16 Resistance Management for Bt Maize and Above-ground Lepidopteran Targets in the USA: From Single Gene to Pyramided Traits

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Summary

Since first being commercialized in 1996, transgenic maize expressing *Bacillus thuringiensis* (Bt) proteins has gained widespread acceptance in the world. In 2013, nearly 50 Mha of Bt maize were planted in 15 countries. In the same year, growers in the USA alone planted *c.*30 Mha of Bt maize, which accounted for 76% of the total Bt maize area of the country. Up to now, Bt maize technology can be classified into two generations. The first generation of Bt maize contains only a single Bt gene for a target. In 2010, the second generation of Bt maize became commercially available and this expresses two or more pyramided Bt proteins. Currently, the pyramided products are predominant in the USA. The major lepidopteran targets of Bt maize in the USA are corn borers (Crambidae), the corn earworm, *Helicoverpa zea*, and the fall armyworm, *Spodoptera frugiperda*. To counter the threat of insect resistance, two resistance management strategies for Bt maize, 'high dose/refuge' and gene pyramiding, have been implemented. The long-term use of Bt maize against the major agricultural pests in North America provides a good opportunity to analyse the effectiveness of the adopted insect resistance management (IRM) plans. Analysis of the available data shows that all corn borer species remain susceptible to Bt proteins and that no field resistance has occurred after nearly two decades of intensive use of Bt maize in the continent. Pyramided Bt maize is effective in controlling corn earworm and fall armyworm, although recent studies indicate that field resistance to single-gene Cry1F maize in the fall armyworm has occurred in the south-east coastal areas of the US mainland. Knowledge of the resistance management gained from the USA should be useful for other countries in their sustainable use of Bt crop technology.

16.1 Introduction

Maize, *Zea mays*, is one of the most widely planted field crops in the world, with a total production of 853 million t in 2012 (National Corn Growers Association, 2013). In the USA, it is the most widely planted field crop, and in 2012, 273.8 million t was produced with a crop value of US$79.8 billion. Many arthropods are associated with maize production, from sowing to harvesting. Chemical and biological control, and host plant resistance, have been common methods used in the management of maize

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pests. Advances in molecular engineering have allowed scientists to transfer foreign genes into species that are not related to one another. One of the most successful bioengineering achievements is the transformation of crop plants with insecticidal genes from the soil bacterium, *Bacillus thuringiensis* (Bt). The resulting transgenic Bt crops produce insecticidal Bt proteins within the plant's tissues that directly kill insects when those plant tissues are consumed. Since their first commercialization in 1996, Bt crops – predominantly maize and cotton – have been rapidly adopted for insect pest management worldwide (James, 2013). In 2013, over 74 Mha of Bt crops were planted in 23 countries, including 49.6 Mha of Bt maize, 22.6 Mha of Bt cotton and 2.2 Mha of Bt soybean (James, 2013) (Table 16.1). The USA has being the leading country in the planting of Bt crops.

The rapid acceptance of Bt crops creates a threat to the long-term durability of the technology. Intensive planting of Bt crops over a wide region can place a high selection pressure on the pest populations and thus accelerate resistance development. To date, field resistance that results in reduced efficacy or failure of pest control (Huang *et al.*, 2011) has been documented in at least four cases (Storer *et al.*, 2010; van Rensburg, 2007; Dhurua and Gujar, 2011; Gassmann *et al.*, 2011); see also Chapters 1 (Tabashnik and Carrière), and 3 (Monnerat *et al.*). In the USA, in order to conserve the susceptibility of pests to Bt crops, insect resistance management (IRM) plans are now mandatory for the planting of these crops (Matten *et al.*, 2012).

Because of the recent occurrence of field resistance in the western corn rootworm, *Diabrotica virgifera virgifera*, to Cry3Bb1

Table 16.1. Global adoption of Bt maize and Bt cotton in 2013.

	Bt maize		Bt cotton		
Country[a]	Area (Mha)	% total maize	Area (Mha)	% total cotton	Total Bt crop area (Mha)
USA	29.94	76	3.03	75	32.97
Brazil	12.35	69	0.33	33	12.68
India	–	–	11.02	95	11.02
China	–	–	4.14	90	4.14
Argentina	3.11	75	0.36	79	3.47
Pakistan	–	–	2.80	86	2.80
South Africa	1.93	71	0.01	95	1.94
Canada	1.25	77	–	–	1.25
Philippines	0.71	57	–	–	0.71
Australia	–	–	0.42	94	0.42
Myanmar	–	–	0.31	85	0.31
Spain	0.13	–[b]	–	–	0.08
Mexico	–	–	0.10	–[b]	0.10
Colombia	0.077	–[b]	0.024	–[b]	0.101
Sudan	–	–[b]	0.062	–[b]	0.062
Chile	0.024	–[b]	–	–	0.024
Honduras	0.018	–[b]	–	–	0.018
Others	0.014 (5 countries)	–[b]	<0.01 (1 country)	–[b]	0.024
Total	49.55		22.62		72.17

[a]Data for the USA are from NASS (2013) and for Canada from Dunlop (2013). All other data were calculated based on James (2013). In addition to Bt maize and Bt cotton, Brazil planted 2.2 Mha of Bt soybean (James, 2013). Other countries not listed above that planted Bt crops with an area of <0.01 Mha in 2013 included Portugal, Cuba, the Czech Republic, Costa Rica, Romania and Slovakia.

[b]A very small percentage.

maize in the USA, intensive studies have been performed on the Bt resistance of these coleopteran pests (Gassmann *et al.*, 2011). In this chapter, a review is presented of the implementation of IRM for Bt maize targeting above-ground lepidopteran pests in the USA over nearly 20 years. The review focuses on the European corn borer (ECB; *Ostrinia nubilalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), sugarcane borer (SCB; *Diatraea saccharalis*), corn earworm (CEW; *Helicoverpa zea*) and fall armyworm (FAW; *Spodoptera frugiperda*) because there is substantial information on these five targets. The long-term use of Bt maize in managing these major agricultural pests in the USA also provides a good opportunity to analyse the effectiveness of the IRM plans that have been adopted.

16.2 Adoption of Bt Maize in the USA

The year 1996 marked the first year that Bt maize was commercially planted in the USA over an area of 1.6 Mha, which accounted for *c.*5% of the nation's total maize planting area (Fig. 16.1). Since then, both the percent and area of Bt maize have increased every year except for 2000 and 2001, which were caused by the StarLink issue (Taylor and Tick, 2000). In 2013, the USA planted nearly 30 Mha of Bt maize, which accounted for 76% of the country's total maize area and 60% of the world's total Bt maize area. The rapid adoption of Bt maize in the USA is revealed with an annual increase rate of 18.8% in the area basis during the past 18 years. Up to date, the commercial Bt maize products that have planted in the U.S. can be classified into two generations. Products of the 1st generation Bt maize produce only a single Bt protein for a target pest, while the second generation expresses ≥2 Bt proteins that act on a same insect.

16.2.1 First-generation Bt maize

Before 2010, all Bt maize products planted in the USA expressed only a single Bt protein for a target pest, and this usually

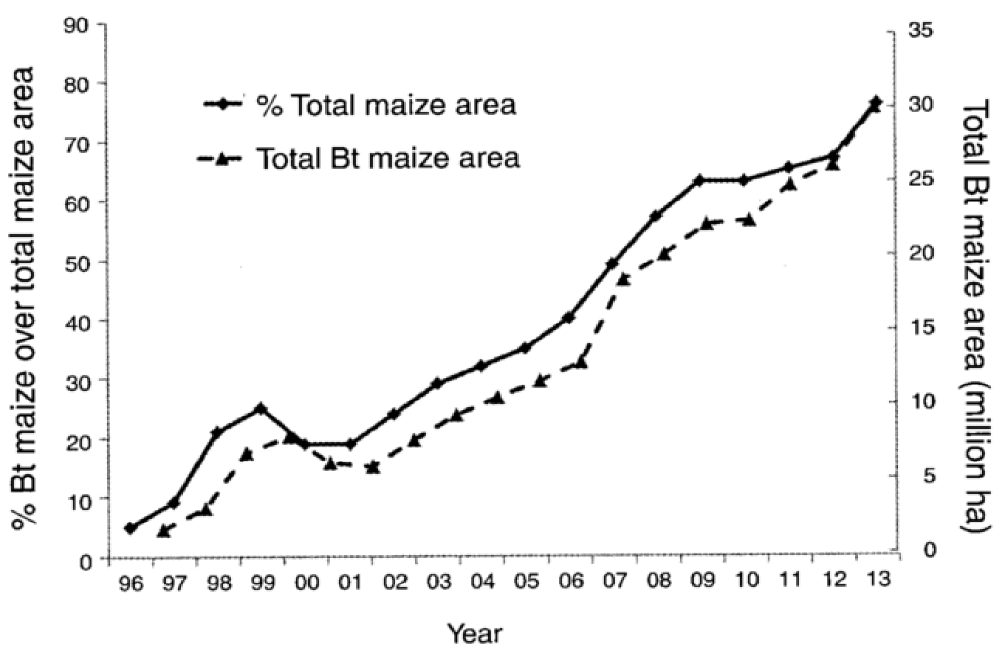

Fig. 16.1. Percentage and total area of maize transformed with genes from *Bacillus thuringiensis* (Bt maize) planted in the USA from 1996 to 2013. Most of the data are based on the annual reports of crop acreage from the USDA National Agricultural Statistics Service (NASS, 1996–2013). Percentage of Bt maize in a year was calculated based on the Bt maize area divided by the total maize area in that year.

Table 16.2. Examples of first-generation Bt maize products.

Bt maize event[a]	Bt gene/s	Year of release	Examples of Bt maize products	Major target insects[b]
MON810	Cry1Ab	1996	YieldGard	Corn borers
Bt11	Cry1Ab	1996	YieldGard (later also called Agrisure Corn Borer)	Corn borers
TC1507	Cry1F	2001	Herculex® I	Corn borers, fall armyworm
MON810 + MON863	Cry1Ab + Cry3Bb1	2003	YieldGard® Plus corn	Cry1Ab for corn borers and Cry3Bb1 for corn rootworms
TC1507 + DAS-59122-7	Cry1F + Cry34/35Ab1	2005	Hexculex® XTRA corn	Cry1F for corn borers and fall armyworm, and Cry34/35Ab1 for corn rootworms
MON810 + MON863	Cry1Ab + Cry3Bb1	2007	YieldGard® VT Triple	Cry1Ab for corn borers and Cry3Bb1 for corn rootworms

[a]An event is a specific genetic modification in a specific species.
[b]Corn borer species may include the European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), sugarcane borer (*Diatraea saccharalis*), southern corn stalk borer (*Diatraea crambidoides*) and lesser corn stalk borer (*Elasmopalpus lignosellus*). Corn rootworm species may include the western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*) and Mexican rootworm (*Diabrotica virgifera zeae*). The fall armyworm is *Spodoptera frugiperda*.

has a relatively narrow insecticidal spectrum (see Table 16.2). For example, YieldGard corn, the most widely used single-gene Bt corn, expresses only the Cry1Ab protein and is effective against some lepidopteran corn borers, such as ECB and SWCB. Some of the first-generation Bt maizes did contain two Bt genes, but these were for different targets. For example, YieldGard Plus corn, a commonly planted first-generation Bt maize, contains the genes for both Cry1Ab and Cry3Bb1, but the Cry1Ab is for controlling lepidopteran corn borers, while the Cry3Bb1 is for managing the coleopteran corn rootworm. The Cry1Ab protein does not have any activity against Coleoptera and the Cry3Ab1 protein is not toxic to Lepidoptera. In the literature, the transfer of more than one foreign gene into a same plant, each gene for different targets/purposes, is often called 'gene stacking' (Huang *et al.*, 2011). An exception to this is the Bt maize Herculex®RW *Rootworm Protection*, which contains two Bt genes, those for the Cry34Ab1 and Cry35Ab1 proteins, which are both active against corn rootworms. However, the two are nearly identical in gene structure and mode of action, so they function like a single gene.

The main above-ground lepidopteran targets of first-generation Bt maize in the USA are stalk borers, including ECB in the entire US Corn Belt (Ostlie *et al.*, 1997), SWCB in the central and southern regions (Trisyono and Chippendale, 2002), SCB in the mid-south (Castro *et al.*, 2004), the southern corn stalk borer, *Diatraea crambidoides*, in the south-east (Reisig and Roberson, 2011) and the lesser corn stalk borer, *Elasmopalpus lignosellus*, in the south (Vilella *et al.*, 2002). FAW is also listed as a main target of the single-gene Cry1F maize (e.g. Herculex®I). In general, the field performance of the first-generation Bt maize has been outstanding for controlling the target pests (Huang *et al.*, 1999; Buschman *et al.*, 2001), and growers in the USA have recognized the great economic and environmental benefits offered by Bt maize (Hutchison *et al.*, 2010). First-generation Cry1Ab maize also can suppress some secondary lepidopteran pests, such as CEW and FAW (Buntin *et al.*, 2004; Chilcutt *et al.*, 2007), but the suppression levels are usually not high (US EPA, 2001). For this reason, neither CEW nor FAW are listed as targets of the first-generation Bt maize, except that FAW is a target of the single-gene Cry1F maize (US EPA, 2005).

16.2.2 Second-generation Bt maize

Second-generation transgenic maize that expresses more than one dissimilar Bt protein targeting above-ground Lepidoptera was first commercially planted in the USA and Canada in 2010. An example is one of the most widely used second-generation products, Genuity® SmartStax™, which contains three dissimilar Cry genes, those for the proteins Cry1A.105, Cry2Ab2 and Cry1F, which all target Lepidoptera. The technique of transferring more than one different Bt gene into the same plant for same target pest/s is usually called 'gene pyramiding' in the literature (Huang *et al.*, 2011). Since the first release of second-generation maize in 2010, many pyramided products have then been commercialized in the USA (see Table 16.3). Most of these products contain multiple Bt genes for managing underground rootworms.

Besides its high efficacy for corn borer control, pyramided maize is also very effective against some noctuid species

Table 16.3. Currently available major Bt maize products targeting above-ground lepidopteran pests in the USA.

Event[a]	Bt genes	Major target insects[b]	Examples of Bt maize products
Singe Bt gene products			
Bt 11	Cry1Ab	CB	Agrisure CB/LL, Agrisure GT/CB/LL, Agrisure 300GT, Agrisure CB/LL/RW, Agrisure Artesian 3011A
MON810	Cy1Ab	CB	YieldGard VT Triple
TC1507	Cry1F	CB, FAW	Herculex® I, Herculex® XTRA; Optimum® AcreMax®1, Optimum® TRIsect
Products containing two pyramided Bt genes			
MON89034	Cry1A.105, Cry2Ab2	CB, CEW, FAW	Genuity® VT Double PRO®, Genuity®VT Triple PRO®
Bt11 and MIR162	Cry1Ab, Vip3A	CB, CEW, FAW	Agrisure Viptera® 3110, Agrisure Viptera® 3111
Bt11 and TC1507	Cry1Ab, Cry1F	CB, CEW, FAW	Agrisure 3122, Agrisure Duracade ™5122
MON810 and TC1507	Cry1Ab, Cry1F	CB, CEW, FAW	Optimum®AcreMax®, Optimum®AcreMax® Xtra, Optimum®AcreMax® Xtreme, Optimum® Intrasect™, Optimum® Intrasect™ Xtra, Optimum® Intrasect™ Xtreme
Products containing three pyramided Bt genes			
MON89034 and TC1507	Cry1A.105, Cry2Ab2, Cry1F	CB, CEW, FAW	Genuity® SmartStax®, SmartStax®
Bt11, TC1507 and MIR162	Cry1Ab, Cry1F, Vip3A	CB, CEW, FAW	Agrisure Duracada™5222

[a]An event is a specific genetic modification in a specific species.
[b]CB, corn stalk borer species, which may include the European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), sugarcane borer (*Diatraea saccharalis*), southern corn stalk borer (*Diatraea crambidoides*) and lesser corn stalk borer (*Elasmopalpus lignosellus*); CEW, corn earworm (*Helicoverpa zea*); FAW, fall armyworm (*Spodoptera frugiperda*).

(Burkness *et al.*, 2010; Siebert *et al.*, 2012; Reay-Jones and Reisig, 2014; Rule *et al.*, 2014) and so CEW and FAW are listed as targets for all pyramided products that control above-ground Lepidoptera (Table 16.3) (DiFonzo and Cullen, 2013). However, there are arguments about the economic benefits of using of Bt maize for controlling CEW, as some studies have shown that there are no significant relationships between maize yield and CEW injury (Reay-Jones and Reisig, 2014).

Since pyramided Bt maize became available in 2010, areas planted with single-gene products in the USA have been reduced rapidly. Although there are no official reports of the actual area planted with pyramided products, it is obvious that predominant type of Bt maize currently planted in the USA contains pyramided traits. Because of the increasing challenge of insect resistance, it is expected that single-gene Bt maize will be completely replaced by pyramided products in the near future.

16.3 Resistance Management for Bt Maize Targeting Above-ground Lepidopteran Pests

Resistance development in target pest populations is a great threat to the sustainable use of Bt maize technology (Tabashnik *et al.*, 2013). To delay the development of resistance, IRM plans are required for the planting of Bt maize in the USA. Basically, two different strategies are currently used for Bt maize IRM: (i) a 'high dose/refuge' strategy; and (ii) a gene pyramiding method.

16.3.1 High dose/refuge strategy

Since Bt maize was first planted in 1996, a high dose/refuge IRM strategy has been used in the USA (Matten *et al.*, 2012). This involves planting/sowing a portion of the maize to high-dose Bt maize that can kill insects carrying only one copy of the resistant gene/s (heterozygotes, RS) (US EPA, 2001). The remaining maize crop is established with non-Bt maize that serves as a refuge for susceptible insects (SS). The relatively large (homozygote) SS populations from refuge plants should then mate with the rare surviving individuals carrying two copies of the resistant gene (homozygotes, RR). Thus, most of their offspring that carry resistance alleles will be RS. Because RS individuals will be killed by high-dose Bt plants, the frequencies of resistance alleles in pest populations should be maintained at low levels for a long period of time (Huang *et al.*, 2011).

The success of the high dose/refuge strategy depends on three key assumptions, that: (i) Bt maize hybrids express a 'high dose'; (ii) the initial frequency of resistance alleles is low (e.g. <0.001); and (iii) a sufficient SS refuge population is available in the nearby environment. A high-dose Bt maize means that the Bt protein concentrations within it are sufficiently high to kill ≥95% of RS individuals (US EPA, 2001). In other words, Bt resistance is functionally recessive. However, the high-dose qualification has not been directly evaluated for most target pests because resistance traits have not been found in those insects. A US Environmental Protection Agency (EPA) Scientific Advisory Panel defined a high dose as 25 times the protein concentration needed to kill SS individuals and recognized five methods to demonstrate that a transgenic crop expresses a high dose of Bt proteins (US EPA, 2001); a Bt plant must meet at least two of these five criteria to qualify as a high-dose type. This definition has been used to evaluate the high-dose qualification of Bt crops against corn borers and other target species.

Currently, the required non-Bt refuge maize is planted in two ways: (i) by structured planting; and (ii) by using a seed mixture. Before 2010, non-Bt refuge maize could only be planted structurally with the Bt maize in an area. For single-gene Bt maize, the high dose/refuge IRM plan requires growers to plant ≥20% structured non-Bt maize as a refuge in the Corn Belt, but they need to plant a minimum of 50% non-Bt maize in the southern region where cotton is planted. The structured non-Bt refuge should be planted within 800 m of the Bt maize field on every farm (Ostlie *et al.*, 1997). Growers' compliance with the structured refuge requirements has been an issue. During the early years, a relatively high rate of compliance (e.g. 86–92%) was reported, but this has declined considerably in recent years (Smith *et al.*, 2012).

Because of the compliance issue with the use of the 'structured refuge', in 2010 the US EPA approved a seed mixture refuge strategy (also called 'refuge-in-the-bag' or RIB) as an alternative for planting pyramid Bt maize in the US Corn Belt (Matten *et al.*, 2012). This strategy has not been approved in the southern region. For RIB, a defined percentage of non-Bt maize seeds is mixed with Bt maize seeds in each bag by seed companies before it is sold to farmers. Hence, compliance will no longer be an issue. The currently used RIB is at a rate of 95% Bt mixed with 5% non-Bt seeds. Mathematical models show that RIB could be an effective strategy (Carroll *et al.*, 2012), though scientific data to support the strategy are still very limited.

With the structured refuge strategy, the dispersal behaviour of adults is important, but with RIB, the major concern is larval movement among Bt and non-Bt plants. For example, movement of susceptible larvae from non-Bt refuge plants to Bt plants in an RIB could cause greater mortality to SS populations than in a structured refuge planting, and so result in a lower refuge population. More importantly, maize is a cross-pollinating crop in which most pollination results from pollen dispersed by wind and gravity (Burkness and Hutchison, 2012). Pollen movement from the surrounded Bt plants to the non-Bt refuge plants in an RIB planting could result in Bt expression in refuge kernels and thereby could directly kill susceptible refuges, especially for kernel feeders such as CEW. Furthermore, pollen movement could also create sublethal exposure and promote selection pressure for resistance by increasing the survival of RS individuals or of individuals carrying minor resistance alleles. All of these essential parameters are largely unknown. The lack of such crucial scientific data is a major reason that has prevented approval of the use of RIB in the southern USA.

16.3.2 Gene pyramiding

The second method currently used for Bt maize IRM is gene pyramiding. In order to be effective, the genes pyramided should lack cross-resistance so that resistance to one Bt protein is not also resistance to others (Zhao *et al.*, 2003; Moar and Anilkumar, 2007). Currently, most pyramided Bt maize products are planted mixed with 5% non-Bt maize in the US Corn Belt (Matten *et al.*, 2012). Compared with the use of single-gene Bt maize, modellings show that resistance development to pyramided Bt maize can be delayed considerably (Carroll *et al.*, 2013).

Published empirical data support the use of gene pyramiding. Zhao *et al.* (2003) conducted a greenhouse study using an artificial population of diamondback moth, *Plutella xylostella*, carrying genes for resistance to Cry1Ac and Cry1C. After 24 generations of selection, resistance to pyramided two-gene plants was significantly delayed compared with the single-gene plants that were deployed in mosaics. Several recent studies have also shown that pyramided Bt maize products could be used for managing insect populations that have developed high levels of resistance to single Bt proteins. For example, SCB populations that are resistant to single-gene Cry1Ab maize are highly susceptible to the pyramided maize products Genuity® VT Double Pro™ or SmartStax™ (Wangila *et al.*, 2012). Similarly, pyramided Bt maize is also effective for controlling Cry1F-resistant FAW (Niu *et al.*, 2014). These results showcase gene pyramiding as a possibly useful tool for delaying resistance development.

16.4 Current Status of Bt Susceptibility of Above-ground Lepidopteran Targets in the USA

As mentioned above, there are several lepidopteran pests targeted by Bt maize in the USA (Tables 16.2 and 16.3). The available data show that corn borer populations in the USA are still susceptible to Bt maize. Pyramided Bt maize is also effective for CEW and FAW. Huang *et al.* (2011) analysed the possible reasons that have contributed to the long-term success of Bt maize for managing corn borers in the North America. They found that the fundamental assumptions of the 'high-dose/refuge' IRM strategy have been met for both ECB and SWCB. However, recent studies have shown that field resistance to single-gene Cry1F maize has occurred in FAW in south-eastern coastal areas of the USA. Below, an analysis is given of the status of Bt susceptibility/ resistance in five lepidopteran targets of Bt maize. Other above-ground lepidopteran targets are minor pests on which there is not much information available.

16.4.1 European corn borer

ECB is the most economically important corn borer and the primary target of Bt maize in the USA, and before the use of Bt maize, annual losses caused by ECB were estimated at US$1 billion (Ostlie *et al.*, 1997). Bt maize was first planted to control ECB in 1996, and its field performance has been excellent (Huang *et al.*, 1999), with no field control failures reported. Monitoring over 10 years (1995–2005) showed that all field populations evaluated have remained susceptible to Cry1Ab across the major maize production areas of the USA (Siegfried *et al.*, 2007). In the past several years, field populations of ECB have been maintained very low in both Bt and non-Bt maize fields (Bohnenblust *et al.*, 2014). A study by Hutchison *et al.* (2010) has shown that the nationwide suppression of ECB is associated with the use of Bt maize, which has offered considerable economic benefits to the US maize growers. In addition, >800 isofemale lines and 131 ECB males collected nationwide have been examined for Cry1Ab resistance using F_2 screens (Andow *et al.*, 1998, 2000; Bourguet *et al.*, 2003; Stodola *et al.*, 2006). No major resistance alleles were found in these populations. Based on the F_2 screens, resistance allele frequency to Cry1Ab in ECB is estimated to be <0.00086 with 95% probability in the USA. The results confirm that the frequency of the Bt resistance allele in ECB is very low in the USA and meets the rare resistance criterion required for the high dose/refuge strategy.

16.4.2 Southwestern corn borer

SWCB is a major corn borer in the central and southern USA (Trisyono and Chippendale, 2002; Castro *et al.*, 2004). Bt maize hybrids are also effective against SWCB (Huang *et al.*, 1999; Buschman *et al.*, 2001). The Bt susceptibility status of SWCB is similar to that of ECB in that no major Bt resistance genes have been found. All Bt maize products targeting Lepidoptera remain effective in controlling SWCB, although compared with ECB, the research associated with SWCB has been limited. An early study using dose-response bioassays indicated that the Cry1Ab susceptibility of the populations collected from several US states did not increase from 1998 to 2000 (Trisyono and Chippendale, 2002). A field population collected in Louisiana in 2005 also remained susceptible to both Cry1Ab protein and Cry1Ab maize plants (Huang *et al.*, 2006). In addition, >400 SWCB individuals collected in Louisiana in 2005 were examined for Cry1Ab resistance using an F_2 screen, but no major resistance was detected (Huang *et al.*, 2007). The results also indicate that Bt resistance allele frequency in SWCB is low and should meet the rare resistance requirement of the high dose/refuge strategy.

16.4.3 Sugarcane borer

SCB is a major corn borer in the mid-southern USA (Castro *et al.*, 2004). It is also a pest of sugarcane, rice and grain sorghum. Compared with ECB and SWCB, SCB is less susceptible to Cry1Ab protein (Huang *et al.*, 2006), but Bt maize hybrids do still remain effective against SCB and field resistance has not occurred (Huang *et al.*, 2012).

Since 2004, >3000 SCB individuals collected from Louisiana, Texas and Mississippi have been examined for Cry1Ab resistance using F_1/F_2 screens. The results showed that Cry1Ab resistance allele frequencies in SCB were low in the populations in Louisiana during 2004–2008 (0.0011), in Texas in 2007 (<0.0016) and in Mississippi in 2009 (<0.061) (Huang *et al.*, 2009, 2012). Laboratory bioassays also showed that 18 field populations collected from Louisiana and Texas during 2004–2006 remained susceptible to Cry1Ab (Huang *et al.*, 2008). However, the resistance frequency in the Louisiana populations increased significantly in 2009, reaching 0.0176 (Huang *et al.*, 2012). Since 2010, SCB populations in maize fields as well as on sorghum and rice in the mid-southern USA have decreased considerably. The reason for this decline is not certain, but it may be related to the area-wide adoption of Bt maize as was reported for the decline of ECB in the North Corn Belt (Hutchison *et al.*, 2010). If the decrease is due to the use of Bt maize, a conservative estimate of the net benefit from planting Bt maize to Louisiana maize growers alone is >US$20 million annually (F. Huang, unpublished data).

16.4.4 Corn earworm

CEW is considered to be the most costly crop pest in North America. The pest has >200 host plants, many of which are economically important crops, such as maize, cotton, grain sorghum and soybean. Damage to maize is mainly caused by larvae feeding on the ear kernels. In the US South, CEW is known to overwinter in the pupal stage, but it cannot usually overwinter in most areas of the Corn Belt. It is a highly mobile insect, capable of long-distance migration (US EPA, 2001). In the South, CEW moves to other hosts such as cotton, grain sorghum and soybean for two to three additional generations after maize has matured. Because CEW is also a major target of Bt cotton in the South, it presents a significant challenge for IRM, as there is the potential for multiple exposures to Bt maize and Bt cotton across generations.

Because the first-generation Bt maize offers only partial control of CEW, this insect was not listed as a target of the single-gene Bt maize and so most studies on Bt resistance in CEW have been associated with Bt cotton (see Chapter 1, Tabashnik and Carrière; Chapter 2, Gao *et al.*; and Chapter 4, Van den Berg and Campagne; and other published articles). Because of the improved efficacy of the second-generation Bt maize, CEW is currently listed as a target for all pyramided Bt maize products (DiFonzo and Cullen, 2013). To date, all pyramided Bt maize remains effective for the control of CEW and no field resistance has been reported in the USA. None the less, there is a major concern in implementing RIB because of the cross-pollinating properties of maize, which can cause Bt proteins to be present in refuge maize kernels in seed mixtures. A recent study showed that cross-pollination in a 95:5% RIB planting of SmartStax™ caused >90% of refuge kernels to express more than one Bt protein (Yang *et al.*, 2014). The expression of Bt proteins in the refuge ears reduced CEW survivorship to only 4.6%, a reduction of 88.1% relative to larval feeding on the ears of pure non-Bt maize plantings. The results demonstrate that at the currently implemented rate, the RIB approach will not be effective in providing refuge for ear-feeding pests such as CEW.

16.4.5 Fall armyworm

FAW is distributed throughout most of the USA and it is believed to be able to overwinter successfully only in south Florida and south Texas in the US mainland. It is also a well-known long-distance migratory insect. This pest also has a wide range of host plants. Similar to CEW, the FAW is also a target of pyramided Bt cotton in the USA.

Most single-gene Bt maize products are not very effective against FAW and so FAW is excluded from the target list for the 1st generation Bt maize except for the Cry1F maize. Unfortunately, after only a few years of intensive use of Cry1F maize in Puerto Rico, field resistance in FAW occurred in the territory (Storer *et al.*, 2010). As a result, Cry1F maize was withdrawn from commercial use in Puerto Rico shortly after the resistance was identified. The pyramided Bt maize is more effective (Burkness *et al.*, 2010; Siebert *et al.*, 2012) and so FAW is listed as a target for this product (DiFonzo and Cullen, 2013).

One study reported that field populations of FAW collected in 2010–2011 in the US mainland, including Florida, were susceptible to Cry1F (Storer *et al.*, 2012). In contrast, unexpected survival of FAW on Cry1F maize plants has been reported in recent years on several occasions in the south-eastern USA and Brazil (see also Chapter 3, Monnerat *et al.*). However, scientific documentation of field resistance has not been reported from anywhere except Puerto Rico (Storer *et al.*, 2010). Since 2011, F_2 screens, diet-incorporated bioassays, greenhouse tests and field studies have been conducted in four south-eastern US states to determine whether this unexpected survival is due to resistance. F_2 screens showed that Cry1F resistance allele frequency in a FAW population collected in 2011 in south Florida reached 0.293 (Huang *et al.*, 2014). Diet-incorporated bioassays showed that populations collected in 2012–2013 from Florida and North Carolina exhibited a significant level of Cry1F resistance. Reduced efficacy and control failure of Cry1F maize for FAW were also documented in field trials in south Florida. Another independent study also found that a population collected from Palm Beach, Florida, in 2011 showed a high Cry1F resistance allele frequency of 0.247 (Vélez *et al.*, 2013). These results documented that field resistance to Cry1F maize has occurred in FAW in the south-east coastal areas of the US mainland.

It is necessary to develop mitigation strategies before this resistance becomes widespread. A study with leaf tissue bioassays showed that the Cry1F-resistant FAW also exhibited a significant level of cross-resistance to three of four pyramided Bt maize products tested (Niu *et al.*, 2013), although the levels of cross-resistance observed in these bioassays were not sufficient enough to allow the resistant FAW to survive on whole plants of pyramided Bt maize (Niu *et al.*, 2014). The results suggest that pyramided Bt maize can be used for managing the Cry1F-resistant FAW. Timely switching from single-gene to pyramided products in the USA should be helpful in maintaining the continued success of Bt maize for managing FAW, at least temporarily.

16.5 Prospects

Since 1996, >275 Mha of Bt maize have been planted in the USA and crop growers have gained considerable economic benefits from planting the crop, which will definitely encourage more countries and more crop growers to adopt the technology. In addition, transgenic RNA interference (RNAi) crops that express different insecticidal toxins are expected to become available soon. The rapid increase in global demand for food/feed and fuel energy makes the need for such technologies even more urgent. Therefore, it is widely expected that the adoption of Bt crops will continue to increase in the future. Thus, IRM will continue to be a great challenge for the sustainable use of those crops.

As in industrialized countries, the use of Bt crops has also delivered substantial benefits in developing countries (James, 2013). During 2013, >40 Mha of Bt crops were planted in 17 developing countries, which accounted for >50% of the world's total Bt crop area (Table 16.1), and as more Bt crops are planted in these countries, the implementation of effective IRM plans will be critical for the sustainability of the technology. The knowledge and experience that has been gained with Bt crops in the USA should be helpful for the implementation of Bt crop IRM in developing countries.

Note

This paper reports research results only. Mention of a proprietary product name does not constitute an endorsement for its use by Louisiana State University Agricultural Center. The article is published with the approval of the Director of the Louisiana Agricultural Experiment Station as manuscript number No. 2014-234-15702.

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