Chapter 17

Resistance of *Spodoptera frugiperda* to *Bacillus thuringiensis* Proteins in the Western Hemisphere

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Abstract Resistance to *Bacillus thuringiensis* (*Bt*) Cry1 insecticidal proteins expressed in genetically modified plants (*Bt* maize and other *Bt* crops) has been documented in the fall armyworm (FAW; *Spodoptera frugiperda* [J.E. Smith]) in South America. The factors that led to the onset of resistance include less-than-optimal product characteristics (dose) and poor compliance with the requirement for structured refuges of non-*Bt* maize. In this article, we review the documented cases of resistance in FAW and explore the path forward to the implementation of effective insect resistance management (IRM) programs to support the sustainable deployment of this technology, particularly in tropical regions. Effective IRM plans require effective product design and management of *Bt* maize technology. Due to the challenges presented in tropical regions, the development of effective *Bt* maize pyramids combining highly effective and novel modes of action is fundamental to a successful IRM strategy. The integration of IRM and business imperatives through the development of a multilayer, multi-stakeholder strategy to ensure the proper use of the technology, and particularly to adequate compliance with refuge requirements, is another critical element of an IRM strategy for *Bt* crops.

Keywords *Bt* maize • Fall armyworm • Insect resistance management • Refuge • *Spodoptera frugiperda*

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Genetically modified plants (GM) containing genes from the soil bacterium *Bacillus thuringiensis* (*Bt*), which produces a number of insecticidal proteins, have been extensively adopted globally. The area planted with crops containing *Bt* traits was approximately 83.7 Mha worldwide in 2015 (James 2015). The benefits realized from adopting *Bt* crops are substantial and include effective management of target insect pests (Wu et al. 2008; Hutchison et al. 2010; Waquil et al. 2013), decreased use of synthetic insecticides (Lu et al. 2012; Tabashnik et al. 2013; Brookes and Barfoot 2016), conservation of beneficial nontarget organisms (natural enemies) (Wolfenbarger et al. 2008; Naranjo 2009; Tian et al. 2012, 2013), and increased crop yield and farm income (Brookes and Barfoot 2016). For these reasons, the use of *Bt* plants in integrated pest management (IPM) programs has been extensively adopted by growers globally (James 2015; Brookes and Barfoot 2016).

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is an important pest of maize and other economically important crops in the Western Hemisphere, particularly in warm-winter areas such as Central and South America (Cruz et al. 2012; Nagoshi et al. 2014, 2015). FAW is a migratory and highly polyphagous pest species that does not have the ability to enter diapause, an inactive state that allows insects to survive prolonged periods of non-conducive conditions such as extreme cold or drought (Luginbill 1928; Sparks 1979). FAW comprises two genetically and behaviorally separate strains that occur sympatrically throughout North and South America (Pashley 1986). The “corn” strain (CS) is associated with maize and sorghum plants, whereas the “rice” strain (RS) is preferentially found in rice and turfgrass (Nagoshi et al. 2007). These “host strains” of FAW are morphologically indistinguishable; however, polymorphisms in the mitochondrial cytochrome oxidase subunit I (COI) gene proved to be capable of consistently differentiating and identifying CS and RS haplotypes (Levy et al. 2002). The COI marker also defined the further subdivision of CS into four haplotype groups (Nagoshi et al. 2007). In the United States, due to the absence of a diapause trait in FAW, this pest species of tropical origin must migrate northward to reinfest cropping areas in temperate areas (Westbrook et al. 2016). FAW is frequently reported as far north as Canada (Mitchell et al. 1991). The primary overwintering regions in the southern portions of Florida and Texas, extending into Mexico, are assumed to be the source of practically all FAW infestations in the United States and Canada (Nagoshi et al. 2014). This knowledge about the biology and ecology of FAW was used to simulate migratory flight of FAW moths from distinct winter-breeding source areas in the United States (Westbrook et al. 2016).

In tropical and subtropical regions of Central and South America, where cropping systems are especially favorable for the buildup of insect pest populations, the use of *Bt* maize technologies has provided an efficient and environmentally safe tool to manage FAW populations and damage (see Table 17.1) (Okumura et al. 2013; Waquil et al. 2013; Trumper 2014). The main threat to the sustained use of *Bt* crops to manage insect populations is the evolution of resistance by target pests (Carrière et al. 2015; Gould 1998; Tabashnik et al. 2013). The risk of resistance evolving to *Bt* crops is frequently linked to the expression of insecticidal proteins in all plant parts throughout the season (constitutive expression), thereby exposing all individuals

Table 17.1 Summary of *Bt* maize events (and stacks) to manage FAW, and respective *Bacillus thuringiensis* insecticidal proteins expressed, approved for cultivation in Brazil and Argentina

	Class of *Bt* proteins		
Event/stack	Cry1	Cry2	Vip3A
MON 810; *Bt*11	Cry1Ab		
TC1507	Cry1F		
MON 89034	Cry1A.105	Cry2Ab2	
MON 89034 × TC1507	Cry1A.105; Cry1F	Cry2Ab2	
MIR162			Vip3Aa20
*Bt*11 × MIR162	Cry1Ab		Vip3Aa20
MON 810 × TC1507 × MIR162	Cry1Ab; Cry1F		Vip3Aa20

Data from ISAAA – International Service for the Acquisition of Agri-Biotech Applications (http://www.isaaa.org/gmapprovaldatabase/)

in local insect pest populations to selection (Caprio et al. 2000). In order to proactively delay the onset of resistance to *Bt* crops, insect resistance management (IRM) programs have been designed and implemented worldwide. The characteristics of tropical regions considerably increase the overall risk for the development of resistance to all tactics used in insect management, including *Bt* proteins expressed by GM plants. For example, the warmer seasonal temperature patterns that allow growers to manage their crops within longer growing seasons (Hollingsworth 2011) also allow for multiple generations of the pest to be exposed to selection every crop season (Martinelli and Omoto 2005). In Brazil, for example, maize is cultivated in two consecutive growing seasons (first crop and second crop). FAW populations are able to build up considerably under such conditions, which favor multiple and overlapping generations of this pest (Omoto et al. 2016). These bioecological factors favor high levels of selection pressure against technologies used to control FAW, including insecticides (Carvalho et al. 2013) and *Bt* proteins expressed in GM plants (Farias et al. 2014a; Bernardi et al. 2015a; Omoto et al. 2016).

In general, the sustained use of *Bt* technologies requires the implementation of refuges coupled with the deployment of *Bt* plants with high killing power over the individuals within an insect pest population (e.g., high-dose plants and/or *Bt* pyramids) (see Sect. 17.2). Despite efforts to effectively delay the evolution of resistance to *Bt* crops, field-evolved resistance has been documented in *Busseola fusca* (Füller) resistant to Cry1Ab maize in South Africa (Van Rensburg 2007), in FAW resistant to Cry1F maize in Puerto Rico and in Brazil (Storer et al. 2010; Farias et al. 2014a), in *Pectinophora gossypiella* (Saunders) resistant to Cry1Ac cotton in India (Dhurua and Gujar 2011), and in *Diabrotica virgifera virgifera* LeConte resistant to Cry3Bb1 maize in the United States (Gassmann et al. 2011). Among the cases of field-evolved insect resistance to *Bt* crops/proteins documented thus far, three common factors are prominent and apparently are the main causes for resistance: (1) deployment of non-high-dose single-mode-of-action *Bt* plants, (2) poor implementation of refuges, and (3) high insect pressure in tropical/subtropical environments. In this chapter, we will discuss the theory behind the management of insect resistance to *Bt* crops and lay out cases of field-evolved resistance to *Bt* crops in FAW documented in the

Western Hemisphere. We will also provide a critical analysis for the likely causes of resistance and indicate the main imperatives regarding product design and usage of *Bt* crops to achieve effective IRM.

17.1 Resistance Management

The definition of insect resistance to *Bt* proteins and crops has been a subject of ongoing debate. The National Research Council (1986) defined insecticide resistance as "genetically heritable changes in a population causing a reduction in susceptibility to a specific insecticide" without addressing on the impact of resistance on product performance at the field level. Tabashnik and colleagues (2008, 2009) and Tabashnik (1994) adapted the definition from NRC to resistance to *Bt* proteins and *Bt* crops and defined field-evolved (or field-selected) resistance as a genetically based decrease in susceptibility of a population to a toxin (e.g., *Bt* protein) caused by exposure of the population to the toxin in the field. However, this definition of field-evolved resistance does not imply loss of economic efficacy of a *Bt* protein and/or *Bt* crop in the field (Tabashnik et al. 2009) because it does not take into consideration factors such as the impact of the magnitude of resistance (temporally or spatially) or the extent of changes in product efficacy. Rather, it is frequently contingent on the reading of laboratory results, without taking into consideration pest ecology and how the interaction between the pest and the *Bt* product influences selection pressure; thus, field-evolved resistance may or may not result in changes in susceptibility on *Bt* crops (Sumerford et al. 2013; Tabashnik et al. 2003). Moreover, according to Sumerford et al. (2013), in order to confirm insect resistance to a *Bt* protein or a *Bt* crop in the field, it is essential to prove (1) the heritable basis for the shift in susceptibility and (2) the ability of individuals possessing the alleles causing the shift in susceptibility to complete their development on plants expressing the *Bt* protein(s), enabling them to inter-mate and transmit resistance alleles to their offspring.

17.2 Management of Insect Resistance to *Bt* Crops

IRM programs for *Bt* crops are designed and implemented to slow the rate at which insect resistance evolves and thus to maximize the effective life of a *Bt* crop, but not to prevent resistance (Head and Greenplate 2012). The rate at which insect resistance may evolve to *Bt* crops is affected by several factors, including the use of a product that results in the mortality of all or nearly all of the heterozygous insects (causing resistance to be functionally recessive), the frequency of the resistance alleles in the insect pest population, and the availability of refuge areas formed by non-*Bt* plants, which provide susceptible insects to contribute to dilution of the resistance alleles through random mating with fully resistant (homozygous) insects (Gould 1998; Roush 1994; Tabashnik et al. 2013). Furthermore, the rate of resistance evolution is significantly influenced by the number of *Bt* proteins (and independent modes of action) expressed in a *Bt* crop and by the level and consistency of expression of each *Bt* protein (Head and Greenplate 2012). For instance, based on mathematical modeling, resistance should evolve more slowly to *Bt* crops with multiple *Bt* proteins than to those with a single *Bt* protein (Roush 1998). Additionally, the ideal expression pattern of each *Bt* protein in a *Bt* crop is season long, at a level high enough to control target insects that are heterozygous for any resistance genes (EPA 1998).

The IRM programs implemented for the first generation of *Bt* crops in the United States (single-mode-of-action products) were based on the "high-dose + refuge" approach. The expectation was that a *Bt* plant that met the "high-dose" standard would be able to control 99.99% of the target insect pest population. The US EPA recommended five different methods, each with its individual practical difficulties, for determining if a *Bt* plant meets the high-dose criterion (Caprio et al. 2000; EPA 1998). Several authors have demonstrated that the value of refuges to resistance management is greater when the high-dose criterion is achieved (Tabashnik et al. 2013). However, several factors can diminish the expected value of the refuge approach for IRM. For example, not all *Bt* plants are capable of fully controlling all of the target species, given the naturally inherited variation in susceptibility to *Bt* proteins found in certain insect species. In that case, resistance is less likely to be functionally recessive. Three other elements that may cause a deviation from the ideal high-dose scenario are the presence of resistance alleles at higher-than-expected frequency, refuges that are not as productive as expected regarding the number of susceptible adults emerging off the non-*Bt* plants, and poor overall refuge compliance or incorrect management of refuge areas (Storer et al. 2012b).

The design of *Bt* plants that expressed two or more distinct *Bt* proteins that control (kill) the same insect pest species (*Bt* pyramids) was proposed to mitigate some of the risk related to deploying single-mode-of-action *Bt* plants (Carrière et al. 2015; Head and Greenplate 2012; Roush 1994; Storer et al. 2012b). In addition, *Bt* pyramids can expand the spectrum of insect control relative to the use of single-toxin *Bt* crops and can therefore be more efficient at controlling target insect species and reducing crop damage. For these reasons, *Bt* pyramids have become increasingly important for IPM and IRM (Carrière et al. 2015). There are three key conditions for *Bt* pyramids to be an effective IRM tactic: (1) high mortality of homozygous susceptible insects is achieved by each component of the pyramid (stack) alone, such that there is "redundant killing"; (2) the probability that cross-resistance between the components is low or absent; and (3) the pattern and expression level of each *Bt* protein in the pyramid (stack) is similar (Storer et al. 2012b). Roush (1994) found that if two insecticidal traits (e.g., *Bt* proteins) each provide >95% control of susceptible insects and there is, for instance, a 10% refuge, the number of generations until resistance occurs can increase 25- to >100-fold relative to a single-trait plant. According to Roush (1998), with the use of *Bt* pyramids, most resistance alleles are eliminated from an insect population when *Bt* proteins control (kill) individuals that are homozygous for susceptibility to one *Bt* protein, even when resistance to the other toxin is not recessive. Thus, the ability of each *Bt* protein in a pyramid to control susceptible insects is more central to IRM than is recessive inheritance of resistance (Roush 1998). As with single-mode-of-action plants, however, deviations from ideal conditions may reduce the IRM value of a *Bt* pyramid. For example, survival of susceptible insects in a *Bt* pyramid that is greater than expected, cross-resistance between the *Bt* proteins in pyramids, and poor compliance with refuge recommendations all have the potential to compromise the effectiveness of a *Bt* pyramid at delaying the onset of insect resistance (Carrière et al. 2015, 2016).

17.3 Documented Cases of Resistance to *Bt* Proteins in FAW

17.3.1 Puerto Rico

The first case of documented field-evolved resistance to *Bt* proteins in FAW came from Puerto Rico (Storer et al. 2010, 2012b). The Cry1F resistance in the FAW population from Puerto Rico was shown to be autosomal and highly recessive, with limited cross-resistance to Cry1Ab and Cry1Ac (Storer et al. 2010). The combination of several factors played a very important role leading to the evolution of resistance to Cry1F protein, expressed in TC1507 maize, in Puerto Rico (Storer et al. 2010, 2012a). The fact that Puerto Rico is an island limits insect migration from external environments and, as a consequence, likely constrains the genetic variability. It also has a tropical climate conducive to year-round cultivation of maize, increasing the number of FAW generations potentially exposed to the selection pressure of TC1507. Drought conditions were experienced in 2006/2007, which reduced the availability of alternative hosts for FAW, limiting the contribution of those plants to the pool of susceptible insects available in the landscape (Storer et al. 2010). Upon an initial confirmation of field resistance in 2006 and as a part of the IRM program for TC1507, the company registrant of that technology stopped the commercial sale of the product (Herculex – TC1507) in Puerto Rico (Storer et al. 2012b). Resistance to Cry1F in FAW in Puerto Rico was found to be stable (Storer et al. 2012b) and was still present even after 4 years without commercial planting of Cry1F maize, with no significant fitness costs associated with resistance (Jakka et al. 2014; Velez et al. 2013). Notwithstanding a prior report of no detectable levels of Cry1F resistance in FAW impacting the US mainland (Storer et al. 2012b), field resistance of FAW to Cry1F maize was documented in the southeastern United States (Huang et al. 2014; Niu et al. 2013, 2014). Although the geographical range and distribution of Cry1F resistance in FAW in the mainland United States remains unclear, the cause for resistance in this case is likely to be migration of Cry1F-resistant FAW from Puerto Rico (Huang et al. 2014).

17.3.2 Brazil

Unexpected damage caused by FAW in TC1507 maize was initially observed in the state of Bahia, Brazil, 3 years after the product was deployed in that country (Farias et al. 2014a). A significant decrease in susceptibility to Cry1F was detected in FAW across Brazil over growing seasons from 2010 to 2013, especially in areas with intensive maize production, high adoption of *Bt* technologies, and poor compliance with refuge recommendations (Farias et al. 2014b). Farias et al. (2015) found that the Cry1F resistance allele in FAW from Bahia was not completely recessive on TC1507 maize when compared with a susceptible laboratory strain. Through leaf tissue dilution bioassays, Farias et al. (2015) concluded that TC1507 was not a high-dose product for FAW. Their results showed high survivorship of susceptible FAW larvae at a 25-fold dilution (i.e., 76.8%). Separately Cry1F-resistant heterozygous larvae exhibited 8.3% survival in a Cry1F leaf bioassay (for the same maize hybrid and growth stage). Similar results were found by Santos-Amaya et al. (2016). These results taken as whole indicate the high likelihood of a significant survivorship of FAW larvae carrying at least a single copy of the Cry1 resistance allele after exposure to TC1507, consequently fueling the evolutionary process that led to field-evolved resistance to Cry1F. No relevant fitness costs were observed in a near-isogenic Cry1F-resistant strain of FAW, indicating stability of resistance to Cry1F protein in the absence of selection pressure (Horikoshi et al. 2015).

Farias et al. (2016) reported that the Cry1F resistance allele was common in several states across Brazil early in 2012 and that the detected frequency of the allele in association with the geographically widespread of resistance suggested a higher-than-anticipated initial frequency of the resistance allele at the time that TC1507 maize was launched there. The estimated resistance allele frequencies varied from 0.009 to 0.277, with the collections from western Bahia (northeast Brazil) showing the highest Cry1F resistance frequencies. Additionally, the frequency of the Cry1F resistance allele increased more than threefold (3×) in the 3 months from the first to the second maize growing season in a particular county in the State of Paraná, Brazil. According to Farias et al. (2016), this result indicated a potential increase in the selection pressure on *Bt* maize associated with the consecutive planting of technologies containing insecticidal proteins with the same or similar modes of action.

17.3.3 Argentina

In Argentina, anecdotal observations of unexpected damage caused by FAW on Cry1F maize (TC1507) were reported in 2012 and 2013 (Trumper 2014). Moreover, Flores and Balbi (2014) documented unexpected survivorship of FAW larvae in laboratory and field trials in Argentina. As in Brazil, low levels of compliance with the structured refuge recommendation were the likely explanation for the shift in the Cry1F susceptibility of FAW populations in Argentina (Trumper 2014).

17.4 Genetics of Cry1F Resistance in FAW

Leite et al. (2016) subjected a field-collected FAW population to four generations (rounds) of laboratory selection, yielding a strain highly resistant to Cry1F. This resistance was demonstrated by the survival of insects reared on leaves of TC1507 maize plants and by the more than 300-fold resistance level measured in bioassays with purified Cry1F protein. Reciprocal crosses between control and Cry1F-selected strains revealed that the resistance was autosomal and incompletely recessive and the response obtained in a backcross of the F1 generation with the resistant strain was consistent with simple monogenic inheritance. Additionally, there were no apparent fitness costs associated with resistance either for survival or larval growth on non-*Bt* maize leaves. These findings provide experimental evidence for rapid evolution of Cry1F resistance in FAW in the laboratory and further reinforce the potential of this species to evolve field resistance to the TC1507 maize and to subsequently impact the efficacy of other Cry1 proteins to due to cross-resistance (see Sect. 17.6).

Santos-Amaya et al. (2016) continuously exposed FAW to the TC1507 event for 11 generations, which resulted in more than 183-fold resistance to Cry1F in the two strains studied. This high resistance level enabled the insects to complete larval development on the *Bt* maize plants. Genetic analyses using concentration-response bioassays with progenies from reciprocal crosses between resistant and susceptible insects indicated that the inheritance of the resistance was autosomal, partially recessive, and without maternal effects (i.e., sex linkage). Backcrosses of the F1 progeny with the resistant parental strains revealed that the resistance in the two selected strains was conferred by a single locus or set of tightly linked loci. These results support some of the assumptions of the strategy in use for management of FAW resistance to *Bt* Cry1F maize, but survival rates of heterozygotes on the *Bt* plants were higher than 5%, supporting the assertion that Cry1F maize (TC1507) does not produce a high dose of the insecticidal protein (i.e., resistance is partially recessive) for FAW.

17.5 Resistance to Cry2Ab2 Protein in FAW

Niu et al. (2016b) detected a major Cry2Ab2 resistance allele using a leaf tissue F_2 screen to screen field-collected population of FAW and estimate the allele frequency. FAW field populations were collected in the United States from Texas, Louisiana, Georgia, and Florida. A conservative estimate of the frequency of major Cry2Ab2 resistance alleles in FAW from the four states was 0.0023 with a 95% CI of 0.0003–0.0064. In addition, six FAW families were considered likely to possess minor resistance alleles at a frequency of 0.0082 with a 95% CI of 0.0033–0.0152. The F_2 screen identified at least 1 (GA-15) out of the 215 two-parent families of FAW from populations collected in the southern United States to possess a major resistance allele to the Cry2Ab2 protein. Larvae from the GA-15 family survived well on whole maize plants expressing Cry2Ab2 protein and demonstrated a significant level (>15-fold) of resistance when fed with the same protein incorporated in an artificial diet. Prior to the study by Niu et al. (2016b), there had been no information available about the frequency of Cry2Ab2 resistance alleles in FAW. The detection of the major resistance allele coupled with the relatively more common "minor" resistance alleles in the field populations of FAW may have important implications for resistance management.

17.6 Studies of Cross-Resistance Among Cry Proteins

Despite early indications that cross-resistance among Cry1 proteins was limited, Hernández-Rodríguez et al. (2013) studied whether the chimeric Cry1A.105 protein had shared binding sites in FAW with Cry1A proteins, with Cry1Fa, or both. The authors concluded that Cry1A.105, Cry1Ab, Cry1Ac, and Cry1Fa competed with high affinity for the same binding sites in FAW, which might explain the cross-resistance among Cry1 proteins. Therefore, cross-resistance among Cry1 proteins may occur through the alteration of shared binding sites. On the other hand, Hernández-Rodríguez et al. (2013) and several other reports indicated that Cry2Ab2 had a different mode of action from that of Cry1F and Cry1A proteins. For that reason, cross-resistance between Cry2Ab2 and Cry1F or Cry1A proteins is not likely (Hernández-Rodríguez et al. 2013). Not surprisingly, low levels of cross-resistance between Cry1F and Cry1A.105 proteins were detected in a Cry1F-resistant FAW strain isolated through an F_2 screen from a field population sampled in south Florida (Huang et al. 2014).

Bernardi et al. (2015a) detected moderate levels of cross-resistance among Cry1 proteins in FAW. The authors tested a Cry1F-resistant FAW strain in bioassays using solubilized proteins (Cry1A.105 and Cry2Ab2) and in leaf tissue trials. The results confirmed moderate levels of cross-resistance among Cry1F, Cry1A.105, and Cry1Ab in FAW. Consistent with other reports, no significant levels of cross-resistance were found between Cry1F and Cry2Ab2, and MON 89034 maize (which expresses Cry1A.105 and Cry2Ab2) in combination with appropriate management practices continues to provide effective control of FAW in Brazil (Bernardi et al. 2015a).

MON 810 maize, which expresses the Cry1Ab protein, significantly contributed to IPM programs for FAW in Brazil (Waquil et al. 2013), but it is not considered a high-dose product for FAW primarily due to the moderate activity of Cry1Ab against this species. The deployment of MON 810 in an environment with widespread resistance to Cry1F documented cross-resistance among Cry1 proteins, and low compliance with structured refuge recommendations led to the documentation of field-evolved resistance to Cry1Ab in FAW in Brazil (Omoto et al. 2016). Results from laboratory (in vitro) and field (in planta) monitoring programs consistently indicated a shift in the susceptibility of FAW to the Cry1Ab protein. According to Omoto et al. (2016), the contribution of the selection pressure of the Cry1Ab protein expressed in MON 810 to the onset of resistance could not be distinguished from impacts from cross-resistance between Cry1Ab and Cry1F proteins, given that resistance to the latter was well documented in Brazil (Farias et al. 2014a, b, 2015, 2016) as was cross-resistance to Cry1Ab (Bernardi et al. 2015a).

Huang et al. (2016) estimated the frequency of Cry1A.105 resistance alleles in field populations of FAW, collected from three locations in the US states of Louisiana and Florida in 2011. A total of 18 FAW families, 4 from Louisiana, and 14 from Florida were identified to be potentially positive families carrying resistance alleles to Cry1A.105. Whole-plant tests confirmed that the four potentially positive families that were evaluated in this test possessed major resistance alleles to Cry1A.105 maize plants. Based on the similar performance of the 18 families in the leaf tissue F_2 screen, the 14 other potentially positive families that were not retested in the whole-plant assay, due to the unsuccessful establishment of progeny populations, most likely possessed major resistance alleles to the Cry1A.105 maize plants. Therefore, all 18 potentially positive families identified in the leaf tissue F_2 screen were also considered as possessing major resistance alleles when calculating resistance allele frequency. The corresponding frequency of alleles for resistance to Cry1A.105 maize was estimated to be 0.0158 with a 95% credibility interval (CI) of 0.0052–0.0323 for the Louisiana populations and 0.0559 with a 95% CI of 0.0319–0.0868 for the Florida populations. These resistance allele frequencies are greater than those estimated for other insect pests and *Bt* proteins. The authors were able to establish two resistant strains in the laboratory (FL32 and FL67), which survived on whole Cry1A.105 maize plants and demonstrated a significant level (>116-fold) of resistance to the Cry1A.105 protein in a diet-incorporated bioassay. These findings suggest that resistance in FAW to single-gene Cry1A.105 maize in the southeast United States is not rare, most likely due to the selection of Cry1F resistance and its cross-resistance to Cry1A.105.

Niu et al. (2016a) evaluated the survival and plant injury of the two Cry1A.105-resistant strains (FL32 and FL67) established by Huang et al. (2016), along with a susceptible population and two F1 heterozygous genotypes, on commercial and experimental *Bt* maize hybrids/lines containing single or pyramided *Bt* genes. These *Bt* maize hybrids/lines consisted of five single-gene *Bt* maize products containing Cry1A.105, Cry2Ab2, Cry1F, or Cry1Ab protein and three pyramided *Bt* maize products expressing Cry1A.105/Cry2Ab2, Cry1A.105/Cry2Ab2/Cry1F, or Cry1Ab/Vip3A. Resistance in FL32 and FL67 on leaf tissues of Cry1A.105 maize was recessive to incompletely recessive, while on whole Cry1A.105 plants, it was moderate to incompletely dominant. This variation in dominance level observed on different test plant materials suggests that careful experimental designs are needed for evaluating the “high-dose” qualification of *Bt* maize against FAW. Their results showed that both Cry1A.105-resistant populations of FAW were highly cross-resistant to Cry1F maize. The cross-resistance was incompletely recessive for FL32 but dominant for FL67 in the leaf tissue bioassay, while it was incompletely dominant in the whole-plant tests for both populations. The non-recessive resistance could be one of the factors that led to the rapid development of resistance to Cry1F maize in some field populations of FAW. The Cry1A.105-resistant strains of FAW were not cross-resistant to Cry2Ab2 or Vip3A.

Yang et al. (2016) investigated whether a Cry1F-resistant FAW strain selected on *Bt* maize was also resistant to *Bt* cotton containing similar or different *Bt* genes. The Cry1F gene in the cotton plants (event DAS-24236-5) is a synthetic, plant-optimized, full length version of the Cry1F gene, whereas in maize (TC1507), it is a plant-optimized version of a truncated Cry1F gene. The authors evaluated the survival, growth, development, and plant injury of the Cry1F maize-susceptible (SS), heterozygous (RS), and resistant (RR) genotypes of FAW on one non-*Bt*, three single *Bt*, and five pyramided *Bt* cotton products. The results showed that Cry1F-maize-resistant FAW was also highly resistant to *Bt* cotton expressing the Cry1Ac and Cry1F proteins. This outcome suggested that the dissimilar forms of the Cry1F gene inserted in the maize and cotton plants apparently did not change the mode of action/binding of the Cry1F protein. These results also demonstrated that the highly resistant FAW selected with *Bt* maize are susceptible to pyramided *Bt* cotton expressing Cry2A and/or Vip3A. Horikoshi et al. (2016) observed similar results, demonstrating that resistance in FAW to Cry1 proteins expressed in *Bt* maize plants can compromise *Bt* cotton technologies expressing similar *Bt* proteins.

17.7 Implications of Cross-Resistance for FAW Resistance Management

The use of *Bt* maize plants with less-than-ideal IRM fit (e.g., less-than-high-dose technologies, components of *Bt* pyramids with cross-resistance to other *Bt* proteins in landscape) combined with low compliance with the structured refuge recommendation seems to be a common theme across the resistance cases and issues documented in FAW across southern South America. A consequence of these findings is a direct reduction in the number of effective modes of action to manage FAW infestations and resistance to *Bt* maize insecticidal proteins. For example, the cross-resistance between Cry1F and Cry1A.105 could significantly reduce the activity of the Cry1A.105 protein in MON 89034 maize, leaving the Cry2Ab2 protein in MON 89034 only partially protected against Cry1F-resistant FAW and stressing the importance of effective implementation of refuges (see Sect. 17.6).

The deployment of MIR162 (Vip3Aa20) maize represents an effective new and unique mode of action added to the maize cropping system. MIR162 achieved the high-dose requirements for FAW (Bernardi et al. 2015b), and the frequency of Vip3Aa20 resistance in Brazilian FAW populations sampled across the country was low (Bernardi et al. 2015c). Pyramided *Bt* maize and *Bt* cotton containing Cry2A and/or Vip3A genes should still provide a means for managing the Cry1F resistance in FAW (Horikoshi et al. 2016). However, there is a high risk of resistance to the current pyramided *Bt* crops evolving in areas where resistance to Cry1F maize is widespread (Horikoshi et al. 2016; Santos-Amaya et al. 2015). Recent studies characterized field-relevant Vip3Aa20 resistance in strains isolated through F_2 screens and showed that the inheritance of Vip3Aa20 resistance was autosomal, recessive, and monogenic (Bernardi et al. 2016). Despite the IRM value of adding Vip3Aa20 to *Bt* pyramids, due to the evolution of resistance to Cry1 proteins and limited activity of Cry2Ab2 against FAW, it is reasonable to assume that the IRM value of *Bt* pyramids combining current available insecticidal proteins is diminished in Brazil and in other areas across South America.

17.8 Conclusions

The current situation highlights the importance of enhancing compliance with the non-*Bt* maize refuge recommendations along with the adoption of best management practices (BMPs) to ensure longer durability of these technologies in tropical and subtropical regions of South America. The low compliance with the refuge recommendations for *Bt* maize technologies in Brazil is connected to an array of factors that highlight the need to effectively engage key stakeholders across the seed supply chain. For instance, the majority of growers in Brazil gain access to *Bt* and non-*Bt* maize seeds and to technical information on the use of these technologies through outside resources such as seed distributors and coops. Therefore, achieving reasonable refuge compliance at the field level requires the proper engagement of key stakeholders through the active implementation of multilayer, multi-stakeholder IRM strategies. Overall, IRM plans should be designed to ensure (1) reasonable refuge seed supply in the marketplace, (2) the existence of marketing programs to incentivize refuge seed sales, and (3) a plan for surveying refuge compliance at the field level. Developing and implementing multilayer, multi-stakeholder programs to ensure reasonable refuge compliance is critical to supporting the use of current *Bt* maize technologies. It is also imperative for the sustainable use of the next generation of GM technologies carrying insect control traits. The design of the next generation of GM technologies for insect control should combine multiple novel insecticidal traits with no cross-resistance to the current Cry1 proteins and with high activity against the same target pests. GM maize products with superior IRM fit in tropical regions, in combination with effective management of these technologies at the field level, will be the foundation for sustainable use of these technologies.

In the light of the global challenges to the implementation of IRM plans, particularly in South America, Excellence Through Stewardship (ETS; http://excellencethroughstewardship.org/), a global industry-coordinated organization that promotes the adoption of stewardship programs for agricultural biotechnology, developed a Guide for Resistance Management for Biotechnology-Derived Plant Products. The goal of the ETS/IRM Guide is to provide guidance to company members for developing and implementing processes around resistance management of *Bt* crops. In addition, the Brazilian Seed Association (ABRASEM) has developed an online system to help growers select appropriate non-*Bt* maize hybrids to be planted as refuge in different regions of Brazil (http://www.abrasem.com.br/sistema-de-selecao-de-cultivares-para-refugio/). The sustained use of *Bt* crops depends upon initiatives like these to ensure all key stakeholders understand their roles in IRM and are committed to the success of IRM strategies.

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