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Successes and Failures of Transgenic Bt Crops: Global Patterns of Field-evolved Resistance

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Summary

Farmers planted genetically engineered crops that produce insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) on a cumulative total of 570 million ha worldwide from 1996 to 2013. These Bt crops kill some key insect pests, yet they are not toxic to most other organisms, including people. Bt crops can suppress pests, reduce the use of insecticide sprays and increase farmer profits, but their benefits are diminished or lost when pests evolve resistance. Here we review data monitoring resistance to seven Bt proteins in 13 major pest species targeting Bt maize and Bt cotton on six continents. Of the 27 sets of monitoring data analysed, seven show severe field-evolved resistance in 2 to 8 years with practical consequences for pest control (i.e. practical resistance), eight show statistically significant but less severe field-evolved resistance and 12 show no evidence of decreased susceptibility after 2 to 15 years. The surge in cases of practical resistance since 2005 is associated with increased planting of Bt crops, increased cumulative exposure of pests to Bt crops and increased monitoring. In addition, practical resistance to Bt crops is associated with a

scarcity of refuges, which consist of host plants that do not produce Bt proteins. To maximize the benefits of Bt crops, we encourage collaboration between growers and scientists in industry, academia and government to implement large refuges of non-Bt host plants, particularly when the inheritance of resistance is not recessive and alleles conferring resistance are not rare.

1.1 Introduction

The widespread bacterium *Bacillus thuringiensis* (Bt) produces proteins that kill some devastating insect pests, but are not toxic to most other organisms, including people (Mendelsohn *et al.*, 2003; Pardo-López *et al.*, 2013). These proteins, called Bt toxins, have been used for decades in sprays to control insects that attack crops, damage forests and vector human diseases (Sanahuja *et al.*, 2011). More recently, to provide another way to control insect pests, the genes encoding Bt toxins have been incorporated in the genomes of maize, cotton and soybean (Tabashnik *et al.*, 2009a; James 2013).

The area planted to transgenic crops producing Bt toxins grew from 1 million ha in 1996 to 76 million ha in 2013 (Fig. 1.1).

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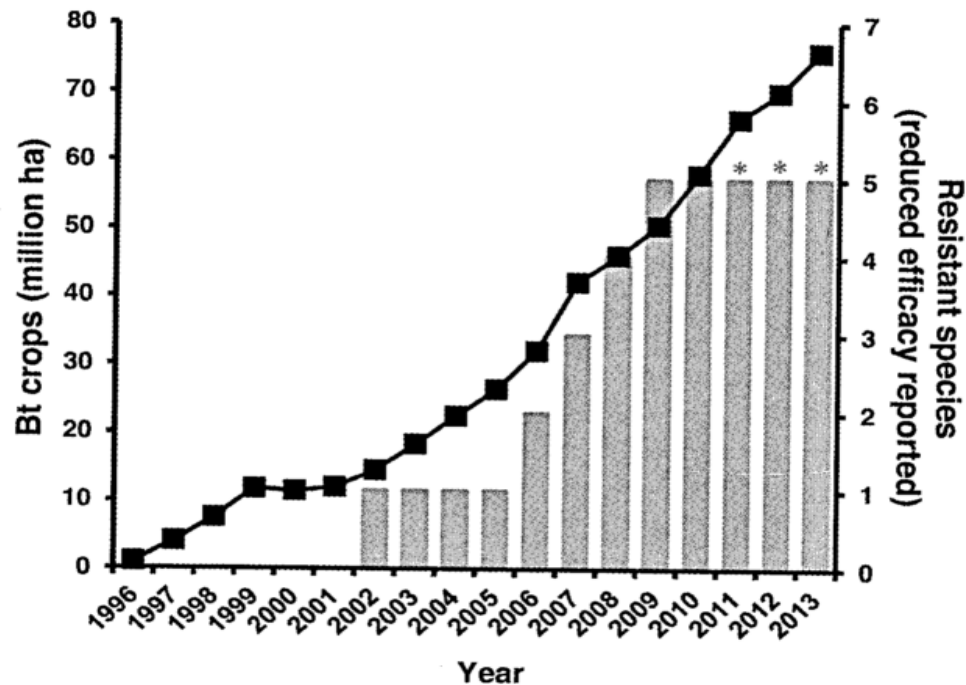


Fig. 1.1. Global area planted to Bt crops (black) and cumulative number of insect species with practical resistance to Bt crops (grey). Adapted from Tabashnik *et al.* (2013). Practical resistance, which entails one or more field populations with >50% resistant individuals and reduced efficacy reported, has been documented in five major target pests (year first detected given in parentheses): *Helicoverpa zea* (2002), *Spodoptera frugiperda* (2006), *Busseola fusca* (2007), *Pectinophora gossypiella* (2008) and *Diabrotica virgifera virgifera* (2009) (see Table 1.1). The asterisks indicate that the number of species with resistant populations may be underestimated for 2011 to 2013 because reports of field-evolved resistance typically are published 2 or more years after resistance is first detected. Although the number of pest species with practical resistance was five from 2009 to 2013, the number of cases of practical resistance increased from five to seven because of *S. frugiperda* resistance to the Cry1F Bt toxin in two countries (Brazil and USA) and *D. v. virgifera* resistance to two Bt toxins (Cry3Bb and mCry3A) (Table 1.1).

The cumulative worldwide total of Bt crops planted from 1996 to 2013 was 570 million ha (1.4 billion acres), with Bt maize and Bt cotton accounting for more than 99% of this total. In 2013, Bt maize accounted for 76% of maize in the USA, while Bt cotton accounted for 75% of cotton in the USA, 90% in China and 95% in India (James, 2013; USDA ERS, 2013). In the first large scale use of Bt soybean, farmers planted 2 million ha of this crop in Brazil during 2013 (James, 2013). In some cases, these Bt crops have suppressed pests, reduced the use of insecticide sprays, increased crop yield and increased farmers' profits (Hutchison *et al.*, 2010; National Research Council 2010,

Tabashnik *et al.*, 2010; Edgerton *et al.*, 2012; Lu *et al.*, 2012). However, these substantial environmental and economic benefits are diminished or even eliminated when pests evolve resistance to Bt toxins (Storer *et al.*, 2012; Tabashnik *et al.*, 2013; Van den Berg *et al.*, 2013).

Here we review the definition of field-evolved resistance, five categories of field-evolved resistance ranging in severity from incipient to practical resistance, the status of resistance or susceptibility of 13 pests to seven toxins used in Bt crops on six continents and factors associated with the successes and failures of Bt crops in the field.

1.2 Definition and Categories of Field-evolved Resistance

Field-evolved (or field-selected) resistance is a genetically based decrease in susceptibility of a population to a toxin that is caused by exposure of the population to the toxin in the field (Tabashnik *et al.*, 2013). Tabashnik *et al.* (2014) and references therein provide detailed discussion of this and other definitions of resistance. Recognizing that field-evolved resistance is not 'all or none', Tabashnik *et al.* (2014) described five categories of field-evolved resistance, which each entail a genetically based decrease in susceptibility to a toxin in one or more field populations:

- incipient resistance, <1% resistant individuals;
- early warning of resistance, 1 to 6% resistant individuals;
- >6 to <50% resistant individuals (no cases reported);
- >50% resistant individuals and reduced efficacy expected, but not reported; and
- practical resistance, >50% resistant individuals and reduced efficacy reported.

Practical resistance is the only category where the practical implications of resistance for reducing the control of pests by Bt crops have been confirmed.

1.3 Global Status of Field-evolved Resistance to Bt Crops

Here we update previous reviews (e.g. Tabashnik *et al.*, 2013) by classifying 27 cases of pest responses to Bt crops (Tables 1.1 and 1.2, Fig. 1.2). Each case represents the responses of one pest species to one Bt toxin used in transgenic crops in one country. For each case, we evaluated the percentage of resistant individuals based on survival in bioassays at a diagnostic concentration of the relevant Bt toxin. A diagnostic concentration of toxin kills all or nearly all susceptible individuals, but few or no resistant individuals (Tabashnik *et al.*, 2014). We categorize the data based on the evidence for the most severe level of

resistance for each case. For example, if practical resistance to a toxin has been detected in one or more populations of a pest in a certain country, while some other populations in the country remain susceptible to that toxin, we classify the case as an example of practical resistance. Thus, even in cases where practical resistance to a Bt toxin occurs in some populations in a given country, the same Bt toxin may still be effective against other populations in that country.

We considered 27 cases based on resistance monitoring data from nine countries for responses to seven Bt toxins by 13 major pest species (12 lepidopterans and one coleopteran). In 12 of 27 (44%) cases, the monitoring data show no statistically significant decrease in susceptibility after 2 to 15 years (median = 8 years) of exposure to Bt crops (Table 1.2). Eleven of these cases are reviewed in Tabashnik *et al.* (2013). The additional case is sustained susceptibility of *Ostrinia nubilalis* (European corn borer) to Cry1F produced by Bt maize in the USA, which reflects a relatively low adoption of maize plants producing only Cry1F (Siegfried *et al.*, 2014). Below we review the 15 cases of field-evolved resistance, including seven cases of practical resistance (Table 1.1).

1.3.1 Incipient resistance and early warning of resistance

All three cases of incipient resistance are from Australia, where a rigorous, proactive monitoring programme enabled the early detection of resistance to Bt toxins in *Helicoverpa punctigera* and *H. armigera* (Downes *et al.*, 2010; Downes and Mahon, 2012a, b) (Table 1.1). Downes *et al.* (2010) used the term 'incipient resistance' to describe a statistically significant increase in the frequency of alleles conferring resistance to Bt toxin Cry2Ab in *H. punctigera* from Australia. Results from the 2008/09 field season showed that the frequency of alleles conferring resistance to Cry2Ab was eight times higher in areas where Bt cotton producing this toxin was grown compared

Table 1.1. Field-evolved resistance to Bt crops: 15 cases in nine pest species from seven countries with severity ranging from incipient to practical resistance. (Adapted from Tabashnik *et al.*, 2013, 2014.)

Pest ^a	Crop	Bt toxin	Country	Years ^b	Reference/s
Incipient resistance: <1% resistant individuals					
<i>Helicoverpa armigera</i>	Cotton	Cry1Ac	Australia	15	Downes and Mahon, 2012b; Tabashnik <i>et al.</i> , 2013
		Cry2Ab	Australia	8	
<i>H. punctigera</i>	Cotton	Cry2Ab	Australia	8	Downes <i>et al.</i> , 2010; Downes and Mahon, 2012a
Early warning of resistance: 1 to 6% resistant individuals					
<i>Diatraea saccharalis</i>	Maize	Cry1Ab	USA	10	Huang <i>et al.</i> , 2012
<i>H. armigera</i>	Cotton	Cry1Ac	China	13	Zhang <i>et al.</i> , 2011, 2012; Jin <i>et al.</i> , 2013
<i>Ostrinia furnacalis</i>	Maize	Cry1Ab	The Philippines	5	Alcantara <i>et al.</i> , 2011
<i>Pectinophora gossypiella</i>	Cotton	Cry1Ac	China	13	Wan <i>et al.</i> , 2012
>50% resistant individuals and reduced efficacy expected^c					
<i>H. zea</i> ^d	Cotton	Cry2Ab	USA	2	Ali and Luttrell, 2007; Tabashnik <i>et al.</i> , 2009a, 2013
Practical resistance: >50% resistant individuals and reduced efficacy reported					
<i>Busseola fusca</i>	Maize	Cry1Ab	South Africa	8	van Rensburg, 2007; Kruger <i>et al.</i> , 2011; Van den Berg <i>et al.</i> , 2013
<i>Diabrotica virgifera virgifera</i>	Maize	Cry3Bb	USA	7	Gassmann <i>et al.</i> , 2011, 2012
<i>D. v. virgifera</i> ^e	Maize	mCry3A	USA	4	Gassmann <i>et al.</i> , 2014
<i>H. zea</i>	Cotton	Cry1Ac	USA	6	Luttrell <i>et al.</i> , 2004; Ali <i>et al.</i> , 2006; Tabashnik <i>et al.</i> , 2008a,b
<i>P. gossypiella</i>	Cotton	Cry1Ac	India	6 ^f	Monsanto, 2010; Dhurua and Gujar, 2011; Sumerford <i>et al.</i> , 2013
<i>Spodoptera frugiperda</i>	Maize	Cry1F	Brazil	2	Farias <i>et al.</i> , 2014; Monnerat <i>et al.</i> (Chapter 3)
<i>S. frugiperda</i>	Maize	Cry1F	USA	3	Storer <i>et al.</i> , 2010, 2012

^a*D. v. virgifera* is a beetle (coleopteran); the other eight species are lepidopterans.

^bYears elapsed between the first year of commercial planting in the region studied and: (i) for the eight cases with either practical resistance or >50% resistant individuals and reduced efficacy expected, the first year of field sampling that yielded evidence of resistance, or (ii) for all other cases, the most recent year of monitoring data reviewed here.

^cReduced efficacy is expected, but has not been reported.

^dMay reflect some cross-resistance caused by selection with Cry1Ac.

^eRegistered October 2006, grown 2007, first resistance reported from populations sampled in 2011; reflects cross-resistance from selection with mCry3A.

^fExcludes years when Bt cotton was grown illegally in India before it was commercialized in 2002. Resistance was first detected in samples collected in 2008, 6 years after commercial planting. If illegal planting started in 2000, the total years elapsed would be 8 (Tabashnik *et al.*, 2013).

Table 1.2. Susceptibility to Bt crops: 12 cases in seven pest species from four countries with monitoring data showing no evidence of resistance. (Adapted from Tabashnik *et al.*, 2013.)

Pest ^a	Crop	Bt toxin	Country	Years ^b	Reference/s
<i>Diatraea grandiosella</i>	Maize	Cry1Ab	USA	6	Huang <i>et al.</i> , 2007
<i>Diabrotica virgifera virgifera</i>	Maize	Cry34/35Ab	USA	5	Gassmann <i>et al.</i> , 2014
<i>Helicoverpa punctigera</i>	Cotton	Cry1Ac	Australia	10	Downes <i>et al.</i> , 2009
<i>Heliothis virescens</i>	Cotton	Cry1Ac	USA	11	Ali <i>et al.</i> , 2006; Blanco <i>et al.</i> , 2009
		Cry1Ac	Mexico	11	Blanco <i>et al.</i> , 2009
		Cry2Ab	USA	2	Ali and Luttrell, 2007
<i>Ostrinia nubilalis</i>	Maize	Cry1Ab	Spain	4	Farinós <i>et al.</i> , 2004
		Cry1Ab	USA	15	Siegfried <i>et al.</i> , 2007; Siegfried and Hellmich, 2012
		Cry1F	USA	8	Siegfried <i>et al.</i> , 2014
<i>Pectinophora gossypiella</i>	Cotton	Cry1Ac	USA	13	Tabashnik <i>et al.</i> , 2010
		Cry2Ab	USA	5	Tabashnik <i>et al.</i> , 2010
<i>Sesamia nonagroides</i>	Maize	Cry1Ab	Spain	7	Andreadis <i>et al.</i> , 2007; Farinós <i>et al.</i> , 2011

^a*D. v. virgifera* is a beetle (coleopteran); the other six species are lepidopterans.

^bYears elapsed between the first year of commercial planting in the region studied and the most recent year of monitoring data reviewed here.

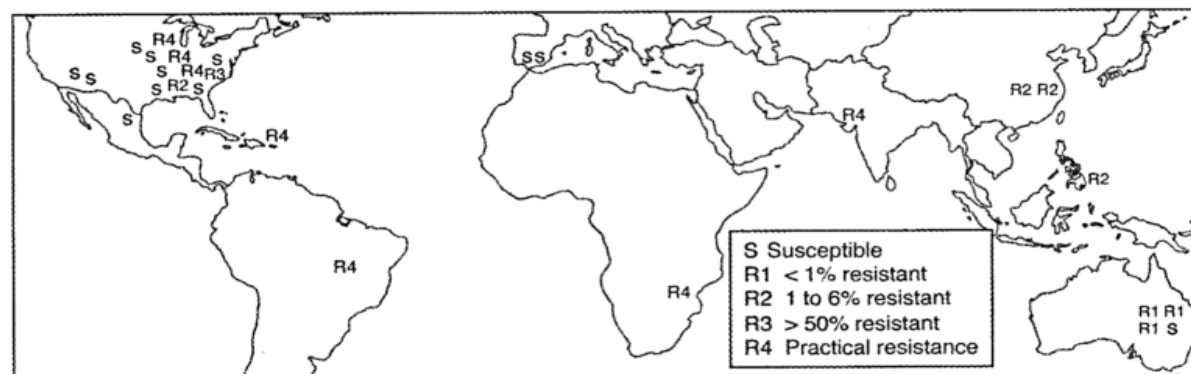


Fig. 1.2. Global status of field-evolved resistance to Bt crops. Each one-letter or letter-number code represents one of 27 cases evaluating the status of field-evolved resistance to one Bt toxin in populations of one pest species from one country (see Tables 1.1 and 1.2). (Adapted from Tabashnik *et al.*, 2013.)

with that in non-cropping areas. Downes *et al.* (2010) also detected an elevenfold increase from 2004/05 to 2008/09 in the frequency of resistance to Cry2Ab in populations exposed to this toxin. However, they estimated that the percentage of resistant individuals – a maximum of 0.2% – was too low to reduce the efficacy of Bt

cotton. Furthermore, from 2008/09 to 2010/11 the frequency of resistance to Cry2Ab did not increase (Downes and Mahon 2012a). This shows that the statistically significant yet small rises in resistance allele frequency characteristic of incipient resistance do not necessarily indicate that further increases in resistance are imminent.

Zhang *et al.* (2011) used the phrase 'early warning' of resistance to describe the statistically significant increase in the percentage of individuals with resistance to Bt toxin Cry1Ac in *H. armigera* from northern China. Their 2010 survey showed that survival at a diagnostic concentration of Cry1Ac was significantly higher for 13 field populations from northern China, where exposure to Bt cotton producing Cry1Ac was extensive, relative to two field populations from northwestern China where exposure to Bt cotton was limited. For the populations from northern China surveyed in 2010, the mean survival at the diagnostic concentration was 1.3% (range: 0 to 2.6%) compared with 0% for the populations from northwestern China and a susceptible laboratory strain (Zhang *et al.*, 2011). Results of screening in 2009 and 2011 also support the conclusion that exposure to Bt cotton increased the frequency of *H. armigera* resistance to Cry1Ac in northern China, with up to 5.4% resistant individuals in a population (Zhang *et al.*, 2012; Jin *et al.*, 2013).

Three other cases also fit the early warning category, with a statistically significant increase in resistance relative to susceptible populations and between 1 and 6% of resistant individuals: resistance to Cry1Ac in Bt cotton of *Pectinophora gossypiella* in China, and resistance to Cry1Ab in Bt maize of *Ostrinia furnacalis* in the Philippines and *Diatraea saccharalis* in the southern USA (Table 1.1). As with incipient resistance, the percentage of resistant individuals in this category is too low to substantially reduce the efficacy of Bt crops. However, field-evolved resistance with >1% resistant individuals detected warrants consideration of enhanced actions to manage resistance, such as increases in monitoring, refuge requirements and alternative methods of control. It remains to be seen what actions, if any, are taken in these four cases of early warning of resistance and how such actions would affect the trajectory of resistance. The absence of cases with >6 to <50% resistant individuals suggests that after the percentage of resistant individuals surpasses 6%, it quickly rises above 50%.

1.3.2 Practical resistance: >50% resistant individuals and reduced efficacy reported

In the seven cases of practical resistance to Bt crops, one or more pest populations had >50% resistant individuals and reduced efficacy of the Bt crop was reported (Table 1.1). These seven cases entail practical resistance to Bt maize in three pests, *Busseola fusca*, *Diabrotica virgifera virgifera* and *Spodoptera frugiperda*; and practical resistance to Bt cotton in two pests, *Helicoverpa zea* and *P. gossypiella*.

Practical resistance to Bt maize

In the US territory of Puerto Rico, *S. frugiperda* (fall armyworm) evolved resistance to Bt maize producing Cry1F in 3 years, which is one of the fastest documented cases of practical resistance to a Bt crop (Table 1.1), and the first case of resistance leading to withdrawal of a Bt crop from the marketplace (Storer *et al.*, 2010, 2012). In 2011, some 4 years after Dow AgroSciences and Pioneer Hi-Bred International voluntarily stopped selling Cry1F maize in Puerto Rico, field populations remained highly resistant (Storer *et al.*, 2012). Despite the opportunity to learn from this failure in Puerto Rico, a similar pattern has been repeated in Brazil, as described by Monnerat *et al.* (Chapter 3). After only a few years of commercial cultivation of Bt maize producing Cry1F, practical resistance of *S. frugiperda* to Cry1F is widespread in Brazil (Farias *et al.*, 2014). The limited gene flow between populations in Puerto Rico and Brazil (Nagoshi *et al.*, 2010) implies that this resistance arose independently in Brazil. It will be intriguing to compare the genetic basis of resistance between the strains of *S. frugiperda* from these two regions.

Practical resistance of *B. fusca* (maize stem borer) to Bt maize producing Cry1Ab occurred in South Africa in 8 years (van Rensburg 2007; Tabashnik *et al.*, 2009a; Van den Berg *et al.*, 2013; Van den Berg and Campagne, Chapter 4), with striking parallels to *S. frugiperda* resistance to Cry1F maize in Puerto Rico. In both cases, proactive resistance monitoring was not conducted

and observations of reduced efficacy in the field preceded the documentation of resistance with bioassays (Storer *et al.*, 2012; Van den Berg *et al.*, 2013). In South Africa, however, Cry1Ab maize was not withdrawn from sales, with >1 million ha planted in 2013 (James, 2013). Continued planting of Cry1Ab maize has yielded widespread resistance and hundreds of reports of product failure during the 2010/11 and 2011/12 seasons (Kruger *et al.*, 2009; Van den Berg *et al.*, 2013). Monsanto, the company that developed the predominant type of Cry1Ab maize grown in South Africa, compensated growers for their insecticide sprays on this Bt maize (Kruger *et al.*, 2009). Large-scale planting of two-toxin Bt maize producing Cry1A.105 (similar to Cry1Ab; Tabashnik *et al.*, 2009a) and Cry2Ab began during the 2012/13 season in South Africa (Van den Berg *et al.*, 2013).

Field and laboratory data indicate practical resistance to Bt maize producing Cry3Bb or mCry3A in some populations of *D. v. virgifera* (Western corn rootworm) from Iowa (Gassmann *et al.*, 2011, 2012, 2014; Gassmann, 2012). Survival in bioassays on Bt maize plants producing either Cry3Bb or mCry3A was significantly higher for nine strains derived in 2011 from 'problem' fields with severe damage to Bt maize producing either of these two toxins compared with survival in eight 'control' laboratory strains that had not been exposed to Bt toxins (Gassmann *et al.*, 2014).

Survival on Cry3Bb maize and mCry3A maize was correlated across the 17 strains of *D. v. virgifera* examined by Gassmann *et al.* (2014). This correlation could reflect cross-resistance, correlated exposure to the two toxins (populations exposed more to Cry3Bb were also exposed more to mCry3A), or both. Previous exposure to Cry3Bb maize was not correlated with exposure to mCry3A maize across the 17 strains ($r = -0.15$, $t = -0.58$, $df = 15$, $P = 0.57$), which leaves cross-resistance as the best explanation for the observed correlation in survival between Cry3Bb maize and mCry3A maize across strains. This cross-resistance is not surprising (Tabashnik and Gould, 2012), because the two toxins have high amino acid

sequence similarity overall (80%) and particularly for domain II (83%), which determines specificity and is associated with cross-resistance in other insects (Tabashnik *et al.*, 1996; Carrière *et al.*, 2015). To put this in perspective, the amino acid sequence similarity between Cry1Aa and Cry1Ac is 80% overall and 81% for domain II, with strong cross-resistance typically seen between these two toxins (Carrière *et al.*, 2015).

Practical resistance to Bt cotton

Despite strong evidence of practical resistance to Bt cotton in *P. gossypiella* from western India and *H. zea* in the south-eastern USA (Table 1.1), these cases have been controversial (Moar *et al.*, 2008; Tabashnik *et al.*, 2008b, 2013, 2014; Luttrell and Jackson, 2012). Resistance of *P. gossypiella* (pink bollworm) to Bt cotton producing Cry1Ac was first detected with laboratory bioassays of the offspring of insects collected from non-Bt cotton fields in 2008 in the state of Gujarat in western India (Dhurua and Gujar, 2011). India ranks second in cotton production, behind only China, and Gujarat accounted for a third of India's cotton production in 2009/10, which is equivalent to about half of the annual cotton production in the USA during 2009 and 2010 (Tabashnik *et al.*, 2014).

Several studies have confirmed widespread resistance of *P. gossypiella* to Cry1Ac in Gujarat, which was associated with an unusually high abundance of larvae on Cry1Ac cotton and moths caught in pheromone traps (Monsanto, 2010; Sumerford *et al.*, 2013; Tabashnik *et al.*, 2013; Fabrick *et al.*, 2014). Fabrick *et al.* (2014) discovered severe disruptions in the transcripts encoding cadherin (a protein that binds Cry1Ac in the midgut of susceptible larvae) associated with resistance to Cry1Ac in the resistant insects from Gujarat and in insects sampled from Bt cotton plants in the neighbouring state of Madhya Pradesh (see Fabrick and Wu, Chapter 7).

Farmers in India have switched to cotton hybrids producing two Bt toxins (Cry1Ac and Cry2Ab), which are effective against pink bollworm larvae resistant to Cry1Ac

(Tabashnik *et al.*, 2002, 2009b). In 2013, they planted two-toxin Bt cotton on 10 million ha, representing 94% of India's cotton (James, 2013). Dhurua and Gujar (2011) concluded that their bioassay results with seed powder from two-toxin cotton incorporated in an artificial diet 'imply slightly reduced susceptibility' to Cry2Ab in a population from Gujarat that was sampled in 2008. In bioassays with plants, however, larvae from a laboratory-selected strain of pink bollworm from Arizona with >200-fold resistance to Cry2Ab did not survive on two-toxin Bt cotton (Tabashnik *et al.*, 2009b).

As with *P. gossypiella* in India, the extensive documentation of the practical resistance of *H. zea* (cotton bollworm) to Cry1Ac in the USA includes reports of decreased efficacy of Bt cotton in the field. Luttrell *et al.* (2004) reported the first evidence of field-evolved resistance to a Bt crop, including data showing that two populations of *H. zea* that inflicted 'unacceptable levels of boll damage' in problem fields of Bt cotton in 2002 were resistant to Cry1Ac in laboratory bioassays (Tabashnik *et al.*, 2008b). The compelling evidence confirming this case of practical resistance includes >50% survival at a diagnostic concentration of Cry1Ac for four strains derived from the field in 2003, a significant association between larval survival on Bt cotton leaves and decreased susceptibility to Cry1Ac in bioassays, as well as Cry1Ac resistance ratios >100 for eight strains derived during 2003 to 2006 from field sources other than Bt crops (Ali *et al.*, 2006; Luttrell and Ali, 2007; Tabashnik *et al.*, 2008b). These resistance ratios were calculated by dividing the concentration of Cry1Ac killing 50% of larvae (LC_{50}) for each field-derived strain by the LC_{50} of a susceptible laboratory strain called UALab (Ali *et al.*, 2006). This robust strain, which was maintained at the University of Arkansas, provides an excellent standard for comparison because the mean LC_{50} of Cry1Ac was virtually identical for UALab and two susceptible laboratory strains from North Carolina State University and the US Department of Agriculture (Ali *et al.*, 2006; Tabashnik *et al.*, 2008b).

One important difference between the two cases of practical resistance to Cry1Ac cotton is that the initial efficacy of this crop was greater against *P. gossypiella* than *H. zea*. For example, the efficacy of Cry1Ac cotton ranged from 77 to 96% against *P. gossypiella* in India from 2002 to 2005 (Bambawale *et al.*, 2004, 2010), but was only 44% against *H. zea* in North Carolina in 2001 (Jackson *et al.*, 2004). This difference in efficacy occurred before field-evolved resistance was detected in either pest; it reflects the 70-fold greater inherent susceptibility to Cry1Ac of pink bollworm relative to *H. zea* (Sivasupramaniam *et al.*, 2008). Thus, the reduction in efficacy associated with resistance to Cry1Ac cotton is almost certainly larger for pink bollworm in India than for *H. zea* in the USA.

Similar to the transition to two-toxin Bt cotton in India described above, cotton farmers in the USA have completely replaced one-toxin Bt cotton producing Cry1Ac with two-toxin Bt cotton, predominantly plants producing Cry1Ac and Cry2Ab (Brévault *et al.*, 2013; Tabashnik *et al.*, 2013). Here, we confirm the classification of *H. zea* resistance to Cry2Ab as >50% resistant individuals detected, with reduced efficacy of the Bt crop expected (Tabashnik *et al.*, 2013). The percentage of *H. zea* populations tested that had >50% survival at a diagnostic concentration of Cry2Ab rose from 0% in 2002 to 50% in 2005, only 2 years after the commercialization of Bt cotton producing Cry2Ab and Cry1Ac (Ali and Luttrell, 2007; Tabashnik *et al.*, 2009a). Three populations sampled from non-Bt plants in Arkansas in 2005 had such low mortality in bioassays that LC_{50} values could not be calculated (Ali and Luttrell, 2007). The decreased susceptibility to Cry2Ab detected in 2005, when cotton producing this toxin was not common, suggests that resistance to Cry1Ac caused some cross-resistance to Cry2Ab, which is consistent with the genetic correlation between resistance to these two toxins (Jackson *et al.*, 2006; Ali and Luttrell, 2007; Tabashnik *et al.*, 2013).

In addition, data from field populations in Arkansas show that mortality caused by a diagnostic concentration of Cry2Ab

decreased substantially in 2010 compared with the previous 4 years (Jackson *et al.*, 2011). This evidence of field-evolved resistance to Cry2Ab coincided with higher abundance of *H. zea* in the field and increased insecticide sprays targeting *H. zea* on Bt cotton in 2010 (Jackson *et al.*, 2011). In the USA from 1999 to 2011, the percentage of Bt cotton producing two toxins increased from 0 to 90%, while the sprays against *H. zea* on Bt cotton tripled (Williams, 2012; Tabashnik *et al.*, 2013).

1.4 Lessons from Global Patterns of Field-evolved Resistance

The number of cases of field-evolved resistance to Bt crops with practical implications for pest control has increased to seven, encompassing the resistance of some populations of five pest species to five Bt toxins in four countries on four continents (Table 1.1 and Fig. 1.2), compared with only one such case as of 2005 (Tabashnik *et al.*, 2008a, 2013). Factors contributing to this surge in documented cases of practical resistance to Bt crops include more extensive monitoring as well as increases in the area planted to Bt crops (Fig. 1.1), the number of pest populations exposed to Bt crops and the cumulative duration of exposure.

Retrospective analyses of global patterns show that, as predicted from theory, abundant refuges of non-Bt host plants, recessive inheritance of resistance and a low initial frequency of resistance can delay the evolution of resistance to Bt crops (Gould 1998; Tabashnik *et al.*, 2008a, 2013; Carrière *et al.*, 2010). Nearly all of the seven cases of practical resistance (Table 1.1) entail a scarcity of refuges. For example, it appears that little or no planting of refuges spurred the rapid evolution of resistance to Bt crops in South Africa, Puerto Rico, India and Brazil (Storer *et al.*, 2012; Tabashnik *et al.*, 2013; Van den Berg *et al.*, 2013; Farias *et al.*, 2014; Monnerat *et al.*, Chapter 3).

Modelling results and empirical evidence suggest that a 'one size fits all' approach to refuge requirements is not optimal. In particular, under the ideal conditions of

recessive inheritance of resistance and low initial resistance allele frequency, refuges that account for a relatively small percentage of a pest's host plants may be sufficient to delay resistance for many years. Conversely, to substantially delay pest adaptation, much larger refuges are needed when resistance is not recessive or resistance alleles are not rare (Tabashnik *et al.*, 2008a, 2013; Carrière *et al.*, 2010). For example, in the Midwestern USA, the required 20% refuge of non-Bt maize has sustained the efficacy of Cry1Ab against *O. nubilalis* for more than a decade, generating billions of dollars in benefits for growers (Siegfried *et al.*, 2007; Hutchison *et al.*, 2010), but has not substantially slowed resistance to Cry3Bb in *D. v. virgifera* (Gassmann *et al.*, 2011, 2014; Tabashnik and Gould, 2012). Selection experiments and extensive screening of field populations show that alleles conferring resistance to Cry1Ab are exceedingly rare in *O. nubilalis* (Siegfried *et al.*, 2007). By contrast, non-recessive alleles conferring resistance to Cry3Bb are not rare in *D. v. virgifera*, yielding rapid evolution of resistance in the laboratory, greenhouse and field (Tabashnik and Gould, 2012).

Although most of the available data on field-evolved resistance to Bt crops involve responses to first-generation plants that each produce a single toxin, Bt plant pyramids producing two or three toxins active against the same pest have become increasingly prevalent during the past decade (Carrière *et al.*, 2015); see also Chapters 13 (Jakka *et al.*) and 16 (Huang). We hypothesize that, as observed with single-toxin Bt crops, pyramids will be extremely effective in delaying resistance under ideal conditions, but decidedly less successful under suboptimal conditions (Brévault *et al.*, 2013; Carrière *et al.*, 2015). For example, two-toxin pyramids are expected to be especially durable when they are used proactively – when resistance to both toxins is rare – as two-toxin cotton producing Cry1Ac and Cry2Ab has been used in Australia against *H. armigera* and *H. punctigera* (Downes *et al.*, 2012a, b). However, the advantages of pyramids may

be diminished or lost when they are used remedially to counter resistance to one of the toxins in the pyramid, as in India, where cotton producing Cry1Ac and Cry2Ab is effectively a single-toxin crop against pink bollworm resistant to Cry1Ac (Tabashnik *et al.*, 2013). In general, the expected benefits of pyramids rely on 'redundant killing', which means that resistance to only one toxin in a pyramid does not increase survival on the pyramid (Brévault *et al.*, 2013; Carrière *et al.*, 2015). The expected benefits of pyramids are undermined by any factors that reduce redundant killing, including the survival of some susceptible individuals on pyramids or cross-resistance between the toxins in pyramids (Brévault *et al.*, 2013; Carrière *et al.*, 2015).

Since 2007, the US Environmental Protection Agency (US EPA) has greatly reduced refuge requirements for Bt crops despite four cases of practical resistance to Bt crops in the USA (Table 1.1), the observed association between the limited planting of refuges and rapid evolution of resistance, and recommendations from public sector scientists to maintain or increase refuge requirements (US EPA, 2002, 2013a; Alyokhin, 2011; Tabashnik and Gould, 2012). These reductions in refuge areas have been spurred, in part, by overly optimistic predictions about the durability of pyramids from computer models simulating ideal conditions (Alyokhin, 2011; Brévault *et al.*, 2013). Currently, in the USA, refuges of non-Bt maize can be as little as 5% of the total area planted to maize (US EPA, 2013b). Refuges of non-Bt cotton are no longer required for Bt cotton in most of the country, primarily because non-Bt host plants other than cotton are considered 'natural' refuges (US EPA, 2007). However, this approach largely ignores variation in host plant distribution that can yield a scarcity of refuges near Bt crops, thereby favouring the emergence and spread of resistance (Sisterson *et al.*, 2005; O'Rourke *et al.*, 2010; Onstad and Carrière, 2014). We hope that the US EPA and other regulatory agencies worldwide will heed one of the lessons learned from the failures and successes during the first 18 years of Bt crops: when

conditions are not optimal, large refuges are needed to sustain the efficacy of Bt crops and maximize their benefits.

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