Surge in insect resistance to transgenic crops and prospects for sustainability

Bruce E Tabashnik & Yves Carrière

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 population[1]. 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 million[4] (**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 secreted[11]. 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 toxin[24].

Although we have previously used up to six categories of susceptibility and resistance to Bt crops[24], 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 control[27]. 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 field[27]. 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

Department of Entomology, University of Arizona, Tucson, Arizona, USA. Correspondence should be addressed to B.E.T. (brucet@cals.arizona.edu).

Received 4 May; accepted 25 August; published online 11 October 2017; doi:10.1038/nbt.3974

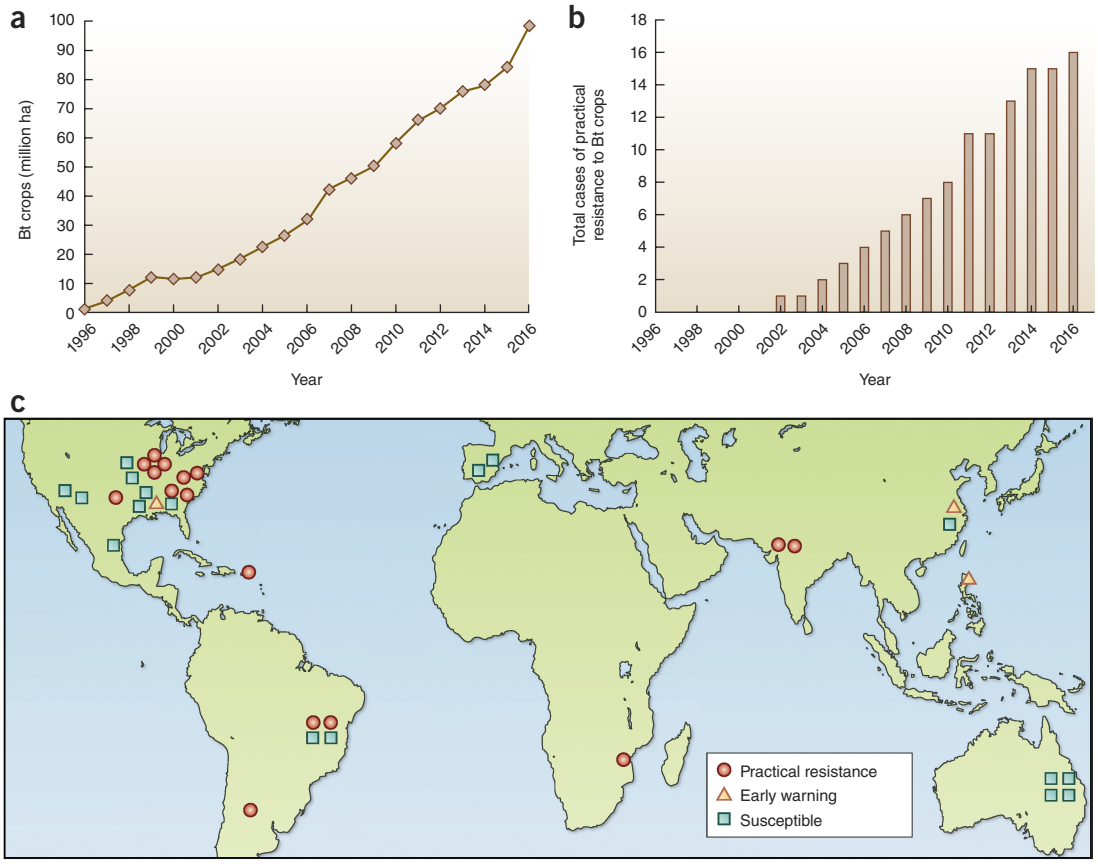


Figure 1 Global status of pest resistance to Bt crops. (**a**) Hectares planted to Bt crops each year. (**b**) Cumulative cases of field-evolved practical resistance to Bt crops. (**c**) Each symbol represents 1 of 36 cases indicating responses of one pest species in one country to one toxin in Bt corn, 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 resistant[27]. Cases fit category 2 when the monitoring data show no statistically significant decrease in susceptibility after field populations have been exposed to a Bt crop[24]. 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 plants[24]. This allows calculation of the dominance parameter *h*, which varies from 0 for completely recessive to 1 for completely dominant[134]. Values of *h* less than 0.05 satisfy the high-dose criterion[24]. Alternatively, several indirect tests measure survival of susceptible insects on Bt plants[135]. The US Environmental Protection Agency (EPA) indicates that Bt plants meet the high-dose standard if they kill at least 99.99% of susceptible insects[135]. 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 adaptation[24]. 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 resistance[24].

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 corn[16]. Also, in China, millions of farmers voluntarily increased planting of non-Bt cotton refuges, apparently to achieve short-term economic gains[124] (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 killed[64]. 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 toxins[137]. 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 China[24]. 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 cotton[46]. 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 China[74] (**Table 2**). The effective refuge percentage including non-cotton host plants was estimated as 56% for *H. armigera* in northern China[74] versus 39% for *H. zea* in Arkansas and Mississippi of the southeastern United States[140]. 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 China[74].

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 pyramids[64]. 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 cotton[124]. 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 suppression[124].

Table 1 Practical resistance to Bt crops

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

[a]First year of commercial planting of a Bt crop in the region monitored. [b]Years 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. [c]Test 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. [d]Each reference provides evidence of practical resistance, data for evaluating the high-dose criterion, or both. [e]Cross-resistance suspected or known as a factor contributing to resistance.

direct evidence of cross-resistance[31]. 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 concomittantly 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 States[41] and *C. includens* exposure to Cry1Ac soy in Brazil[42]. 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							
C. includens	Soy[d]	Cry1Ac	Brazil	2013[d]	1[d]	No	42,109
D. grandiosella	Corn	Cry1Ab	USA	1999	6	?	110
H. armigera	Cotton	Cry1Ac	Australia	1996	19	No	111–113
H. armigera	Cotton	Cry2Ab	Australia	2004	11	Yes	12,114
H. punctigera	Cotton	Cry1Ac	Australia	1996	19	?	111
H. punctigera	Cotton	Cry2Ab	Australia	2004	11	Yes	12,114
H. virescens	Cotton	Cry1Ac	Mexico	1996	11	?	115
H. virescens	Cotton	Cry1Ac	USA	1996	11	Yes	98,115,116
H. virescens	Cotton	Cry2Ab	USA	2003	2	Yes	32,117–119
O. nubilalis	Corn	Cry1Ab	Spain	1998	15	?	120,121
O. nubilalis	Corn	Cry1Ab	USA	1996	15	No	43,122
O. nubilalis	Corn	Cry1Fa	USA	2003	8	No	41,123
P. gossypiella	Cotton	Cry1Ac	China	2000	15	Yes	124
P. gossypiella	Cotton	Cry1Ac	USA	1996	12	Yes	17
P. gossypiella	Cotton	Cry2Ab	USA	2003	5	Yes	17,125,126
S. frugiperda	Corn	Vip3Aa	Brazil	2010	5	Yes	10
S. nonagroides	Corn	Cry1Ab	Spain	1998	15	Yes	127
Early warning of resistance[e]							
D. saccharalis	Corn	Cry1Ab	USA	1999	10	No	24,128,129
H. armigera	Cotton	Cry1Ac	China	1997	16	No	74
O. furnacalis	Corn	Cry1Ab	Philippines	2003	6	No	130,131

[a]First year of commercial planting of a Bt crop in the region monitored. [b]For 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. [c]Test 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. [d]The 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. [e]The 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 corn[43]. However, this evaluation of the high-dose criterion is based on survival after only 15 days, which could overestimate dominance[43].

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 equivalent[44]. 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
D. v. virgifera	Corn	USA	Cry3Bb, eCry3.1Ab, mCry3A, Cry34/35Ab	None[b]
P. gossypiella	Cotton	India	Cry1Ac, Cry2Ab	None[b]
H. zea	Corn and cotton	USA	Cry1Ab, Cry1Ac, Cry1A.105, Cry2Ab	Vip3Aa
S. frugiperda	Corn	Brazil	Cry1Ab, Cry1F	Cry2Ab, Vip3Aa[c]

[a]See **Table 1**. [b]In 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*. [c]The 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 toxins[11], which could be a 'natural pyramid strategy' (**Box 1**). Although four Vip families with a total of >100 toxins are known[11], Vip3Aa is the only Vip in commercialized transgenic crops. Because Vip3Aa19 in Bt corn and Vip3 Aa20 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].

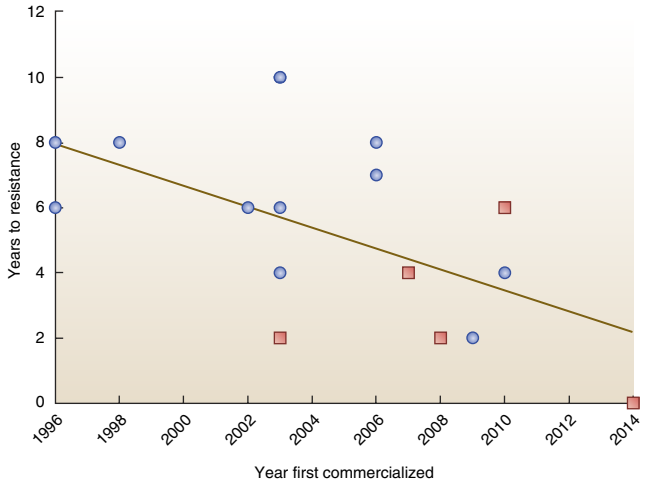


Figure 2 Increasingly rapid evolution of pest resistance to Bt crops. For the 16 cases of practical resistance to Bt crops (**Table 1**), the time from the first commercial planting of a Bt crop to the first evidence of resistance (years to resistance) decreased over the past two decades (linear regression: $y = -0.32x + 643$, $R^2 = 0.35$, 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 corn[10], 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 Brazil[10] 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 Vip3A (**Supplementary Table 5**). In four comparisons between related strains of *H. virescens*, 2,000-fold resistance to Vip3A caused up to sevenfold cross-resistance to Cry1Ab or Cry1Ac[56]. 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 Cry1F[60]. For *H. armigera*, a Cry2Ab-resistant strain generated by CRISPR–Cas9 knockout of the ABC transporter gene *HaABCA2* was not cross-resistant to Vip3Aa[13]. 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 strains[9].

Pyramiding Vip3Aa and Cry toxins diminishes the risk of resistance in pest populations that are susceptible to both types of toxin[34] (**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 resistance[12].

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 another[61]. Corn producing Vip3Aa alone does not meet the high-dose criterion against *H. zea*[62,63]. Therefore, if corn producing Vip3A 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 Vip3Aa[53]. 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 toxins[66].

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 orders[71].

The LC_{50} of DvSn7 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 RNAi[72]. Although no significant correlation occurred between susceptibility to Cry3Bb and DvSn7 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 DvSn7 dsRNA either alone, with Cry3Bb, or with Cry3Bb + Cry34/35Ab; but this difference was statistically significant only for DvSn7 dsRNA with Cry3Bb[72]. In field tests where resistance to Cry3Bb was likely, DvSn7 dsRNA reduced emergence of *D. v. virgifera* adults by about 80–95% (ref. 68). Corn producing DvSn7 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 Agency[68].

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 organisms[73]. JHA cotton suppresses JH acid methyltransferase, which is crucial for JH synthesis, while JHB cotton suppresses JH binding protein, which transports JH to organs[69]. In bioassays with cotton leaves, mortality of *H. armigera* larvae was 57–64% for JHA cotton and 66–70% for JHB cotton[69]. 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 percentage[69]. 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% refuge[69]. 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 efficacy[69].

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 toxins[75] (**Table 1**). Cotton producing Vip3Aa alone had minimal efficacy against this pest[61], 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 years[68]. However, this new trait does not meet the high-dose standard[68], 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 RNAi[78].

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.

ACKNOWLEDGMENTS

This work was supported by USDA Biotechnology Risk Assessment Grant 2014-33522-22214.

COMPETING FINANCIAL INTERESTS

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

1. Godfray, H.C.J. *et al.* Food security: the challenge of feeding 9 billion people. *Science* **327**, 812–818 (2010).
2. Sanahuja, G., Banakar, R., Twyman, R.M., Capell, T. & Christou, P. *Bacillus thuringiensis*: a century of research, development and commercial applications. *Plant Biotechnol. J.* **9**, 283–300 (2011).
3. Pardo-López, L., Soberón, M. & Bravo, A. *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. *FEMS Microbiol. Rev.* **37**, 3–22 (2013).
4. James, C. Global status of commercialized of biotech/GM crops: 2016. *ISAAA Briefs* **52** (ISAAA, Ithaca, NY, 2016).
5. National Academies of Sciences, Engineering, and Medicine. *Genetically Engineered Crops: Experiences and Prospects* (National Academies Press, Washington, DC, 2016).
6. Mendelsohn, M., Kough, J., Vaituzis, Z. & Matthews, K. Are Bt crops safe? *Nat. Biotechnol.* **21**, 1003–1009 (2003).
7. Comas, C., Lumbierres, B., Pons, X. & Albajes, R. No effects of *Bacillus thuringiensis* maize on nontarget organisms in the field in southern Europe: a meta-analysis of 26 arthropod taxa. *Transgenic Res.* **23**, 135–143 (2014).
8. Nicolia, A., Manzo, A., Veronesi, F. & Rosellini, D. An overview of the last 10 years of genetically engineered crop safety research. *Crit. Rev. Biotechnol.* **34**, 77–88 (2014).
9. Mahon, R.J., Downes, S.J. & James, B. Vip3A resistance alleles exist at high levels in Australian targets before release of cotton expressing this toxin. *PLoS One* **7**, e39192 (2012).
10. Bernardi, O. *et al.* Frequency of resistance to Vip3Aa20 toxin from *Bacillus thuringiensis* in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations in Brazil. *Crop Prot.* **76**, 7–14 (2015).
11. Chakroun, M., Banyuls, N., Bel, Y., Escriche, B. & Ferré, J. Bacterial vegetative insecticidal proteins from entomopathogenic bacteria. *Microbiol. Mol. Biol. Rev.* **80**, 329–350 (2016).
12. Downes, S., Walsh, T. & Tay, W.T. Bt resistance in Australian insect pest species. *Curr. Opin. Insect Sci.* **15**, 78–83 (2016).
13. Wei, Y., Wu, S., Yang, Y. & Wu, Y. Baseline susceptibility of field populations of *Helicoverpa armigera* to *Bacillus thuringiensis* Vip3Aa toxin and lack of cross-resistance between Vip3Aa and Cry toxins. *Toxins (Basel)* **9**, 127 (2017).
14. Wu, K.M., Lu, Y.H., Feng, H.Q., Jiang, Y.Y. & Zhao, J.Z. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. *Science* **321**, 1676–1678 (2008).
15. Carpenter, J.E. Peer-reviewed surveys indicate positive impact of commercialized GM crops. *Nat. Biotechnol.* **28**, 319–321 (2010).
16. Hutchison, W.D. *et al.* Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science* **330**, 222–225 (2010).
17. Tabashnik, B.E. *et al.* Suppressing resistance to Bt cotton with sterile insect releases. *Nat. Biotechnol.* **28**, 1304–1307 (2010).
18. Edgerton, M.D. *et al.* Transgenic insect resistance traits increase corn yield and yield stability. *Nat. Biotechnol.* **30**, 493–496 (2012).
19. Kathage, J. & Qaim, M. Economic impacts and impact dynamics of Bt (*Bacillus thuringiensis*) cotton in India. *Proc. Natl. Acad. Sci. USA* **109**, 11652–11656 (2012).
20. Lu, Y., Wu, K., Jiang, Y., Guo, Y. & Desneux, N. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* **487**, 362–365 (2012).
21. Gould, F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu. Rev. Entomol.* **43**, 701–726 (1998).
22. Heckel, D.G. Ecology. Insecticide resistance after *Silent Spring*. *Science* **337**, 1612–1614 (2012).
23. Wu, Y. Detection and mechanisms of resistance evolved in insects to Cry toxins from *Bacillus thuringiensis*. *Adv. Insect Physiol.* **47**, 297–342 (2014).
24. Tabashnik, B.E., Brévault, T. & Carrière, Y. Insect resistance to Bt crops: lessons from the first billion acres. *Nat. Biotechnol.* **31**, 510–521 (2013).
25. Tabashnik, B.E., Gassmann, A.J., Crowder, D.W. & Carriére, Y. Insect resistance to Bt crops: evidence versus theory. *Nat. Biotechnol.* **26**, 199–202 (2008).
26. Huang, F., Andow, D.A. & Buschman, L. Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. *Entomol. Exp. Appl.* **140**, 1–16 (2011).
27. Tabashnik, B.E., Mota-Sanchez, D., Whalon, M.E., Hollingworth, R.M. & Carrière, Y. Defining terms for proactive management of resistance to Bt crops and pesticides. *J. Econ. Entomol.* **107**, 496–507 (2014).
28. Andow, D.A. *et al.* Early detection and mitigation of resistance to Bt maize by western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **109**, 1–12 (2016).
29. Tabashnik, B.E. Tips for battling billion-dollar beetles. *Science* **354**, 552–553 (2016).
30. Jakka, S.R.K., Shrestha, R.B. & Gassmann, A.J. Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). *Sci. Rep.* **6**, 27860 (2016).
31. Zukoff, S.N. *et al.* Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. *J. Econ. Entomol.* **109**, 1387–1398 (2016).
32. Ali, M.I. & Luttrell, R.G. Susceptibility of bollworm and tobacco budworm (Lepidoptera: Noctuidae) to Cry2Ab2 insecticidal protein. *J. Econ. Entomol.* **100**, 921–931 (2007).
33. Tabashnik, B.E., Van Rensburg, J.B.J. & Carrière, Y. Field-evolved insect resistance to Bt crops: definition, theory, and data. *J. Econ. Entomol.* **102**, 2011–2025 (2009).
34. Carrière, Y., Crickmore, N. & Tabashnik, B.E. Optimizing pyramided transgenic Bt crops for sustainable pest management. *Nat. Biotechnol.* **33**, 161–168 (2015).
35. Wei, J. *et al.* Cross-resistance and interactions between Bt toxins Cry1Ac and Cry2Ab against the cotton bollworm. *Sci. Rep.* **5**, 7714 (2015).
36. Welch, K.L. *et al.* Cross-resistance to toxins used in pyramided Bt crops and resistance to Bt sprays in *Helicoverpa zea*. *J. Invertebr. Pathol.* **132**, 149–156 (2015).
37. Liu, L. *et al.* Resistance to *Bacillus thuringiensis* toxin Cry2Ab and survival on single-toxin and pyramided cotton in cotton bollworm from China. *Evol. Appl.* **10**, 170–179 (2017).
38. Farias, J.R. *et al.* Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. *Crop Prot.* **64**, 150–158 (2014).
39. Monnerat, R. *et al.* Evidence of field-evolved resistance of *Spodoptera frugiperda* to Bt corn expressing Cry1F in Brazil that is still sensitive to modified Bt toxins. *PLoS One* **10**, e0119544 (2015).
40. Omoto, C. *et al.* Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. *Pest Manag. Sci.* **72**, 1727–1736 (2016).
41. Siegfried, B.D. *et al.* Estimating the frequency of Cry1F resistance in field populations of the European corn borer (Lepidoptera: Crambidae). *Pest Manag. Sci.* **70**, 725–733 (2014).
42. Yano, S.A.C. *et al.* High susceptibility and low resistance allele frequency of *Chrysodeixis includens* (Lepidoptera: Noctuidae) field populations to Cry1Ac in Brazil. *Pest Manag. Sci.* **72**, 1578–1584 (2016).
43. Crespo, A.L.B. *et al.* On-plant survival and inheritance of resistance to Cry1Ab toxin from *Bacillus thuringiensis* in a field-derived strain of European corn borer, *Ostrinia nubilalis*. *Pest Manag. Sci.* **65**, 1071–1081 (2009).
44. Downes, S. *et al.* Adaptive management of pest resistance by *Helicoverpa* species (Noctuidae) in Australia to the Cry2Ab Bt toxin in Bollgard II® cotton. *Evol. Appl.* **3**, 574–584 (2010).
45. US Environmental Protection Agency. Biopesticides registration action document. *Bacillus thuringiensis* plant-incorporated protectants. http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm (2001).
46. US Environmental Protection Agency. *Pesticide News Story: EPA Approves Natural Refuge for Insect Resistance Management in Bollgard II Cotton.* http://www.epa.gov/oppfead1/cb/csb_page/updates/2007/bollgard-cotton.htm (2007).

47. Storer, N.P., Kubiszak, M.E., Ed King, J., Thompson, G.D. & Santos, A.C. Status of resistance to Bt maize in *Spodoptera frugiperda*: lessons from Puerto Rico. *J. Invertebr. Pathol.* **110**, 294–300 (2012).
48. Tabashnik, B.E. & Gould, F. Delaying corn rootworm resistance to Bt corn. *J. Econ. Entomol.* **105**, 767–776 (2012).
49. Van den Berg, J., Hilbeck, A. & Bøhn, T. Pest resistance to Cry1Ab Bt maize: Field resistance, contributing factors and lessons from South Africa. *Crop Prot.* **54**, 154–160 (2013).
50. Mohan, K.S., Ravi, K.C., Suresh, P.J., Sumerford, D. & Head, G.P. Field resistance to the *Bacillus thuringiensis* protein Cry1Ac expressed in Bollgard(®) hybrid cotton in pink bollworm, *Pectinophora gossypiella* (Saunders), populations in India. *Pest Manag. Sci.* **72**, 738–746 (2016).
51. Argentinian Association of Seed Companies. *Preguntas y Respuestas Sobre Cultivos Bt y Manejo de Resistencia de Insectos*. First edition. http://fundacion-antama.org/preguntas-y-respuestas-sobre-cultivos-bt-y-manejo-de-resistencia-a-insectos/ (2016).
52. Horikoshi, R.J. *et al.* Effective dominance of resistance of *Spodoptera frugiperda* to Bt maize and cotton varieties: implications for resistance management. *Sci. Rep.* **6**, 34864 (2016).
53. Yang, F., Kerns, D.L., Head, G., Brown, S. & Huang, F. Susceptibility of Cry1F-maize resistant, heterozygous, and susceptible *Spodoptera frugiperda* to Bt proteins used in the transgenic cotton. *Crop Prot.* **98**, 128–135 (2017).
54. Barkthade, U.P. & Thakare, A.S. Protease mediated resistance mechanism to Cry1C and Vip3A in *Spodoptera litura*. *Egypt. Acad. J. Biol. Sci.* **3**, 43–50 (2010
55. Bernardi, O. *et al.* Selection and characterization of resistance to the Vip3Aa20 protein from *Bacillus thuringiensis* in *Spodoptera frugiperda*. *Pest Manag. Sci.* **72**, 1794–1802 (2016).
56. Pickett, B.R., Gulzar, A., Ferré, J. & Wright, D.J. *Bacillus thuringiensis* Vip3A toxin resistance in *Heliothis virescens* (Lepidoptera: Noctuidae). *Appl. Environ. Microbiol.* **83**, e03506 (2017).
57. Chakroun, M. *et al.* Characterization of the resistance to Vip3Aa in *Helicoverpa armigera* from Australia and the role of midgut processing and receptor binding. *Sci. Rep.* **6**, 24311 (2016).
58. Jackson, R.E., Marcus, M.A., Gould, F., Bradley, J.R. Jr. & Van Duyn, J.W. Cross-resistance responses of CrylAc-selected *Heliothis virescens* (Lepidoptera: Noctuidae) to the *Bacillus thuringiensis* protein Vip3Aa. *J. Econ. Entomol.* **100**, 180–186 (2007).
59. An, J. *et al.* Vip3Aa tolerance response of *Helicoverpa armigera* populations from a Cry1Ac cotton planting region. *J. Econ. Entomol.* **103**, 2169–2173 (2010).
60. Huang, F. *et al.* Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt maize. *PLoS One* **9**, e112958 (2014).
61. US Environmental Protection Agency. *Bacillus thuringiensis* modified Cry1Ab (SYN-IR67B–1) and Vip3Aa19 (SYN-IR102–7) insecticidal proteins and the genetic material necessary for their production in COT102 X COT67B cotton. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006529_12-Aug-08.pdf (2008).
62. US Environmental Protection Agency. *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (*via* elements of vector pNOV1300) in event MIR162 maize. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006599_3-Apr-09.pdf (2009).
63. Burkness, E.C., Dively, G., Patton, T., Morey, A.C. & Hutchison, W.D. Novel Vip3A *Bacillus thuringiensis* (Bt) maize approaches high-dose efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae) under field conditions: Implications for resistance management. *GM Crops* **1**, 337–343 (2010).
64. Carrière, Y., Fabrick, J.A. & Tabashnik, B.E. Can pyramids and seed mixtures delay resistance to Bt crops? *Trends Biotechnol.* **34**, 291–302 (2016).
65. Difonzo, C. Handy Bt trait table for U.S. corn production. Updated 15 March, 2017. http://msue.anr.msu.edu/news/handy_bt_trait_table (2017).
66. Roush, R.T. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? *Pestic. Sci.* **51**, 328–334 (1997).
67. Kim, Y.H., Soumaila Issa, M., Cooper, A.M.W. & Zhu, K.Y. RNA interference: Applications and advances in insect toxicology and insect pest management. *Pestic. Biochem. Physiol.* **120**, 109–117 (2015).
68. Head, G.P. *et al.* Evaluation of SmartStax and SmartStaxPRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. *Pest Manag. Sci.* **73**, 1883–1899 (2017).
69. Ni, M. *et al.* Next-generation transgenic cotton: pyramiding RNAi and Bt counters insect resistance. *Plant Biotechnol. J.* **15**, 1204–1213 (2017).
70. Bolognesi, R. *et al.* Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *PLoS One* **7**, e47534 (2012).
71. Bachman, P.M. *et al.* Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte). *Transgenic Res.* **22**, 1207–1222 (2013).
72. Moar, W. *et al.* Cry3Bb1-resistant western corn rootworm, *Diabrotica virgifera virgifera* (LeConte) does not exhibit cross-resistance to DvSnf7 dsRNA. *PLoS One* **12**, e0169175 (2017).
73. Bellés, X., Martín, D. & Piulachs, M.D. The mevalonate pathway and the synthesis of juvenile hormone in insects. *Annu. Rev. Entomol.* **50**, 181–199 (2005).
74. Jin, L. *et al.* Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. *Nat. Biotechnol.* **33**, 169–174 (2015).
75. Kranthi, K.R. Pink bollworm strikes Bt-cotton. *Cotton Statistics & News*. http://www.cicr.org.in/pdf/Kranthi_art/Pinkbollworm.pdf (2015).
76. Tabashnik, B.E. *et al.* Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. *PLoS One* **8**, e80496 (2013).
77. US Environmental Protection Agency. Framework to delay corn rootworm resistance. https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/framework-delay-corn-rootworm-resistance#q1 (2016).
78. Shukla, J.N. *et al.* Reduced stability and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects. *RNA Biol.* **13**, 656–669 (2016).
79. Roush, R.T. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? *Philos. Trans. R. Soc. Lond., B* **353**, 1777–1786 (1998).
80. Alyokhin, A. Scant evidence supports EPA's pyramided Bt corn refuge size of 5%. *Nat. Biotechnol.* **29**, 577–578 (2011).
81. Tabashnik, B.E. *et al.* Efficacy of genetically modified Bt toxins against insects with different genetic mechanisms of resistance. *Nat. Biotechnol.* **29**, 1128–1131 (2011).
82. Badran, A.H. *et al.* Continuous evolution of *Bacillus thuringiensis* toxins overcomes insect resistance. *Nature* **533**, 58–63 (2016).
83. Schellenberger, U. *et al.* A selective insecticidal protein from *Pseudomonas* for controlling corn rootworms. *Science* **354**, 634–637 (2016).
84. Sampson, K. *et al.* Discovery of a novel insecticidal protein from *Chromobacterium piscinae*, with activity against Western Corn Rootworm, *Diabrotica virgifera virgifera*. *J. Invertebr. Pathol.* **142**, 34–43 (2017).
85. Bates, S.L., Zhao, J.Z., Roush, R.T. & Shelton, A.M. Insect resistance management in GM crops: past, present and future. *Nat. Biotechnol.* **23**, 57–62 (2005).
86. Gassmann, A.J., Petzold-Maxwell, J.L., Keweshan, R.S. & Dunbar, M.W. Field-evolved resistance to Bt maize by western corn rootworm. *PLoS One* **6**, e22629 (2011).
87. Van Rensburg, J.B.J. First report of field resistance by stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *S. Afr. J. Plant Soil* **24**, 147–151 (2007).
88. Van Rensburg, J.B.J. Evaluation of Bt-transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *S. Afr. Tydskr. Plant Ground* **16**, 38–43 (1999).
89. Blanco, C.A. *et al.* Current situation of pests targeted by Bt crops in Latin America. *Curr. Opin. Insect Sci.* **15**, 131–138 (2016).
90. Grimi, D.A., Ocampo, F., Martinelli, S. & Head, G.P. Detection and characterization of *Diatraea saccharalis* resistant to Cry1A.105 protein in a population of northeast San Luis province in Argentina. Congreso Argentino de Entomología; Posadas, Misiones, Argentina (2015).
91. Murúa, M.G. *et al.* Situation and perspectives of insect resistance management (IRM) in Bt crops in Argentina. XXV International Congress of Entomology, Orlando, Florida (2016).
92. Gassmann, A.J. *et al.* Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae): Root injury in the field and larval survival in plant-based bioassays. *J. Econ. Entomol.* **109**, 1872–1880 (2016).
93. Ludwick, D.C. *et al.* Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. *J. Appl. Entomol.* **141**, 28–40 (2017).
94. Gassmann, A.J. *et al.* Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. *Proc. Natl. Acad. Sci. USA* **111**, 5141–5146 (2014).
95. Dively, G.P., Venugopal, P.D. & Finkenbinder, C. Field-evolved resistance in corn earworm to Cry proteins expressed by transgenic sweet corn. *PLoS One* **11**, e0169115 (2016).
96. Storer, N.P., Van Duyn, J.W. & Kennedy, G.G. Life history traits of *Helicoverpa zea* (Lepidoptera: Noctuidae) on non-Bt and Bt transgenic corn hybrids in eastern North Carolina. *J. Econ. Entomol.* **94**, 1268–1279 (2001).
97. Luttrell, R.G. *et al.* in *Proceedings of the 2004 Beltwide Cotton Conferences*, San Antonio, Texas, January 5–9, 2004 (ed. Richter, D.A.) 1373–1383 (National Cotton Council of America, Memphis, TN; 2004).
98. Ali, M.I., Luttrell, R.G. & Young, S.Y. III Susceptibilities of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) populations to Cry1Ac insecticidal protein. *J. Econ. Entomol.* **99**, 164–175 (2006).
99. Unglesbee, E. Worm woes. *Progressive Farmer* Winter 2017. http://dtnpf-digital.com/article/Worm+Woes/2664522/368510/article.html.
100. Dhurua, S. & Gujar, G.T. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Manag. Sci.* **67**, 898–903 (2011).
101. Fabrick, J.A. *et al.* Alternative splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to Bt cotton in India. *PLoS One* **9**, e97900 (2014).
102. Nair, R., Kamath, S.P., Mohan, K.S., Head, G. & Sumerford, D.V. Inheritance of field-relevant resistance to the *Bacillus thuringiensis* protein Cry1Ac in *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) collected from India. *Pest Manag. Sci.* **72**, 558–565 (2016).

103. Ostrem, J.S. *et al.* Monitoring susceptibility of western bean cutworm (Lepidoptera: Noctuidae) field populations to *Bacillus thuringiensis* Cry1F protein. *J. Econ. Entomol.* **109**, 847–853 (2016).
104. DiFonzo, C. *et al.* An open letter to the Seed Industry regarding the efficacy of Cry1F against western bean cutworm. *Cornell Field Crop News*, October 2016. http://blogs.cornell.edu/ccefieldcropnews/2016/10/04/an-open-letter-to-the-seed-industry-regarding-the-efficacy-of-cry1f-bt-against-western-bean-cutworm-october-2016/.
105. Peterson, J. *et al.* Begin scouting for western bean cutworm eggs in corn. *Cropwatch*. http://cropwatch.unl.edu/2016/begin-scouting-western-bean-cutworm-eggs-corn (July 8, 2016).
106. Eichenseer, H., Strohbehn, R. & Burks, J. Frequency and severity of western bean cutworm (Lepidoptera: Noctuidae) ear damage in transgenic corn hybrids expressing different *Bacillus thuringiensis* Cry toxins. *J. Econ. Entomol.* **101**, 555–563 (2008).
107. Farias, J.R. *et al.* Dominance of Cry1F resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on TC1507 *Bt* maize in Brazil. *Pest Manag. Sci.* **72**, 974–979 (2016).
108. Storer, N.P. *et al.* Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. *J. Econ. Entomol.* **103**, 1031–1038 (2010).
109. Bernardi, O. *et al.* Assessment of the high-dose concept and level of control provided by MON 87701 × MON 89788 soybean against *Anticarsia gemmatalis* and *Pseudoplusia includens* (Lepidoptera: Noctuidae) in Brazil. *Pest Manag. Sci.* **68**, 1083–1091 (2012).
110. Huang, F. *et al.* Frequency of alleles conferring resistance to *Bacillus thuringiensis* maize in Louisiana populations of southwestern corn borer (Lepidoptera: Crambidae). *Entomol. Exp. Appl.* **122**, 53–58 (2007).
111. Downes, S. 2015–16 End of Season Resistance Monitoring Report. http://www.cottoninfo.com.au/publications/end-season-resistance-monitoring-conventional-insecticide-testing-report.
112. Bird, L.J. & Akhurst, R.J. Relative fitness of Cry1A-resistant and -susceptible *Helicoverpa armigera* (Lepidoptera: Noctuidae) on conventional and transgenic cotton. *J. Econ. Entomol.* **97**, 1699–1709 (2004).
113. Bird, L.J. & Akhurst, R.J. Fitness of Cry1A-resistant and -susceptible *Helicoverpa armigera* (Lepidoptera: Noctuidae) on transgenic cotton with reduced levels of Cry1Ac. *J. Econ. Entomol.* **98**, 1311–1319 (2005).
114. Walsh, T.K. *et al.* Dual Cry2Ab and Vip3A resistant strains of *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae); testing linkage between loci and monitoring of allele frequencies. *J. Econ. Entomol.* **107**, 1610–1617 (2014).
115. Blanco, C.A. *et al. Bacillus thuringiensis* Cry1Ac resistance frequency in tobacco budworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **102**, 381–387 (2009).
116. Gould, F. *et al.* Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. *Proc. Natl. Acad. Sci. USA* **94**, 3519–3523 (1997).
117. Adamczyk, J.J. Jr., Adams, L.C. & Hardee, D.D. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. *J. Econ. Entomol.* **94**, 1589–1593 (2001).
118. US Environmental Protection Agency. *Bacillus thuringiensis* Cry2Ab2 protein and its genetic material necessary for its production in cotton. Amended. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006487_4-Mar-03.pdf (2003).
119. Sivasupramaniam, S. *et al.* Toxicity and characterization of cotton expressing *Bacillus thuringiensis* Cry1Ac and Cry2Ab2 proteins for control of lepidopteran pests. *J. Econ. Entomol.* **101**, 546–554 (2008).
120. Farinós, G.P., de la Poza, M., Hernández-Crespo, P.F., Ortego, F. & Castañera, P. Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt maize cultivation in Spain. *Entomol. Exp. Appl.* **110**, 23–30 (2004).
121. European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms. Scientific opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2013. *EFSA J.* **13**, 4039 (2015).
122. Siegfried, B.D. & Hellmich, R.L. Understanding successful resistance management: the European corn borer and Bt corn in the United States. *GM Crops Food* **3**, 184–193 (2012).
123. Pereira, E.J.G., Storer, N.P. & Siegfried, B.D. Inheritance of Cry1F resistance in laboratory-selected European corn borer and its survival on transgenic corn expressing the Cry1F toxin. *Bull. Entomol. Res.* **98**, 621–629 (2008).
124. Wan, P. *et al.* Hybridizing transgenic Bt cotton with non-Bt cotton counters resistance in pink bollworm. *Proc. Natl. Acad. Sci. USA* **114**, 5413–5418 (2017).
125. Tabashnik, B.E. *et al.* Asymmetrical cross-resistance between *Bacillus thuringiensis* toxins Cry1Ac and Cry2Ab in pink bollworm. *Proc. Natl. Acad. Sci. USA* **106**, 11889–11894 (2009).
126. Fabrick, J.A. *et al.* Multi-toxin resistance enables pink bollworm survival on pyramided Bt cotton. *Sci. Rep.* **5**, 16554 (2015).
127. Castañera, P., Farinós, G.P., Ortego, F. & Andow, D.A. Sixteen years of Bt maize in the EU hotspot: Why has resistance not evolved? *PLoS One* **11**, e0154200 (2016).
128. Huang, F. *et al.* Extended monitoring of resistance to *Bacillus thuringiensis* Cry1Ab maize in *Diatraea saccharalis* (Lepidoptera: Crambidae). *GM Crops Food* **3**, 245–254 (2012).
129. Ghimire, M.N., Huang, F., Leonard, R., Head, G.P. & Yang, Y. Susceptibility of Cry1Ab- susceptible and -resistant sugarcane borer to transgenic corn plants containing single or pyramided *Bacillus thuringiensis* genes. *Crop Prot.* **30**, 74–81 (2011).
130. Alcantara, E., Estrada, A., Alpuerto, V. & Head, G. Monitoring Cry1Ab susceptibility in Asian corn borer (Lepidoptera: Crambidae) on Bt corn in the Philippines. *Crop Prot.* **30**, 554–559 (2011).
131. Zhang, T. *et al.* Inheritance patterns, dominance and cross-resistance of Cry1Ab- and Cry1Ac-selected *Ostrinia furnacalis* (Guenée). *Toxins (Basel)* **6**, 2694–2707 (2014).
132. Liu, Y.-B. & Tabashnik, B.E. Experimental evidence that refuges delay insect adaptation to *Bacillus thuringiensis*. *Proc. R. Soc. Lond. B* **264**, 605–610 (1997).
133. Carrière, Y. *et al.* Sources, sinks, and zone of influence of refuges for managing insect resistance to Bt crops. *Ecol. Appl.* **14**, 1615–1623 (2004).
134. Liu, Y. & Tabashnik, B.E. Inheritance of resistance to *Bacillus thuringiensis* toxin Cry1C in the diamondback moth. *Appl. Environ. Microbiol.* **63**, 2218–2223 (1997).
135. US Environmental Protection Agency. *Final Report of the Subpanel on Bacillus thuringiensis (Bt) Plant-Pesticides and Resistance Management, February, 1998.* https://archive.epa.gov/scipoly/sap/meetings/web/pdf/finalfeb.pdf.
136. Carrière, Y., Crowder, D.W. & Tabashnik, B.E. Evolutionary ecology of insect adaptation to Bt crops. *Evol. Appl.* **3**, 561–573 (2010).
137. Bourguet, D. *et al.* Heterogeneity of selection and the evolution of resistance. *Trends Ecol. Evol.* **28**, 110–118 (2013).
138. Cui, J., Luo, J., Van Der Werf, W., Ma, Y. & Xia, J. Effect of pyramiding Bt and CpTI genes on resistance of cotton to *Helicoverpa armigera* (Lepidoptera: Noctuidae) under laboratory and field conditions. *J. Econ. Entomol.* **104**, 673–684 (2011).
139. Zhao, J.-Z. *et al.* Concurrent use of transgenic plants expressing a single and two *Bacillus thuringiensis* genes speeds insect adaptation to pyramided plants. *Proc. Natl. Acad. Sci. USA* **102**, 8426–8430 (2005).
140. Gustafson, D.I., Head, G.P. & Caprio, M.A. Modeling the impact of alternative hosts on *Helicoverpa zea* adaptation to Bollgard cotton. *J. Econ. Entomol.* **99**, 2116–2124 (2006).

Supplementary Information

Surge in Insect Resistance to Transgenic Crops and Prospects for Sustainability

Bruce E Tabashnik & Yves Carrière

Department of Entomology, University of Arizona, Tucson, Arizona, USA.

Address correspondence to Bruce Tabashnik: brucet@cals.arizona.edu.

This Supplementary Information contains:

Nature Biotechnology: doi:10.1038/nbt.3974

Supplementary Methods

Distinguishing between incomplete resistance and heterogeneous populations: Calculating the minimum percentage of resistant individuals in a potentially heterogeneous population when some susceptible individuals survive in a bioassay

Ideally, a diagnostic test with either a plant or diet bioassay kills 100% of susceptible individuals, but 0% or close to 0% of resistant individuals. If so, the percentage of resistant individuals is readily calculated as the survival in the diagnostic test adjusted for control mortality on either non-Bt plants or untreated diet.

Here we provide a formula to calculate the minimum percentage of resistant individuals in a potentially heterogeneous population when the survival of susceptible individuals tested separately using the same bioassay is substantially greater than zero. We also apply this formula to the data from Minnesota of Ludwick *et al.*[93] for western corn rootworm seedling and greenhouse bioassays with Cry34/35Ab corn, and use the conceptual framework provided by the formula to clarify the evidence for resistance to Cry34/35Ab in both Iowa[92] and Minnesota[93].

We begin with an equation for total survival (V_T) (adjusted for control mortality) observed in a bioassay of a potentially heterogeneous population:

$V_T = (P_S \text{ X } V_S) + (P_R \text{ X } V_R)$

where P_S and P_R are the proportion of phenotypically susceptible and resistant individuals in the tested population, respectively, and V_S and V_R are the survival (adjusted for control mortality) of the susceptible and resistant individuals, respectively. (Note: Values of $V_R < 1$ indicate incomplete resistance, whereas $P_R < 1$ indicates the population includes some susceptible individuals.)

To estimate the minimum percentage of resistant individuals, we assume the survival of resistant individuals (V_R) = 1. This assumpiton maximizes the contribution of each resistant individual to total survival (V_T), which yields the minimum proportion of resistant individuals needed to achieve any given value of V_T.

Because $P_S + P_R = 1$, we can substitute $1- P_R$ for P_S. By substitution and rearrangement we get

$P_R = (V_T - V_S)/(1- V_S)$

which enables calculation of P_R from any values of V_T and V_S.

From the greenhouse bioassays of Ludwick *et al.*[93], $V_T = 0.63$ and $V_S = 0.25$, which yields $P_R = 0.51$. From their seedling bioassays, $V_T = 0.68$ and $V_T = 0.27$, which yields $P_R = 0.56$. Thus, in the potentially heterogeneous population tested, the results of the greenhouse and seedling bioassays show that at least 51% and 56% of the individuals were resistant, respectively.

In the greenhouse bioassays, larval weight was about 65% lower on Cry34/35Ab corn than non-Bt corn for both the resistant Minnesota population and a control population[93]. Given that >50% of the population was resistant based on the survival data noted above, we infer these survivors had incomplete resistance because larval weight on Cry34/35Ab was not significantly higher for this population than for the control population.

In laboratory plant bioassays, survival of larvae from the Iowa populations with resistance to Cry34/35Ab was 72 to 73% lower on Cry34/35Ab corn relative to non-Bt corn[92]. The root injury data from the populations in Iowa show no difference between Cry34/35Ab corn and non-Bt corn[92], implying all or nearly all individuals were resistant. This high proportion of resistant individuals coupled with the significantly lower survival on Cry34/35Ab corn than non-Bt corn also indicates incomplete resistance.

Nature Biotechnology: doi:10.1038/nbt.3974

Supplementary Note 1

Practical resistance to Bt corn in *D. v. virgifera* and *S. albicosta*

Practical resistance of *D. v. virgifera* has been documented to all four coleopteran-active Bt toxins produced by corn: Cry3Bb, mCry3Aa, eCry3.1Ab, and Cry34/35Ab (**Table 1**). Resistance to Cry3Bb, which was first detected in 2009, causes strong cross-resistance to mCry3Aa and eCry3.1Ab, but not to Cry34/35Ab[28-31,85,94]. Two papers report evidence of practical resistance to Cry34/35Ab in five field populations sampled in 2013, four from central and eastern Iowa[92] and one from Minnesota[93]. At all five sites, growers or crop consultants complained of greater than expected damage to Bt corn producing Cry34/35Ab alone (two sites) or pyramids of Cry34/35Ab plus either Cry3Bb or mCry3Aa (three sites). Larval survival on Cry34/35Ab corn relative to non-Bt corn was significantly higher for the progeny of adults collected from the five problem field sites than for 11 unselected populations tested as controls[92,93]. These results show that the reduced efficacy observed in the field was associated with genetically based, field-evolved resistance.

The field-selected resistant population from Minnesota had 63 to 68% survival on Cry34/35Ab corn relative to non-Bt corn[93], which indicates >50% of the individuals in this population were resistant to Cry34/35Ab (**Supplementary Methods**). For three of the four sites in Iowa, root injury data were obtained for non-Bt corn (control) and Bt corn producing Cry34/35Ab either alone or in pyramids with Cry3Bb and mCry3Aa. At these three sites, the mean root injury was not lower for the Bt corn (1.59) than the non-Bt corn (1.57), indicating that Cry34/35Ab (as well as Cry3Bb and mCry3Aa) provided no protection against rootworm damage[92]. These results suggest that all or nearly all of the individuals in these three populations were resistant to Cry34/35Ab.

Relative to their performance on non-Bt corn, the populations with resistance to Cry34/35Ab suffered disadvantages on Cry34/35Ab corn, including lower survival[92,93]. The observed disadvantages on Cry34/35Ab corn relative to non-Bt corn imply that these populations included some susceptible individuals, resistance in homozygous resistant individuals was incomplete, or both (**Box 1** and **Supplementary Methods**). The evidence points to incomplete resistance in both the Minnesota and Iowa populations with practical resistance to Cry34/35Ab (**Supplementary Methods**).

For *S. albicosta* (western bean cutworm), the evidence of practical resistance to Cry1F produced by Bt corn includes laboratory bioassay data and widespread reports of reduced efficacy in the field[104-107]. The LC_{50} of Cry1F for a population sampled from Texas in 2013 was 2000 times higher than the LC_{50} values for both the most susceptible field population tested in 2013 and a laboratory strain[104]. Overall, a statistically significant, 5.2-fold increase in the LC_{50} of Cry1F occurred in data pooled from 13 bioassays of populations sampled in 2013 and 2014 from Nebraska, New Mexico and Texas relative to 19 bioassays of populations sampled from Iowa and Nebraska in 2003 and 2004 (ref. 104).

Based on extensive field trials in Iowa, Nebraska, Colorado, Texas and New Mexico from 2002 to 2006, the mean percentage of ears damaged by *S. albicosta* was reduced by about 85% in corn producing Cry1F relative to non-Bt corn ($P < 0.001$) (ref. 107). In July 2016, five extension entomologists from Nebraska concluded, "When first introduced to the market, Cry1F provided approximately 80% control of western bean cutworm. However, recent research has shown that its effectiveness has decreased in some areas, such as parts of southwest and central Nebraska, in the last 10 years"[106]. Moreover, extension entomologists from six other states wrote an open letter to the seed industry in October 2016 declaring, "Wherever Cry1F is challenged by WBC [western bean cutworm], it fails to provide observable benefit to producers."[105].

Nature Biotechnology: doi:10.1038/nbt.3974

Supplementary Note 2

Early warning of resistance

Zhang *et al.*[142] used the term 'early warning' of resistance to describe the statistically significant increase in the percentage of individuals with resistance to Cry1Ac in *H. armigera* from northern China. Their 2010 survey showed that mean survival at a diagnostic concentration of Cry1Ac was significantly higher for 13 field populations from northern China (1.3%), where exposure to Bt cotton producing Cry1Ac was extensive, relative to two field populations from northwestern China (0%) where exposure to Bt cotton was limited. Subsequent monitoring in northern China showed further increases in the mean survival at the diagnostic concentration, which by 2013 had increased to 5.5% (range: 0.3 to 11.3%)[73]. As in the other cases of early warning of resistance described below, reduced efficacy of Bt plants in the field has not been reported.

For *D. saccharalis* in Louisiana, the frequency of alleles conferring resistance to Cry1Ab in Bt corn increased 9-fold in 2009 relative to 2004 to 2008 (ref. 129). Based on the 2009 monitoring data and related work showing non-recessive inheritance of *D. saccharalis* resistance to Cry1Ab[129,130], the maximum percentage of resistant individuals was estimated as 2.4% (ref. 24). During 2009 field trials in Louisiana, Huang *et al.*[143] observed a substantial number of live larvae and injury to multi-toxin Bt corn. However, during 2010 and 2011, abundance of this pest in the region was so low that not enough individuals were sampled for screening[129].

For *O. furnacalis* in the Philippines, where Bt corn producing Cry1Ab was commercialized in 2003, the maximum survival at a diagnostic concentration of Cry1Ab increased 14-fold from 0.38% in 2007-2008 to 5.5% in 2009 (ref. 131). Also, field populations with >1% survival at the diagnostic concentration increased from 0% (0 of 11) in 2007-2008 to 62% (5 of 8) in 2009 (Fisher's exact test, P = 0.0048) (ref. 131). During 2010, efficacy in 198 fields based on mean percentage of damaged plants for Bt corn relative to non-Bt corn was 49% (including corn with and without herbicide tolerance)[144]. Meanwhile, cultivation of Bt corn in the Philippines increased from 400,000 ha in 2009 to 760,000 ha in 2014, with over 80% adoption in some areas[132,145].

References

142. Zhang, H. *et al.* Early warning of cotton bollworm resistance associated with intensive planting of Bt cotton in China. *PLoS ONE* **6**, e22874. (2011).

143. Huang, F., Ghimire, M.N., Leonard, B.R., Zhu, Y.-C. & Head, G.P. Susceptibility of field populations of sugarcane borer from non-Bt and Bt maize plants to five individual Cry toxins. *Insect Sci.* **19**, 570-578 (2012).

144. Afidchao, M. M., Musters, C.J.M., Wossink, A. & Balderama, O.F. Analysing the farm level economic impact of GM corn in the Philippines. *NJAS – Wageningen J. Life Sci.* **70-71**, 113-121 (2014).

145. Adlemita, R.R., Villena, M.M.C.A. & James, C. *Biotech Corn in the Philippines: A Country Profile.* Los Baños, Laguna: ISAAA and Southeast Asian Regional Center for Graduate Study and Research in Agriculture - Biotechnology Information Center (2015).

Supplementary Table 1. The number of generations per year pests fed on Bt crops for the 16 cases of practical resistance.

Insect	Crop	Toxin	Country	Gens. per year[a]	Reference
B. fusca	Corn	Cry1Ab	S. Africa	2	146
D. saccharalis	Corn	Cry1A.105	Argentina	3.5	147
D. v. virgifera	Corn	Cry3Bb	USA	1	28
D. v. virgifera	Corn	Cry34/35Ab	USA	1	28
D. v. virgifera	Corn	mCry3A	USA	1[b]	28
D. v. virgifera	Corn	eCry3.1Ab	USA	1[b]	28
H. zea	Corn	Cry1Ab	USA	2	148
H. zea	Corn	Cry1A.105	USA	2[b]	148
H. zea	Cotton	Cry1Ac	USA	3	141
H. zea	Cotton	Cry2Ab	USA	3[b]	141
P. gossypiella	Cotton	Cry1Ac	India	7	149
P. gossypiella	Cotton	Cry2Ab	India	7	149
S. albicosta	Corn	Cry1Fa	USA	1	150
S. frugiperda	Corn	Cry1Ab	Brazil	11[b]	38
S. frugiperda	Corn	Cry1F	Brazil	11	38
S. frugiperda	Corn	Cry1F	USA	10	47

[a]Generations per year that the pest fed on the Bt crop in the region monitored. When a range was reported, we used the midpoint of the range (i.e., 3.5 represents the range of 3-4 generations reported).

[b] Cross-resistance suspected or known as a factor contributing to resistance.

References

146. Van Rensburg, J.B.J. *et al.* Geographical variation in the seasonal moth flight activity of the maize stalk borer, *Busseola fusca* (Fuller) in South Africa. *S. African J. Plant Soil* **2**, 123-126 (1985).

147. Moré, M. *et al.* Influence of corn, *Zea mays*, phenological stages in *Diatraea saccharalis* F. (Lep. Crambidae) oviposition. *J. Appl. Entomol.* **127**, 512-515 (2003).

148. Storer, N.P. *et al.* Spatial processes in the evolution of resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae) to Bt transgenic corn and cotton in a mixed agroecosystem: a biology-rich stochastic simulation model. *J. Econ. Entomol.* **96**,156-172 (2003).

149. Mohan, K.S. An area-wide approach to pink bollworm management on Bt cotton in India – a dire necessity with community participation. Curr. Sci. **112**, 1988-1989 (2017).

150. Hanson *et al.* Degree-day prediction models for the flight phenology of western bean cutworm (Lepidoptera: Noctuidae) assessed with the concordance correlation coefficient. *J. Econ. Entomol.* **108**,1728-1738 (2015).

Nature Biotechnology: doi:10.1038/nbt.3974

Supplementary Table 2. Multiple regression testing the effects on years to evolve practical resistance of two factors: cross-resistance and the number of generations per year that pests fed on Bt crops.

Source	Estimate (SE)	P value
Intercept	5.76 (0.79)	<0.0001
Cross-resistance	-1.86 (0.55)	0.005
Generations per year	-0.31 (0.14)	0.049

The fit of the model including both main effects (cross-resistance and generations per year) is significant ($P = 0.007$, $R^2 = 0.53$). None of the interactions between factors is significant. The multiple regression analysis was performed in JMP 12, SAS Institute Inc. 2015. Cary, NC.

Nature Biotechnology: doi:10.1038/nbt.3974

Supplementary Table 3. The number of generations per year pests fed on Bt crops for the 17 cases of sustained susceptibility.

Insect	Crop	Toxin	Country	Gens. per yr. [a]	References[d]
C. includens	Soy	Cry1Ac	Brazil	3	151
D. grandiosella	Corn	Cry1Ab	USA	3.5	152
H. armigera	Cotton	Cry1Ac	Australia	4	25
H. armigera	Cotton	Cry2Ab	Australia	4	25
H. punctigera	Cotton	Cry1Ac	Australia	3	153
H. punctigera	Cotton	Cry2Ab	Australia	3	153
H. virescens	Cotton	Cry1Ac	Mexico	6	154
H. virescens	Cotton	Cry1Ac	USA	4	25
H. virescens	Cotton	Cry2Ab	USA	4	25
O. nubilalis	Corn	Cry1Ab	Spain	2.5	155
O. nubilalis	Corn	Cry1Ab	USA	3	25
O. nubilalis	Corn	Cry1Fa	USA	3	25
P. gossypiella	Cotton	Cry1Ac	China	3	125
P. gossypiella	Cotton	Cry1Ac	USA	5	25
P. gossypiella	Cotton	Cry2Ab	USA	5	25
S. frugiperda	Corn	Vip3Aa	Brazil	11	38
S. nonagroides	Corn	Cry1Ab	Spain	3	25

[a]Generations per year that the pest fed on the Bt crop in the region monitored. When a range was reported, we used the midpoint of the range (i.e., 3.5 represents the range of 3-4 generations reported).

References

151. Palma, J. *et al.* Molecular variability and genetic structure of *Chrysodeixis includens* (Lepidoptera: Noctuidae), an important soybean defoliator in Brazil. *an J. Plant SoilPLoS ONE* **10**(3): e0121260 (2015).

152. Baldwin, J.L. *et al.* Corn borer pests in Louisiana corn. LSUAg. Pub. 2947. http://www.lsuagcenter.com/NR/rdonlyres/A626E99C-CF02-480C-97D1-1F922408A2CC/23629/pub2947cornborerLOWRES.pdf

153. Baker, G.H. & Tann, C.R. Long-term changes in the numbers of *Helicoverpa punctigera* (Lepidoptera: Noctuidae) in a cotton production landscape in northern New South Wales, Australia. *Bull. Entomol. Res.* **107**, 174-187 (2016).

154. Molina-Ochoa, J. *et al.* Current status of *Helicoverpa zea* and *Heliothis virescens* within a changing landscape in the southern United States and Mexico. *Southwestern Entomol.* **35**, 347-354 (2010).

155. Farinós, G.P. *et al.* Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt maize cultivation in Spain. *Entomol. Exp. Appl.* **110**, 23-30 (2004).

Nature Biotechnology: doi:10.1038/nbt.3974

Supplementary Table 4. Planting of transgenic corn and cotton producing Vip3Aa.

Country	Crop	Other Bt toxins[a]	Year	%[b]	References
Australia[c]	Cotton	Cry1Ac, Cry2Ab	2015	7.6	156-158
Australia[c]	Cotton	Cry1Ac, Cry2Ab	2016	92	156-158
Brazil[d]	Corn	Cry1Ab	2015	<5	10
USA	Corn	Cry1Ab, Cry1F	2011	1.1	159
USA	Corn	Cry1Ab, Cry1F	2013	3.5	160
USA	Cotton	Cry1Ac, Cry1Fa	2014	<0.01	161
USA	Cotton	Cry1Ac, Cry1Fa	2015	0.48	162
USA	Cotton	Cry1Ac, Cry1Fa	2016	0.65	163

[a]For the years indicated above, the lepidopteran-active Bt toxins listed were produced in combination with Vip3Aa in all of the Vip3Aa cotton and in some types of Vip3Aa corn. Although some corn hybrids produce coleopteran-active Bt toxins in combination with Vip3Aa, only the lepidopteran-active Bt toxins are listed.

[b]Percentage of the total area planted with the crop listed that produced Vip3Aa. For example, in Australia in 2015, cotton producing Vip3Aa accounted for 7.6% of all cotton planted.

[c]Sown in the year listed (2015 or 2016), full seasons are 2015-16 and 2016-17, respectively.

[d]Bernardi *et al.*[10] indicates that Vip3Aa corn was approved for commercial planting in 2009 and "was planted in less than 5% of the total corn-growing area in Brazil (Syngenta information)."

References

156. Queensland Country Life. Aust cotton growers rush to use Bollgard 3. http://www.queenslandcountrylife.com.au/story/4245922/bollgard-3-dominates-aust-cotton-crop/ (2016).

157. Cotton Australia. Statistics. http://cottonaustralia.com.au/cotton-library/statistics (2017)

158. Monsanto Australia. Anthony May email April 9, 2017.

159. Christensen, P. Chinese approval of Syngenta Agrisure Viptera. Seed in Context Blog. http://www.intlcorn.com/seedsiteblog/?p=268 (2012).

160. Christensen, P. Viptera could have been approved for importation into China, but was not. Seed in Context Blog. http://www.intlcorn.com/seedsiteblog/?p=1891 (2014).

161. U.S. Department of Agriculture, Agricultural Marketing Service. Cotton varieties planted 2014. crop.https://search.ams.usda.gov/mndms/2014/09/CN20140912AVAR.PDF

162. U.S. Department of Agriculture, Agricultural Marketing Service. Cotton varieties planted 2015. crophttps://search.ams.usda.gov/mndms/2015/09/CN20150915AVAR.PDF

163. U.S. Department of Agriculture, Agricultural Marketing Service. Cotton varieties planted 2016. crop. https://www.ams.usda.gov/mnreports/cnavar.pdf

Nature Biotechnology: doi:10.1038/nbt.3974

Supplementary Table 5. Cross-resistance between Vip3A and Cry1 toxins in related strains of five species of noctuid lepidopteran pests.

Insect	Unselected strain	Related selected strain	Selected with	Tested for cross-resistance to	Metric[a]	RR[b]	CRR[c]	log(CRR)	Ref.
H. armigera	96S	Cry1Ac-R	Cry1Ac	Vip3Aa	LC_{50}	2970	1.7	0.23	164
H. armigera	SCD-r1	SCD	Cry1Ac	Vip3Aa	LC_{50}	440	1.0	-0.01	13
H. virescens	YDK	YHD2	Cry1Ac	Vip3Aa	LC_{50}	20,000	1.2	0.068	57
H. virescens	Vip-Unsel	Vip-Sel G15	Vip3Aa	Cry1Ab	LC_{50}	2000	3.2	0.51	55
H. virescens	Vip-Unsel	Vip-Sel G18	Vip3Aa	Cry1Ab	LC_{50}	2000	6.7	0.83	55
H. virescens	Vip-Unsel	Vip-Sel G15	Vip3Aa	Cry1Ac	LC_{50}	2000	7.1	0.85	55
H. virescens	Vip-Unsel	Vip-Sel G18	Vip3Aa	Cry1Ac	LC_{50}	2000	1.0	0.00	55
H. zea	SC	AR	Cry1Ac	Vip3Aa	LC_{50}	100	0.94	-0.027	165
H. zea	GA	GA-R	Cry1Ac	Vip3Aa	EC_{50}	57	1.6	0.20	36
S. frugiperda	SS	RR	Cry1F	Vip3Aa	LC_{50}	>62	1.5	0.18	52
S. frugiperda	SS	RR	Cry1F	Vip3Aa	IC_{50}	930	0.6	-0.26	52
T. ni [d]	SS	RR	Cry1Ac	Vip3Aa	IC_{50}	2054	2.1	0.33	166
T. ni	SS	RR	Cry1Ac	Vip3AaAc	IC_{50}	2054	1.8	0.26	166
T. ni	SS	RR	Cry1Ac	Vip3Ac	IC_{50}	2054	1.02	0.0078	166
T. ni	SS	RR	Cry1Ac	Vip3AcAa	IC_{50}	2054	3.2	0.51	166

[a]Concentrations causing 50% response in tested insects based on mortality (LC_{50}), efficacy (EC_{50}), or growth inhibition (IC_{50}).

[b]Resistance ratio (RR) is the LC_{50} (or EC_{50} or IC_{50}) of a toxin for the resistant strain that was selected with that toxin divided by the LC_{50} (or EC_{50} or IC_{50}) of the same toxin for a related, unselected strain.

[c] Cross-resistance ratio (CRR) is the LC_{50} (or EC_{50} or IC_{50}) of a toxin not used for selection (e.g., Vip3Aa) for a strain selected with another toxin (e.g., Cry1Ac), divided by the LC_{50} (or EC_{50} or IC_{50}) of the toxin not used for selection for a related, unselected control strain. The expected value of CRR is 1 if cross-resistance is absent and >1 if cross-resistance is present.

[d]*Trichoplusia ni*

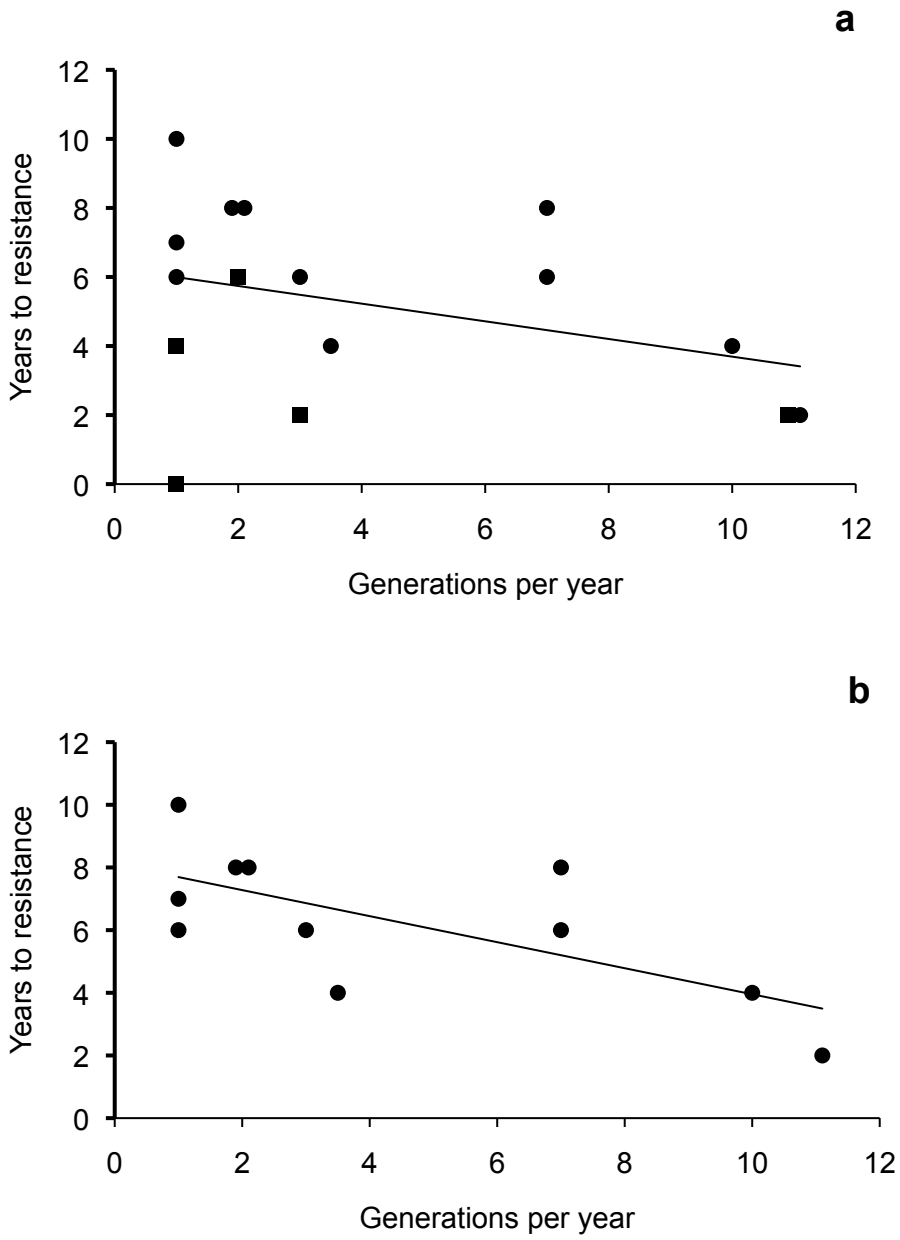
References

164. Zhang, Q., Chen, L.-Z., Lu, Q., Zhang, Y., Liang, G.-M. Toxicity and binding analyses of *Bacillus thuringiensis* toxin Vip3A in Cry1Ac-resistant and -susceptible strains of *Helicoverpa armigera* (Hü bner). *J. Integr. Agric.* **14**, 347–354 (2015).

165. Anilkumar, K. J. *et al.* Production and characterization of *Bacillus thuringiensis* Cry1Ac-resistant cotton bollworm *Helicoverpa zea* (Boddie). *Appl. Environ. Microbiol.* **74**, 462-469 (2008).

166. Fang, J. *et al.* Characterization of chimeric *Bacillus thuringiensis* Vip3 toxins. *Appl. Env. Microbiol.* **73**, 956-961 (2007).

Nature Biotechnology: doi:10.1038/nbt.3974



Supplementary Figure 1. Negative association between time to practical resistance and the number of generations per year (GPY) that pests fed on Bt crops. (**a**) With all 16 cases, this association is not significant (linear regression: R^2 = 0.12, df = 14, P = 0.19). Squares represent the five cases where cross-resistance is suspected or known to have shortened the time to resistance. (**b**) The negative association is significant when considering only the 11 cases where cross-resistance is not involved (y = -0.42x + 8.1, R^2 = 0.46, df = 9, P = 0.022). Multiple regression for all 16 cases shows a significant negative relationship between time to practical resistance and GPY (P = 0.049) after accounting for the significant effects of cross-resistance (P = 0.005) (**Supplementary Table 2**).

Nature Biotechnology: doi:10.1038/nbt.3974