in Bt corn and with Cry1 and Cry2A toxins in Bt cotton^{34,52}.

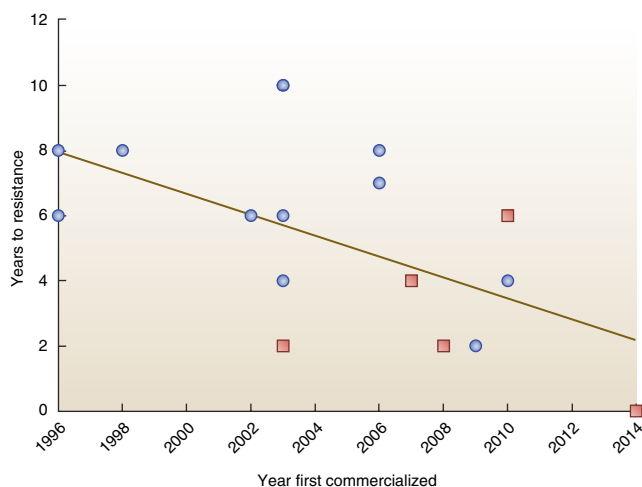


Figure 2 Increasingly rapid evolution of pest resistance to Bt crops. For the 16 cases of practical resistance to Bt crops (Table 1), the time from the first commercial planting of a Bt crop to the first evidence of resistance (years to resistance) decreased over the past two decades (linear regression: $y = -0.32x + 643$, $R^2 = 0.35$, $df = 14$, $P = 0.016$). The squares indicate five cases where cross-resistance is suspected or known to have shortened the time to resistance, including western corn rootworm resistance to eCry3.1Ab detected in the field in 2014 before plants producing this toxin were grown commercially.

In the United States, corn and cotton producing Vip3Aa were first registered in 2008 (ref. 33) and first grown commercially in 2011 and 2014, respectively (Supplementary Table 4). In the United States, Vip3Aa corn accounted for roughly 1.1% and 3.5% of all corn planted in 2011 and 2013, respectively, while Vip3Aa cotton was less than 1% of all cotton planted annually from 2014 to 2016 (Supplementary Table 4). The only type of Vip3Aa cotton grown commercially in the United States from 2014 to 2016 also produces Cry1F and Cry1Ac (Widestrike 3) (Supplementary Table 4); introduction is expected in 2017 for cotton producing Cry1Ac + Cry2Ab + Vip3Aa (Bollgard 3) and Cry1Ab + Cry2Ae + Vip3Aa (Twinlink Plus)⁵³.

In Brazil, Vip3Aa corn was approved in 2009 and has accounted for <5% of all corn¹⁰, while Vip3Aa cotton has not been grown commercially. In Australia, Bt corn is not grown and the percentage of hectares planted with cotton producing Cry1Ac + Cry2Ab + Vip3Aa increased from about 7.6% in the 2015–2016 season to >90% in the 2016–2017 season (Supplementary Table 4). Aside from Australia, the low adoption of Vip3Aa crops to date suggests their value is primarily for managing resistance, rather than providing immediate economic benefits.

The genetic potential for field-evolved resistance to Vip3Aa is demonstrated by laboratory selection with Vip3Aa that yielded 285- to >3,000-fold resistance to this toxin in five major lepidopteran pests (*H. armigera*, *H. punctigera*, *H. virescens*, *S. frugiperda*, and *Spodoptera litura*)^{9,54–57}. Based on F_2 screens conducted from 2009 to 2013, before field populations in Australia were exposed to crops producing Vip3Aa, the estimated frequency of alleles conferring resistance to Vip3Aa was higher than expected: 0.034 for *H. armigera*¹¹ and 0.010 for *H. punctigera*¹². A similar study in Brazil¹⁰ estimated the frequency of Vip3Aa resistance alleles in *S. frugiperda* as 0.0009 in 2013 to 2014.

Although Cry and Vip toxins have the same general mode of action, they have no structural homology and bind to different sites in the insect midgut, so cross-resistance between them is predicted to be

low or nil^{11,34,57}. The experimental evidence supports this hypothesis. In 11 evaluations of cross-resistance based on comparisons between related strains of five pest species, 57- to 20,000-fold resistance to Cry1Ac or Cry1F caused at most 3.2-fold cross-resistance to Vip3Aa (Supplementary Table 5). In four comparisons between related strains of *H. virescens*, 2,000-fold resistance to Vip3Aa caused up to sevenfold cross-resistance to Cry1Ab or Cry1Ac⁵⁶. Analysis of all 15 experiments mentioned above reveals weak, but statistically significant cross-resistance between Vip3Aa and Cry1Ab, Cry1Ac, or Cry1F (mean = 1.8-fold cross-resistance, $n = 15$, one-sample t -test of log-transformed resistance ratios, $P = 0.01$).

Comparisons between unrelated insect strains or populations, which can be influenced by differences in genetic background and are less rigorous than the aforementioned comparisons between related strains, also show no strong cross-resistance between Vip3Aa and Cry1Ac^{9,13,58,59}. For 142 families of *S. frugiperda* produced by single pairs of field-collected adults, survival on corn producing Vip3Aa was not correlated with survival on corn producing Cry1F⁶⁰. For *H. armigera*, a Cry2Ab-resistant strain generated by CRISPR–Cas9 knockout of the ABC transporter gene *HaABCA2* was not cross-resistant to Vip3Aa¹³. Likewise, strains of *H. armigera* and *H. punctigera* selected for >200-fold resistance to Vip3Aa had either increased susceptibility to Cry2Ab or at most 1.7-fold cross-resistance to Cry2Ab relative to unrelated susceptible strains⁹.

Pyramiding Vip3Aa and Cry toxins diminishes the risk of resistance in pest populations that are susceptible to both types of toxin³⁴ (Box 1). For example, in Australia, cotton producing Cry1Ac, Cry2Ab, and Vip3Aa was introduced when populations of *H. armigera* and *H. punctigera* were susceptible to all three toxins, which greatly lowers the risk of resistance¹².

Conversely, in the United States, some populations of *H. zea* are already resistant to the Cry1 and Cry2A toxins used in combination with Vip3Aa in Bt corn and cotton (Table 1 and Supplementary Table 4). Moreover, while plants producing Vip3Aa are gradually being more widely adopted in the United States, resistance of *H. zea* to Cry1 and Cry2A toxins is likely to become more widespread. This increases the chances that in the near future, Vip3Aa will be the only toxin in commercialized Bt plants that is effective against some populations of *H. zea* (Table 3), which markedly raises the risk of resistance. In addition, exposure of *H. zea* to Vip3Aa in both corn and cotton increases selection for resistance to this toxin. According to the US Environmental Protection Agency, cotton producing Vip3Aa alone met the high-dose standard against *H. zea* in one test, but not in another⁶¹. Corn producing Vip3Aa alone does not meet the high-dose criterion against *H. zea*^{62,63}. Therefore, if corn producing Vip3Aa is widely deployed, large refuges will be needed to delay resistance in *H. zea*.

For *S. frugiperda*, resistance to Cry1 toxins (Table 1) increases the risk of resistance to corn and cotton producing Cry1 toxins in combination with Vip3Aa⁵³. Yet, corn producing Vip3Aa meets the high-dose standard against *S. frugiperda*^{52,55,62,63}, which lowers the risk of resistance. Also, field-evolved resistance to Cry2A toxins has not been reported for *S. frugiperda*, and Cry2A toxins are effective against strains of *S. frugiperda* resistant to Cry1F or Vip3Aa^{52,53,60}. Therefore, the cotton pyramids producing Vip3Aa together with Cry2Ab or Cry2Ae could help to delay resistance. In corn, however, some current hybrids produce either Vip3Aa or Cry2Ab^{64,65}, but recently developed hybrids producing both of these toxins have not been widely adopted. Thus, in effect, Cry1-resistant populations on corn may be selected by a mosaic of single-toxin plants exposing some larvae to Vip3Aa and others to Cry2Ab (Table 3), which is considered the least durable way to deploy two toxins⁶⁶.

Next-generation insect control

RNAi offers great promise as an alternative, or complement, to Bt toxins in transgenic crops for managing insect pests^{67–69}. In RNAi, small double-stranded RNA (dsRNA) causes sequence-specific suppression of target gene expression. To achieve safe and effective pest control with RNAi, the goal is to reduce expression of genes encoding proteins that are essential to pests, but not to other organisms. Because the mode of action differs markedly between Bt toxins and RNAi, strong cross-resistance between them is not expected.

Transgenic crops under development that kill pests with a combination of Bt toxins and RNAi include corn targeting *D. v. virgifera*⁶⁸ and cotton targeting *H. armigera*⁶⁹. The corn produces two Bt toxins (Cry3Bb and Cry34/35Ab) active against *Diabrotica* species and a dsRNA transcript. The dsRNA transcript contains a 240-base pair fragment of the *D. v. virgifera* gene encoding a protein (DvSnf7) vital for intracellular protein sorting^{68,70}. In diet bioassays, the DvSnf7 dsRNA killed larvae of *D. v. virgifera* and *Diabrotica undecimpunctata howardi*, but not larvae from five other genera of beetles or insects from seven species representing three other orders⁷¹.

The LC₅₀ of DvSnf7 dsRNA was 2.7-fold higher for a Cry3Bb-resistant strain (Gass-R) than a related Cry3Bb-susceptible strain (Gass-S) of *D. v. virgifera*, indicating that resistance to Cry3Bb caused statistically significant, but weak cross-resistance to this form of RNAi⁷². Although no significant correlation occurred between susceptibility to Cry3Bb and DvSnf7 dsRNA across eight unrelated strains of *D. v. virgifera*⁷², this type of analysis is less rigorous than the comparison between related strains noted above, because differences among strains in genetic background could mask weak cross-resistance. In greenhouse bioassays, beetle emergence was higher for Gass-R than Gass-S on corn producing DvSnf7 dsRNA either alone, with Cry3Bb, or with Cry3Bb + Cry34/35Ab; but this difference was statistically significant only for DvSnf7 dsRNA with Cry3Bb⁷². In field tests where resistance to Cry3Bb was likely, DvSnf7 dsRNA reduced emergence of *D. v. virgifera* adults by about 80–95% (ref. 68). Corn producing DvSnf7 dsRNA, Cry3Bb, and Cry34/35Ab for rootworm control, along with Cry1A.105, Cry1Fa, and Cry2Ab for control of caterpillar pests is under consideration for registration by the US Environmental Protection Agency⁶⁸.

In related work, Ni *et al.*⁶⁹ developed two kinds of transgenic cotton plants (JHA and JHB) producing dsRNA that kills larvae of *H. armigera* by interfering with their juvenile hormone (JH). JH is critical for insect development, yet absent from most other organisms⁷³. JHA cotton suppresses JH acid methyltransferase, which is crucial for JH synthesis, while JHB cotton suppresses JH binding protein, which transports JH to organs⁶⁹. In bioassays with cotton leaves, mortality of *H. armigera* larvae was 57–64% for JHA cotton and 66–70% for JHB cotton⁶⁹. Mortality caused by JHA and JHB cotton did not differ significantly between a Cry1Ac-resistant strain and a related susceptible strain of *H. armigera*, indicating no cross-resistance. For cotton plants protected by both RNAi (either JHA or JHB) and a Bt toxin similar to Cry1Ac, these two traits acted independently and caused 92–93% mortality of a susceptible strain.

Results from modeling suggest that combining RNAi-mediated protection with one or more Bt toxins can delay the evolution of resistance, but the gain in durability depends on the refuge percentage⁶⁹. For example, under realistic assumptions, the predicted delay for resistance to evolve to a cotton combining both Bt and RNAi (Bt+RNAi) relative to Bt cotton is 4 years with a 5% refuge versus 14 years with a 50% refuge⁶⁹. For Bt+RNAi corn, a 5% refuge was simulated, and the increase in durability for Bt+RNAi corn relative to Bt corn was 1 to 5 years under some realistic scenarios (fig. 5b,d in ref. 68). In northern

China where Bt cotton is grown extensively, abundant non-Bt host plants provide an effective refuge estimated as 56% for *H. armigera*⁷⁴. By contrast, for many populations of *D. v. virgifera* in the midwestern United States, the refuge percentage may be close to 5%, which is the minimum under current regulations^{48,65}.

Because resistance to RNAi has not been reported yet in the laboratory or field, the assumptions in models about this adaptation remain to be tested. Nonetheless, the qualitative effects of refuge percentage were similar across a broad range of assumptions, which suggests that these trends are robust and larger refuges can greatly extend efficacy⁶⁹.

Outlook for managing resistance

When the first Bt crops were commercialized more than 20 years ago, strategies for delaying pest resistance relied entirely on theoretical projections from modeling. Since then, global monitoring has documented both remarkable successes and disappointing failures in terms of managing pest resistance to Bt crops (Tables 1 and 2). In the best cases, despite high adoption of Bt crops, pest resistance has been delayed for close to two decades, with excellent prospects for continued pest suppression. These successes include sustained susceptibility of *H. armigera* and *H. punctigera* in Australia; *H. virescens*, *O. nubilalis*, and *P. gossypiella* in the United States; and *P. gossypiella* in China (Table 2).

Conversely, for the 16 cases of practical resistance, the average time for evolution of resistance was only 5.2 years (Table 1). In four situations, practical resistance has reduced the number of Bt toxins that are available in commercialized transgenic crops and still effective against some pest populations to two, one, or none (Table 3). In India, no transgenic cotton is available now or expected to be available in the next several years to control *P. gossypiella* populations resistant to Cry1 and Cry2 toxins⁷⁵ (Table 1). Cotton producing Vip3Aa alone had minimal efficacy against this pest⁶¹, and cotton has not been engineered to produce the genetically modified Cry toxins that kill *P. gossypiella* resistant to Cry1 and Cry2 toxins (B.E.T. *et al.*)⁷⁶. In the United States, practical resistance to each of the four coleopteran-active Bt toxins produced by corn has been documented for some populations of *D. v. virgifera* (Table 1 and Supplementary Note 1). Transgenic corn protected from this pest by RNAi might be available commercially in a few years⁶⁸. However, this new trait does not meet the high-dose standard⁶⁸, and its efficacy may be short-lived unless refuge requirements are markedly increased, and the transgenic corn is used with other control measures such as crop rotation^{48,77}. We also caution that, despite the encouraging results with Bt+RNAi cotton against *H. armigera*⁶⁹, it has generally been difficult to control lepidopteran pests with RNAi⁷⁸.

Modeling results and empirical evidence show that refuge requirements must be tailored to each pest–transgenic-crop combination. For plants producing a single Bt toxin, when the high-dose standard is met and resistance is rare, refuges accounting for as little as 20% of a pest's host plants may be sufficient to delay resistance for a decade or more. Conversely, when the high-dose standard is not met and resistance is not rare, larger refuges (e.g., 50%) are needed to substantially delay pest adaptation^{21,24,25,48}. Similar principles apply to pyramids. Modeling and empirical evidence suggest that refuges of 10% can be effective for delaying resistance of pest populations that are highly susceptible to each of two or more independently acting toxins or traits in a pyramid^{12,44,79}. However, smaller refuges are risky even under optimal conditions^{79,80}, and much larger refuges are needed to substantially delay resistance if each of the toxins or traits in a pyramid is not highly effective, either inherently or because of field-evolved resistance^{34,64}.

Insects are remarkably adaptable and are expected to evolve resistance to any control method, including transgenic plants with combinations of protective traits as different as Bt toxins and RNAi. Innovations such as genetically modified Bt toxins that kill pests resistant to native Bt toxins^{81,82} and discovery of insecticidal proteins from bacteria other than Bt^{83,84} will continue to provide new tools for pest control. In turn, pests will adapt. The analyses of global patterns of field-evolved resistance to transgenic crops presented here provide empirical support for a framework to effectively manage pest resistance to current and future transgenic crops.

The primary lesson from the past two decades is that abundant refuges can delay pest resistance to transgenic crops. In practical terms, transgenic crops are most durable when used in combination with other control tactics in integrated pest management^{17,64,77,85,86}. The sustainability of transgenic crops for pest control depends largely on the will to implement this knowledge.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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COMPETING FINANCIAL INTERESTS

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

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