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MINI REVIEW

Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America

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Abstract

Transgenic maize and cotton expressing *Bacillus thuringiensis* (Bt) toxins were first commercialized in 1996. By 2009, Bt crops were planted on ca. 47.6 Mha in 22 countries worldwide, with the USA and Canada accounting for 54% of this area. Resistance (virulence) development in target insect pests is a major threat to the sustainable use of Bt crops. Four major target pests of Bt crops in the USA and Canada – European corn borer, *Ostrinia nubilalis* (Hübner), southwestern corn borer, *Diatraea grandiosella* Dyar (both Lepidoptera: Crambidae), tobacco budworm, *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae), and pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) – remain susceptible to Bt toxins after 15 years of intensive use of Bt maize and Bt cotton. The success in sustaining susceptibility in these major pests is associated with successful implementation of the 'high-dose/refuge' insecticide resistance management (IRM) strategy: (i) Bt crop cultivars express a 'high dose', (ii) initial frequency of resistance alleles is very low, and (iii) a refuge is maintained nearby in the environment. Field resistance (including control failure) to a Bt crop has been clearly documented in three situations: fall armyworm [*Spodoptera frugiperda* JE Smith] in Puerto Rico, African stem borer [*Busseola fusca* Fuller (Lepidoptera: Noctuidae)] in South Africa, and *P. gossypiella* in India. Factors associated with these cases of field resistance include: failure to use high-dose Bt cultivars and lack of sufficient refuge. These observations support the claim that implementation of the 'high-dose/refuge' IRM strategy has been successful in substantially delaying field resistance to Bt crops.

Introduction

The ability to transfer genes among unrelated species is a major technological advance for modern agriculture. Transgenic insect-resistant maize, *Zea mays* L. (Poaceae), and cotton, *Gossypium* spec. (Malvaceae), expressing *Bacillus thuringiensis* Berliner (Bt) proteins have been adopted rapidly worldwide since they were first commercialized in 1996. Approximately 47.6 Mha of Bt crops were planted in 22 countries worldwide in 2009 (James, 2009). This included 32.4 Mha of Bt maize planted in 15 countries and 15.2 Mha of Bt cotton planted in 10 countries (Table 1). The USA has been the leading country in adopting Bt crops. In 2009 the USA planted 22 Mha of Bt maize, accounting for 63% of its total maize area, and 2.4 Mha of Bt cotton, accounting for 65% of its total cotton area (NASS, 2009). Canada has also been a major adopter of Bt maize, with an adoption rate similar to that in the USA. Other countries that planted >1 Mha of Bt maize in 2009 included Brazil (5 Mha), Argentina (1.96 Mha), and South Africa (1.67 Mha) (James, 2009). Bt cotton is also widely planted in India, China, and Australia (Table 1).

In North America, the major lepidopteran targets of Bt maize (e.g., Cry1Ab or Cry1F maize) have been the European corn borer [*Ostrinia nubilalis* (Hübner)], southwestern corn borer [*Diatraea grandiosella* Dyar (both

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Table 1 Global adoption of transgenic Bt maize and Bt cotton in 2009

Country	Crop	Year of first planting of Bt crops	Common transgenes in commercial Bt crops before 2010	Million ha (% of total planting area)	Major lepidopteran pest species
North America					
USA[1]	Maize	1996	Cry1Ab, Cry1F, Cry1A.105/Cry2Ab2	22.20 (63)	Stalk borers[2], rootworms[3], *Helicoverpa zea*, armyworms[2], cutworms[2]
	Maize	2003	Cry3Bb1, Cry34Ab1/Cry35Ab1		Rootworms[3]
	Cotton	1996	Cry1Ac, Cry2Ab2, Cry1F, Vip3A	2.40 (65)	*Heliothis virescens*, *Pectinophora gossypiella*, *H. zea*, loopers[2], armyworms[2]
Canada	Maize	1996	Cry1Ab, Cry1F, Cry1A.105/Cry2Ab2	1.05 (74)	*Ostrinia nubilalis*, *Diabrotica virgifera virgifera*, *Diabrotica barberi*, *H. zea*, *Spodoptera frugiperda*, *Agrotis ipsilon*, *Striacosta albicosta*
Asia					
India	Cotton	2002	Cry1Ac, Cry2Ab2	8.40 (87)	*Helicoverpa armigera*, *P. gossypiella*, *Earias vittella*
China	Cotton	1997	Cry1Ac, Cry1Ac/Cry1Ab	3.67 (68)	*H. armigera*, *P. gossypiella*, *Ostrinia furnacalis*, *Spodoptera exigua*, *Earias cupreoviridis*, *Earias fabil*, *Earias insulana*
Philippines	Maize	2003	Cry1Ab	0.38 (19)	*O. furnacalis*
Central and South America					
Brazil	Maize	2008	Cry1Ab	5.00 (39)	*Diatraea saccharalis*, *S. frugiperda*, *H. zea*
	Cotton	2002	Cry1Ac	0.15 (18)	*Alabama argillacea*, *P. gossypiella*, *H. virescens*
Argentina	Maize	1998	Cry1Ab	1.96 (76)	*D. saccharalis*, *Elasmopalpus lignosellus*, *H. zea*
	Cotton	1998	Cry1Ac	0.25 (70)	*Helicoverpa gelotopoeon*, *H. zea*, *H. virescens*, *A. argillacea*, *P. gossypiella*
Africa					
South Africa	Maize	1998/1999	Cry1Ab	1.61 (67)	*Busseola fusca*, *Chilo partellus*
Burkina Faso	Cotton	2008	Cry1Ac	0.12 (29)	*H. armigera*, *Earias* spp., *Diparopsis watersi*
Australia					
Australia	Cotton	1995/1996	Cry1Ac/Cry2Ab2	0.17 (86)	*H. armigera*, *Helicoverpa punctigera*
Total				>47.7	

Countries listed planted a minimum 100 000 ha of Bt crops in 2009. Data for the USA were from NASS (2009), for Canada were based on the report from Dunlop (2009), and others were based on James (2009). Additional countries that planted <100 000 ha in 2009 included Uruguay, Spain, Mexico, Colombia, Honduras, Egypt, Czech Republic, Portugal, Poland, Romania, Slovakia, and Puerto Rico.

[1] Area for maize is for all Bt products combined. A small area of Bt potato expressing Cry3A has also been planted in the USA for controlling *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae).

[2] Maize stalk borer species may include *O. nubilalis*, *D. grandiosella*, *Papaipema nebris* Guenée (Lepidoptera: Noctuidae), *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), *D. crambidoides*, *D. saccharalis*, *D. lineolata*, and *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae). Armyworm species may include *S. frugiperda*, *Pseudaletia unipunctata* (Haworth) (Lepidoptera: Noctuidae), and *S. ornithogalli*. Cutworm species may include *Agrotis ipsilon* (Hufnagel), *Agrotis gladiaria* Morrison, *Loxagrotis albicosta* (Smith), *Lacinipolia renigera* (Stephens), *Nephelodes minians* Guenée, *Felita jaculifera (= ducens)* (Guenée), *Apamea devastator* (Brace), *Spodoptera ornithogalli* (Guenée), *Euxoa ochrogaster* (Guenée), *Euxoa detersa* (Walker), *Xestia* spp., and *Peridroma saucia* Hübner (all Lepidoptera: Noctuidae). Looper species may include *Pseudoplusia includens* (Walker) and *Trichoplusia ni* (Hübner) (both Lepidoptera: Noctuidae). Major target species can be different depending on the geographical areas (Steffey et al., 1999).

[3] Maize rootworm species are Chrysomelidae (Coleoptera) and may include *D. virgifera virgifera*, *D. barberi*, and *Diabrotica virgifera zeae* Krysan & Smith.

Crambidae)], and bollworm [*Helicoverpa zea* (Boddie) (Noctuidae)]. The major targets of Bt cotton in the USA are the tobacco budworm [*Heliothis virescens* Fabricius (Lepidoptera: Noctuidae)], bollworm (*H. zea*), and pink bollworm [*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)]. In Asia and Australia, the primary target pest of Bt cotton has been *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Table 1). In general, the field performance of commercialized Bt crop varieties has been outstanding for controlling the target pests.

Growers in North America have recognized this advantage and have been eager to take the advantage of the economic and environmental benefits offered by Bt crops (Hutchison et al., 2010; National Research Council, 2010). However, adoption in Europe and many developing countries has been uneven (Andow et al., 2008; Wu et al., 2008; James, 2009; Fonseca & Zambrano, 2010).

The rapid adoption of Bt crops is a threat to the long-term durability of Bt crops. Widespread planting of Bt crops places strong selection pressure on the pest populations, which could result in development of resistance to Bt (virulence) and control failure (Alstad & Andow, 1995; Ostlie et al., 1997; Gould, 1998; Tabashnik et al., 2008). In the plant resistance literature, resistance is the property expressed by the plant to the insect, and virulence is the property expressed by the insect to the plant. We employ the word 'resistance' as a synonym for 'virulence' throughout this review for convenience. Control failure or reduced efficacy of Bt crops due to resistance has been clearly documented in three cases in the world. However, after 15 years of intensive use of Bt maize and Bt cotton in North America, Bt crops remain effective against their major target pest species, *O. nubilalis*, *D. grandiosella*, *H. virescens*, and *P. gossypiella*, and there is no sign of resistance in these four pests.

To delay resistance development, government agencies in the USA and Canada have adopted an insecticide resistance management (IRM) plan, known as the 'high-dose/refuge' strategy, for planting Bt crops (Ostlie et al., 1997; Gould, 1998; Baute, 2004). This strategy involves, first, using 'high-dose' Bt plants that can kill ≥95% of the heterozygotes for Bt resistance. This prevents heterozygous insects on Bt plants from transmitting the resistance alleles to the next generation. The 'high/dose refuge' strategy requires, second, farmers to plant a specified proportion of their crop to a non-Bt variety of the crop to serve as a refuge for hosting susceptible insects. Bt-susceptible insects should emerge from refuge areas and mate with the rare potentially resistant homozygous individuals that might emerge from the Bt crop. If the frequency of resistance is low enough, typically ca. 0.001, most offspring will be heterozygous and thus be killed by the high-dose Bt plants. Consequently, resistance allele frequencies in field populations should remain low for a long period of time. Mathematical modeling has shown that the 'high-dose/refuge' IRM strategy should delay resistance development in target pest populations if the three fundamental requirements of the strategy are met (Alstad & Andow, 1995; Onstad & Gould, 1998; US EPA, 2001; Onstad et al., 2002; Caprio et al., 2004; Tyutyunov et al., 2008).

Several reviews on Bt resistance management have been published (Tabashnik, 1994; Gould, 1998; Tabashnik & Carrière, 2007; Andow, 2008; Andow et al., 2008; Ferré et al., 2008; Tabashnik et al., 2008, 2009). In this review, we discuss the management of resistance in the major lepidopteran pests of Bt crops in North America and compare this to cases of field resistance of Bt crops elsewhere in the world. We focus this review on *O. nubilalis*, *D. grandiosella*, *H. virescens*, and *P. gossypiella* because there is substantial information on these four pests. The long-term use of Bt crops against these major agricultural pests in the USA and Canada provides an excellent opportunity to evaluate the effectiveness of the 'high-dose/refuge' IRM strategy. Information on other minor target pests at this time is still too limited for analysis of success/failure of resistance management strategies. In North America, there are two additional target pests, the northern and western corn rootworms, *Diabrotica barberi* Smith & Lawrence and *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Table 1). Bt maize expressing Cry3Bb1 or Cry34/35Ab1 has been deployed to manage these below-ground pests. These Bt maize technologies are not high-dose technologies, and even though they have just been recently commercialized, the resistance risk is considerably higher (Cook et al., 2004; Oyediran et al., 2005; Meihls et al., 2008). The objective of this review is to gain a better understanding of the circumstances that led to these successes and failures of resistance management so that improved IRM strategies can be developed and problems in the future can be avoided.

Definition of resistance

There is considerable disagreement in scientific literature on the definition of resistance, especially 'field resistance' (Andow, 2008; Tabashnik et al., 2009). The National Research Council (1986) proposed that insecticide resistance be defined as an individual trait, which is an inherited ability of an insect to tolerate doses of a toxicant that would prove lethal to the majority of individuals in the normal population of the species. Andow (2008) suggested that a major resistance allele to a Bt crop is present when homozygous-resistant individuals can grow and mature on the Bt crop and can mate and produce viable offspring. Such major resistance genes have been detected in several target pests of Bt crops (see reviews in Tabashnik et al., 2008; Meihls et al., 2008; Downes et al., 2009; Liu et al., 2010). However, finding major resistance alleles in field insect populations does not mean that field resistance is present.

The Insecticide Resistance Action Committee (2010) defined field resistance to be when there is 'repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species'. Therefore, field resistance is a characteristic of a population and depends on many factors including resistance allele frequency, fitness of resistant individuals, and population densities in the field. The differentiation between 'individual' resistance and field resistance is necessary because individual resistance will not always translate to field resistance; field resistance occurs only when individual resistance is common. Based on this definition of field resistance, resistance to a Bt crop is signified when there is field control failure or reduced efficacy of the Bt crops. After 15 years of use of Bt crops in the world, field resistance or control failure has been clearly documented in three cases, which are discussed below.

Factors contributing to the long-term success of Bt crops for management of major target pests in North America

The 15 years of success of transgenic Bt crops in managing *O. nubilalis*, *D. grandiosella*, *H. virescens*, and *P. gossypiella* in North America without any signs of resistance is not unexpected. Here we review information to show that the fundamental requirements of the 'high-dose/refuge' IRM strategy have applied to these pests, and suggest that this is the primary reason for their continued susceptibility. We also consider several other factors that might have contributed to the long-term success of Bt crops in North America.

High dose (recessive resistance) of commercial Bt crop cultivars against target pests

The success of the 'high-dose/refuge' strategy requires that Bt plants express a sufficiently high concentration of Bt proteins to ensure that ≥95% of the heterozygous individuals carrying one copy of a major resistance allele can be killed (Andow & Hutchison, 1998; US EPA, 2001).

The high-dose qualification has not been directly evaluated for most target pests of Bt maize or cotton because major resistance traits have not been found in most of these species, particularly at the time when applications were made for unregulated status (Bourguet et al., 2003). Therefore, some indirect criteria of 'high dose' have been proposed. The US EPA Scientific Advisory Panel (SAP) on Bt Plant-Pesticides and Resistance Management (US EPA-SAP, 1998; US EPA, 2001) used empirical data to suggest that a working definition of the high dose should be 'a dose 25 times the toxin concentration needed to kill Bt-susceptible larvae.' Caprio et al. (2000), based on a literature review, suggested 50 times the toxin concentration needed to kill 50% of the Bt susceptible larvae as a better standard. This indirect definition of 'high dose' has been used to evaluate the high-dose status of Bt crops in the USA (US EPA, 2001). The SAP recognized five methods to demonstrate that a transgenic crop expresses a high dose of Bt proteins (US EPA-SAP, 1998; US EPA, 2001). A high-dose Bt plant for one pest species is not necessarily a high dose against another target pest. Published data have shown that all commercial Bt crop cultivars are very effective against their major target pest species, *O. nubilalis* and *D. grandiosella* for Bt maize, and *H. virescens* and *P. gossypiella* for Bt cotton. Based on these results, all Bt maize (except for the Cry1Ac maize which was planted for only a few years in the 1990s) and Bt cotton varieties that have been commercially available in North America are presumed to meet the high-dose requirement of the IRM strategy for these four pests (US EPA, 2001).

In some cases, major resistance alleles have been found, so high-dose can be evaluated directly by measuring the survival of heterozygotes (genetic dominance). The major resistance alleles to Bt cotton in laboratory-selected strains of *H. virescens* (Gould et al., 1995; Jurat-Fuentes et al., 2000; Heckel et al., 2007) and *P. gossypiella* (Liu et al., 2001; Tabashnik et al., 2002) are recessive and the genetic dominance is <5% (Table 2). For example, resistance to Cry1Ac in the laboratory-selected YHD2 strain of

Table 2 Fitness of resistance genotypes on Bt and non-Bt plants assumed for the 'high-dose/refuge' IRM strategy, and more general conditions

		Fitness		
Assumption	Plant type	Susceptible homozygote	Heterozygote	Resistant homozygote
High-dose/refuge	Bt plants	K	$(1-d_r)K + d_r$	1
	Non-Bt plants	1	1	1
General	Bt plants	K	$(1-d_r)K + d_r(1-L)$	1−L
	Non-Bt plants	1	$(1-d_c) + d_c(1-C)$	1−C

K = efficacy of Bt plant, d_r = genetic dominance of resistance ($d_r = 0$ is a recessive resistance), L = measure of incomplete resistance, C = measure of cost of resistance, and d_c = dominance of cost of resistance ($d_c = 1$ is a dominant cost).

H. virescens with 230 000-fold resistance rate survived on commercial Cry1Ac cotton (US EPA, 2002) and was inherited recessively (Gould et al., 1995; Jurat-Fuentes et al., 2000; Gahan et al., 2001; Heckel et al., 2007). Bt resistance in laboratory-selected strains of *P. gossypiella* was also recessive at the high concentration of Cry1Ac in Bt cotton (Liu et al., 2001; Tabashnik et al., 2002). Partially recessive major resistance has been identified to Cry1F maize in a laboratory-selected Cry1F-resistant strain of *O. nubilalis* (Pereira et al., 2008). Although the authors found >3 000-fold resistance to Cry1F toxin in this strain, survival of heterozygous larvae on Cry1F maize had a dominance of 0.23 on vegetative and 0.10 on reproductive stage maize. However, when larval survival and growth were considered together, dominance was reduced to 0.07 and 0.004 on vegetative and reproductive stage maize, respectively (table 1 in Pereira et al., 2008). Because larval mortality was measured at the 15th day after inoculation of neonates in the study, the dominance could be reduced more if mortality were checked later. Therefore it appears that Cry1F maize should qualify as high dose for *O. nubilalis*. In addition, a field-developed resistance in the fall armyworm, *Spodoptera frugiperda* JE Smith (Noctuidae), to Cry1F maize is also partially recessive, but not high dose (Storer et al., 2010). Dominance of Cry1F resistance in *S. frugiperda* was reported to be 0.07 and 0.14 for larval growth inhibition and survival, respectively (Storer et al., 2010). Major resistance alleles to Bt crops have been found to be partially recessive in *H. armigera* (Bird & Akhurst, 2004; Xu et al., 2005), *D. saccharalis* (Wu et al., 2009a), and species on other crops besides maize and cotton (Heckel et al., 2007), or even partially dominant in *H. armigera* (Bird & Akhurst, 2005).

Initial Bt resistance allele frequency in field insect populations

Studies have provided sufficient data to document that the frequencies of Bt resistance allele in field populations of *O. nubilalis*, *D. grandiosella*, *H. virescens*, and *P. gossypiella* in North America are very low (Table 3) and meet the rare resistance allele requirement of the 'high-dose/refuge' strategy. A 10-year resistance monitoring study in *O. nubilalis* showed that there were no detectable changes in Cry1Ab gross susceptibility among populations across the major maize production areas in the USA (Siegfried et al., 2007). In addition, >3 000 isoline families of *O. nubilalis* from the USA and Europe have been examined for Cry1Ab resistance using F_2 screens (Andow & Alstad, 1998; Andow et al., 1998, 2000; Bourguet et al., 2003; Farinós et al., 2004; Stodola et al., 2006; Engels et al., 2010). Only a few minor resistance alleles were detected, but no major Cry1Ab resistance allele has been found in the numerous populations that have been examined. Bt resistance allele frequency has been estimated to be <0.0004 with 95% probability in *O. nubilalis* in the USA (Bourguet et al., 2003). The situation for *D. grandiosella* is similar to that for *O. nubilalis* in that no major gene for resistance to Bt maize has been found, although the search has been more limited for *D. grandiosella* than for *O. nubilalis* (Trisyono & Chippendale, 2002; Huang et al., 2007a). Estimated resistance allele frequency in *D. grandiosella* in the USA mid-south region is <0.0035 with 95% credibility (Huang et al., 2007a).

Similarly, resistance monitoring in *H. virescens* populations from 2002 to 2008 showed that there were no significant changes in gross susceptibility to Cry1Ac and Cry2Ab2 in field populations across the USA Cotton Belt (Ali et al., 2006; Ali & Luttrell, 2007; Luttrell & Ali, 2009).

Table 3 Summary of the application of the three fundamental requirements of the 'high-dose/refuge' strategy to Cry1Ab maize and Cry1Ac cotton in the USA, and Bt crops and localities with documented (grey boxes) or suspected (light grey boxes) field resistance

Bt crop and locality	Pest	Recessive resistance (high dose)[1]	Resistance allele <0.001[2]	Refuge[1]
Cry1Ab maize in USA	*Ostrinia nubilalis*	+	<0.0004	+
Cry1Ab maize in USA	*Diatraea grandiosella*	+	<0.0035	+
Cry1Ab maize in South Africa	*Busseola fusca*	–	?	–
Cry1F maize in Puerto Rico	*Spodoptera frugiperda*	–	?	–?
Cry1Ac cotton in USA	*Heliothis virescens*	+	0.0004	+
Cry1Ac cotton in USA	*Pectinophora gossypiella*	+	<0.0003	+
Cry1Ac cotton in USA	*Helicoverpa zea*	–	?	+
Cry1Ac cotton in India	*P. gossypiella*	+	?	–?
Cry1Ac cotton in China	*Helicoverpa armigera*	+/–	?	–?

[1] '+' = meets the requirement of the 'high-dose/refuge' IRM strategy; '–' = does not meet the requirement; '+/–' = meets the requirement in the vegetative plant stages but not for the reproductive stages; '?' = information is unavailable.

[2] Bt resistance allele frequencies are based on Bourguet et al. (2003) for *O. nubilalis*, Huang et al. (2007a) for *D. grandiosella*, Blanco et al. (2009) for *H. virescens*, and Tabashnik et al. (2006) for *P. gossypiella*. Inequalities provide upper range of the 95% credibility interval for *O. nubilalis* and *D. grandiosella*, and a 95% confidence interval for *P. gossypiella*. The value for *H. virescens* is an expected frequency.

During 1993, Gould et al. (1997) examined >1 000 feral individuals of *H. virescens*, collected from Mississippi, Louisiana, Texas, and North Carolina (all USA) and found three males that had a major resistance allele to Cry1Ac. The Bt resistance allele frequency was estimated at 0.0019 (range 0.0005–0.0043) (Blanco et al., 2009). A mutation of the cadherin gene was found to be associated with resistance to Cry1Ac in this species (Gahan et al., 2001). A subsequent DNA screen of >7 000 *H. virescens* individuals collected from Louisiana and Texas during 1996–2002 found no individuals carrying that mutation (Gahan et al., 2007). In addition, an F_2 screen of >1 000 isoline families of *H. virescens* collected from the USA Cotton Belt during 2006 and 2007 also did not find major resistance alleles, and the estimated resistance allele frequency of 0.0004 (range 0–0.0025) was not significantly different from that estimated during 1993 (Blanco et al., 2009). This suggests that resistance in *H. virescens* to Cry1Ac has not increased after 15 years of intensive use of Bt cotton in the USA.

For *P. gossypiella*, statewide resistance monitoring to Cry1Ac toxin has been conducted in Arizona (USA) since 1997 (Patin et al., 1999; Tabashnik et al., 2000). An early survey reported that resistance allele frequency to Cry1Ac in 10 field populations of *P. gossypiella* collected from Bt and non-Bt cotton plants in 1997 was high: 0.16 (range 0.05–0.26) (Tabashnik et al., 2000). The estimated resistance allele frequencies in this survey could be overestimated if the results were applied to natural populations because three of the 10 populations were collected from Bt plants. In addition, a low discriminating dose, 10 μg of Cry1Ac per ml of diet, was used to identify the resistance. In fact, a later study using a DNA screening method revised the resistance allele frequency in *P. gossypiella* to be <0.0003 with 95% confidence in Arizona, California, and Texas combined (Tabashnik et al., 2006).

Refuges in the USA and Canada

The refuge plan in the 'high-dose/refuge' IRM strategy has generally been implemented successfully for all Bt maize varieties in the USA and Canada. This has required a significant effort from government agencies, crop growers, extension personnel, biotech industries, scientists, and others who are interested in Bt technology. During the early commercialization of Bt maize, grower compliance to refuge requirements was reported to be high. For example, an early survey showed that >85% maize growers in the USA complied with both the size and placement requirements for refuges (ABSTC, 2002). From 2001 to 2006, compliance rates between 72 and 96% were reported (Jaffe, 2003a,b; ABSTC, 2005; Goldberger et al., 2005), with the industry reporting 96% (ABSTC 2005). However, by 2007 and 2008, ABSTC was reporting only 74–80% compliance (US EPA, 2010). Similarly, a 2005 Canadian survey reported that >80% growers followed the refuge requirements and that the reported compliance did not change significantly compared with the survey results in 2001 (Canadian Corn Pest Coalition, 2005). A more recent survey reported that the compliance rate was 61% in 2009 (Dunlop, 2009). The Canadian survey also showed that nearly 23% of the non-compliant growers had planted some refuge, 11% of growers had planted between 15 and 20% refuge, and only 17% of growers had planted no refuge at all. This relatively high compliance rate in the USA and Canada has undoubtedly contributed to the long-term success of Bt maize in North America.

About 65% of cotton in the USA was Bt cotton during 2009 (Table 1). Nationwide compliance rates for Bt cotton refuge requirements are not available. Carrière et al. (2005) measured the compliance with refuge requirements for Bt cotton in six Arizona regions from 1998 to 2003 using the geographical information system technology. Their survey showed an overall compliance rate of >88% in 5 of the 6 years.

Natural refuges may also play an important role in the long-term success of Bt cotton in southeastern USA. Tobacco and soybean may be important refuges for *H. virescens* in parts of the southeast (Abney et al., 2007). Consequently, in 2007, the US EPA allowed unstructured, natural refuges for planting pyramided Bt cotton varieties (e.g., Bollgard II$^{®}$) in southeastern USA (US EPA, 2006; Dow AgroSciences, 2007). Because *H. zea* is a polyphagous species with many other host plants (US EPA, 2001; Dow AgroSciences, 2007), alternate hosts should play a critical role as Bt cotton refuges for *H. zea* throughout the southwestern USA (Gould et al., 2002; Jackson et al., 2008; Head et al., 2010).

Incomplete resistance and fitness cost

The long-term success of Bt crops for managing resistance in *O. nubilalis*, *D. grandiosella*, *H. virescens*, and *P. gossypiella* may be also associated with incomplete resistance and costs of resistance, factors that are not usually included in IRM planning. These factors can delay resistance evolution, but require identification and evaluation of major resistance alleles, which typically are not available when the IRM plan is being constructed. For example, major resistance alleles for Cry1Ab have still not been found for *O. nubilalis* or *D. grandiosella*, so risk-averse assumptions are used, and it is assumed that resistance is complete (Table 2, high-dose/refuge assumptions; $L = 0$) and there is no cost of resistance (Table 2; $C = 0$).

Incomplete resistance is when resistant homozygous insects on Bt plants have lower fitness than susceptible insects on refuge plants (Table 2; $L>0$) (Tabashnik & Carrière, 2007). Incomplete resistance will slow down resistance evolution by reducing the selective advantage of resistance. It is typically observed when major resistance alleles have been found. Resistant insects on Bt plants often develop more slowly, have a greater mortality, and/or produce fewer offspring than susceptible insects on non-Bt plants. For example, survival of a Cry1F-resistant strain of *O. nubilalis* on Cry1F maize plants at vegetative stages was about 17% of that observed for susceptible larvae on the non-Bt isoline plants (Pereira et al., 2008). Cry1Ac-resistant *P. gossypiella* on Bt cotton plants required an average of 5.7 days longer to develop compared to on non-Bt cotton (Liu et al., 1999) and the resistant strain had a lower survival rate and a lower pupal weight (Carrière et al., 2006). Similarly, the survival of the Cry1Ac resistant strain of *H. virescens* (YHD2) on Cry1Ac cotton exhibited less than half of the survivorship on conventional cotton plants (US EPA, 2002), also implying that the resistance was incomplete. Incomplete resistance also appears to be very common for resistance to Bt crops in other target pests. For example, Bird & Akhurst (2004) reported that growth and development of resistant *H. armigera* larvae on Bt cotton were significantly delayed when compared to the susceptible strain on non-Bt plants. The overall intrinsic rate of increase of *H. armigera* on Bt cotton was reduced >50% in the resistant strain compared with the susceptible strain on non-Bt cotton. Huang et al. (2007b) also observed that resistant neonates of the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), took 20–30 more days to reach the pupal stage on Cry1Ab maize than susceptible insects on non-Bt plants (Huang et al., 2007b). Incomplete resistance can evolve to become complete resistance (McKenzie, 1996), although this has not been examined for any of the Bt resistance traits.

A fitness cost of resistance is when the homozygous resistant larvae on a non-Bt plant have lower fitness than the susceptible larvae on the same non-Bt plant (Table 2; $C > 0$) (Gassmann et al., 2009). Resistance can be delayed or halted in field populations if there is a fitness cost. In most cases, major Bt resistance alleles have some detectable fitness costs (Gassmann et al., 2009). For example, Cry1Ac-resistant *P. gossypiella* had a significant fitness cost in larval survival (Carrière et al., 2001, 2005), and sometimes in larval development time (Carrière et al., 2001, 2006), but not in larval mass (Carrière et al., 2006). Compared to the susceptible insects, larval survival of two resistant strains of *P. gossypiella* was reduced by 52% on non-Bt cotton (Carrière et al., 2001). Growth rate of Cry1Ac-resistant *H. virescens* was less than that of susceptible larvae on diets (Gahan et al., 2005; Jackson et al., 2007), but no fitness costs were detected in larval survival on non-Bt cotton plants (Johnson et al., 1997; Jackson et al., 2007). Available data have shown that major resistance to Bt cotton is associated with fitness cost in several other target pests including *H. armigera* (Bird & Akhurst, 2004, 2005, 2007; Liang et al., 2008) and *H. zea* in laboratory assays (Burd et al., 2003; Jackson et al., 2006; Anilkumar et al., 2008). However, there are some exceptions; for example, a resistant strain of *D. saccharalis* survives and completes larval development on Cry1Ab maize, with no observable fitness cost associated with the resistance (Wu et al., 2009b).

Fitness costs may have particularly strong evolutionary effects if they are non-recessive (Table 2; $d_c>0$ is non-recessive; $d_c = 1$ is dominant and implies fitness of heterozygotes on non-Bt plants = $1-C$) (Carrière et al., 2006; Gassmann et al., 2009). When the frequency of resistance is low, most individuals carrying resistance alleles are heterozygotes, so a non-recessive fitness cost will lower heterozygote fitness on non-Bt plants relative to their susceptible counterparts. Dominance of a fitness cost is frequently not measured. However, in *P. gossypiella*, all well-designed measures of dominance levels showed that fitness costs in this pest were recessive (Carrière et al., 2001, 2005, 2006). Resistance to Cry1Ac cotton in laboratory-selected strains in *P. gossypiella* was associated with three resistance alleles (r1, r2, r3) of a cadherin gene (Morin et al., 2003). To determine the dominance of fitness costs of Cry1Ac resistance in *P. gossypiella*, Carrière et al. (2006) used two hybrid strains of *P. gossypiella* and polymerase chain reaction amplifications to test the association between cadherin genotype and fitness components for individuals sharing a common genetic background. Greenhouse studies showed that on non-Bt cotton plants, larval development time of homozygotes was significantly greater than that of the susceptible strain, while the development time was similar between susceptible strain and heterozygous genotypes (Carrière et al., 2006). These results suggested that the fitness cost of the Cry1 Ac-resistance in *P. gossypiella* was recessive. In contrast, in *H. armigera*, a mixture of recessive, dominant and overdominant costs were observed (Bird & Akhurst, 2004, 2005, 2007).

Field resistance to Bt crops and potential reasons for the resistance

Reasons for the development of field resistance to Bt crops are still unclear, but the lack of implementation of the 'high-dose/refuge' IRM strategy seems to be a primary cause. In all three cases that field resistance has been clearly documented, adoption of Bt crops was rapid and high, but no more so than in many regions of mainland USA, where field resistance has clearly not yet occurred in the four major target pests and the 'high-dose/refuge' IRM strategy has been implemented. Specifically there are at least two factors that may have involved in the three documented cases of field resistance: (i) use of non-high-dose Bt cultivars, and (ii) lack of refuge planting.

Puerto Rico

The first case of field resistance to Bt crops is the resistance of *S. frugiperda* to Cry1F maize in Puerto Rico (US EPA, 2007a; Matten et al., 2008; Storer et al., 2010). Cry1F maize became commercially available in Puerto Rico in 2003 to control *S. frugiperda*. By 2006 there were reports of unexpected damage to Cry1F maize by *S. frugiperda* in three regions on the island, which were identified as field resistance (US EPA, 2007b). Laboratory bioassays in 2007 documented that insect populations collected from two of these regions (Santa Isabel and Salinas, about 10 km apart from each other) were >160-fold more tolerant to Cry1F toxin than a laboratory susceptible strain (US EPA, 2007b; Storer et al., 2010). Cry1F maize was withdrawn from commercial use in Puerto Rico (US EPA, 2007a; Storer et al., 2010). The Cry1F resistance in the Puerto Rico populations of *S. frugiperda* was shown to be autosomally inherited and partially recessive (Storer et al., 2010), but not recessive enough to be considered high dose for Cry1F maize (Siebert et al., 2008; Hardke et al., 2011).

In Puerto Rico, Cry1F maize was grown in a few of the narrow coastal plains around the island, and this created a series of relatively small isolated populations around the island (US EPA, 2007a,b, Storer et al., 2010). A small portion of the land is irrigated with continuous maize production all year. *Spodoptera frugiperda* pressure is high in and around these irrigated areas. During the dry season nearly all *S. frugiperda* would be restricted to these small isolated irrigated maize fields. The adoption rate for Bt maize in Puerto Rico is not available, but it is believed to have been very high with little or no refuge in the irrigated regions where resistance to Bt maize occurred in *S. frugiperda*. This kind of environment would be expected to increase selection pressure and speed up resistance evolution. In irrigated tropical regions, *S. frugiperda* can have up to 12 generations per year (Andrews, 1980), although 4–6 may be more common in non-irrigated regions (EPPO, 1990). Hence, during the 3-year period of commercial use prior to the control failure there could have been as many as 36 generations of *S. frugiperda* under high selection for Cry1F resistance with little refuge.

South Africa

The second case is the resistance of an African stem borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae), to Cry1Ab maize (e.g., YieldGard® maize) in South Africa. Bt maize (Cry1Ab) was commercialized in the 1998/1999 cropping season for controlling a maize stalk-borer complex of *B. fusca* and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (van Wyk et al., 2009). Of the two, *B. fusca* was much less susceptible to Cry1Ab maize than *C. partellus* (van Rensburg, 2007). After its introduction, adoption of Bt maize in South Africa increased rapidly, approaching 1.61 Mha in 2009, which accounted for 67% of South Africa's total maize area (Table 1; van Rensburg, 2007). Cry1Ab maize hybrids were effective against *B. fusca* in the vegetative growth stages of maize during the first growing season (1998/1999). Later that same growing season, damaged plants and diapausing larvae of *B. fusca* were observed on various Bt maize hybrids (van Rensburg, 2007), which is a good indication that Cry1Ab maize was not a high dose for *B. fusca.* During the 2004/2005 growing season, severe damage to vegetative stages of Bt maize plants from *B. fusca* was recorded in several locations in the Vaalharts irrigation scheme, a 32 000-ha irrigated valley near the Vaals River. Field and greenhouse tests documented that survival and weight gain of the larvae collected from Bt maize plants in this area were considerably greater than those from non-Bt maize areas (van Rensburg, 2007), confirming that the damage to Bt maize was due to resistance in *B. fusca*. During 2005/2006, an additional resistant population of *B. fusca* was recorded in another area of the Vaalharts irrigation scheme (Kruger et al., 2009). Information on inheritance, cross-resistance, and fitness of these Cry1Ab-resistant *B. fusca* is not yet available.

The Genetically Modified Organism Act of South Africa directs seed companies to develop contracts with farmers that require a planting of 20% refuge for IRM (Kruger et al., 2009; Monsanto, 2010a). However, for several reasons maize producers disprefer planting non-Bt refuge maize on irrigated land. In the Vaalharts irrigation scheme, adoption of Bt maize reached 95% (Kruger et al., 2009), and nearly all fields had a history of continuous Bt maize production. This suggests that there was considerable noncompliance with the refuge requirements. In addition, even the very limited area of non-Bt maize may not have functioned well as a refuge because it was too far from most of the Bt maize (Kruger et al., 2009). The lack of refuge planting would place a very high selection pressure on the field population of *B. fusca* and thus lead to resistance (van Rensburg, 2007).

India

The third case of field resistance is in *P. gossypiella* to Cry1AC cotton in Gujarat, India. Gujarat is the second largest cotton-producing state in India with 2.4 Mha during 2007/2008 (Karihaloo & Kumar, 2009). India first commercialized Cry1Ac Bt cotton in 2002/2003 with 0.5 Mha. Since then, adoption of Bt cotton has been rapid and reached 8.4 Mha in 2008/2009 (Table 1). At the same time, its total cotton area has also expanded from 7.7 Mha in 2002/2003 to 9.4 Mha in 2008/2009 (Karihaloo & Kumar, 2009). During the 2008/2009 cropping season, unexpected survival of *P. gossypiella* in several Bt cotton fields was observed in Gujarat (Monsanto, 2010b). Although there are still some disagreements, recent laboratory bioassays have confirmed that the field survival was associated with major resistance to Cry1Ac (Tabashnik & Carrière, 2010; Dhurua & Gujar, 2011). The exact spatial extent of field failures is not known, but its simultaneous occurrence in four different areas of Gujarat indicates that it is widespread.

About 83% of the Cry1Ac cottons available in India contain Mon531 or Mon15985 (Karihaloo & Kumar, 2009). These varieties and their Bt progeny are known to express a high dose against *P. gossypiella*. There is some concern that the extensive illegal Bt cotton seed produced and grown in Gujarat may express low doses of toxin (Monsanto, 2010b). About 26% of the cotton grown in Gujarat during the 2007/2008 season was believed to be illegally produced seed (Business Standard, 2008; Karihaloo & Kumar, 2009). The composition and expression of the illegal seed is not known. However, the dominant market position of Mon531 and Mon15985 might suggest that these events comprise the majority of the illegal seed, and that seed mixes of Bt and non-Bt plants is the more likely illegal product than low dose expression.

The Indian government requires each Cry1Ac cotton field to be surrounded by a non-Bt refuge of a popular non-Bt hybrid or pigeon pea (Karihaloo & Kumar, 2009). The refuge size is supposed to be larger than five rows or a minimum of 20% of the field area. Compliance is unknown; however, it is believed that the refuge requirement has not been followed in India (Stone, 2004; APCoAB, 2006; Karihaloo & Kumar, 2009). Indeed, based on the previously cited statistics (Table 1), country-wide adoption of Bt cotton was near 90% during 2008/2009, which implies extensive noncompliance in the country.

Suspected field resistance

Field resistance to Bt crops has been suggested in two additional cases, *H. zea* to Cry1Ac cotton in southcentral USA (Tabashnik et al., 2008, 2009), and *H. armigera* to Cry1Ac cotton in China (Liu et al., 2010). Current observations suggest that field resistance might have occurred, but these observations have not yet been confirmed. For both cases, as reviewed below, the 'high-dose/refuge' IRM plan has not been implemented.

In 7 years of resistance monitoring in southcentral and southeastern USA, 5% of the populations of *H. zea* collected from Bt crop fields were found to be >100-fold more resistant to Cry1Ac toxin than a susceptible laboratory strain (Luttrell & Ali, 2009), indicating a significant increase in resistance allele frequencies. A slight increase in resistance to Cry1Ac was also reported in *H. zea* during the first 3 years of commercial use of Bt cotton in the Mississippi Delta area (Hardee et al., 2001). Laboratory bioassays have shown a considerable variability in Cry1Ac susceptibility in *H. zea* among field populations collected from different locations and at different times (Ali et al., 2006, 2007; Luttrell & Ali, 2009), but the observed monitoring results are greater than the natural variability. In addition, field populations of *H. zea* collected from Bt cotton plants in USA have shown some level of reduced susceptibility to Cry2Ab2, one of the two Bt proteins expressed in Bollgard II® cotton (Ali & Luttrell, 2007; Luttrell & Ali, 2009). Field resistance (control failure) has not yet been clearly documented (Moar et al., 2008; Luttrell & Ali, 2009), however, during 2010 there were preliminary reports of field resistance to Bt cotton in *H. zea* (F Huang & LL Buschman, pers. comm.) that still need verification to confirm development of field resistance. Cry1Ac cotton (e.g., Bollgard® I) does not express a high dose against *H. zea* (US EPA, 2001; Jackson et al., 2003). Compared to *H. virescens*, *H. zea* is more broadly polyphagous, so there will be more natural refuge for *H. zea* than *H. virescens*. Hence the refuge requirements implemented for *H. virescens* may suffice for *H. zea* as well.

Field resistance to Cry1Ac cotton is also suspected in *H. armigera* in China. Commercial planting of Cry1Ac Bt cotton began in 1997 with <0.1 Mha and expanded to 3.67 Mha in 2009 (Table 1; Wu, 2007). Bt cotton has accounted for ca. two-thirds of the country's total cotton area since 2003. In 9 years (1999–2007) of resistance monitoring, the frequency of the Cry1Ac resistance allele in *H. armigera* populations in Qiuxian County (northern China) increased from 0.0058 in 1999 to 0.091 in 2007 (Xu et al., 2009; Liu et al., 2010). Bioassays with purified Cry1Ac toxin have also demonstrated that populations collected from Bt cotton plants in 2007 were significantly less susceptible to Cry1Ac toxin than a laboratory susceptible colony. In addition, field surveys showed that the numbers of *H. armigera* eggs laid on Bt cotton plants increased consistently from 2003 to 2007 (Liu et al., 2010). However, field control failures due to resistance have not yet been documented from this area. Cry1Ac cotton is high dose for the first generation of *H. armigera*, but low dose for the second generation (Bird & Akhurst, 2004, 2005). In addition, there is no refuge requirement, and it is not known whether naturally occurring refuges are sufficient. It is believed, however, that there is insufficient refuge in at least some areas (Wu, 2007).

Increase in resistance frequencies

Because resistance is an individual trait, the frequency of resistance in a population will increase prior to consequent field resistance. Hence, an increase in the frequency of resistance does not indicate a failure or reduced efficacy of a control strategy. In a rather unfortunate confusion of terminology, Tabashnik et al. (2008, 2009) focused on ‘field-evolved resistance,’ which is defined as an increase in resistance frequency in a naturally occurring population. ‘Field-evolved resistance’ is readily confused with the term ‘field resistance.’ We shall refer to ‘increase in resistance frequency’ instead of ‘field-evolved resistance.’ An increase in resistance frequency indicates that evolution of resistance is occurring. There are three additional cases where resistance frequencies appear to be increasing in natural populations. These include *H. armigera* and *H. punctigera* on Bt cotton in eastern Australia and *D. saccharalis* on Bt maize in mid-southern USA.

The frequency of Cry2Ab resistance alleles in the Australian populations of *H. armigera* and *H. punctigera* may be increasing. Cry1Ac Bt cotton was commercialized in Australia in 1995/1996 with a required 50% refuge because it was not high dose for the second generation of *H. armigera* (Cotton CRC Extension Team, 2009). During the 2004/2005 growing season, Cry1Ac Bt cotton was completely replaced by the pyramided Bollgard® II cotton, which expresses Cry1Ac and Cry2Ab proteins. In 2009, Australia planted 172 000 ha of Bollgard® II cotton, accounting for 86% of its total cotton area (Table 1). F_2 screens showed that the resistance allele frequency in *H. armigera* to Cry2Ab was low, 0.0033 (range 0.0017–0.0055), during the 2002/2003–2005/2006 seasons (Mahon et al., 2007). However, the frequency estimated with an F_1 screen appeared to indicate an increase to 2% in the populations collected during the 2008/2009 season (Cotton CRC Extension Team, 2009). Similarly, the resistance allele frequency in *H. punctigera* was estimated to be 0.0018 (range 0.0005–0.0040) during 2002/2003 to 2006/2007, but appeared to increase to 5% for the populations collected during the 2008/2009 season (Downes et al., 2009, 2010). Selection only for Cry2Ab resistance could occur during the second generation of these pests on Bollgard® II cotton. There are no suspected cases of field resistance to Bollgard® II in Australia, but the increased frequency of resistance to Cry2Ab is a point of concern (Cotton CRC Extension Team, 2009; Downes et al., 2010).

In the mid-south region of the USA, *D. saccharalis* is an important maize stalk-boring pest (Huang et al., 2007b). In Louisiana, Bt maize was first commercially planted in 1999 and now is the primary tool for managing stalk boring pests. During 2004–2009, a total of 2 230 feral individuals of *D. saccharalis* were collected from four geographical locations in Louisiana and examined for resistance alleles to Cry1Ab maize using F_1/F_2 screening methods. Resistance allele frequency to Cry1Ab maize was low with an average of 0.0011 (range 0.0003–0.002) for the combined population collected during 2004–2008, but increased considerably in 2009 to 0.018 (range 0.008–0.031) (Huang et al., 2011).

In addition, studies showed that Cry1Ab maize hybrids commonly planted in Louisiana express a ‘high dose’ for *D. saccharalis* during the vegetative plant stages but not during the reproductive stages (Wu et al., 2007; Ghimire et al., 2011). The Cry1Ab resistance in *D. saccharalis* was not associated with any fitness costs (Wu et al., 2009b) and the resistance was stable after 24 generations without selection (Huang et al., 2011). Field resistance has not been documented in *D. saccharalis*, but these results suggest that, relative to *O. nubilalis* or *D. grandiosella*, there appears to be a high risk for development of resistance in *D. saccharalis* if Cry1Ab maize continues to be widely used in the USA mid-south region.

Conclusions and future directions

Since 1996, transgenic Bt crops have become an important tool for managing the major insect pests of maize and cotton, especially in the USA. Yet the development of resistance remains a major threat to the sustainable use of Bt crops. Management of resistance to Cry1Ab maize and Cry1Ac cotton in the USA and Canada has rested on the implementation of the ‘high-dose/refuge’ strategy, which has been projected to delay resistance evolution substantially. Field resistance has not occurred where the ‘high-dose/refuge’ strategy has been implemented (Table 3). In this review, we have shown that *O. nubilalis*, *D. grandiosella*, *H. virescens*, and *P. gossypiella* all have been managed with the ‘high-dose/refuge’ strategy (Table 3). None of these species has shown any indication of field resistance despite 15 years of intensive use of Cry1Ab maize and Cry1Ac cotton in the USA and Canada. For all four species (Table 3), (i) the Bt crop expressed a high dose of Cry toxin, (ii) the initial resistance allele frequency was sufficiently low (<0.001), and (iii) sufficient non-Bt refuges were maintained.

In contrast, field resistance to Bt crops occurred rapidly when the requirements for the ‘high-dose/refuge’ strategy were not met (Table 3). The three cases of documented field resistance occurred when there was insufficient refuge and the Bt crop was not high dose. Unfortunately, nothing is known about the initial resistance allele frequencies in any of these three cases. In addition, the two cases of suspected field resistance also show that when the Bt plant is not high dose (*H. zea* in the USA and *H. armigera* in China) and/or a refuge is not appropriately implemented (*H. armigera* in China), field resistance may be eminent.

The rapid development of field resistance in *P. gossypiella* in India, compared to the absence of resistance in this pest in the USA is particularly telling (Table 3). In the USA, Bt cotton is high dose, resistance is rare, and the refuge has been implemented. In India, Bt cotton may be a seed blend of high-dose and non-expressing plants, resistance may or may not have been rare, and the refuge has likely not been implemented. Clearly, successful implementation of the 'high-dose/refuge' strategy in the USA has delayed field resistance, and the absence of the 'high-dose/refuge' strategy in India has allowed rapid emergence of field resistance.

The rapid field resistance in *S. frugiperda* on Cry1F maize in Puerto Rico also contrasts sharply with the absence of resistance in this pest in the USA (Table 3). In both localities, Cry1F maize is not high dose, but in Puerto Rico, adoption was high and refuges were not properly implemented, while in the USA, adoption was low and refuges were implemented. The initial frequency of resistance has not yet been reported, but it may have been high. The lack of implementation of the 'high-dose/refuge' strategy in Puerto Rico is clearly associated with rapid field resistance. The probability of field resistance by *S. frugiperda* in Cry1F maize might also be very high in the USA, but the low adoption rate of Cry1F maize has delayed field resistance.

A gene-pyramiding strategy using transgenic plants that express two or more Bt proteins against the same target will likely be useful in delaying resistance development (Roush, 1998). Pyramided Bt maize technology has only recently become commercially available in North America. However, Bollgard II®, which contains pyramided genes of Cry1Ac and Cry2Ab, has been used for several years in the USA and Australia without field resistance. Current evidence suggests that the gene-pyramiding strategy appears to be a useful supplement for IRM. Although it does not eliminate the need for implementing some version of a 'high-dose/refuge' IRM strategy, it may allow relaxation of some of the quantitative characteristics of the strategy.

Several other major crops (e.g., rice, soybean) have been engineered to express Cry toxins, and may become commercially available in the near future (James, 2009). IRM will continue to be a major challenge for the future success of Bt crop technologies. Implementation of IRM plans in developing countries faces more challenges than it does in the industrialized world (Andow, 2008). Continuing efforts in developing gene-pyramided Bt crops that have independent modes of action will be important for developing countries. The knowledge and experience gained in the past 15 years should provide a solid foundation to develop and implement more effective IRM strategies to ensure the continued success of Bt crop technologies in the future.

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