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Adaptive Potential of Fall Armyworm (Lepidoptera: Noctuidae) Limits Bt Trait Durability in Brazil

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Abstract

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith 1797) (Lepidoptera: Noctuidae), is the most important corn pest in South America. Larvae feed mostly on leaves, but also ears when population densities are high. This pest has been historically controlled with insecticide applications, but many cases of resistance have limited their efficacy. Transgenic corn varieties expressing *Bacillus thuringiensis* proteins (Bt corn) have been a widely adopted alternative to insecticides and, in the past 8 yr, have been the primary technology for fall armyworm control in Brazil. Because transgenic varieties require 10–15 yr to be developed and fall armyworm has quickly evolved resistance to most commercially released Bt corn hybrids, strategies for Bt trait durability are paramount. Most of the Bt corn hybrids lost their ability to control fall armyworm in just 3 yr after their release in Brazil. Here we summarize what is known about Bt resistance in fall armyworm in Brazil, a phenomenon perhaps never seen before in any part of the world. Furthermore, we suggest that the interactions between management practices adopted (or not adopted, e.g., refuge compliance) to delay the evolution of resistance and the ecological and evolutionary characteristics of fall armyworm are driving the rapid evolution of resistance to Bt corn in Brazil. As newer products emerge in the market, careful consideration will be needed to maximize trait durability.

Key words: *Spodoptera frugiperda*, insect resistance management, Bt crop, Bt resistance

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith 1797) (Lepidoptera: Noctuidae), is the major corn pest in Brazil and throughout South America (Blanco et al. 2016). In the past 4 yr, Brazilian cotton and corn fields have experienced high fall armyworm infestations causing large economic losses. Corn yield reductions caused by this pest can reach 34–38% (Carvalho 1970). When late instars act as seedling cutworms, corn losses can reach up to 100% (Ávila et al. 1997). Such population outbreaks are, in part, due to increased cultivation during the second corn season (corn planted immediately after soybean—soybean September to January; corn February to June; Valicente 2008). In 2015, the area of second corn season in Brazil was 65.5% of the total crop land, whereas the traditional summer season was 34.5% (Companhia Nacional de Abastecimento [CONAB] 2016). As a consequence, fall armyworm populations that used to peak during the summer (first corn season) are now found throughout the extended growing period facilitated by so-called "green bridges"—plant hosts where fall armyworm migrate from one crop to another in the same region, maintaining high populations.

This species has complete development cycle (eggs, larvae, pupae, and adults; Fig. 1); however, the control is focused only on larva stage. Chemical control has been widely used in the past three decades to manage fall armyworm infestations, although this tactic is not without concerns. Growers lack or do not adopt economic thresholds for chemical applications, and multiple immigrations are common in early stage corn fields. In addition, females usually lay eggs on the upper layer of the corn leaves and, after hatching, the neonates tend to quickly move to the whorl of the plant. Once hidden in the whorl, the exposure, and therefore efficacy, of chemical controls is limited (Young 1979). Thus, most insecticides are applied on a weekly basis to prevent neonates from moving into the plant whorl (Cruz 1995). After neonates move to the whorl, one of the strategies used by growers is to increase the liquid volume and application doses to increase fall armyworm mortality. However, aerial applications are frequently used across large farms, which use very low liquid volume and leads to sublethal dosage exposure. The interaction between larval behavior and chemical application may have promoted rapid resistance evolution of this species to many insecticides in Brazil (Diez-Rodríguez and Omoto 2001). The Arthropod Pesticide Resistance Database listed in 2016 fall armyworm resistance to 24 different active ingredients. Field resistance has been

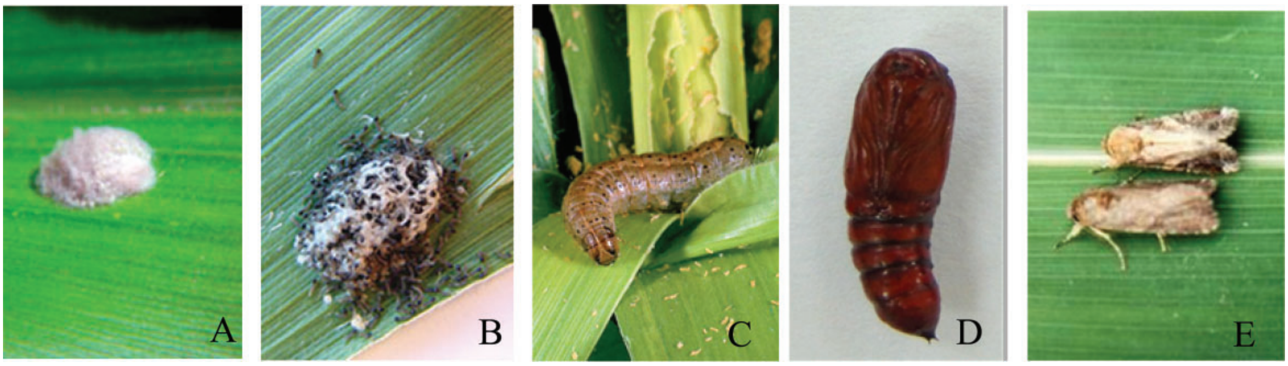

Fig. 1. Fall armyworm life cycle stages. (**A**) Eggs. (**B**) Early instar larva. (**C**) Late instar larva. (**D**) Pupa. (**E**) Adults (above male and below female). Pictures A, B, C, and E: Paulo Henrique Soares da Silva (EMBRAPA). Picture D: Caroline Sakuno (Syngenta Crop Protection).

documented in 45 different locations among eight countries. Brazil includes 25 cases, representing 55.5% of the total worldwide cases.

To help to overcome control challenges imposed by fall armyworm, transgenic corn hybrids expressing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have been the most widely adopted technology in South America and across the world (National Academies of Sciences, Engineering, and Medicine 2016). The rapid adoption of genetically modified crops has been driven by various benefits provided by this technology including effective insect control, reduced agricultural inputs (i.e., chemical pesticides), and positive economic impact for growers (National Academies of Sciences, Engineering, and Medicine 2016). For example, between 1996 and 2011, genetically modified crops provided an economic benefit of US$98.2 billion; in 2011 alone, it was estimated at US$19.8 billion. An additional benefit was the significant increase of corn productivity, which increased by 195 million tons globally (Brookes and Barfoot 2013). Brazil represents 8.8% (15.43 million hectares) of the total corn production in the world (U.S. Department of Agriculture [USDA] 2013), of which 12.1 million hectares were planted with genetically modified maize tolerant to insects (Bt corn; International Service for the Acquisition of Agri-Biotech Applications [ISAAA] 2013). From 2008 to 2015, five different Bt proteins and five different pyramid products (those containing more than one Bt protein targeting fall armyworm) were launched in Brazil for Lepidoptera control (Table 1; National Technical Committee on Biosafety [CTNBio] 2016).

Similar to insecticides, fall armyworm can also develop resistance to Bt crops in response to the strong selection pressure that this technology imposes over field populations (Storer et al. 2012). To manage resistance and ensure trait durability, insect resistance management (IRM) practices for Bt crops must be a priority. IRM strategies include refuge (i.e., non-Bt crops planted with Bt corn), the use of high-dose, and pyramid products. The primary IRM strategy for insect resistance management is the adoption of refuge. The refuge should then produce a preponderance of susceptible insects (genotype *ss*) which will mate with potential homozygous resistant (*rr*) selected for in the Bt area. Assuming resistance is recessive, the offspring generated would be heterozygous (*rs*), and controlled by a Bt crop (Gould and Tabashnik 1998, Matten et al. 2008, Tabashnik et al. 2009).

Refuges are most effective when used in concert with a high-dose protein. Under high-dose, homozygous recessive (*ss*) and heterozygous (*rs*) insects cannot survive when exposed to the product; only homozygous resistant (*rr*) insects might survive (Tabashnik et al. 2004, Crespo et al. 2009). The high dose strategy has been somewhat successful in other Bt crops such as cotton expressing Cry1Ac to control *Heliothis virescens* (F.) and *Pectinophora gossypiella* (Saunders); these pests became resistant only 11 and 13 yr, respectively, after introduction of Bt cotton (Tabashnik et al. 2013). Perhaps the best case of refuge and high-dose success has been with *Ostrinia nubilalis*, European corn borer. Once the most damaging corn pest in North America, this lepidopteran has been substantially controlled by several Bt proteins (Hutchison et al. 2010).

Bt traits that are not high dose events might require multiple control tactics to maintain low resistance frequency. This strategy assumes that insect resistance to all tactics is unlikely, and at least one toxin will provide mortality. Pyramiding of Bt proteins is one such example adopted to delay insect resistance to Bt crops. Successful cases of pyramiding were reported in cotton expressing Cry1Ac + Cry2Ab to control *Helicoverpa armigera* Hübner and *Helicoverpa punctigera* Wallengren in Australia and since its introduction in 2005, there has been no reported of resistance for either species (Tabashnik et al. 2013). However, success of IRM will depend on other conditions such as initial low frequency of resistance for each protein, recessive resistance for each protein, fitness cost and incomplete resistance, lack of cross resistance, refuge strategy (block compared to integrated), and refuge compliance (Tabashnik et al. 2013, Carriére and Tabashnik 2001, Gassmann et al. 2009).

Unfortunately, IRM for fall armyworm has been challenging. In Brazil, multiple Bt proteins are labelled for fall armyworm control, either in single or pyramid combinations. Cry1Ab was first introduced in 2008, but unexpected damage by fall armyworm occurred three years later. Similarly, Cry1F was introduced in 2009 and growers reported unexpected damage in several different regions of the country as early as 2012. A pyramid Bt product containing Cry1A.105 + Cry2Ab2 was introduced in 2010, but yet unexpected damage in the field was first noticed in 2013. Nowadays, this product can have as many as three insecticide applications in some regions of Brazil to achieve adequate fall armyworm control, while in other regions, the product continues to offer good suppression for fall armyworm as well as controlling secondary Lepidoptera pests. In addition, fall armyworm resistance emerged in other South American countries including Argentina, Paraguay, Uruguay, and Colombia evidenced by growers reports of unexpected damage (data not published) where the same products expressing the same Cry proteins are grown. Of all the Bt proteins, fall armyworm remains susceptible to only Vip3Aa20, a toxin produced in the vegetative stage of Bt (as opposed to a Cry endotoxin). Introduced in 2010, Vip3Aa20 has no reports of unexpected damage or product failures against fall armyworm in the field. However, rapid adaptation of fall armyworm to insecticides and almost all Bt Cry proteins makes Brazil a most challenging environment for the durability of any Bt technology.

Table 1. Commercial Bt corn for Lepidoptera control in Brazil, regulatory approval timelines, and initial failure seen in the field

Company	Traits	Event name	Trade name	Approval year	Field failure started
Syngenta	Cry1Ab	SYN-BT011-1	Agrisure TL	2008	2011
	Vip3Aa20	SYN-IR162-4	Agrisure Viptera	2009	NF
	Cry1Ab + Vip3Aa20 + Gli	SYN-BT011-1 x SYN-IR162-4 x MON-00021-9	Agrisure Viptera3	2010	NF
Monsanto	Cry1Ab	MON-00810-6	YieldGard	2008	2011
	Cry1A.105 + Cry2Ab2	MON-89034-3	YieldGard VT Pro	2009	2013
Dow Agrosciences	Cry1F	DAS-01507-1	Herculex I	2008	2011
	Cry1A.105 + Cry2Ab2 + Cry1F	MON-89034-3 x DAS-01507-1 x MON-00603-6	PowerCore	2013	2014
DuPont	Cry1F	DAS-01507-1 x MON-00603-6	Herculex I	2009	2011
	Cry1F + Cry1Ab	DAS-01507-1 x MON-00810-6 x MON-00603-6	Optimum Intrasect	2011	2012
	Cry1F + Cry1Ab + Vip3Aa20 + Gli + Glu	DAS-01507-1 x MON-00810-6 x SYN-IR162-4xMON-00603-6	Leptra	2015	NF

NF—No failure reported.

Field failure started—Based on growers reports and observations (J.C.F., unpublished data).

In this paper, we discuss fall armyworm resistance evolution to Bt toxins in Brazil within a framework of three interacting factors: 1) genetics; 2) biology and ecology; and 3) implementation of resistance management tactics. We suggest that these three factors enabled fall armyworm to overcome Bt crops in an unexpected and unprecedented period of time in Brazil.

Genetics Characteristics of Fall Armyworm and Its Impact on Resistance Evolution to Bt Crops

Resistance Allele Frequencies: Assumptions and Empirical Estimations

Resistance evolution tends to be faster when the initial frequency of resistance alleles is high in insect populations (Georghiou and Taylor 1977, Tabashnik and Croft 1982, Tabashnik 1994, Roush 1997). Due to its contribution for resistance evolution, the estimation and predictions of resistance through mathematical modeling could be substantially improved if the frequency of resistance was empirically estimated (Gould et al. 1997). In most cases, the estimation of resistance frequencies is usually absent and only assumed. Current methods of estimating frequencies are extremely laborious and usually performed only after the technology is launched and may not reflect true frequencies before the product introduction (Génissel et al. 2003). With F_2 screens (for methodology see Andow and Alstad (1998)), frequencies have been estimated in a limited number of studies, mostly in Lepidoptera: *H. armigera* (Wu et al. 2002), *H. virescens* (Gould et al. 1997), *Helicoverpa zea* Boddie (Burd et al. 2003), *O. nubilalis* (Andow et al. 1998, Bourguet et al. 2003), *Scirpophaga incertulas* Walker (Bentur et al. 2000), and *P. gossypiella* (Tabashnik et al. 2000). The frequency of fall armyworm resistance to Vip3Aa20 in Brazil was estimated in 2013 and 2014 (Bernardi et al. 2015a), 3–4 yr after its initial release. Data for Cry proteins were unknown before or immediately after proteins introduction, potentially jeopardizing models predicting resistance evolution. Data on frequency of resistance alleles to Cry1F were published 8 yr after product registration in Brazil (Farias et al. 2015).

The initial frequency of a new allele is calculated by the mutation rate and the population size (1/2*N*, for a diploid organism, where *N* is the population size). The reproductive output of fall armyworm is quite high, with one female able to produce an average of 1,688 offspring (Barros et al. 2010). Hence, fall armyworm is expected to have very large populations after one generation. However, large population sizes also influence genetic and allelic diversity and reduce the potential of rare alleles (i.e., resistance) to be lost through random genetic drift. Species with higher population sizes are generally more adaptable and have a greater evolutionary response toward shifting selection pressures (Wright 1932). Integrated pest management (IPM) strategies, when deployed at a regional level, can enforce a population reduction, decreasing diversity and adaptation potential (Hutchison et al. 2010). However, in terms of IPM strategies, population reduction is often accompanied with a strong selection pressure such as insecticides or Bt traits. Before product failures, therefore, we may expect a genetic and population bottleneck, not due to random processes (e.g., environmental), but a selective bottleneck retaining only resistant individuals. The question remains whether or not Bt resistance is a novel mutation or a preexisting polymorphism. Nonetheless, given the reproductive output and strong selection pressure of fall armyworm, resistance can spread quite quickly.

High initial frequency of resistance alleles, provided mainly by a high frequency of homozygous resistant (*rr*) insects, can neutralize the benefit from any resistance management practices adopted. For example, the most useful strategies are a high dose associated with refuge implementation; however, if high in frequency, *rr* insects will also reside in refuge, and even potentially outnumber susceptible (*ss*). Additional control tactics in the refuge could control *rr* insects, but *ss* insects will likely suffer the same fate. It is possible that the rapid evolution of fall armyworm resistance to Cry proteins in Brazil may be associated with higher initial frequency of resistance alleles in the field populations than originally assumed.

The Fall Armyworm Resistance as a Dominant Trait Facilitates Rapid Evolution

Assuming that resistance is based on a single gene or allele, extended Bt trait durability depends on the inheritance of resistance, e.g., recessive or dominant (Storer et al. 2003). Trait inheritance of insect resistance to Bt crops can be measured by the heritability (*h*), which varies from 0 (completely recessive) to 1 (completely dominant; Gould and Tabashnik 1998). This parameter can also be indirectly estimated through crossing experiments of resistant and susceptible individuals (Gould and Tabashnik 1998, Tabashnik et al. 2004). Generally speaking, if resistance is recessive, then an insect will need two copies of the resistant allele. Resistance is functionally recessive when the dose of protein expressed in the Bt plant is sufficient to kill all heterozygous insects (*rs*); however, some survivorship of resistant homozygous insects (*rr*) is expected (Tabashnik et al. 2004, Crespo et al. 2009). Alternatively, functional dominance is seen when heterozygous (*rs*) are more likely to survive on Bt crops (i.e., needing one copy of the resistance allele).

As the dominance of resistance is classified as “functionally” completely dominant or completely recessive, it may also depend on protein expression of plant tissues (e.g., dosage). Cry protein expression in cotton and corn can be reduced as the plant matures (Dutton et al. 2004). Bt cotton expresses less Cry1Ac during the reproductive stages than in vegetative stages; thus, resistance could appear as recessive if insects feed on plants in vegetative stage but partially dominant during reproductive stages (Showalter et al. 2009). Alternatively, cotton plants expressing Vip3Aa19 tends to have stable expression throughout the plant’s life cycle (Llewellyn et al. 2007).

Fall armyworm resistance to Cry proteins appears to be dominant, with substantial heterozygote survival (Cry1F—Farias et al. 2015; Cry1Ab—Jakka et al. 2016). The fall armyworm genetics combined with insufficient protein concentration to kill all heterozygous (*rs*) individuals (e.g., high-dose) likely facilitated the persistence of resistant alleles in heterozygotes and enabled fall armyworm resistance evolution in Brazil.

Absence or Reduced Fitness Costs Promotes Survival of Resistant Fall Armyworm

A fitness cost is a biological or ecological penalty that organisms encounter for carrying the resistance allele. The intensity of this penalty is influenced by the environment and genetics of the target pest (Carrière et al. 2010). In regards to the high dose and refuge strategy, fitness costs have most impact when resistant (*rr*) insects are less fit compared to susceptible individuals (*ss*) in the refuge (e.g., *ss* individuals are more likely to survive and reproduce on non-Bt plants than *rr* individuals). As the rationale of the refuge is to support the production of *ss* individuals, any survival of *rr* can potentially increase resistance evolution (Carriére and Tabashnik 2001). Bt durability can be very successful when resistance comes with a high fitness cost, is recessive, and refuges are abundant, even if the initial frequency of resistance is high (Carriére and Tabashnik 2001).

Assuming the initial frequency of resistance alleles is low and likely carried by heterozygous insects (*rs*), resistance evolution in this stage is largely governed by the difference of fitness between susceptible individuals (*ss*) and heterozygous (*rs*) feeding on refuge areas (assuming before widespread Bt adoption). Thus, the strength of the fitness cost is critical for individuals that carry the *r* allele to transmit resistance to the next generation. Data on the strength of fitness costs will help to better understand the resistance risks and develop improved IRM strategies. Unfortunately for fall armyworm resistance to Cry proteins, fitness costs are reduced or are absent when resistant larvae are fed non-Bt corn (Vélez 2013, Jakka et al. 2014, Bernardi et al. 2015b, Santos-Amaya et al. 2016, Souza et al. 2016) or when resistant larvae are fed on different host plants, limiting the effectiveness of the refuge. The absence of a fall armyworm fitness cost likely maintained resistant alleles in the population, hastening Bt resistance.

Biology and Ecology of Fall Armyworm and Its Influence on Resistance Evolution

A species’ life history traits have a strong influence on resistance evolution; these include developmental rate, sex ratio, generations per year, and the timing and rate of reproduction. Evolution of resistance, for example, is directly correlated to the number of generations per year (Tabashnik and Croft 1982). The fall armyworm is highly reproductively efficient in tropical areas, where the warmer temperature allows the more generations per year compared to temperate areas that may have two of fewer generations in a year. In some tropical and subtropical regions (areas without frost) fall armyworm can produce up to 10 generations during a year (Metcalf et al. 1965). In Brazil, this species can have, an average of 8.3 generations under field conditions (Busato et al. 2005). Rapid generation turnover is facilitated by the presence of multicrop systems where different crops are grown at the same time and in succession year-round, which maintains high fall armyworm density.

As previously mentioned, this pest has a high reproductive output, which is also related to different host plants. A study on the reproductive capacity on different host plants showed that the number of eggs per females ranged from 1,342 up to 1,844 when larvae were fed millet or corn leaves, respectively, and 1,844 and 1,839 eggs when fed on soybean and cotton, respectively (Barros et al. 2010). Furthermore, this study confirmed a high oviposition capacity, especially on corn and cotton, which are two hosts that provide selection pressure for Bt resistance. The net reproductive rate of female or female per generation (R0) is statistically equal when larvae are grown on soybean (422 ± 107), millet (331 ± 42.4), cotton (372 ± 81), but higher in corn (502 ± 42).

Despite these differences, fall armyworm females do not appear to exhibit a preference among host plants to lay eggs (Barros et al. 2010). Assuming eight generations in the field, one reproductive female could be responsible for >14,000 offspring in a year (1,844 eggs × 8 generations). Resistant females, therefore, can rapidly produce resistant offspring, increasing resistance alleles, assuming no fitness costs exist.

High Migration Capacity of Fall Armyworm Increases Resistance Allele Dispersal

Migratory capacity and gene flow influence the dispersal of Bt resistance alleles when species are not genetically structured and share similar environments across its range, such as common agronomic crop production practices and management tactics (Fuentes-Contreras et al. 2004). The speed of dispersal will depend on the initial frequency of the resistance alleles in the population as well as the dispersion characteristics of adults. In addition to a large reproductive capacity described above, fall armyworm adults are able to migrate hundreds of kilometers after mating but before laying eggs (Metcalf et al. 1965). Meteorological synoptic maps have recorded long-distance dispersal of adults, detecting individuals migrating from Mississippi, USA, to Canada within 30 h (Johnson 1995).

Adult insects have the potential to rapidly spread resistance alleles among regional and continental populations. However, some geographic barriers to migration of this species do exist, such as the Appalachian Mountains in eastern North America (Nagoshi et al. 2015). The fall armyworm experiences Bt crops across much of its range (see below), expanding not only the distribution range but the area that potentially favors *rr* individuals.

Fall Armyworm Polyphagy Increases Exposure to Similar Bt Proteins in Multiple Crop Hosts

The fall armyworm is a polyphagous species that feeds on >80 species of plants, including the most important commercial crops of corn, cotton, and soybean (Pogue 2002, Capinera 2008). The latter three crops are also used in succession or concomitant in some regions. The fall armyworm life cycle is very similar among those host crops (24.2 d—corn; 27.4 d—cotton; 26.4 d—soybean; 24.5 d—millet; Barros et al. 2010). Individuals resistant to Cry1F do not appear to have a fitness cost when fed corn, soybean, or cotton (Jakka et al. 2014). These results support the potential for those crops to keep multiple and likely overlapping generations of fall armyworm, including those resistant to Bt crops, maintaining large population sizes in all Brazilian regions. In addition to commercial and cash crops, the larvae can feed on several host plants used for cover crops, occupying >90% of crop area. While none of these crops include Bt varieties, this practice likely maintains large fall armyworm populations year-round (De Sá et al. 2009, Prasifka et al. 2009, Barros et al. 2010). Despite a wide range of alternative host which could serve as a natural refuge, this has not been sufficiently effective to manage resistance of fall armyworm in Brazil. There may be mating asynchrony between individuals selected in Bt fields and susceptible individuals generated in alternative hosts.

Complex Detoxification System From Adaptation to Multiple Host Plants

Fall armyworm feeding on different host plants may have facilitated adaptation to overcome many plant allelochemicals including expressing plant protease inhibitors (Jongsma et al. 1995, Brioschi et al. 2007, Dunse et al. 2010, Chikate et al. 2013), developing cell detoxification systems including cytochrome P450s and ABC transporters (Xie et al. 2012, Dermauw et al. 2013), and increasing metabolism of toxic compounds (Wadleigh and Yu, 1988, Sasabe et al. 2004, Li et al. 2007).

Detoxification is a major physiological trait related to host-plant evolution (Ehrlich and Raven 1964). Cytochrome P450s are a class of a detoxification enzymes metabolizing xenobiotic compounds in insects (Li et al. 2004, Sasabe et al. 2004, Rupasinghe et al. 2007). In general, insects with a wide host range have a diverse set of P450s compared with specialists (Giraudo et al. 2015). For example, fall armyworm has 100–120 P450 genes (Giraudo et al. 2015) while *Drosophila sechellia*, a specialist insect, has around 70 P450s (Good et al. 2014). Interestingly, P450s are also known as one of the main enzymes responsible for metabolizing many insecticide compounds (Rupasinghe et al. 2007), which could explain the capacity of fall armyworm to rapidly adapt to several active ingredients under selection pressure. Their role in Bt resistance has yet to be determined.

ABC transporters are also used in the detoxification process of host plant allelochemicals and have frequently been associated with resistance to insecticides through the detoxification process (increased expression of ABC transporter genes). More recently, resistance to Bt crops was also associated to the ABC transporter system, due to mutations that decreased their expression (*H. virescens* to Cry1Ac, Gahan et al. 2010; *P. xylostella*, Baxter et al. 2011, Guo et al. 2015; *Spodoptera exigua*, Park et al. 2014; *Bombyx mori* to Cry1Ab, Atsumi et al. 2012; *H. armigera* to Cry1Ac and Cry1Ab, Xiao et al. 2014). However, mechanisms of fall armyworm insecticide or Bt resistance have yet to be determined.

Fall Armyworm Larva Movement Is Density Dependent and Influenced by Strong Cannibalism

Pronounced fall armyworm cannibalism is an additional ecological factor that plays a crucial role in population regulation. Despite the large reproductive output, it is common to find only one late-stage larva per corn plant. Cannibalism is affected by several factors, with insect density associated with host plant architecture being the most important (Fischer 1961, Istock 1966, Sikand and Ranade 1975, Tschinkel 1978). On corn, females lay eggs in the upper surface of new leaves emerging from the whorl. After eclosing, neonates drop into the whorl where they remain agglomerated until they are induced to disperse, avoiding being easy prey from intraspecifics.

Larval movement plays a considerable role in insect resistance evolution to Bt crops, especially when considering integrated refuges (e.g., refuge in a bag). Larval movement from Bt to refuge plants may lead to selection with sublethal doses. However, in Brazil, integrated refuges have not been implemented for this very reason. Nonetheless, late instars carrying resistance alleles can survive on Bt plants even if there is a “high dose” event (i.e., no movement, Miraldo et al. 2016). Most of the Cry proteins are not “high dose” for fall armyworm (Santos-Amaya et al. 2015, Farias et al. 2015), and the potential of late instar survivorship is very high, accelerating resistance evolution. Assuming 50% of maize plants are infested with one larva, and planted at 65,000 plants per hectare, around 32,500 larvae per hectare can be produced in the first generation.

Agroecosystem Landscape in Tropical Brazil Favors Resistance

Brazil’s tropical climate associated with abundant rain in important agronomic crop growing areas promotes very intensive land use. Two crop seasons per year is common for almost all regions, with some able to produce a third crop under pivot irrigation. Crop succession forms a year-round mosaic that includes many fall armyworm hosts such as soybean, corn, wheat, cotton, or millet, with Cry proteins found in corn, cotton, and soybean. For example, in Bahia State, some farms grow corn in the summer (from September to January), then cotton (January to June) and, after cotton harvest, then plant soybean; all three hosts can express similar Cry proteins and place intense and consistent selection pressure in fall armyworm for 10 mo of the year (Fig. 2). The ecological interactions between fall armyworm, Cry protein availability in multiple crops year-round, and the large expanse of agronomic crop production in the Brazilian landscape poses one of the biggest challenges to delaying fall armyworm resistance.

The fast resistance evolution of this species to Bt corn expressing Cry proteins in Brazil might also be influencing the evolution of resistance in other South American countries that grow the same products. Interestingly, unexpected damage for Cry proteins has been observed in Argentina, Paraguay, Uruguay, and Colombia soon after the failures in Brazil (Blanco et al. 2016). We raise here two different, but not mutually exclusive, hypotheses for such “regional” resistance in several different countries in a short period of time: 1) resistance evolution developed locally due to intense selection

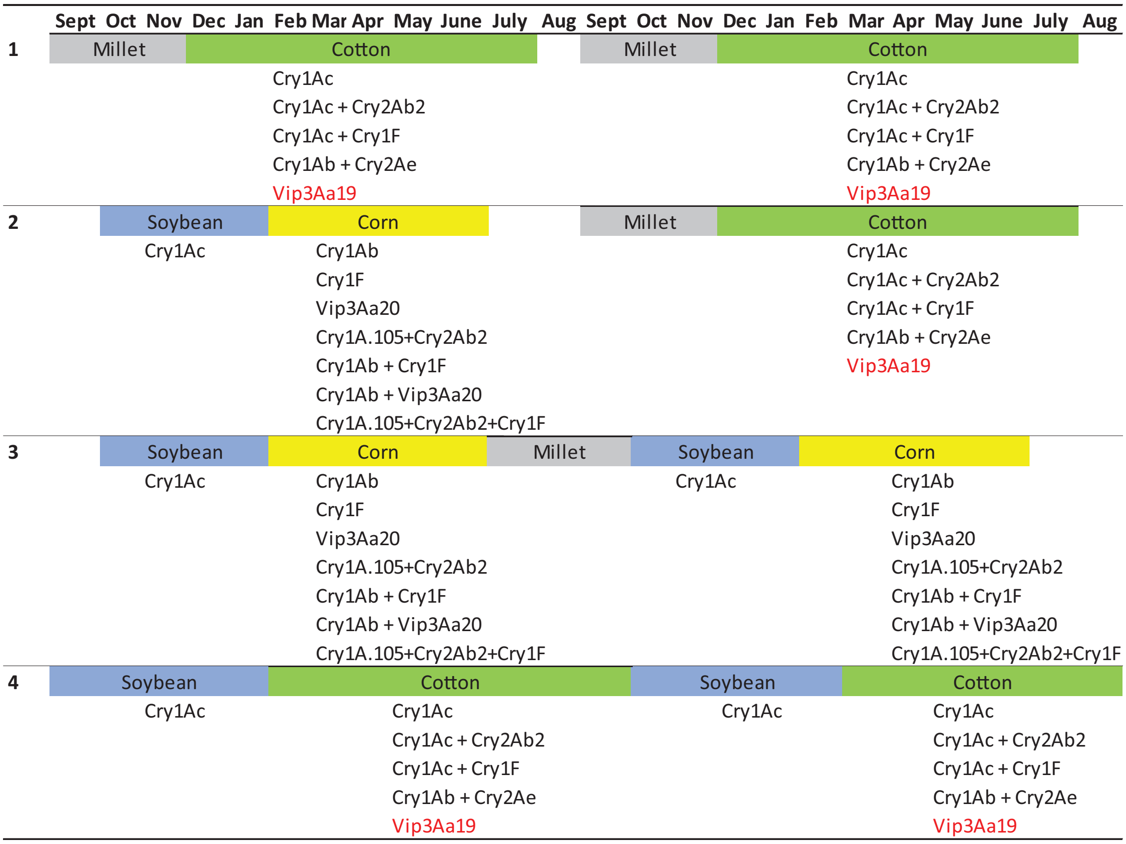

Fig. 2. Four most common scenarios of agriculture production for Bt crops in Bahia and Mato Grosso States, Brazil. Intensive use of Cry proteins occurs concomitantly or in succession. Vip3Aa19 (red) in combination with other Cry protein is expected to be available by 2019 depending on regulatory approval. Figure from Prof. Celso Omoto from University of São Paulo University, ESALQ, with his permission.

pressures or 2) long-distance migration of fall armyworm spread resistant alleles across South America.

A landscape genetic approach could help test these two hypotheses as well as to understand the metapopulation dynamics of fall armyworm. The question remains whether Brazil, with 16 million hectares of corn (not to mention other Bt crops), is a potential source of fall armyworm Bt resistance for other South American countries where production is significantly smaller (Argentina: 4 million ha, Paraguay: 700,000 ha, Uruguay: 83,200 ha, Colombia: 68,000 ha) with shorter season (Fig. 3). If this hypothesis is supported, fall armyworm may adhere to the "Mainland-island Model" of metapopulation dynamics (Hanski and Gyllenberg 1997). This could allow industry to implement strategies toward reducing emigration from sources and help manage Bt resistance evolution across South America.

Lack of Proper Adoption of IRM Strategies to Prevent Fall Armyworm Resistance

Among the three factors influencing resistance evolution (pest genetics, biology and ecology, and resistance management), only resistance management is amenable to human intervention. Accordingly, industry, academic and government researchers (e.g., EMBRAPA) have developed and advertised the best practices for IRM for Bt crops in Brazil (Brazilian Biotechnology Industry Association [Agrobio] 2016).

The primary IRM strategy recommended is the refuge, which will reduce the selection pressure for a proportion of the population. However, refuge compliance in Brazil has been one of the biggest challenges agricultural industry has faced since Bt crops launched. While informal surveys have been done, compliance data have not been published. However, expectations are that compliance is < 20% (Brazilian Seeds Industry Association [ABRASEM]—data not published). In addition, resistance for Cry proteins launched since 2008 is not consistently recessive (Cry1F—Farias et al. 2015, Cry1Ab—Jakka et al. 2016), thus not adhering to the high dose and refuge strategy. Vip3Aa20 expressed in Agrisure Viptera hybrids is a unique Bt corn event that adheres to high dose concept for fall armyworm (Bernardi et al. 2016). This product has been on the market for over five years and in six different countries of South America (Argentina, Paraguay, Uruguay, Brazil, and Colombia) and, to date, unexpected damage has not been reported in the field or in laboratory screening efforts.

The refuge effectiveness also depends on the biology and ecological factors of each target pest. For example, when fall armyworm larvae feed on corn leaves, volatiles are released that deter additional oviposition (De Moraes et al. 2001, Harmon et al. 2003). The refuge, under high fall armyworm pressure, can be extensively damaged by larvae from the first adult migration into the field. Additional adult females that migrate will then likely prefer to lay eggs on Bt corn instead of the refuge (Téllez-Rodríguez et al. 2014). Furthermore, the refuge would likely generate susceptible adults only in the first migration of fall armyworm, which may result in asynchronous mating times between adults emerging from Bt and refuge fields. A potential recommendation would be to increase refuge size. Also, refuge could be planted with hybrids that support higher plant density (e.g., plant architecture), thereby increasing the

Fig. 3. Countries in South America that cultivate Bt corn and area of hybrids market (not including white corn for human consumption). COL, Colombia; BRA, Brazil; PY, Paraguay; UY, Uruguay; AR, Argentina. Red circles represent the estimated size of potential Bt corn area (USDA 2016) and red arrows represent potential migration of fall armyworm carrying resistant alleles.

number of susceptible insects being generated as only one late instar per whorl is common due to cannibalism.

A different strategy would include a partial insecticide spray program to protect some of the new corn leaves in the refuge. In Brazil, IRAC (Insecticide Resistance Action Committee—Brazil [IRAC-BR] 2016) recommends to plant refuge and make no more than two foliar applications until V6 corn stage when 20% of the plants reach a fall armyworm damage score of 3 (Davis Scale; Fig. 4). This recommendation can generate a reasonable number of adults in early corn stages and could give some protection of at least two growth of new leaves which would be suitable for new females to lay eggs in later growth stages of the refuge (IRAC-BR 2016). This could make the refuge more effective for longer time periods. Unfortunately, this recommendation was released in 2015, after which wide-scale resistance to some Cry proteins had already occurred.

The pyramiding of multiple Bt proteins is also strongly recommended to manage resistance in Bt fields and has been used worldwide. The pyramid strategy discussed earlier uses two or more genes expressing different Bt proteins targeting the same pest in the same hybrid. Extended durability is achieved when each single protein from that combination is able to control all susceptible individuals (Roush 1998, Brévault et al. 2013). When Bt corn was first launched in Brazil, only a single toxin expressed in hybrids was available, and the technology adoption was close to 75% in the first two years (Céleres 2013). Thus, the selection pressure imposed by a single product was very high. When the first pyramids were launched, the selection pressure had already occurred, increasing the frequency of resistance alleles to the pyramid. For example, Cry1F protein was commercialized in Brazil for at least five years as single toxin. In 2011, a pyramid containing Cry1A.102 + Cry2Ab2 was released. One year later, the combination Cry1F + Cry1A.105 + Cry2Ab2 was also introduced. Both pyramids had unexpected observations of fall armyworm feeding within 2–3 yr after launch (Table 1).

Fig. 4. Threshold for insecticide application in refuge area for fall armyworm control. Limitation to two insecticide applications until V6 corn stage. Insecticide Resistance Action Committee—Brazil 2016. Photo: IRAC-BR.

The rapid evolution of resistance to pyramids might be also explained by the potential for cross resistance between proteins. Some of the proteins compete for the same binding sites in the receptor of the fall armyworm midgut, causing unstable binding when one protein is in presence of the other, reducing the efficacy of both (Hernández-Rodríguez et al. 2013, Sena et al. 2009). Such potential for cross resistance might be given by high sequence similarity between proteins. For example, Cry1A.105 has 99% of its amino acid sequence similar to Cry1F for domain III and is identical to Cry1Ab for domain I (Tabashnik et al. 2013). The multi-Bt crop system combined with cross resistance among Cry proteins is further aggravated by the reduced refuge compliance. Thus, pyramid strategy for manage resistance of fall armyworm to Bt crops in Brazil expressing Cry proteins has not realized expected benefits.

Another option for IRM is the use of chemical treatment in Bt corn. The use of insecticide to manage resistance in Bt crops can have positive (if only Bt field is treated), negative (if only refuge is treated), or neutral impact (if both refuge and Bt field are treated; Insecticide Resistance Action Committee—International [IRAC-International] 2016). The rationale of spraying the Bt field would be to kill any potential survivors selected on Bt areas (i.e., *rr* insects). However, similar challenges in application efficacy, such as protection in the ear, still remain, and *rr* genotypes could still emerge, with the added detriment of increased insecticide selection.

In conclusion, Bt crops are the fastest technology ever adopted for agronomic crop production and has brought benefits not only to growers but also to the environment, consumers, and food security (National Academies of Sciences, Engineering, and Medicine 2016). The high adoption of such technology has strongly contributed to a reduction of important crop pests in several regions and crops. Indeed, after Bt corn was first introduced in Brazil to control fall armyworm, larvae practically disappeared from corn fields. However, Bt trait efficacy diminished quickly in Brazil and in other South American countries due to fast resistance evolution of this pest to Cry proteins.

Rapid resistance evolution seems to be influenced by several different factors including genetics and biology and ecology characteristics of the species, the agricultural landscape, and especially, lack of resistance management practice. Immense and rapid reproduction, large-scale adult dispersal, lack of fitness costs and non-high–dose products, and poor refuge compliance have created a perfect storm that facilitated Bt resistance in fall armyworm. Further research and implementation on IRM strategies would help understand the potential risk for resistance evolution for new products, allowing industry, academic, and government researchers to propose and improve proactive resistance management strategies.

Development of a perfect IRM system is challenging due to the interaction of biology, ecology, and even society (e.g., refuge compliance). Decision of any IRM strategy implementation starts on the farm and will largely influence trait durability. The refuge, which is a basic recommendation and has consensus among the scientific community and agriculture industry as one of the best practices, is poorly adopted in Brazil. Academic, industry, and government researchers and regulators should continue their collaborations to develop solid, science-based recommendations and transfer these through the product chain to extend the durability of Bt technologies in the field. These groups should also invest in education and training of farmers and crop consultants for proper refuge implementation. As only one Bt product remains effective (Vip3Aa20), all agronomic groups have a vested interest in extending its durability.

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