# SUSCEPTIBILITY OF THE FALL ARMYWORM, *Spodoptera frugiperda* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE) TO *Bacillus thuringiensis* (Bt) PROTEINS AND SYNTHETIC INSECTICIDES FROM DIFFERENT CORN PRODUCTION SYSTEMS IN MEXICO AND PUERTO RICO

By

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**ABSTRACT**

SUSCEPTIBILITY OF THE FALL ARMYWORM, *Spodoptera frugiperda* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE) TO *Bacillus thuringiensis* (Bt) PROTEINS AND SYNTHETIC INSECTICIDES FROM DIFFERENT CORN PRODUCTION SYSTEMS IN MEXICO AND PUERTO RICO

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The strength of the factors favoring the evolution of resistance to insecticides in agriculture may vary in different production regions. The assumption that lower susceptibility to insecticides is to be observed in populations from areas with higher deployment of insecticides cannot be assumed. The main goal of this dissertation is to provide relevant information to better understand the impact that different pest management strategies have in the development of insecticide resistance in fall armyworm, specifically the use of synthetic insecticides alone or in combination with *Bacillus thuringiensis* (Bt) crop technology. Also, the potential capacity of fall armyworm to recover and complete development after long-term exposure to Bt proteins was addressed in laboratory experiments. Mexico and Puerto Rico have contrasting corn production systems, but fall armyworm is the number one pest in both territories. Mexico has a high diversity of corn production systems, varying from self-subsistence to commercial fields, where the amount of insecticides sprays against fall armyworm varies. Puerto Rico has a very intense corn production system, dominated by hybrid and seed research development, where there is virtually zero tolerance for pest damage. Therefore, fall armyworm is subjected to different levels of selection pressure by insecticides used in these two territories. While in Mexico, synthetic insecticides are the

main control tool, in Puerto Rico, fall armyworm populations are managed with both synthetic insecticides and Bt corn. To determine the baseline susceptibility to synthetic insecticides and Bt proteins in field-collected fall armyworm populations from Mexico and Puerto Rico, bioassays were performed with insecticides of different modes of action. In general, fall armyworm from Mexico showed low resistance ratio values in comparison with a susceptible reference population. Only three Mexican populations displayed resistance ratios above 10-fold of the $LC_{50}$ of the susceptible population: Sonora to chlorpyrifos, Sinaloa to flubendiamide and Oaxaca to permethrin. Fall armyworm from Puerto Rico showed the highest resistance ratios to flubendiamide, chlorantraniliprole, chlorpyrifos, thiodicarb, methomyl, triflumuron, spinetoram, permethrin, deltamethrin and zeta-cypermethrin. Only spinosad, abamectin and emamectin benzoate insecticides resulted in resistance ratios below 10-fold. Diet overlay bioassays were carried out with the Bt proteins Cry1F, Cry1A.105, Cry2Ab2 and Cry1Ac. All of the fall armyworm populations from Mexico were susceptible to Bt proteins. However, fall armyworm from Puerto Rico showed high resistance to Cry1F and Cry1Ac. Bioassays were performed with Cry1F and Cry1Ac on the susceptible reference strain and the population from Puerto Rico. Cry1F exposure in the fall armyworm susceptible population showed reduced survival with the highest concentration (31%). Both Cry1F and Cry1Ac caused asynchrony in development and adult emergence between the control and treatments in the susceptible population, but not in the Puerto Rico population. These results have serious implications for Bt resistance management because fall armyworm shows high capacity to recover after long Bt protein exposure

*Romans 11:36*

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## KEY TO ABBREVIATIONS

Bt – *Bacillus thuringiensis*

GE – Genetically Engineered

IRAC – Insecticide Resistance Action Committee

IPM – Integrated Pest Management

IRM – Integrated Resistance Management

RIB – Refuge in the bag

# CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

## Introduction

Food security is a worldwide concern as the world population increases (FAO 2015); according to the United Nations, it is estimated that by 2050 there will be 9.8 billion people in the world (UN DESA 2017). Agriculture is the main economic activity that ensures continued food supply, and is described as one of the key factors that can also increase economic growth, especially in developing countries (FAO 2015). This concern for food security drove the “Green Revolution” era that was characterized by the genetic improvement of crops through conventional plant breeding, which boosted global food production (Borlaug 1983).

Crop protection was also revolutionized during this era, with the incorporation of chemical pesticides that helped farmers increase productivity at relatively low costs (Bottrell 1979). However, increasing agricultural productivity resulted in unforseen problems: increased water use, $CO_2$ emmissions and adverse environmental effects, such as extensive land conversion to agriculture and pesticide-driven pollution (Conway and Barbier 2013, Strassburg et al. 2014). In addition, the indiscriminate use of synthetic pesticides led to the rapid evolution of pesticide resistance in pests, plus a long list of non-desired detrimental effects, such as the pollution of the environment, human health hazards, harm to non-target species and the rise of secondary pests (Carson 1963, Ehler 2006).

The early concept of “integrated pest control” was born as an effort to decrease the reliance on synthetic insecticides by including the use of natural enemies and cultural practices to suppress pest populations (Stern et al. 1959). This outlook changed the focus from complete eradication of crop pests to that of managing their populations to avoid economic damage, serving as the foundation for the development of the "integrated pest management" (IPM) concept (Higley and Pedigo 1996). The following decades were marked by a boom in research, policy-making and regulations to incorporate IPM practices in the field (Frazier 2017).

Unfortunatelly, the evolution of insecticide resistance is an ongoing threat to the sustainability of agricultural systems. After the first recorded case of resistance to an insecticide in the San Jose scale to sulphur lime sprays (Melander 1914), extensive research was conducted to understand the factors involved in this phenomenon. Some insect species have demostrated an outstanding capacity to adapt to synthetic and biological toxins. For example, populations of the diamondback moth (*Plutella xylostella*) (Tabashnik et al. 1990, 1997, Ferré et al. 1991, Vasquez 1995, Díaz-Gomez et al. 2000, Baxter et al. 2005, Balasubramani et al. 2008), and the colorado potato beetle (*Leptinotarsa decemlineata*) (Mota-Sanchez et al. 2006, Alyokhin et al. 2008, 2015) have developed resistance to synthetic and biological insecticides through diverse resistance mechanisms.

The fall armyworm (*Spodoptera frugiperda*) has become notorious, not only for the increasing number of resistance cases to synthetic insecticides (Yu 1991, 2003, Morillo and Notz 2007, Leon-Garcia et al. 2012, Nascimento et al. 2015a, 2015b), but also for high levels of resistance to the insecticidal proteins expressed by genetically engineered crops such as corn and cotton (Blanco et al. 2010; Storer et al. 2010; Farias et al. 2014; O. Bernardi et al. 2014; F. Huang et al. 2014; Niu, Qureshi, et al. 2016), Omoto et al. 2016). While considered a secondary pest in cotton, *S. frugiperda* is the number one pest in corn in Latin America (Blanco et al. 2016). Furthermore, it is involved in the first case of field-evolved resistance to Bt corn, which resulted in removal of hybrids expressing Cry1F from field use in Puerto Rico (Storer et al. 2010, 2012).

Importance and production of corn

Corn is one of the most important crops in the world and together with wheat and rice these crops comprise 60% of the total food calorie intake worldwide (FAO 2017). The center of origin and domestication of corn is in Mexico, from its ancestor, the "teosinte", corn domestication occurred gradually through human selection, resulting in an outstanding diversity of varieties with distinctive sizes, colors, flavors and geographical adaptations (Kato et al. 2009, Matsuoka et al. 2002, Ramos-Madrigal et al. 2016).

In 2015/2016 an estimated 178.54 million hectares of corn was grown worldwide. If total grain production is considered, corn is the largest crop grown with 969.49 million metric tons produced during 2015/2016. Preliminary data for 2017/2018 indicates that total corn producing area will increase to 183.76 million hectares, with a corresponding increase in production to 1,070.51 million metric tons worldwide (USDA FAS 2017).

During 2015-2016 period, the top seven countries that produce 80% of the total corn worldwide are the United States, China, Brazil, European Union, Mexico, Ukraine and India (USDA FAS 2017). The US grows approximately 36% of the total world production of corn, followed by China with 23%. However, in terms of total area planted with this crop, China leads with 38.12 million hectares, followed by the US with 32.68 million hectares; this discrepancy is because the yield obtained in the US (10.57 metric tons per hectare) is almost double the yield obtained in China (5.89 metric tons per hectare) (USDA FAS 2017).

As the world leader in corn production, United States agricultural systems are characterized by high technology input, including genetically engineered (GE) hybrids, fertilizers, synthetic pesticides, and advanced machinery (USDA ERS 2017a, USDA ERS 2017b). In contrast with the majority of other corn producing countries, most of the US corn production (96%) is destined to livestock feed, as grain or silage. Another important purpose is the industrial production of ethanol biofuels. Human consumption represents a smaller percentage of the corn usage in this country, mainly for secondary products such as sweeteners and oil (USDA ERS 2017a). Finally, about 15% of the US corn production is exported to countries like Mexico, Japan and China, representing around 40% of the total corn trade worldwide (USDA ERS 2017c).

Puerto Rico

Corn production in Puerto Rico is focused on parent seed and hybrid research, carried out by several agricultural biotechnology (AgBio) companies; corn production for silage and forage is on a smaller scale (Storer et al. 2010, Belay et al. 2012). According to the Puerto Rico Agricultural Biotechnology Industry Association (PRABIA 2017), AgBio research uses less than 1.5% of Puerto Rico's territory dedicated for agriculture and all the seed produced is exported. As a result, corn fields have extremely low tolerance for pests and rely on synthetic insecticides to achieve high levels of pest control (Belay et al. 2012).

Mexico

Corn is a staple food and crucial part of the culture in Mexico, which ranks fifth in world production (25.97 million metric tons) and sixth in terms of total planted area (7.21 million hectares) (USDA FAS 2017). Corn yields in Mexico averages 3.6 metric tons per hectare, almost three times lower than the average in the US (10.57 metric tons/hectare) (USDA FAS 2017). Thus, according to the Mexican Secretariat of Economy (SE 2012), Mexico produces only 77% of its national requirement of corn and the rest is supplied by imports from the United States.

After the domestication of corn by pre-Columbian civilizations in what is now southern Mexico, corn was gradually distributed throughout the world and further selected for adaptations to specific climates, soil compositions and management practices, among other needed characteristics (Kato et al. 2009, Matsuoka et al. 2002, Ramos-Madrigal et al. 2016). However, Mexico is the country with the highest diversity of corn landraces and corn production remains very heterogeneous throughout the country, ranging from self-subsistence by using various landraces and more traditional agricultural practices, to the high technology systems that use corn hybrids, fertilizers, synthetic insecticides and automated machinery (Keleman et al. 2009, Blanco et al. 2014). This heterogeneity in Mexican corn production systems have been described as the main factor responsible for the low yield averages (SE 2012). Blanco et al (2014) classified them as “commercial” and “self-subsistence”, indicating that these differences also make the incorporation of IPM programs difficult throughout the country.

Bt corn

During the Green Revolution, corn was one of the most researched crops, and a large number of corn hybrids were developed to increase yields and adapt to specific weather conditions and geographical areas. With the inception of modern biotechnology, genetically engineered (GE) corn varieties included traits that conferred herbicide tolerance, resistance to insects and environmental stressors, and improved nutritional value (Fernandez-Cornejo J. 2004). Since their commercial release in 1996, GE crops have benefitted farmer's competitiveness and health (Huang et al. 2005, Qaim 2010), prompting gradual and consistent adoption rates, so that by 2016, GE corn represented 33% of the global corn production (ISAAA 2017).

GE crops with the ability to produce insecticidal proteins from the bacteria *Bacillus thuringiensis* (Bt), have become fundamental in agriculture in many developed and developing countries (Smyth et al. 2015). In some of these regions, this technology has helped decrease the amount of synthetic insecticides deployed in the field, which benefited non-target organisms such as natural enemies and wildlife, plus decreased pollution and human health hazards (Huang et al. 2002, Qaim and Zilberman 2003, Bennett et al. 2006, Qaim 2010).

Cry1 δ-endotoxins are produced by *Bacillus thuringiensis* (Bt) as crystal inclusions during the bacterial sporulation stage, hence the common name "Cry" proteins. These proteins have an insecticidal effect against specific insect orders (Gould 1988, Luo et al. 1999, Tabashnik et al. 2009 and Pardo-López et al. 2012). Genes from Bt that encode for specific Cry proteins were isolated and inserted into crops such as corn and cotton. Modified plants then produce either a single or a combination of Bt proteins (Höfte et al. 1989). The mode of action of these endotoxins can be summarized in the following steps: 1) after Cry protein ingestion, it is activated in the midgut by proteases under high pH conditions, 2) activated protein binds to specific receptors in the insect midgut and 3) the toxin forms pores in the midgut membrane, releasing midgut contents into the body cavity and eventually killing the insect (Bravo et al. 1992).

Unfortunatelly, the long-term sustainability of Bt crops is threatened by the evolution of resistance to Bt proteins in target pests (Tabashnik et al. 2013). Several cases of field-evolved resistance to Bt technology were documented in the last decade: 1. Cotton bollworm (*Helicoverpa armigera*) resistance to Cry1Ac in China (Liu et al. 2010 and Zhang et al. 2011); 2. Stem borer (*Busseola fusca*) resistance to Cry1Ab in South Africa (Van Rensburg 2007); 3. Western corn rootworm (*Diabrotica virgifera virgifera*) resistance to Cry3Bb1 and mCry3A in the U.S. (Gassmann et al. 2011; 2014); 4. Pink bollworm (*Pectinophora gossypiella*), resistance to Cry1Ac in India (Dhurua and Gujar 2011); 5. Fall armyworm (*Spodoptera frugiperda*) resistance to Cry1F and Cry1Ac protein in Puerto Rico (Blanco et al. 2010, Storer et al. 2010), to Cry1F in southern US (Huang et al. 2014, Li et al. 2016) and to Cry1F, Cry1Ab and reduced susceptibility to Cry1Ac in Brazil (Bernardi et al. 2014, Farias et al. 2014 and Omoto et al. 2016); 9. Corn earworm (*Helicoverpa zea*), resistance to Cry1Ab and Cry1A.105+Cry2Ab2 containing Bt corn hybrids in the US (Dively et al. 2016); and 10. Western bean cutworm (*Striacosta albicosta*) resistance to Cry1F in Ontario, Canada (Smith et al. 2017)

GE corn in Mexico

Mexico is a pioneer in the adoption of insect and herbicide resistant GE cotton and herbicide resistant GE soybeans, both of which benefit farmers and the environment (Traxler et al. 2003, Otero 2015). However, GE corn is considered a special case. Because of Mexico's status as the center of origin of corn, many activists believe that GE corn is a threat to the diversity of corn landraces and to Mexican culture (Kato et al. 2009, Baltazar et al. 2015). Protest by anti-GE groups resulted in the legal suspension of GE corn permits from September 2013 until August 2015, when the ban was lifted for lack of scientific basis; yet, the commercial production of GE corn is still prohibited throughout the country (Vargas-Parada 2014, Otero 2015).

Mexico has a comprehensive legal framework for GE organisms, the Biosafety Law, which includes measures for experimentation, pilot and commercial plantings, product labeling and protection of important centers of species diversity (DOF 2005). Nevertheless, despite the positive experience with GE cotton (i.e. the dramatic reduction of pesticide use and increased yields) the public opinion towards GE corn in Mexico has more sentimental roots (Otero 2015). Other factors, like the general distrust in governmental decisions and a lack of knowledge concerning GE crops (López Montesinos et al. 2016), further complicate the adoption of GE corn in this country in the forseeable future.

Integrated Resistance Management (IRM) strategies in Bt crops

There has been extensive theoretical and experimental analysis of the current IRM strategies designed to delay the evolution of resistance to GE Bt crops (Gould 1994, 1998, 2000, Vacher et al. 2003, Bourguet et al. 2005, Tabashnik 2008a, 2008b, Huang, Andow, and Buschman 2011, Carroll et al. 2013, Tabashnik et al. 2013, Yang et al. 2014 and Garcia et al. 2016). The most popular strategy worldwide is the "high-dose/refuge", in which farmers are required to plant a percentage of non-Bt acreage to serve as refuge for susceptible insects. This strategy is predicated on a low frequency of resistance alleles in the population, the few resistant survivors mate with susceptible insects from the refuge, their heterozygous offspring would be killed by the high-dose Bt protein produced by the GE plants, delaying population-level resistance (Andow and Hutchinson, 1998 and Huang et al. 2011).

The success of the “high-dose/refuge” strategy relies on the following assumptions: 1) resistance is conferred by recessive alleles, 2) low initial frequency of resistant alleles 3) crops produce a high dose of the Bt protein, killing at least 95% of heterozygote individuals, 4) random mating between resistant and susceptible insects, 5) there are fitness costs associated with resistance to Bt proteins and 6) oviposition occurs indiscriminately on both Bt and non-Bt plants (Gould 1994, Huang et al. 2011, Gryspeirt and Grégoire 2012, Tabashnik et al. 2013).

The development of second-generation pyramided GE crops producing multiple toxins, allowed the re-configuration of the refuge from separate blocks or rows, to an integrated design. Now Bt and non-Bt seeds are included in the seed mix, in a strategy known as “refuge-in-the-bag” (RIB). Since RIB simplifies agronomic practices, the most important advantage is ensuring farmer’s compliance with the refuge component of IRM programs (Rule et al. 2014, Fei et al. 2015). The key conditions for the efficacy of RIB are: 1) no cross resistance between Bt proteins produced by the GE hybrid, 2) no single toxin crops are grown in proximity to the pyramided Bt field and 3) limited movement of the target pest’s larval stages from plant to plant (Fei et al. 2015).

The efficacy of the RIB strategy in delaying the evolution of Bt resistance in target pests has been controversial in the scientific community (Chilcutt and Tabashnik

2004, Onstad 2014). Key conditions for the success of RIB can be broken, potentially accelerating the evolution of Bt resistance. For example, corn pollination is wind mediated, and the risk of cross-pollination between Bt and non-Bt hybrids is higher when the plants are closer together (Burkness and Hutchison 2012). Up to 94% of refuge ears in RIB fields express single or combination of Bt proteins at different concentrations (Yang et al. 2014). This increases the evolution of resistance in two ways: 1) by reducing the pool of susceptible insects, as they may not survive feeding on refuge corn kernels expressing lethal doses of Bt proteins and 2) by increasing the survival of heterozygote insects that feed on kernels in ears with patchwork with lower concentrations of Bt proteins, increasing the frequency of resistance alleles in the pest population (Chilcutt and Tabashnik 2004, Yang et al. 2014).

Fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

Habits and biology

Fall armyworm displays high genetic diversity among populations from different regions within its natural distribution (Monnerat et al. 2006, (Rodney N Nagoshi and Meagher 2008). Two strains of fall armyworm have been described using molecular markers that have a certain level of host specificity to corn and rice. The corn strain is more specific than the rice strain, showing high levels of preference for corn plants (Pashley 1986, 1988; Pashley and Martin 1987, Meagher et al. 2004, Prowell et al. 2004 and Groot et al. 2016), whereas the rice strain shows a weaker preference for rice.

The complete life cycle of fall armyworm averages 30 days, but can be up to 90 days during colder seasons (Capinera 2000). Adult moths are nocturnal; males and females mate from dusk until midnight. Females oviposit on the host leaves. On average, females produce 1,500 eggs and lay them in groups of variable number, usually about 200 (Sparks 1979, Andrews 1988, Ashley et al. 1989 and Capinera 2000). In general, fall armyworm has six larval instars, although seven have been reported under laboratory conditions (Santos et al. 2003). After hatching, larvae feed first on the eggshells and then move to the host foliage. Some larvae can move to neighboring plants. Because fall armyworm is cannibalistic, it is rare to encounter more than one or two full grown larvae feeding on the same plant (Sparks 1979, Capinera 2000 and Pannuti et al. 2015). On corn, later instars can be found in the whorl. Before pupation, larvae move off the plant and pupate in the soil, where they complete development until adulthood (Sparks 1979 and Capinera 2000).

Distribution and migration

Fall armyworm is endemic to the tropical - subtropical regions of the American continent (Sparks 1979, Pitre 1988, Capinera 2000) and it has recently been detected in West and Central Africa (Goergen et al. 2016). It can be found throughout the year in warmer regions; however, since fall armyworm cannot go through diapause, it is unable to survive cold winters in northern US and Canada (Sparks 1979, Nagoshi et al. 2012).

Fall armyworm displays an outstanding migratory capacity, its migration patterns within the US mainland, northern Mexico and the Caribbean island of Puerto Rico have been widely studied, and explain how fall armyworm is able to reach northern territories of the Western Hemisphere during warm seasons (Nagoshi et al. 2012, 2014; Westbrook et al. 2016). This phenomenon is very relevant from a pest management perspective, since fall armyworm movement between regions can facilitate genetic exchange between populations, this could mean that individuals resistant to an insecticide would increase the frequency of resistance alleles in a different population, or susceptible insects could migrate into a resistant population, reducing the frequency of resistance alleles (Nagoshi et al. 2010).

Damage in corn

Fall armyworm is reported feeding on 186 host plants and has become a major pest in economically important crops such as corn, sorghum and rice (Casmuz et al. 2010); it is a sporadic pest in cotton, where it can cause significant damage as it prefers feeding on the reproductive parts of the plants instead of the foliage, directly affecting crop productivity (Barros et al. 2010).

In corn, fall armyworm larval infestations can be found on leaves and corn ears, later instars can mainly be found feeding in the corn whorl (Capinera 2000, Murúa et al. 2009). In Mexico, fall armyworm is present through corn vegetative development (V2-12), but in some areas it can also infest corn at "silking" and "blister" stages (Blanco et al. 2014). Fall armyworm has the potential to cause 45% yield reduction in maize (Hruska and Gladstone 1988), but in some tropical areas it could reach 100% if left uncontrolled (personal communication with Henry Teran-Santofimio). In the United States, corn losses caused by fall armyworm were estimated to average $300 million annually (Knipling 1980).

Management in corn

For decades, the main tool for fall armyworm control has been the use of synthetic insecticides (Young 1979, Yu 1991, 1992, Al-Sarar et al. 2006, Blanco et al. 2010). However, in the US, since the commercialization of genetically engineered (GE) plants producing *Bacillus thuringiensis* (Bt) insecticidal proteins, synthetic insecticide use has dropped 47.8% where GE seeds are available (Brookes and Barfoot 2017). Nevertheless, this is not the case in countries where Bt technology is not available or regions where fall armyworm has begun to develop field-evolved resistance to the Bt proteins and synthetic insecticide sprays are still necessary to manage this pest (Blanco et al. 2016).

Fall armyworm has developed field-evolved resistance to 21 insecticidal active ingredients within six modes of action in seven countries in the Americas (Mota-Sanchez and Wise 2017). These compounds include major chemical families of conventional insecticides such as organochlorinates, organophosphates, pyrethroids and carbamates. Also, this list includes microbial disruptors of insect midgut membranes. Fall armyworm have developed resistance to the Bt proteins Cry1F, Cry1Ac and Cry1Ab in Puerto Rico (Blanco et al. 2010, Storer et al. 2010), Cry1A.105 in the continental United States (Huang et al. 2014; 2016) and Cry1F, Cry1Ab and reduced susceptibility to Cry1Ac in Brazil (Bernardi et al. 2014, Farias et al. 2014 and Omoto et al. 2016). Hence, fall armyworm is currently the lepidopteran pest with the highest number of field-evolved resistance cases in the American continent.

Survival and recovery potential after Bt protein exposure

Few studies have tested the recovery potential of a Lepidopteran pest after long-term exposure to Bt proteins. Two studies from more than 40 years ago analyzed the survival of the tobacco budworm (*Heliothis virescens*) after different periods of exposure to the HD-1 isolate of *Bacillus thuringiensis* (Dulmage and Martinez 1973; Dulmage, Graham, and Martinez 1978). In the first study, *H. virescens* development was recorded during continuous exposure to the Bt, which resulted in reduced larval and pupal weights, plus a lower survival to adulthood (Dulmage and Martinez 1973). In the second study, *H. virescens* larvae were exposed for 24, 48, 72 hours or continuously before transfer to untreated diet, resulting in lower larval recovery as exposure time increased (Dulmage, Graham, and Martinez 1978).

Hardke et al. (2011) conducted a survivorship study on a fall armyworm strain collected from the field and maintained in the laboratory for 2-3 years. On Cry1F expressing corn larval survival was reduced by almost 50% and developmental time increased in survivors. On Cry1Ab expressing corn, there was also a delay in comparison with non-Bt plant treatments. A recent study by Binning et al (2014) found that fall armyworm exhibits an aversive response against Cry1F corn after previous ingestion and low levels of larval survival after a 14-day period of exposure to Cry1F.

## Conclusion

Fall armyworm has demonstrated an remarkable capacity to develop field-evolved resistance to insecticides, with cases of resistance to about 21 active ingredients across 6 modes of action, including Bt proteins produced by Bt crops (Mota-Sanchez and Wise 2017). Thus, fall armyworm is currently one of the hardest pests to manage in both conventional and Bt corn producing regions (Blanco et al. 2016). For instance, corn growers from Mexico, a country with no commercial production of Bt corn, report the need to spray synthetic insecticides 2-3 times for fall armyworm control per crop cycle (Blanco et al. 2014). In contrast, fall armyworm management in Bt corn fields in Puerto Rico rely on both Bt technology and conventional insecticides, with up to 25 sprays in some crop seasons (Belay et al. 2012).

Chapter 2 updates the status of synthetic insecticide susceptibility in fall armyworm populations in corn fields from Mexico and Puerto Rico, which differ in their pest management systems, describes intoxication symptoms, and analyzes the role that different biological, geographical and ecological factors play in the evolution of insecticide resistance. Chapter 3 updates the status of fall armyworm susceptibility to four Bt proteins currently being produced by Bt corn: Cry1F, Cry1Ac, Cry1A.105 and Cry2Ab2 in field populations from regions with and without Bt corn. Chapter 4 tests the recovery potential of a susceptible and a resistant fall armyworm population after being treated with high-doses of Cry1F and Cry1Ac in the laboratory, and discusses the implications under field conditions.

# CHAPTER 2: FIELD-EVOLVED RESISTANCE OF THE FALL ARMYWORM *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) TO SYNTHETIC INSECTICIDES IN MEXICO AND PUERTO RICO


## Abstract

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is one of the main pests of corn in many areas of the American continents. The reliance on pesticides to control fall armyworm has led to the development of insecticide resistance in many regions. We determined the resistance levels of fall armyworm to insecticides of different modes of action in fall armyworm populations from Puerto Rico and from several Mexican states with different insecticide use patterns. Mexican populations that expressed higher resistance ratios were: Sonora (20-fold to chlorpyriphos), Oaxaca (19-fold to permethrin), and Sinaloa (10-fold to flubendamide). The Puerto Rico population exhibited a remarkable field-evolved resistance to many pesticides. The $RR_{50}$ to the insecticides tested were: flubendiamide (500-fold), chlorantraniliprole (160-fold), methomyl (223-fold), thiodicarb (124-fold), permethrin (48-fold), chlorpyriphos (47-fold), zeta-cypermethrin (35-fold), deltamethrin (25-fold), triflumuron (20-fold), spinetoram (14-fold). Spinosad (8-fold), emamectin benzoate and abamectin (7-fold) displayed lower resistance ratio. However, these compounds are still effective to manage fall armyworm resistance populations in Puerto Rico.

Fall armyworm populations from Mexico show different levels of susceptibility, which may reflect the heterogeneity of the pest control patterns in this country. The status of

insecticide resistance in the fall armyworm from Puerto Rico indicates a challenging situation for the control of this pest with these insecticides in the close future.

## Introduction

Fall armyworm is the most important corn pest in Latin America. Mexican corn farmers report that fall armyworm is the hardest pest to manage and it is estimated that ~3,000 tons of insecticides per year are used to manage it (Blanco et al. 2014). Chemical control is the main management strategy, but insecticide use patterns against fall armyworm may vary depending on the agricultural region, since corn production in Mexico is diverse (Blanco et al. 2014). States with the largest farms and most intensive corn production are mainly located in the north and central areas of the country, and rely heavily on synthetic insecticides to control fall armyworm (Blanco et al. 2014). In contrast, the southern states, corn production systems are less intense and some small growers in these regions still use traditional cropping systems, where synthetic insecticides are less used (Kato et al. 2009).

Acetylcholinesterase (AChE) inhibitors (organophosphates and carbamates), and sodium channel modulators (pyrethroids), are among the older insecticides used in Mexico to control fall armyworm (Albert 2006, Cortez-Mondaca and Rodriguez-Cota 2012). Several products still used against fall armyworm include chlorpyrifos (organophosphate), methomyl (carbamate), and pyrethroids such as cypermethrin, permethrin and lambda-cyhalothrin (Maya-Hernandez 2017, personal communication and Posos 2017, personal communication). Active ingredients with novel modes of action that have been introduced for fall armyworm control in Mexico include: novaluron (inhibitor or chitin biosynthesis), flubendiamide, chlorantraniliprole and cyantraniliprole (ryanodine receptor allosteric modulators). However, low corn prices and high cost of agricultural supplies such as fertilizers and pesticides push farmers to choose cheaper insecticides that are usually within older chemistries.

In Puerto Rico, fall armyworm has become the primary pest of corn, especially in the economically important industry of hybrid seed research and production, which has a very low tolerance for kernel damage. There is high pressure of fall armyworm year-round in corn on the island due to several factors, including the tropical climate, the sequential planting of cornfields (corn is always available) and the attraction to, and concentration of moths into fields during the dry season. Also, during dry seasons fall armyworm has limited migration within corn fields; this relative isolation contributed to the quick development of field-evolved resistance to the Bt proteins Cry1F, Cry1Ac and Cry1Ab (Storer et al. 2010, 2012). Thus, current control strategies rely on both Bt proteins and synthetic insecticides, with up to 28 applications per season to control larval populations (Belay et al. 2012).

To understand the development of pesticide resistance, it is necessary to consider the impact that different management strategies have on speeding up or slowing down its evolution. Since both Mexico and Puerto Rico rely on insecticides to control fall armyworm, resistance is a key issue. Yet, the frequency of insecticide use and the consequent pest selection pressure in Puerto Rico is heavier than in the most intense corn growing regions in Mexico. Still, insecticide use against fall armyworm in Mexico varies between regions and from one production season to another, which makes it hard to predict the development of resistance.

Thus, it is of critical importance to monitor the susceptibility of fall armyworm to synthetic insecticides in different agricultural regions and to determine the baseline susceptibility to both common and new chemistries deployed in the field. Such information is essential to design effective Integrated Pest Management (IPM) and Insecticide Resistance Management (IRM) strategies. If selection pressure by insecticide applications were considered alone, we hypothesize that fall armyworm from Puerto Rico will show reduced susceptibility to the main active ingredients used against this pest. On the other hand, we expect to see more variation in the susceptibility to insecticides in the populations collected from different regions of Mexico. Therefore, the objective of this study was to determine the susceptibility of the fall armyworm to synthetic insecticides in populations collected in different corn production regions under different production systems in Mexico and Puerto Rico.

## Materials and Methods

### Insect Populations

Fall armyworm populations were collected during larval development ($4^{th}$ to $6^{th}$ instars) directly from infested plants in commercial fields. Table 1 shows fall armyworm collection information: location, date of collection and number of insects collected. Fall armyworm populations are abbreviated as follows: from Mexico, northwest = Chihuahua (Chi), Sinaloa (Sin), Sonora (Son); northeast = Tamaulipas (Tam), Coahuila (Coa); west-central= Jalisco (Jal); southwest = Oaxaca (Oax); from Puerto Rico = PR. Additionally, Monsanto Company provided a laboratory susceptible (SUS) strain from their rearing facilities in Union City, Tennessee. Larvae were placed in 10 ml plastic cups with 4-6ml of artificial fall armyworm diet (Southland Products Inc., Lake Village, AR) and shipped to the laboratory to complete development under controlled conditions. Upon arrival in the laboratory, larvae were visually confirmed as fall armyworm using morphological features. Emerging adult moths were further reconfirmed as fall armyworm by examining the forewing of males.

**Table 1.** Information on the source of the field fall armyworm populations.

| Country | State | Locality | Date[a] | n[b] |
|---|---|---|---|---|
| Mexico | Tamaulipas | Gustavo Diaz Ordaz | 4/2013 | 300 |
| | Chihuahua | Cuauhtemoc | 6/2013 | 300 |
| | Coahuila | Torreon | 6/2013 | 300 |
| | Sinaloa | Culiacan | 4/2013 | 110 |
| | | Los Mochis | 5/2015 | 220 |
| | Sonora | Huatabampo | 5/2013 | 300 |
| | | San Luis Rio Colorado | 9/2015 | 250 |
| | Oaxaca | San Pablo Huitzo | 9/2013 | 220 |
| | | | 8/2015 | 250 |
| | Jalisco | San Martin Hidalgo | 4/2015 | 545 |
| Puerto Rico | | Ponce | 6-11/16 | 600 |

[a]Collection date (month/year), [b]number of insects collected.

<u>Insect rearing</u>

Larvae were held at 27±1 ºC and a photoperiod of 16:8 hours light: dark. Insect development was supervised every other day, when pupae turned from orange-red to dark red, they were placed in groups of 40 inside collapsible insect cages (Bioquip, Rancho Dominguez, CA) to provide room for emerging adults to fly and mate. A sponge with a solution of 10% sugar in a petri dish was provided as food source. After three days of adult emergence, moths from each cage were placed in 1L paper brown bags and food was added. Every other day moths were transferred to a new paper bag, and eggs masses laid in the brown paper bag were placed in a plastic container with moist paper towels. Neonate larvae were transferred to bioassay trays (Frontier Agricultural Sciences, Newark, DE) with 1 ml of artificial diet per well.

Bioassays

Bioassays were conducted using technical grade material provided by US EPA National Pesticide Standard Repository (Fort Meade, MD). The 13 active ingredients tested, their modes of action and purity (%) are listed in Table 2. Bioassays on Mexican fall armyworm populations - were conducted at the Colegio de Postgraduados in Montecillo, Mexico (2013) and at Michigan State University (MSU) in East Lansing, Michigan (2014-16). Bioassays on the PR population were done at MSU in June to November 2016. The PR and SUS populations were screened against all 13 insecticides, while the Mexican populations were screened against a subset of five active ingredients representing five different modes of action (Table 2).

Topical bioassays were performed on second instars 24h after they molted. Three to five replications per dose were conducted. Insecticides were serially diluted in acetone and five to seven different concentrations of each that covered between 0 to 100% mortality were used. One µl of solution was applied on the dorsum of each second instar using a 50 µl micro-syringe (Hamilton Company, Reno, NV) coupled with a micro-applicator (PB600-1 Repeating dispenser, Hamilton Company), as in previously published procedures (Alava 1976, Brewer et al. 1989, McCaffery et al. 1991). A control treatment consisted of 1µl of acetone per larvae. Treated larvae were placed individually in 10 ml plastic cups (Gordon Food Service) with 2 ml of diet. Mortality was assessed 72 h after treatment. Mortality individuals were considered dead if they did not respond after being touched with a small brush, or when they showed severe intoxication symptoms (slow movement, twitching, halted molting, and severe growth inhibition).

Recording of intoxication symptoms

Observations were made in the SUS population at 24 h and 72 h after insecticide exposure. Insecticide treated insects were compared with their corresponding controls, and the following parameters were considered: Insects: shape, size, evidence of molting (by searching for remains of previous exoskeleton and head capsule), movement after prodding with a small paintbrush; diet: signs of feeding on diet (feeding marks on diet), excretion abundance and coloration. Our observations were compared with those reported in available literature (Bloomquist and Miller 1985, Chandler et al. 1992, Salgado 1998, Gunning et al. 1999, Martin et al. 2003, Tohnishi et al. 2005, Ahmad et al. 2009, Carvalho et al. 2013).

Statistical analysis

Mortality data were corrected using Abbot's formula (Abbott 1925). Probit analysis (Finney 1971) was conducted using SAS version 9.3 (SAS Institute, Cary, NC) to calculate values of slope, the $LD_{50}$, $LD_{90}$ and fiducial limits (95%) for each population. Resistant ratios (RR) were calculated by dividing the $LD_{50}$, $LD_{90}$s values of the field fall armyworm populations by the $LD_{50}$, $LD_{90}$s values of the susceptible (SUS) population. Log dose responses were plotted using Origin® (OriginLab Corp, Northampton, MA). Relative potency ratios (RP) were calculated by dividing the $LD_{50}$ of the least toxic active ingredient by the $LD_{50}$ of each active ingredient for the susceptible population and the Puerto Rico population.

**Table 2.** Active ingredients used against fall armyworm.

| Active ingredient | Mode of action[a] | IRAC group[a] | Purity (%)[b] | Population[c] |
|---|---|---|---|---|
| thiodicarb | Acetylcholinesterase (AChE) inhibitors | 1A | 99.8 | PR |
| methomyl | | 1A | | MX, PR |
| chlorpyrifos | | 1B | | MX, PR |
| permethrin | Sodium channel modulators | 3A | 49.5 | MX, PR |
| deltamethrin | | | 97.2 | PR |
| zeta-cypermethrin | | | 92 | PR |
| flubendiamide | Ryanodine receptor (RyR) modulators | 28 | 98.7 | MX, PR |
| chlorantraniliprole | | | 99.2 | PR |
| spinetoram | Nicotine acetylcholine receptor (nAChR) allosteric modulators | 5 | 85.8 | MX, PR |
| spinosad | | | 90 | PR |
| triflumuron | Inhibitor of chitin biosynthesis | 16 | 98 | PR |
| abamectin | Glutamate-gated chloride channel (GLUCL) allosteric modulators | 6 | 80 | PR |
| emamectin benzoate | | | 97.2 | PR |

[a]Mode of action and group numbers are those of IRAC, the Insecticide Resistance Action Committee (http://www.irac-online.org/)
[b]Concentration of active ingredient in the technical grade standard insecticide formulation
[c]MX= Mexican and PR = Puerto Rico fall armyworm populations

## Results

Relative potencies of active ingredients

Figure 1 shows the relative potency ratios in the SUS and PR populations. Low relative potency ratio values mean lower toxicity of the active ingredient to second instar larvae and high relative potency ratios mean higher toxicity. In general, there was variation in toxicity among and within IRAC mode of action groups in both SUS and PR populations. Abamectin was the least toxic compound in both populations and the LD50 values were used to calculate all relative potency ratios. Acetylcholinesterase inhibitors displayed low toxicity: the carbamates, thiodicarb and methomyl showed lower relative potency ratios than the organophosphate chlorpyriphos. Emamectin benzoate displayed the highest relative potency ratio (RP= 5,000) in both populations.

The PR population showed lower relative potency ratios than the SUS population, and only two active ingredients showed a relative potency ratio above a 1,000 in the PR population: spinetoram (RP= 1,000) and emamectin benzoate (RP= 5,000). In contrast, in the SUS population five active ingredients showed potency ratios equal of higher than 1,000-fold: flubendiamide (RP= 1,000), spinetoram and chlorantraniliprole (RP= 3,000) and emamectin benzoate (RP= 5,000). More importantly, new chemistries such as ryanodine receptor modulators showed a dramatic reduction in toxicity in the PR population in relation to the relative potency ratio obtained in the SUS reference: chlorantraniliprole showed a 24-fold decrease in relative potency ratio, and flubendiamide exhibited a 77-fold decrease in toxicity.

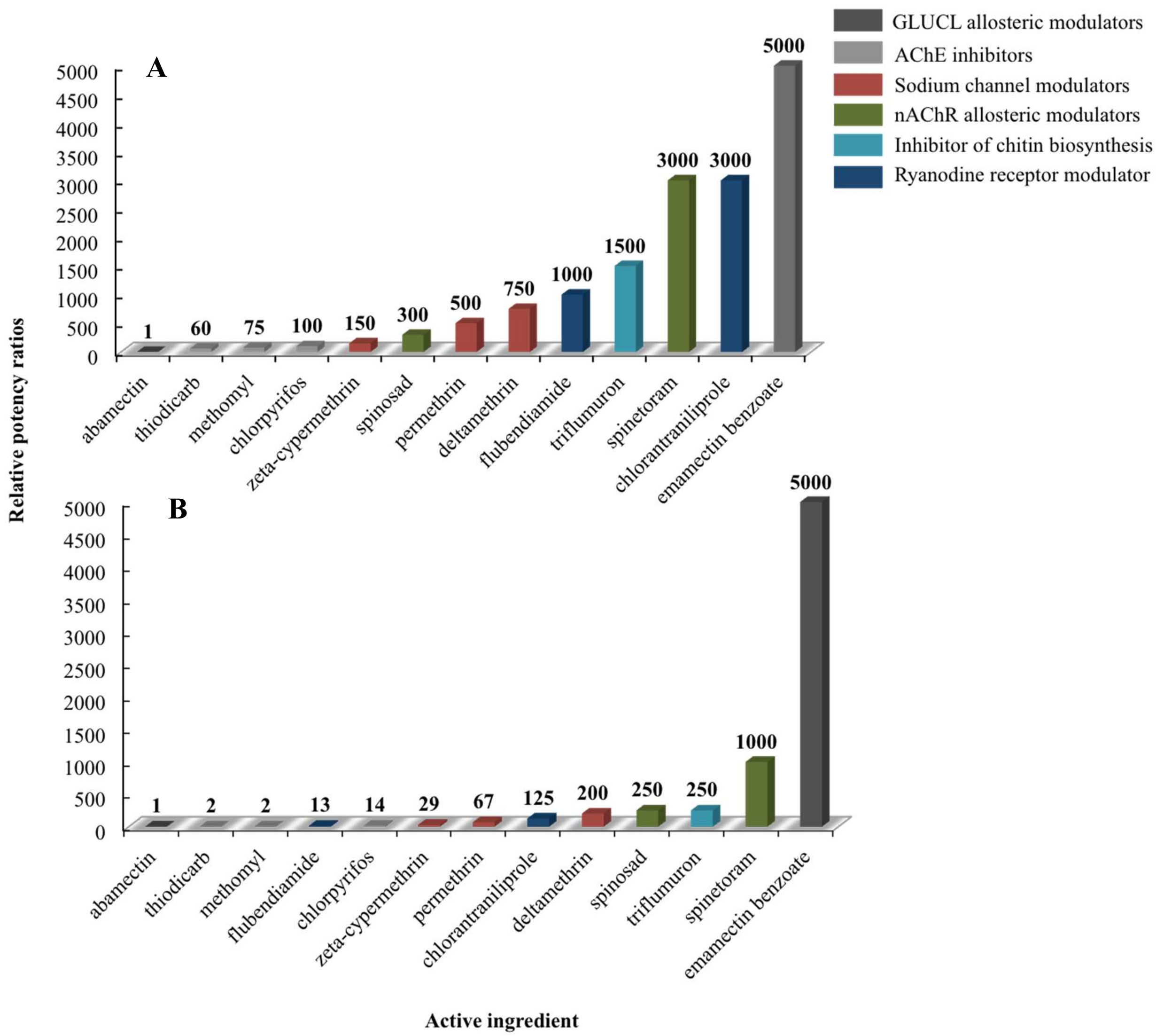


**Figure 1.** Relative potency ratios of the active ingredients used against fall armyworm: A) Susceptible reference population and B) Puerto Rico population.

Intoxication symptoms

In general, with all tested insecticides, field populations showed a slightly different behavioral response than the susceptible population: field collected larvae contorted more aggressively when the pesticide drop was placed on their dorsal area. Field collected PR larvae started excreting seconds after pesticide application. In contrast, the susceptible reference strain was paralyzed for a couple seconds (±4) and didn't show excretion behavior immediately after the insecticides' application. Overall, intoxication symptoms appeared 24 h after pesticide exposure with the exception of triflumuron (benzoylurea), for which symptoms did not appear until the third day, or molting phase.

*AChE inhibitors and sodium channel modulators.* Both toxicological groups displayed similar intoxication symptoms in both Mexican and PR fall armyworm insects. In general, severely affected larvae slowed down feeding and 24 h after treatment larvae were smaller than control individuals. Additionally, intoxicated larvae responded to prodding by lifting up their head and part of the thorax, remaining in that position for several seconds until prodded again. In contrast, larvae from the control treatment returned to the original position immediately or after prodding slightly with the brush.

*nAChR allosteric modulators.* After 24 h there were no differences between the larval sizes of the insecticide-treated and control larvae. Intoxication symptoms showed only after prodding: affected larvae moved slightly, their bodies were straight and stiff; if turned upside down, affected larvae would twitch but not recover to the original position. After 72 h affected insects would have reduced or completely halted feeding, depending on the intoxication severity, affected insects were up to 1-3 instars behind the control. Dead larvae were smaller and stiff, but no color changes or shrinking were observed.

*Inhibitors of chitin biosynthesis.* There were no evident intoxication symptoms until the first molting after treatment from second to third instar (±48 h after treatment). Insecticide-intoxicated larvae failed to detach their old head capsule from the new one, halted shedding the old exoskeleton and constraining of the body at different lengths were observed. Dead larvae looked severely deformed.

*Ryanodine receptor modulators*. Intoxicated larvae with flubendiamide and chlorantraniliprole showed reduced feeding after ±24 h. Heavy intoxication was characterized by complete halted feeding as observed by the lack of larvae droppings and unconsumed diet. After 72 h, the bodies of affected larvae appeared contracted with darker coloration in comparison with control larvae.

Bioassay results

Table 3 summarizes the bioassay results obtained with fall armyworm from Mexico, PR and the SUS populations. Fall armyworm from PR displayed the highest resistance ratios at both $LD_{50}$ and $LD_{90}$ levels to all pesticides in comparison with the field populations from Mexico and the susceptible reference population.

*AChE inhibitors.* Fall armyworm from PR showed the highest resistance ratio to methomyl (223-fold) followed by thiodicarb 124-fold, and chlorpyrifos (47-fold). These results indicate the occurrence of resistance within acetylcholinesterase inhibitors in this population. For the Mexican populations only the strain from the state of Son (2013 field collection) displayed a high resistance ratio to the organophosphate chlorpyriphos ($RR_{50}$=20-fold). However, no significant resistance was found in the population collected in 2015 ($RR_{50}$=2.3-fold).

*Sodium channel modulators.* Fall armyworm from PR showed the highest $LD_{50}$ an $LD_{90}$ values to permethrin, zeta-cypermethrin and deltamethrin. When compared with the SUS population the highest resistance ratios 50 and 90 were obtained with permethrin. The log dose-probit regression slopes obtained with the three compounds indicate that there is similar phenotypic variation in the response to this mode of action in the PR population.

Fall armyworm from Oaxaca in 2013 was the only population from Mexico that displayed high resistance ratios to permethrin ($RR_{50}$=19-fold and $RR_{90}$=46-fold). However, the Oaxaca population collected in 2015 showed lower $LD_{50}$ and $LD_{90}$ values, which resulted in lower resistance ratios to this product for this year ($RR_{50}$ =3-fold and $RR_{90}$9-fold).

*nAChR allosteric modulators.* Fall armyworm from PR displayed the highest resistance ratio to spinetoram ($RR_{50}$ = 14-fold and $RR_{90}$ = 82-fold), spinosad was also tested against this population showing low resistance ratios ($RR_{50}$ 8-fold and $RR_{90}$=6-fold).. Fall armyworm Mexican populations displayed resistance ratios 50s and 90s below 5-fold for spinetoram, and their overlapping fiducial limits indicate that these field populations are not statistically different in their response to this active ingredient.

*GLUCL allosteric modulators.* Fall armyworm from PR showed the lowest resistance ratios at the $LD_{50}$ and $LD_{90}$ levels to this group's active ingredients: 7-fold for both emamectin benzoate and abamectin. However, fall armyworm exhibited a $RR_{90}$ of 10-fold to emamectin benzoate and just 2-fold to abamectin. Log dose-probit regression slopes for these two active ingredients for fall armyworm PR are similar: emamectin benzoate (1.7±0.5) and abamectin (2±0.5).

*Inhibitor of chitin biosynthesis.* Fall armyworm from PR displayed a $RR_{50}$ of 20-fold to triflumuron, but the $RR_{90}$ was very high (490-fold). The log dose-probit regression slope for this population (0.7±0.2) was smaller than the slope of the SUS strain (2.5±0.5).

*Ryanodine receptor modulators.* Fall armyworm from PR exhibited the highest resistance ratios to the diamide flubendiamide at both $LD_{50}$ and $LD_{90}$ levels (500-fold and 2,019-fold respectively). Resistance ratios with chlorantraniliprole were also high $RR_{50}$=160-fold and $RR_{90}$=500-fold. Additionally, the log dose-probit regression slopes were similar between these two compounds: flubendiamide (1±0.2) and chlorantraniliprole (1.1±0.2). Figure 2 shows the log dose-probit plot for the toxicity of diamides in the SUS population in comparison with the PR population.

Fall armyworm from Sinaloa was the only Mexican population that showed 10-fold resistance to flubendiamide. Although the Coa population showed a $RR_{50}$= 7-fold, the fiducial limits at $LD_{50}$ and $LD_{90}$ levels overlapped with the Sin population. Both Jal and Oax populations displayed resistance ratios below 5-fold at both $LD_{50}$ and $LD_{90}$ levels relative to the SUS population.

**Table 3.** Dose mortality response of fall armyworm from several Mexican states and Puerto Rico to conventional pesticides.

| Active ingredient | Population /year of bioassay | n | Slope ±SE [a] | $LD_{50}$(95% FL)[b] | $LD_{90}$(95% FL)[b] | $RR_{50}$[c] | $RR_{90}$[c] |
|---|---|---|---|---|---|---|---|
| **Acetylcholinesterase inhibitors** | | | | | | | |
| chlorpyrifos | PR 2016 | 154 | 2.7 ±0.5 | 1.4 (1.1 , 1.9) | 1.6 (1.2 , 3.1) | 47 | 60 |
| | SON 2013 | 350 | 2.1 ±0.2 | 0.6 (0.5 , 0.7) | 2.3 (1.7 , 3.9) | 20 | 35 |
| | COA 2013 | 240 | 2 ±0.5 | 0.3* | 1.1* | 9 | 17 |
| | OAX 2013 | 210 | 1.7 ±0.3 | 0.2 (0.1 , 0.2) | 0.9 (0.5 , 2.8) | 5 | 13 |
| | OAX 2016 | 238 | 2.7 ±0.8 | 0.1 (0.03 , 0.4) | 0.3 (0.2 , 43,920) | 4 | 5 |
| | SON 2015 | 90 | 6.3 ±1.7 | 0.07 (0.06 , 0.08) | 0.1 (0.08 , 0.2) | 2 | 2 |
| | SIN 2015 | 126 | 2.1 ±0.5 | 0.04 (0.03 , 0.06) | 0.2 (0.1 , 0.5) | 1 | 3 |
| | JAL 2015 | 280 | 2.4 ±0.3 | 0.03 (0.03 , 0.04) | 0.1 (0.08 , 0.2) | 1 | 2 |
| | SUS | 270 | 3.8 ±0.5 | 0.03 (0.03 , 0.04) | 0.07 (0.06 , 0.09) | 1 | 1 |
| methomyl | PR 2016 | 214 | 0.9 ±0.2 | 8.9 (4.9 , 27.5) | 201 (50.9 , 6,331) | 223 | 157 |
| | OAX 2016 | 126 | 0.7 ±0.2 | 0.2 (0.07 , 1.2) | 9.3 (1.2 , 12,271) | 5 | 7 |
| | COA 2013 | 220 | 1.1 ±0.2 | 0.2 (0.1 , 0.3) | 3 (1.7 , 8.8) | 5 | 2 |
| | JAL 2016 | 259 | 1.3 ±0.4 | 0.09 (0.005 , 2.3) | 0.7 (0.2 , 1.34E+10) | 2 | 1 |
| | SIN 2015 | 126 | 2.1 ±0.4 | 0.09 (0.06 , 0.1) | 0.4 (0.2 , 1) | 2 | 0.3 |
| | CHI 2013 | 210 | 1.4 ±0.19 | 0.09 (0.06 , 0.1) | 0.8 (0.5 , 1.8) | 2 | 1 |
| | OAX 2013 | 140 | 1.6 ±0.8 | 0.03 | 0.2 | 1 | 0.2 |
| | SUS | 510 | 0.9 ±0.1 | 0.04 (0.03,0.06) | 1.3 (0.66 , 3.29) | 1 | 1 |
| thiodicarb | PR 2016 | 120 | 1.4 ±0.3 | 12.4 (7.5 , 26.5) | 99 (40 , 869.2) | 124 | 198 |
| | SUS | 126 | 1.8 ±0.3 | 0.1 (0.06 , 0.2) | 0.5 (0.3 , 1.3) | 1 | 1 |

[a]SE = standard error, [b]$LD_{50}$ and $LD_{90}$= Lethal dose expressed as micrograms of active ingredient per microliter, *no fiducial limits could be calculated, [c]RR= resistant ratio, $LD_{50}$, or $LD_{90}$ of field population over the $LD_{50}$, or $LD_{90}$ of the susceptible population

**Table 3.** (cont'd)

| Active ingredient | Population /year of bioassay | n | Slope ±SE[a] | $LD_{50}$(95% FL)[b] | $LD_{90}$(95% FL)[b] | $RR_{50}$[c] | $RR_{90}$[c] |
|---|---|---|---|---|---|---|---|
| **Sodium channel modulators** | | | | | | | |
| Permethrin | PR 2016 | 141 | 1.6 ±0.4 | 0.3 (0.02 , 3) | 2.2 (0.6 , 13,961,081) | 48 | 220 |
| | OAX 2013 | 210 | 2 ±0.3 | 0.1 (0.09 , 0.2) | 0.5 (0.3 , 1.5) | 19 | 46 |
| | JAL 2015 | 126 | 3.4 ±0.7 | 0.05 (0.04 , 0.06) | 0.1 (0.1 , 0.2) | 8 | 9 |
| | SON 2013 | 280 | 2.4 ±0.3 | 0.03 (0.03 , 0.04) | 0.1 (0.08 , 0.2) | 5 | 9 |
| | TAM 2013 | 300 | 2.9 ±0.3 | 0.03 (0.02 , 0.03) | 0.07 (0.06 , 0.1) | 5 | 6 |
| | COA 2013 | 290 | 2.6 ±0.4 | 0.03 (0.03 , 0.04) | 0.09 (0.07 , 0.1) | 5 | 8 |
| | CHI 2013 | 230 | 2.1 ±0.5 | 0.03 (0.01 , 0.06) | 0.1 (0.06 , 1.2) | 5 | 9 |
| | OAX 2016 | 120 | 1.8 ±0.3 | 0.02 (0.01 , 0.02) | 0.08 (0.04 , 0.3) | 3 | 9 |
| | SIN 2015 | 126 | 2 ±0.5 | 0.004 (0.003 , 0.006) | 0.02 (0.01 , 0.07) | 1 | 2 |
| | SUS | 480 | 5.1 ±0.7 | 0.006 (0.006 , 0.007) | 0.01 (0.01 , 0.02) | 1 | 1 |
| Zeta-cypermethrin | PR 2016 | 120 | 1.9 ±0.3 | 0.7 (0.5 , 1.1) | 3.1(1.9 , 7.5) | 35 | 62 |
| | SUS | 240 | 3.3 ±0.4 | 0.02 (0.02 , 0.02) | 0.05 (0.04 , 0.07) | 1 | 1 |
| Deltamethrin | PR 2016 | 120 | 1.9 ±0.4 | 0.1 (0.08 , 0.22) | 0.6 (0.3 , 2.1) | 25 | 30 |
| | SUS | 140 | 1.9 ±0.4 | 0.004 (0.003 , 0.005) | 0.02 (0.01 , 0.04) | 1 | 1 |

[a]SE = standard error, [b]$LD_{50}$ and $LD_{90}$= Lethal dose expressed as micrograms of active ingredient per microliter, *no fiducial limits could be calculated, [c]RR= resistant ratio, $LD_{50}$, or $LD_{90}$ of field population over the $LD_{50}$, or $LD_{90}$ of the susceptible population.

**Table 3.** (cont'd)

| Active ingredient | Population /year of bioassay | n | Slope ±SE [a] | $LD_{50}$(95% FL)[b] | $LD_{90}$(95% FL)[b] | $RR_{50}$[c] | $RR_{90}$[c] |
|---|---|---|---|---|---|---|---|
| **Nicotine acetylcholine receptor allosteric modulators** | | | | | | | |
| Spinetoram | PR 2016 | 124 | 1 ±0.3 | 0.02 (0.003 , 0.05) | 0.5 (0.2 , 3.9) | 14 | 82 |
| | JAL 2015 | 210 | 2.6 ±0.6 | 0.005 (0.002 , 0.01) | 0.01 (0.007 , 0.2) | 4 | 3 |
| | SIN 2015 | 231 | 3.7 ±1 | 0.004 (0.001 , 0.008) | 0.009 (0.005 , 0.3) | 3 | 2 |
| | OAX 2016 | 127 | 2.3 ±0.4 | 0.003 (0.002 , 0.004) | 0.01 (0.007 , 0.02) | 2 | 2 |
| | SUS | 450 | 2.1 ±0.3 | 0.001 (0.001 , 0.002) | 0.006 (0.004 , 0.009) | 1 | 1 |
| Spinosad | PR 2016 | 141 | 2 ±0.4 | 0.08 (0.06 , 0.13) | 0.4 (0.2 , 1) | 8 | 6 |
| | SUS | 299 | 1.6 ±0.2 | 0.01(0.008 , 0.01) | 0.07 (0.04 , 0.1) | 1 | 1 |
| **Glutamate-gated chloride channel allosteric modulators** | | | | | | | |
| Emamectin benzoate | PR 2016 | 122 | 1.7 ±0.5 | 0.004 (0.001 , 0.006) | 0.02 (0.01 , 0.13) | 7 | 10 |
| | SUS | 286 | 2.4 ±0.4 | 0.0006 (0.0004 , 0.0009) | 0.002 (0.001 , 0.005) | 1 | 1 |
| Abamectin | PR 2016 | 120 | 2 ±0.5 | 20 (11.6 , 29) | 88 (54.1 , 280.3) | 7 | 2 |
| | SUS | 184 | 1 ±0.3 | 3 (1.4 , 10.7) | 54 (13.9 , 2,302) | 1 | 1 |
| **Inhibitor of chitin biosynthesis** | | | | | | | |
| Triflumuron | PR 2016 | 120 | 0.7 ±0.2 | 0.08 (0.007 , 0.2) | 4.9 (1.4 , 611.1) | 20 | 490 |
| | SUS | 126 | 2.9 ±0.5 | 0.004 (0.003 , 0.005) | 0.01 (0.007 , 0.02) | 1 | 1 |

[a]SE = standard error, [b]$LD_{50}$ and $LD_{90}$= Lethal dose expressed as micrograms of active ingredient per microliter, *no fiducial limits could be calculated, [c]RR= resistant ratio, $LD_{50}$, or $LD_{90}$ of field population over the $LD_{50}$, or $LD_{90}$ of the susceptible population.

**Table 3.** (cont'd)

| Active ingredient | Population /year of bioassay | n | Slope ±SE[a] | $LD_{50}$(95% FL)[b] | $LD_{90}$(95% FL)[b] | $RR_{50}$[c] | $RR_{90}$[c] |
|---|---|---|---|---|---|---|---|
| **Ryanodine receptor modulators** | | | | | | | |
| Flubendiamide | PR 2016 | 126 | 1 ±0.2 | 1.5 (0.8 , 5.2) | 32.3 (7.9 , 1,056) | 500 | 2,019 |
| | SIN 2015 | 147 | 1.5 ±0.7 | 0.03 (0.02 , 1.5) | 0.2 (0.07 , 7,342,799) | 10 | 15 |
| | COA 2015 | 120 | 2.5 ±0.8 | 0.02 (0.02 , 0.1) | 0.08 (0.04 , 2.6) | 7 | 5 |
| | JAL 2015 | 125 | 1.5 ±0.5 | 0.005 (0.0006 , 4.36) | 0.04 (0.009 , 2.35E+25) | 2 | 2 |
| | OAX 2015 | 126 | 1.2 ±0.3 | 0.004 (0.002 , 0.007) | 0.04 (0.02 , 0.5) | 1 | 3 |
| | SUS | 210 | 2 ±0.3 | 0.003 (0.003,0.005) | 0.02 (0.01 , 0.03) | 1 | 1 |
| Chlorantraniliprole | PR 2016 | 120 | 1.1 ±0.2 | 0.16 (0.06 , 0.32) | 2.5 (1.2 , 10.2) | 160 | 500 |
| | SUS | 120 | 2.1 ±0.4 | 0.001 (0.0007 , 0.002) | 0.005 (0.003 , 0.01) | 1 | 1 |

[a]SE= standard error, [b]$LD_{50}$ and $LD_{90}$= Lethal dose expressed as micrograms of active ingredient per microliter, *no fiducial limits could be calculated, [c]RR= resistant ratio, $LD_{50}$, or $LD_{90}$ of field population over the $LD_{50}$, or $LD_{90}$ of the susceptible population.

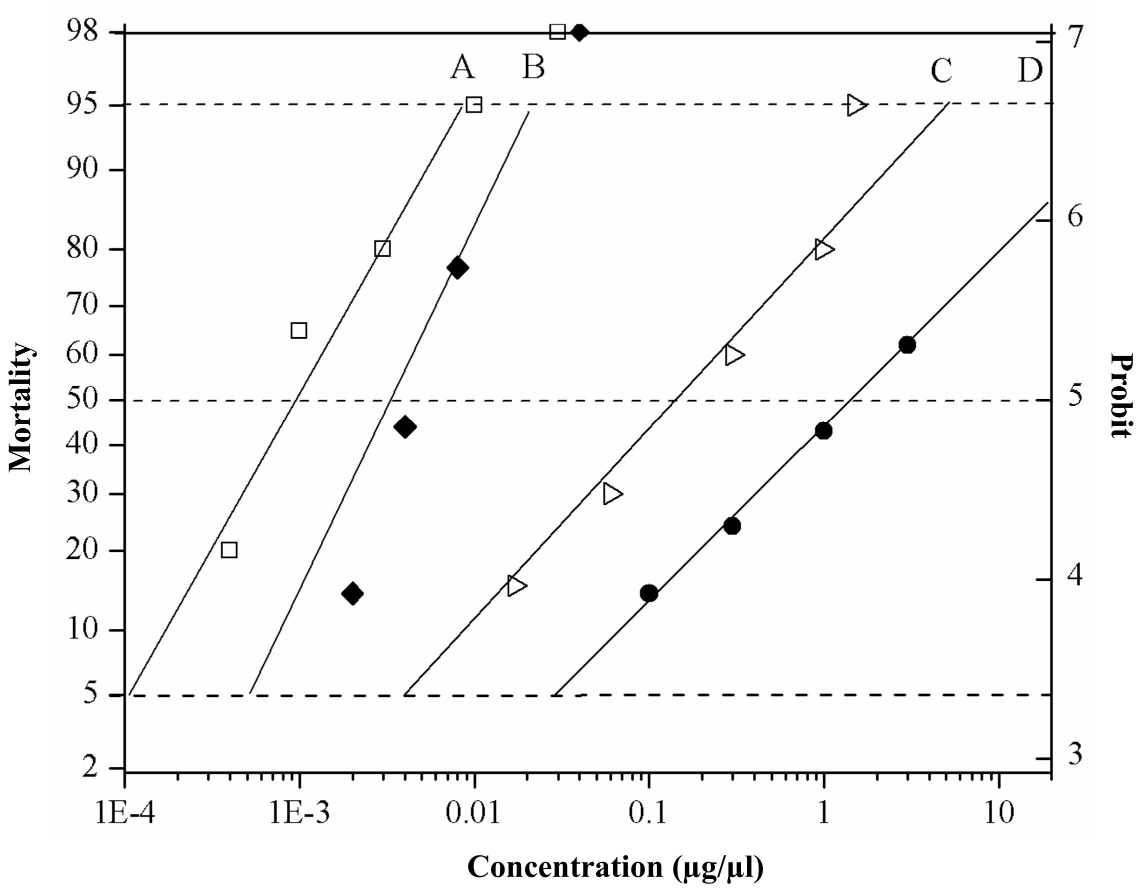


**Figure 2**. Mortality response of fall armyworm to diamides. Susceptible reference population: A) chlorantraniliprole, B) flubendiamide. Puerto Rico: C) chlorantraniliprole and D) flubendiamide.

## Discussion

The intoxication symptoms of fall armyworm described in this study for each mode of action are consistent with those reported in the literature. For instance, experiments with AChE inhibitors and sodium channel modulators in different insect species have found that both mode of action reduce locomotion abilities on affected individuals, causing loss of coordination and balance (Bloomquist and Miller 1985). In fact, one of the criteria for determining mortality in bioassays involving these compounds are slow or uncoordinated motor movements (Gunning et al. 1999; Martin et al. 2003, Ahmad et al. 2009, Carvalho et al. 2013).

Symptoms caused by the nAChR allosteric modulator spinosad in several insect species indicates that this compound disrupts motor neuron activity, causing involuntary flexion and eventually stiffness due to exhaustion (Salgado 1998). Intoxication with benzoylureas (inhibitors of chitin biosynthesis), is characterized by molt disruption and malformations, which have been reported for *Helicoverpa zea* and fall armyworm larvae after treatment with diflubenzuron and teflubenzuron (Chandler et al. 1992). A unique intoxication symptom caused by ryanodine receptor modulators is gradual body contractions without convulsions in affected Lepidoptera larvae, until larvae appear smaller than the untreated individuals (Tohnishi et al. 2005).

In this study, we report field-evolved resistance in a fall armyworm population from Puerto Rico to five major modes of action: AChE inhibitors (chlorpyriphos, methomyl and thiodicarb), sodium channel modulators (permethrin, deltametrin and zeta-cypermethrin), nAChR allosteric modulators (spinetoram), inhibitors of chitin biosynthesis (triflumuron), and RyR modulators (flubendiamide and chlorantraniliprole). These results are consistent with low performance reports of these active ingredients in the field in Puerto Rico (Unpublished data, personal communication with Henry Teran-Santofimio), or “practical resistance” where the insecticide is not effective to control a pest (Tabashnik et al. 2014). Furthermore, through this work we found field-evolved resistance in the following Mexican field fall armyworm populations: chlorpyriphos in Son (2013), flubendiamide in Sin (2015) and permethrin in Oax (2013).

**AChE inhibitors.** The $LD_{50}$ values displayed by the fall armyworm PR population were very high for the carbamates methomyl and thiodicarb, resistance ratios obtained at both the $LD_{50}$ and $LD_{90}$ levels were above a 100-fold for both carbamates. Resistance ratio to the organophosphate chlorpyriphos was lower, but the slope value was very high. This could indicate that there is phenotypic variation within this mode of action group in fall armyworm from Puerto Rico, with less variation for the organophosphates than carbamates (Chilcutt and Tabashnik 1995). Moreover, there is evidence that fall armyworm from Puerto Rico resistant to two Bt proteins and an organophosphate (acephate) has higher activity of esterases and gluthathione-S-transferases (GST) in comparison with a susceptible fall armyworm strain. This suggests that resistance mechanisms to different of modes of action may have evolved side by side (Zhu et al. 2015).

With the exception of the Sonora population (2013 field collection), the rest of the Mexican fall armyworm populations displayed resistance ratios below 10-fold to AChE inhibitors, regardless of the agricultural area where they were collected. Yet, we found a 10-fold lower resistance ratio to chlorpyrifos in the Son population collected in 2015 in comparison with the 2013. These two populations were collected in different locations: 2013 was collected in Huatabampo, which is in the southern border of the state, while the 2015 population was collected in San Luis Rio Colorado, located in the north, bordering with the United States. Although these two locations are distant from each other, the official recommendations to control fall armyworm in corn in this Mexican state only includes two major modes of action, AChE inhibitors and GABA-gated chloride channel blockers (SAGARPA 2015f). Hence, resistance to chlorpyrifos was expected, but the differences found between locations and collection years cannot be explained with insecticide use patterns alone. Immigration of susceptible alleles could be playing a significant role in reducing the frequency of resistance alleles to this mode of action. Fall armyworm migration has been described as an important factor in carrying resistance alleles to mainland US from Puerto Rico, so the reverse phenomenon may be a plausible explanation (Huang et al. 2014 and Camargo et al. 2017). Unfortunately, there are no studies on fall armyworm movement in these regions, so it is critical to take a proactive approach to manage the potential evolution of resistance to the insecticides that are available to control this pest. This should include rotation of insecticides with different modes of action and periodical screenings of the status of susceptibility of fall armyworm to the compounds used.

**Sodium channel modulators.** Fall armyworm from Puerto Rico also displayed the highest resistance ratios to the sodium channel modulators tested. Permethrin was the least effective compound, with the highest resistance ratio ($RR_{50}$=48-fold). Furthermore, we found that the PR population displayed high resistance levels to deltamethrin and zeta-cypermethrin.

Unexpectedly, the $LD_{50}$ and fiducial limits obtained with permethrin in the Oax 2013 population overlap with the values obtained with permethrin in the PR population. Both Oax 2013 and 2015 were collected from the same locations, where farmers reported zero pesticide applications. San Pablo Huitzo and San Lorenzo Cacaotepec Etla belong to the Valles Centrales region of Oaxaca, where corn production in general has low technology input and fertilizers, machinery and pesticides are rarely used (SAGARPA 2015d). In addition, the population Oax 2015 showed a significantly lower

$LD_{50}$ value, and non-overlapping fiducial limits than its 2013 counterpart. This variation could be an indicator of fall armyworm migration; in 2013, individuals carrying resistance alleles could have moved from other agricultural regions where pesticides are used.

**Ryanodine receptor modulators.** Fall armyworm PR population displayed the highest resistance ratios at both the $LD_{50}$ and $LD_{90}$ level to flubendiamide. The Puerto Rican population showed typical intoxication symptoms reported in the literature and observed in the SUS and Mexican field populations: halted feeding and uncontrolled muscle activity (Tohnishi et al. 2005). Nevertheless, many fall armyworm PR insects were able to recover and only 62% mortality was obtained with the highest dose. Chlorantraniliprole was slightly more toxic to fall armyworm from PR than flubendiamide, but a resistance ratio of 160-fold may imply a generalized loss of susceptibility to compounds of this mode of action. This result is supported by the similar values of the log dose-probit regression slope between flubendiamide and chlorantraniliprole, suggesting similar phenotypic variability in the response of this population to both pesticides. Furthermore, these results correspond with field failures of these products for fall armyworm control in Puerto Rico (Unpublished data, personal communication with Henry Teran-Santofimio).

From the fall armyworm Mexican populations, only Coa 2015 and Sin 2015 displayed $LD_{50}$ values significantly different from the SUS to flubendiamide, but only Sin 2015 showed a resistance ratio of 10-fold at the $LD_{50}$ level to this active ingredient. However, no field failures (practical resistance-Tabashnik et al 2014) of the commercial product have been reported to date, but it is important to continue monitoring the resistance of fall armyworm to ryanodine receptor modulators in Sinaloa.

**nAChR allosteric modulators.** Spinetoram showed a medium resistance ratio in the PR population. However, the other nAChR allosteric modulator spinosad, expressed low levels of resistance and it is actually a key component of the fall armyworm management in the island because its field efficacy against this pest. Mexican fall armyworm field populations displayed resistance ratios below 5-fold to spinetoram, indicating high potential for this mode of action for fall armyworm control in these regions.

**GLUCL allosteric modulators.** We found that fall armyworm from Puerto Rico remains sensitive to GLUCL allosteric modulators emamectin benzoate and abamectin. Both emamectin benzoate and abamectin displayed low $LD_{50}$ values, resulting in negligible resistance ratios.

A case of negative cross-resistance has been reported in a laboratory-selected strain of *Helicoverpa armigera* highly resistant to Cry1Ac. This resistance resulted in an increased susceptibility to the bacterial-derived insecticides abamectin and spinosad, but had no effect for other synthetic insecticides (Xiao et al. 2016). In our study fall armyworm from Puerto Rico has developed high levels of field-evolved resistance to Cry1F and Cry1Ac (Blanco et al. 2010 and Storer et al. 2010, 2012, Gutierrez-Moreno unpublished), and low levels of resistance to abamectin and spinosad. Perhaps high resistance to Cry1Ac resulted in low resistance levels to abamectin. Xiao et al. (2016) propose that a mutation in the ATP-binding cassette (ABCC2) is responsible for both resistance to Cry1Ac and increased susceptibility to abamectin in *H. armigera*. This is a plausible explanation since ABC transporters have been reported as playing a crucial role in xenobiotic excretion (Merzendorfer 2014). In *Helicoverpa armigera* and

*Helicoverpa punctigera*, Bird and Downes (2014) reported that Cry2Ab resistant strains showed a slight increase in susceptibility to the AChE inhibitors chlorpyriphos and methomyl, but no interaction was detected for other toxicological groups; concluding that the development of resistance is independent between Cry2Ab and certain groups of synthetic insecticides.

The evolution of resistance in fall armyworm from Puerto Rico to a wide range of insecticides seems to have a generalized mechanism. Detoxification enzymes have been described as the main mechanism of resistance, including cytochrome P450, esterases and glutathione-S-transpherases in many polyphagous Lepidopteran species. These enzymes were responsible for fall armyworm resistance to growth regulators, pyrethroids, organophosphates and carbamates (Yu 1983, 1991, 1992, 2003; Adamczyk et al. 1997, Ahmad et al. 2006, 2009; Zhao et al. 2006, Saleem et al. 2008, Ahmad et al. 2009 and Giraudo et al. 2015). Additionally, target-site insensitivity has also been described to play a key role as a resistance mechanism in conjunction with metabolic resistance to organophosphates and pyrethroids (Yu 1991, Carvalho et al. 2013). Genetic studies have indicated that the inheritance of insecticide resistance in fall armyworm to the benzoylurea lufenuron and the pyrethroid lambda-cyhalothrin is autosomal and incompletely recessive, suggesting the action of a single major gene in addition to the effect of minor genes, which can lead to resistance through multiple modes of action (Diez-Rodríguez and Omoto 2001, Nascimento et al. 2015).

Ryanodine receptor modulators are a relatively new class of insecticides. Active ingredients belonging to this group have shown high selectivity to insect ryanodine receptors over mammalian, especially toxic against Lepidopteran species. Ryanodine receptor modulators are intracellular calcium channels located in the sarcoplasmic and endoplasmic reticula in muscles and neurons; they are responsible for the release of calcium necessary for muscle movement (Sattelle et al. 2008 and Zalk et al. 2015). Flubendiamide and chlorantraniliprole have been commercially available since 2006 and 2007, respectively (Troczka et al. 2016). Up to now resistance and cross-resistance between these two active ingredients have been reported extensively on *Plutella xylostella* (Lepidoptera: Plutellidae), *Tuta absoluta* (Lepidoptera: Gelchiidea), *Spodoptera litura* and *Spodoptera exigua* (Lepidoptera: Noctuidae) in China, indicating that the main resistance mechanism is target site insensitivity, rather than the action of detoxification enzymes (Su et al. 2012, Che et al. 2013, Adams et al. 2016).

Genetic studies have indicated that the inheritance of insecticide resistance in fall armyworm to the benzonylurea, lufenuron and the pyrethroids, lambda-cyhalothrin is autosomal and incompletely recessive, suggesting the action of a single major gene in addition to the effect of minor genes, which can lead to resistance to multiple modes of action (Diez-Rodríguez and Omoto 2001, Nascimento et al. 2015). In fall armyworm populations from Venezuela resistant to both lambda-cyhalothrin and methomyl, Morillo and Notz (2007) suggested that the resistance mechanisms for these two compounds are different, while pyrethroid resistance is likely to be caused by the loss of sensitivity in the target site. In contrast, resistance to methomyl might be caused by a metabolic mechanism. Therefore, although the mechanisms of resistance to different active ingredients may be different, they might be rose at the same time through strong selection pressure in the field (Morillo and Notz, 2007).

The evolution rate of pesticide resistance depends mainly on the strength of the selection pressure and the extent of the genetic variation in the resistant trait (Conner and Hartl 2004). Table 4 shows the factors that play a key role in the evolution of pesticide resistance in fall armyworm from the different agricultural regions comprised in this study. They are analyzed in order of importance for each region.

Factor 1 - Pesticide deployment. The selection pressure by intense insecticide deployment is especially strong in Puerto Rico. Pesticide use remains fundamental for fall armyworm control in corn, particularly in parent seed production of Bt corn, where pest tolerance is very low. After the reports of fall armyworm resistance to Bt technology (Cry1F protein) were confirmed in the island, these fields have been continually sprayed with conventional pesticides (Storer et al. 2012).

As previously stated, fall armyworm from Mexico was collected from four different regions. Northwest: Chihuahua, Sinaloa and Sonora, Northeast: Tamaulipas and Coahuila, West-central: Jalisco and Southwest: Oaxaca. About 23% of the total area planted with corn in Mexico are comprised within the states in the first three regions, with 1.9 out of 8.2 million hectares, and about 43% of the total corn production, with 16.5 out of 38.5 million tons (SIAP 2017). These states are characterized by the use of corn hybrids, high technology input and heavy reliance in conventional insecticides to control crop pests. However, corn production in the southwestern state of Oaxaca has a total of 569,000 ha planted and a total production of 666,614 tons, representing 0.1% of the total production of forage corn and 7.4% of corn grains produced in Mexico (SIAP 2017). Corn production in Oaxaca is dominated by more traditional production systems, with very low, or zero pesticide use depending on the region in the state.

**Table 4.** Factors that play a role in resistance evolution to synthetic insecticides in the fall armyworm in different agricultural regions.

| | Region | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Factor | Puerto Rico | Sinaloa | Jalisco | Sonora | Tamaulipas | Coahuila | Chihuahua | Oaxaca |
| Pesticide selection pressure | ++++ | +++ | +++ | +++ | +++ | +++ | +++ | --- |
| Generations | ++++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Geographical isolation | ++++ | --- | --- | --- | --- | --- | --- | --- |
| Migration | --- | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Host availability | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Extreme weather conditions (draught, hurricanes, etc.) | +++ | + | + | + | + | + | + | + |

As previously stated, fall armyworm from Mexico was collected from four different regions. Northwest: Chihuahua, Sinaloa and Sonora, Northeast: Tamaulipas and Coahuila, West-central: Jalisco and Southwest: Oaxaca. About 23% of the total area planted with corn in Mexico are comprised within the states in the first three regions, with 1.9 out of 8.2 million hectares, and about 43% of the total corn production, with 16.5 out of 38.5 million tons (SIAP 2017). These states are characterized by the use of corn hybrids, high technology input and heavy reliance in conventional insecticides to control crop pests. However, corn production in the southwestern state of Oaxaca has a total of 569,000 ha planted and a total production of 666,614 tons, representing 0.1% of the total production of forage corn and 7.4% of corn grains produced in Mexico (SIAP

2017). Corn production in Oaxaca is dominated by more traditional production systems, with very low, or zero pesticide use depending on the region in the state.

The variability of pesticide deployment for fall armyworm control in Mexico doesn't follow an established pattern, control tactics and chemistries used for pest control may vary greatly depending on the agricultural systems (Hernández et al. 2017). The lack of IRM programs in corn production, with a sound use of the different chemistries and modes of action can speed up the evolution of pesticide resistance, especially in the Northern regions of the country where higher number of sprays are applied. For example in Jalisco, there are at least three pesticide applications per crop cycle (Posos, personal communication), but other regions report up to 12 sprays per cycle (Blanco et al. 2016). Farmers in the Oaxaca Central Valleys where our fall armyworm populations were collected reported zero pesticide applications; therefore these fall armyworm populations were not subjected to selection pressure. In theory, 2013 and 2015 fall armyworm from Oaxaca should have remained susceptible to synthetic insecticides, if we assume zero migration of insects from other regions with pesticide deployment.

Factor 2 - Number of generations. Fall armyworm's life cycle may be completed in 30 days in warm regions, while in cooler temperatures it can take up to 90 days (Capinera, 2000). However, in some tropical regions fall armyworm was reported to have eight generations per year, while in Brazil there were records of 10 generations per year (Busato et al. 2005, Farias et al. 2014). In the presence of a key selecting agent (pesticides), higher numbers of generations per year have the potential to fix resistance alleles in a population at an accelerated rate, assuming that a single major recessive gene confers resistance. After heavy pesticide deployment has selected against the dominant susceptible gene, if selection pressure is continuous, with frequent application of pesticides, resistant individuals will display higher fitness over non-resistant individuals.

Puerto Rico’s climate is sub-tropical maritime, characterized by warm temperatures, with an average of 25°C, but can reach temperatures up to 31°C and precipitation levels that can vary from 800 to 4,000 mm per year (Puerto Rico Climate Change Council 2013). These weather conditions are very favorable for fall armyworm development so that there have been reports of approx. 10 fall armyworm generations per year in this island (Storer et al. 2012). This is also tightly associated with constant sources of food throughout the year (Factor 5).

Corn producing areas in Northwestern and Northeastern Mexican states are dominated by dry/very dry weather conditions, with the exceptions of a coastal region in southern Sinaloa with warm sub-humid weather and most of the coastal region of Tamaulipas, classified as temperate-humid (INEGI 2017). Temperatures in these regions may reach their highest peaks during the summer months from June to August, ranging from 36°C (Chihuahua 2011) to 40°C (Sonora 2014). Annual precipitation varies between these regions: Tamaulipas received the largest amount of rain between these states, averaging 835 mm/year from May to August (2011 to 2016) and Coahuila was the driest state, with an average annual precipitation of 408 mm from May to September (2011 to 2016) (Servicio Meteorológico Nacional 2017). With these dry weather conditions, the presence and optimal development of fall armyworm is benefited by the use of irrigated crop production systems, which ensure the continuum presence of host plants in the warm summer seasons. This is consistent with previous reports of higher fall armyworm adult incidence during the month of June in the irrigated corn-growing region in Rio Grande Valley in southern Texas and northern Tamaulipas (Raulston et al. 1986). Additionally, there are studies that indicate a negative correlation between relative humidity and fall armyworm male captures in light traps, while a positive correlation was established between dry seasons and male captures (Rojas et al. 2004 and Vilarinho et al. 2011).

The state of Jalisco, representing the Mexican West-Central, has two main weather regions: warm sub-humid and temperate sub-humid (INEGI 2017). Between a period of six years, from 2011 to 2016, temperatures ranged from a low of 6.4°C (January 2011) during the winter months, to a high of 35°C (May 2016) during the summer months. The rainy season in this state runs from June to September, with an average precipitation of 898 mm/year, the highest precipitation in the past six years was registered in 2015 (1,145 mm/year) (Servicio Meteorológico Nacional 2017).

It is relevant to mention that Jalisco is a leading corn grower in Mexico, in 2015 alone 694 thousand hectares were harvested, representing 9% of the total harvested area and a total production of 7.1 million tons, representing 18% of the total production of forage, seed and grain in the country (SIAP 2017). Fall armyworm was collected in the warm sub-humid region of the state, this is a high intensity agricultural area where corn is grown in the Spring-Summer season, with plantings starting from April through September and harvesting occurring from June and until March of the next year.

The state of Oaxaca, representing the Southwest of Mexico, has three main distinct weather regions: warm sub-humid, dry and temperate sub-humid (INEGI 2017).

Fall armyworm was collected from a geographical region called the Oaxaca Central Valley, with a predominantly dry weather. In this state, temperatures range from a low of 13.6 °C (Dec 2011) to a high of 35 °C (May 2016). The annual precipitation averages 1,194 mm/year in the state (data from 2011 to 2016), but this amount is lower in the dry Central Valleys (Servicio Meteorológico Nacional 2017). Most of the corn grown in this region is rain-fed, but there are about 191 ha with irrigated corn farms in the municipalities where fall armyworm was collected (SIAP 2017). The majority of the corn production is conducted between the months of May and June, to take advantage of the rainy season that runs from June to September/October, but irrigated corn is produced twice a year, the only months without corn are December and January (Martinez, personal communication).

Factor 3 - Geographic isolation. In Puerto Rico, fall armyworm is isolated from the continental territories, but within this island there are mountains that block the free movement of the insect in between agricultural areas (Storer et al. 2010). This factor is not as strong in the Mexican regions included in this study. Although there are some limits for fall armyworm dispersal between the west, center and east of the country due to the mountainous systems, fall armyworm could move along the coastal lines of the Pacific and Gulf of Mexico and within the central states. This process will be further analyzed in the next factor “Migration”.

Factor 4 - Migration. This factor is tightly associated with the previous one, especially in Puerto Rico, where the geographical isolation of fall armyworm has limited potential influx of new individuals that may increase the frequency of susceptible alleles. In contrast, gene flow is one of the potential explanations for the low variability in the response of fall armyworm to conventional pesticides between the Mexican populations. In a previous study, López-Edwards et al. (1999) described the presence of at least two main groups of fall armyworm populations in Mexico, “Gulf coast” and “Pacific coast”, which are separated by two mountain ranges that divide the country (Sierra Madre Oriental and Sierra Madre Occidental) and have developed a level of reproductive isolation. Moreover, Perez-Zubiri et al. (2016) analyzed the genetic variability of different fall armyworm populations from the northern Mexican states of Coahuila, Durango, Sinaloa, and the west-central state of Jalisco, using molecular markers they found that this variability is higher within than among populations, which implies significant levels of gene flow between them. Hence, the Oaxaca population could be considered as belonging to the “Pacific coast” region, with fall armyworm populations from Jalisco, Sinaloa, Sonora and Chihuahua. Nevertheless, further studies on fall armyworm migration patterns within Mexico are necessary to understand how growers can cope with this pest in the diverse agricultural systems that characterize corn production systems in Mexico (Blanco et al. 2014).

Factor 5 - Alternative host availability. For fall armyworm from Puerto Rico, this factor is also very significant in the evolution of pesticide resistance and it is linked to extreme weather conditions, which reduce the availability of host plants (see Factor 6 description).

In the Mexican regions included in this study, even though corn is grown in different seasons between northwest, northeast and west, fall armyworm has other host crops available to survive, such as cotton in Chihuahua (SIAP 2017) or alfalfa, sugar cane and oatmeal in Jalisco (OEIDRUS 2017), which are also grown in irrigated systems. In Oaxaca, fall armyworm could use alternative wild hosts in the dry seasons when host crops are not available. The wild vegetation in the region where fall armyworm was collected is characteristic of the xerophilus shrubs, with a few wild fall armyworm hosts: red-jasmin (*Plumeria rubra*), various ficus trees (*Ficus* spp) and the castor bean (*Ricinus communis*) (García-Mendoza et al. 2004 and Martínez-Martínez, 2015). Furthermore, the castor bean has been identified as a suitable host for fall armyworm development and reproduction, comparable to corn (Martínez-Martínez, 2015).

In addition, several weed species that grow in all of these regions are considered fall armyworm hosts, for example: bermuda grass (*Cynodon dactylon*) and field bindweed (*Convolvulus arvensis*) (Cazmus et al. 2010 and CONABIO 2017). Since wild hosts are not sprayed with pesticides, they can also provide a refuge for susceptible individuals to develop and contribute to slowing down the evolution of pesticide resistance.

Factor 6. Extreme weather conditions. Puerto Rico has a dry season that generally runs from December to March (Puerto Rico Climate Change Council 2013). During these months, fall armyworm's food sources become scarce and can be pushed to the irrigated cornfields, where they will be subjected to selection pressure with both pesticides and Bt proteins. This was indicated to be one of the leading factors that sped the development of resistance to Cry1F in Puerto Rico (Storer et al. 2010, 2012); severe droughts in this island were reported to dramatically reduce alternative hosts for fall armyworm in the years previous to the first report of resistance to Cry1F (Storer et al. 2010, 2012).

Dry seasons are a common occurrence in the Northern states of Mexico, the government has a classification system that comprises four drought levels: D0. Abnormally dry, D1. Moderate drought, D2. Severe drought, D3. Extreme drought and D4. Exceptional drought (Servicio Meteorológico Nacional 2017). In the past ten years, there has been one drought season, from March 2011 to June 2012 that ranged from levels D2 to D4 throughout the country. The impact was heavier in the Mexican northern states, which registered droughts on levels D3 to D4. There is no apparent direct correlation between this phenomenon and the resistance cases we report here in fall armyworm from Mexico, our experiments started with fall armyworm collected in 2013 and we didn't detect resistance to the major modes of action used for fall armyworm control. Still, the extremely dry conditions during 2011 and 2012 could be a factor with a reduced impact in these agricultural regions, and it's effect can be affected by the interaction with other factors, such as migration or alternative host plant availability.

## Conclusion

The variability of pesticide use for fall armyworm control in Mexico doesn't follow an established pattern. Control tactics and chemistries used for pest control may vary significantly depending on the agricultural systems and even individual grower choices (Blanco et al 2014, Hernández et al. 2017). This agricultural diversity in Mexico, plus the availability of many cultivated and wild alternative hosts may reasonably be the factors behind the overall lower levels of pesticide resistance in fall armyworm. However, given the size of the country and area allocated for corn production the fall armyworm populations collected from Mexico and tested for susceptibility might be a just a small sample size and a bigger number of populations are required to get a comprehensive assessment of resistance development in Mexico. In addition, IPM and IRM strategies that take advantage of these conditions should be developed to prolong the lifespan of the available control tools.

The selection pressure by intense insecticide use is especially strong in Puerto Rico, where pesticide use remains fundamental for fall armyworm control in corn. This is particularly important in hybrid seed research, where pest tolerance is very low. Therefore, the status of pesticide resistance of fall armyworm in this island deserves special attention. We found two pesticide modes of action (GLUCL and nAChR allosteric modulators) that show high potential to control this pest.

Field-evolved resistance in crop pests is a continuous threat to the sustainability of agriculture worldwide. Thus, it is crucial to monitor the susceptibility of this pest to the control strategies available, considering factors such as fall armyworm host preferences, migration, management practices and the potential movement of these insect populations between agricultural regions, in order to detect any changes on time to develop and update IPM and IRM programs.

# CHAPTER 3: SUSCEPTIBILITY STATUS OF THE FALL ARMYWORM *Spodoptera frugiperda* (JE SMITH) (LEPIDOPTERA: NOCTUIDAE) TO BT PROTEINS IN MEXICO AND PUERTO RICO


## Abstract

During the last decade, the fall armyworm fall armyworm has become the main pest of Bt corn in many areas of the American continent. Fall armyworm is the most important insect pest in corn production in Mexico and although Bt corn is not yet grown in that country, it is necessary to establish the baseline susceptibility of this pest to the main Bt proteins used in Bt corn varieties to determine the potential life span of this technology in the region. Additionally, after a decade since the first detection of fall armyworm resistance to Cry1F and Cry1Ac in Puerto Rico, it is crucial for current IPM programs used against fall armyworm to keep monitoring the status of this pest's susceptibility to the current Bt proteins included in the pyramided Bt corn hybrids presently grown in this region.

We conducted bioassays to determine the current susceptibility status to the Bt proteins Cry1A.105, Cry2Ab2, Cry1F and Cry1Ac on three different populations of fall armyworm in Mexico and one population from Puerto Rico. Fall armyworm from Puerto Rico showed the highest $LC_{50}$ values for all Bt proteins tested: less than 3% mortality was achieved with the highest Cry1F concentration (10,000 ng/cm$^2$) and 65% mortality was the maximum achieved with the highest Cry1Ac concentration (6,000 ng/cm$^2$). In contrast, Cry1A.105 and Cry2Ab2 exhibited low resistance ratios, below 2-fold in comparison with the $LC_{50}$ of the susceptible population. In contrast, all Mexican fall

armyworm populations displayed remarkable low $LC_{50}$s and resistance ratios were below 1-fold.

## Introduction

Pest control in Mexican corn production faces challenges due to the heterogeneous composition of the corn agricultural systems throughout the country; this heterogeneity complicates the development of effective Integrated Pest Management (IPM) programs (Blanco et al. 2014). Fall armyworm is the main pest in corn in most of the producing regions in Mexico and is reported to be the hardest to control with the available tools (Blanco et al. 2014). Currently, farmers rely heavily on synthetic insecticides, with two or three insecticide sprays per crop cycle (Blanco et al. 2016). However, some growers have reported the need to spray up to 12 times to control fall armyworm (Mota-Sanchez, unpublished).

Genetically engineered (GE) corn with the ability to produce *Bacillus thuringiensis* (Bt) proteins has not yet been approved for commercialization in Mexico. This country has experienced the benefits of GE technology, with the adoption of Bt and herbicide tolerant cotton and herbicide tolerant soybeans (Otero 2015), but corn is a controversial crop, due to its relevance in Mexican culture as staple food. However, Bt technology could provide a suitable tool to manage fall armyworm, which could potentially reduce the use of synthetic insecticides.

In Puerto Rico, fall armyworm is the most important pest in corn production, which is focused toward seed and hybrid research, with very low pest tolerance (Storer et al. 2010; 2012). The first report to damage in Bt corn by fall armyworm in Puerto Rico was reported 10 years after the introduction of Bt maize (TC1507) producing Cry1F

(Storer et al. 2010 and Blanco et al. 2010). The second case was reported in Brazil, where fall armyworm developed resistance to Cry1F after only three years of use in the field (Farias et al. 2014). The third confirmed case of field-evolved resistance to Cry1F in fall armyworm was detected in southern United States, 18 years after commercialization (Huang et al. 2014). Additionally, some fall armyworm populations display inherently low susceptibility to the Bt proteins Cry1Ab and Cry1Ac (Luttrell et al. 1999, Hardke et al. 2011 and Huang et al. 2014), or developed field-evolved resistance to both proteins (Bernardi et al. 2014 and Omoto et al. 2016).

The first two cases of Cry1F resistance (Puerto Rico and Brazil) shared certain common factors: continuous Bt corn production, fall armyworm in both systems has >10 generations per year, with relatively limited insect migration. All of these factors increase selection pressure (Storer et al. 2010, 2012 and Farias et al. 2014). However, in the case of fall armyworm resistance in southern US, these factors have yet to be identified (Huang et al. 2014).

Monitoring the susceptibility of target pests to control tools is a key component in IPM programs, as it allows for changes in management tactics before resistance is fixed in a population (Tabashnik et al. 2013). Knowing the level of susceptibility of a pest to a pesticide before the technology is deployed in the field can help strategically plan its application. In this chapter we determine the susceptibility of fall armyworm from Mexican populations collected in corn fields from three regions, two regions where synthetic insecticides are the main control strategy and one collected from a location where no pesticides are used. Also, we determine the susceptibility of fall armyworm from Puerto Rico to Cry1A.105 and Cry2Ab2, which are currently being deployed in pyramided Bt corn on the island. Finally, we evaluated the susceptibility of fall armyworm from Puerto Rico to Cry1F and Cry1Ac, which have been reported as ineffective against fall armyworm from this territory.

## Materials and Methods

Populations

Fall armyworm populations from Mexico were collected during summer 2015 in cornfields from the following locations: northwest: Los Mochis, Sinaloa (Sin), west central: San Martin de Hidalgo, Jalisco (Jal) and southwest: San Pablo Huitzo and San Lorenzo Cacaotepec Etla, Oaxaca (Oax). Insects from Puerto Rico (PR) were collected in corn from June to November 2016. Monsanto Company provided a fall armyworm laboratory susceptible (SUS) population from their rearing facilities in Union City, Tennessee. Field fall armyworm populations were collected during larval development directly from affected corn plants. Insects were placed in 10 ml plastic cups with 4-6 ml of artificial diet (Southland Products Incorporated: fall armyworm diet). In 2013, larvae were sent to the laboratories of Colegio de Postgraduados, Mexico and for the 2014-2016 period, larvae were sent to our laboratory at Michigan State University, East Lansing, Michigan, United States.

Insect rearing

Larvae were held at 27±1 ºC and a photoperiod of 16:8 hours light: dark. Insect development was supervised every other day, when pupae turned from orange-red to dark red, they were placed in groups of 40 inside collapsible insect cages (Bioquip, Rancho Dominguez, CA) to provide room for emerging adults to fly and mate, a sponge with a solution of 10% sugar in a petri dish was provided as food source. After three days of adult emergence, adults from each cage were placed in 1L paper brown bags and a wet sponge with a solution of 10% sugar in a petri dish was added. Every other day moths were transferred to a new paper bag; eggs masses laid in the brown paper bag were separated and placed in a plastic container with moist paper towels. Neonate larvae were transferred to bioassay trays (Frontier Agricultural Sciences, Newark, DE) with 1 ml of artificial diet per well.

Bt proteins

Monsanto Company (St. Louis, MO) provided the following Bt proteins in 1 ml aliquots: Cry1A.105: 80% purity, with a concentration of 1mg/ml. The protein arrived diluted in 25 mM CAPS buffer, pH 10.3 and 1 mM benzamidine-HCl, 0.1 EDTA and 0.2 mM DDT. Cry2Ab2: 87% purity, with a concentration of 0.31 mg/ml. The protein arrived diluted in 50 mM CAPS buffer, pH 11 and 2 mM DTT. Additionally, Monsanto Company provided both buffers to make the dilutions used in the bioassays. Both proteins and buffers arrived at our laboratory in dry ice and were immediately placed on a -80°C freezer.

Dow Agrosciences (Indianapolis, IN) provided lyophilized Cry1F and Cry1Ac and 10 mM CAPS buffer (3-(cyclohexylamino)-1-propane sulfonic acid), diluted in deionized water, pH 10.5. Proteins were kept in a desiccator (Fisher Scientific rectangular Desi-Vac Container™), both proteins and buffer were placed in a refrigerator at 4°C.

Diet overlay bioassays

Experiments with fall armyworm from Mexico were conducted from July 2015 until November 2016. Bioassays with SUS were performed from June 2014 until

February 2017. Bioassays with the PR population were carried out from June 2016 until February 2017.

Bioassays were conducted using the procedure described by Storer et al. (2010) using 128-well bioassay trays (Frontier™ Agricultural Sciences) with 1ml of fall armyworm artificial diet per well. The surface area of the diet in each well was 1.5cm$^2$. A total of 100µl of each concentration was applied over the diet surface on each well, and let to air dry for about three hours. A single repetition of each dose consisted of 16 wells with one neonate per well. Table 3 shows the dose range used for each population and each protein. Three to five repetitions were conducted per Bt protein, covering 0 to 100% mortality, except with Cry1F and Cry1Ac with the fall armyworm PR population, in which we were unable to reach 100% mortality. Two controls were used: 1) diet alone and 2) diet plus the respective CAPS buffer. One neonate larva was placed per well with a small paintbrush. Only healthy, actively moving neonates were chosen for the experiments. Mortality and weight were evaluated seven days after treatment, larvae that failed to move after prodding with a small paint brush, failed to molt to second instar, and larvae weighing ≤0.1 mg were considered dead (Bernardi et al. 2014, Farias et al. 2014, Vélez et al. 2014).

**Table 5.** Bt protein concentrations used in dose-response mortality bioassays.

| Population | Bt protein | Concentration range (ng/cm$^2$) |
|---|---|---|
| Susceptible | Cry2Ab2 | 10 - 1,000 |
| | Cry1A.105 | 10 - 1,000 |
| | Cry1F | 7.4 - 1,000 |
| | Cry1Ac | 7.4 - 1,300 |
| Jalisco Oaxaca | Cry2Ab2 | 0.7 - 200 |
| | Cry1A.105 | 0.02 - 67 |
| | Cry1F | 7.4 - 1,000 |
| | Cry1Ac | 7.4 - 1,000 |
| Sinaloa | Cry2Ab2 | 22.2 - 1,300 |
| | Cry1A.105 | 22.2 - 1,300 |
| | Cry1F | 7.4 - 1,000 |
| | Cry1Ac | 22.2 - 1,300 |
| Puerto Rico | Cry2Ab2 | 7.4 - 6,000 |
| | Cry1A.105 | 7.4 - 6,000 |
| | Cry1F | 22.2 - 10,000 |
| | Cry1Ac | 22.2 - 6,000 |

Statistical analysis

Mortality data was corrected using Abbot's formula (Abbott 1925). Probit analysis (Finney 1971) was conducted using SAS version 9.3 (SAS Institute 2011) to calculate values of slope, lethal doses fifty ($LC_{50}$), lethal doses ninety ($LC_{90}$), and fiducial limits (95%) for each population. Resistance ratios were obtained by dividing the $LC_{50}$, $LC_{90}$ values obtained with the field fall armyworm populations by the $LC_{50}$, $LC_{90}$ values obtained with the susceptible colony. Log dose responses were plotted using Origin® (OriginLab 2007).

Effective concentrations are expected to reduce larval weight by 50% ($EC_{50}$) and confidence intervals (CI) were estimated using a non-linear regression analysis in SAS version 9.3 (SAS Institute 2011), using the logistic model described by Sims et al. (1996):

$$\text{Weight} = W_0 / [1 + (\text{dose}/EC_{50})^b]$$

## Results

Fall armyworm from Mexico

Field fall armyworm populations from Mexico displayed low $LC_{50}$ values to Cry1F, Cry1Ac, Cry2Ab2 and Cry1A.105 in comparison with the SUS population (Table 5). Resistance ratios (RR) were below 1-fold of the $LC_{50}$, with the exception of Oaxaca with Cry1Ac that showed a $RR_{50}$= 1.3-fold. For the Sinaloa population, with Cry1A.105 and Cry2Ab2, mortality of 100% was achieved at the lower doses, so $LC_{50}$ values couldn't be calculated. Cry1A.105 was the most toxic against fall armyworm from Jalisco, both $LC_{50}$ and $EC_{50}$ values obtained with this population were the lowest among all the field populations (Table 5).

**Table 6.** Concentration-mortality response (ng/cm$^2$) of fall armyworm from several Mexican states and Puerto Rico to Bt proteins.

| Bt protein | Pop[a] | n[b] | $LC_{50}$[c] | 95% FL[d] | $RR_{50}$[e] | $LC_{90}$[c] | 95% FL[d] | $RR_{90}$[e] | Slope ±SE[f] | $X^2$[g] | EC50[h] | SE[i] | 95% CI[j] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cry1F | SUS | 338 | 174.4 | 135.7 , 222.6 | 1.0 | 849 | 606.6 , 1,349 | 1.0 | 1.9 ±0.2 | 0.9 | 11 | 1.3 | 8.2 , 13.4 |
| | SIN | 624 | 29.2 | 22.6 , 36.7 | 0.2 | 201 | 145 , 309.) | 0.2 | 1.5 ±0.1 | 2.8 | 16 | 1.4 | 12.5 , 18.7 |
| | JAL | 384 | 42.8 | 29.8 , 59.8 | 0.2 | 463 | 275.4 , 1,015 | 0.5 | 1.2 ±0.2 | 4.3 | 5 | 1.2 | 2.3 , 7.4 |
| | OAX | 384 | 26.5 | 0.03 , 122 | 0.2 | 502 | 113 , 1.92E+12 | 0.6 | 1 ±0.3 | 2.5 | 7 | 0.8 | 5.2 , 8.8 |
| | PR | 256 | >10,000 | ne[h] | ne | >10,000 | ne | ne | ne | ne | >10,000 | ne | ne |
| Cry1Ac | SUS | 770 | 148.2 | 120 , 183.2 | 1.0 | 1,050 | 750.3 , 1,635 | 1.0 | 1.5 ±0.1 | 4.0 | 5 | 1.1 | 2.4 , 6.9 |
| | SIN | 512 | 15.3 | 8 , 22 | 0.1 | 78 | 57 , 125 | 0.1 | 1.8 ±0.3 | 0.3 | 23 | 0.2 | 22.1 , 23.5 |
| | JAL | 380 | 34.2 | 20.5 , 52.3 | 0.2 | 817.7 | 397.9 , 2,781 | 0.8 | 0.9 ±0.1 | 1.5 | 1.6 | 0.6 | 0.4 , 2.8 |
| | OAX | 385 | 188.8 | 132 , 280 | 1.3 | 3,262 | 1,669 , 8,983 | 3.1 | 1 ±0.1 | 3.4 | 3 | 0.6 | 1.5 , 4 |
| | PR | 624 | 1,815 | 934.5 , 5,374 | 12.2 | 705,419 | 95,917 , >100,000 | 671.8 | 0.5 ±0.08 | 1.4 | 68 | 10.8 | 43 , 92 |
| Cry1A.105 | SUS | 641 | 201.9 | 150.4 , 266.3 | 1 | 859 | 586.6 , 1,576 | 1 | 2 ±0.3 | 4.5 | 31 | 5 | 19.6 , 42.8 |
| | SIN | 383 | ne | ne | ne | ne | ne | ne | ne | ne | ne | ne | ne |
| | JAL | 256 | 4.6 | 3.2 , 6.4 | 0.02 | 28.3 | 17.8 , 57.5 | 0.03 | 1.6 ±0.2 | 4.5 | 1.3 | 0.2 | 0.9 , 1.8 |
| | OAX | 560 | 14.5 | 3.4 , 139.7 | 0.07 | 164.4 | 37.8 , >100,000 | 0.2 | 1.2 ±0.3 | 5.8 | 1.4 | 0.3 | 0.8 , 2.2 |
| | PR | 303 | 273.8 | 172.5 , 442.8 | 1.4 | 3,234 | 1,542 , 12,547 | 3.8 | 1.2 ±0.2 | 1.4 | 73.1 | 9.3 | 51.7 , 94.4 |
| Cry2Ab2 | SUS | 640 | 173.2 | 130.8 , 214.1 | 1 | 469 | 373.5 , 649.9 | 1 | 3 ±0.4 | 5 | 111 | 27 | 52.4 , 169.6 |
| | SIN | 381 | ne | ne | ne | ne | ne | ne | ne | ne | ne | ne | ne |
| | JAL | 256 | 49.7 | 30.3 , 94 | 0.2 | 1,040 | 398.9 , 5,369 | 2.7 | 0.9 ±0.1 | 3.2 | 5.4 | 1.4 | 2.3 , 8.5 |
| | OAX | 410 | 13.3 | 3.1, 165.5 | 0.1 | 1,762 | 150.3 , >100,000 | 4.6 | 0.6 ±0.2 | 2.4 | 1.1 | 0.2 | 0.7 , 1.6 |
| | PR | 253 | 119.2 | 68.5 , 187 | 0.5 | 2,092 | 1,091 , 6,065 | 5.5 | 1 ±0.2 | 0.4 | 24.7 | 5.8 | 11.7 , 37.7 |

[a]Fall armyworm population
[b]total number of insects used
[c]lethal concentration that is expected to kill 50% of the population, expressed in nanograms of active ingredient per square centimeter of diet (ng/cm$^2$)
[d]fiducial limits
[e]resistance ratio= LC of field collected fall armyworm over the LC of the susceptible reference population

[f]slope ± standard error
[g]chi-square value
[h]effective concentration of Bt protein that is expected to reduce larval weight by 50%
[i]standard error
[j]Confidence intervals
ne= not estimated due to insufficient mortality or weight reduction

EC50 values obtained for the fall armyworm Mexican populations were low, indicating that these populations are highly susceptible to Bt proteins (Table 5). Overall, all fall armyworm Mexican field populations followed the same weight reduction trend as the SUS population, with the most dramatic weight drops obtained with the lower concentrations (Figures 3-6).

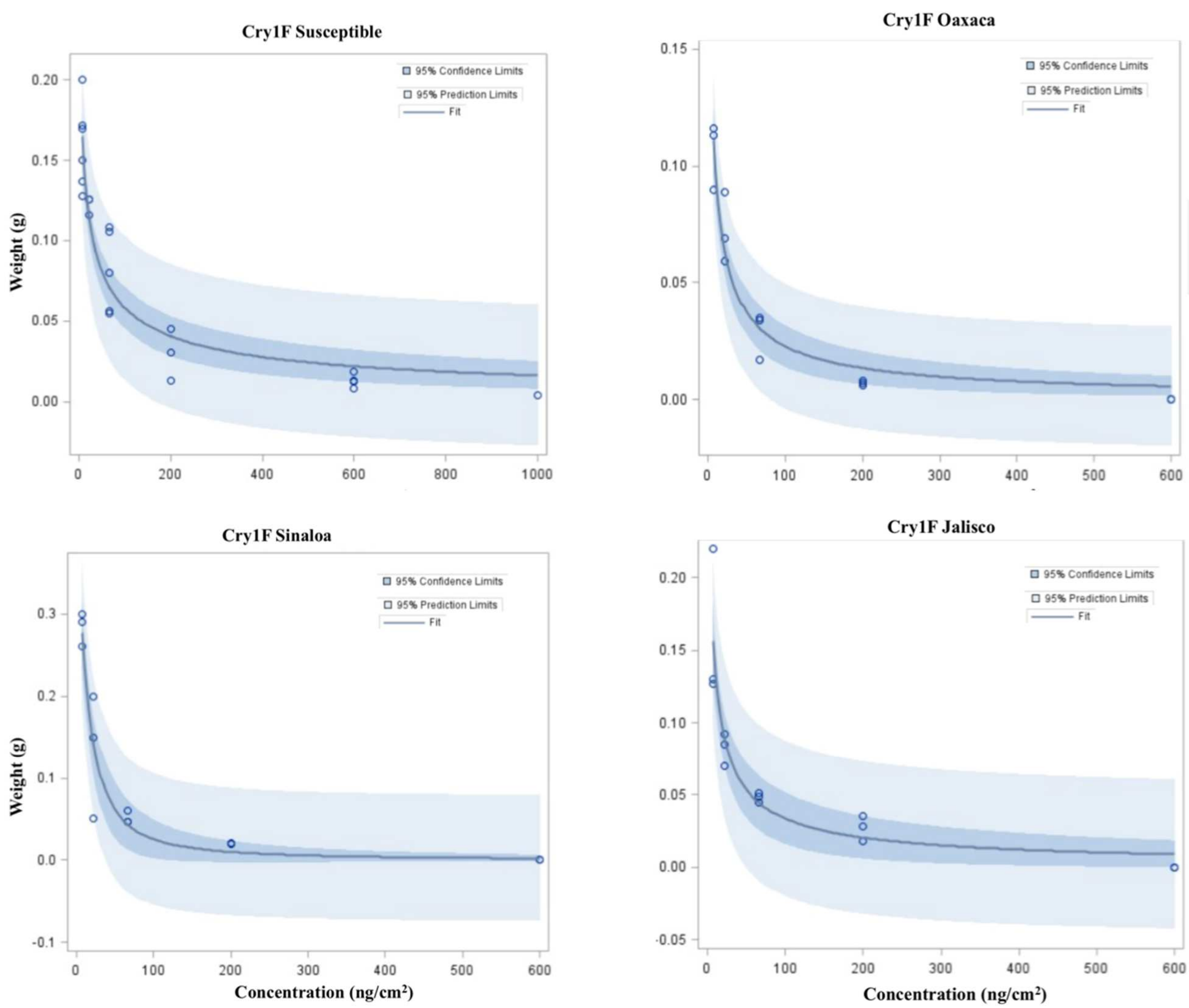


**Figure 3.** Concentration – larval weight reduction response to Cry1F of fall armyworm from Mexico and the SUS reference population.

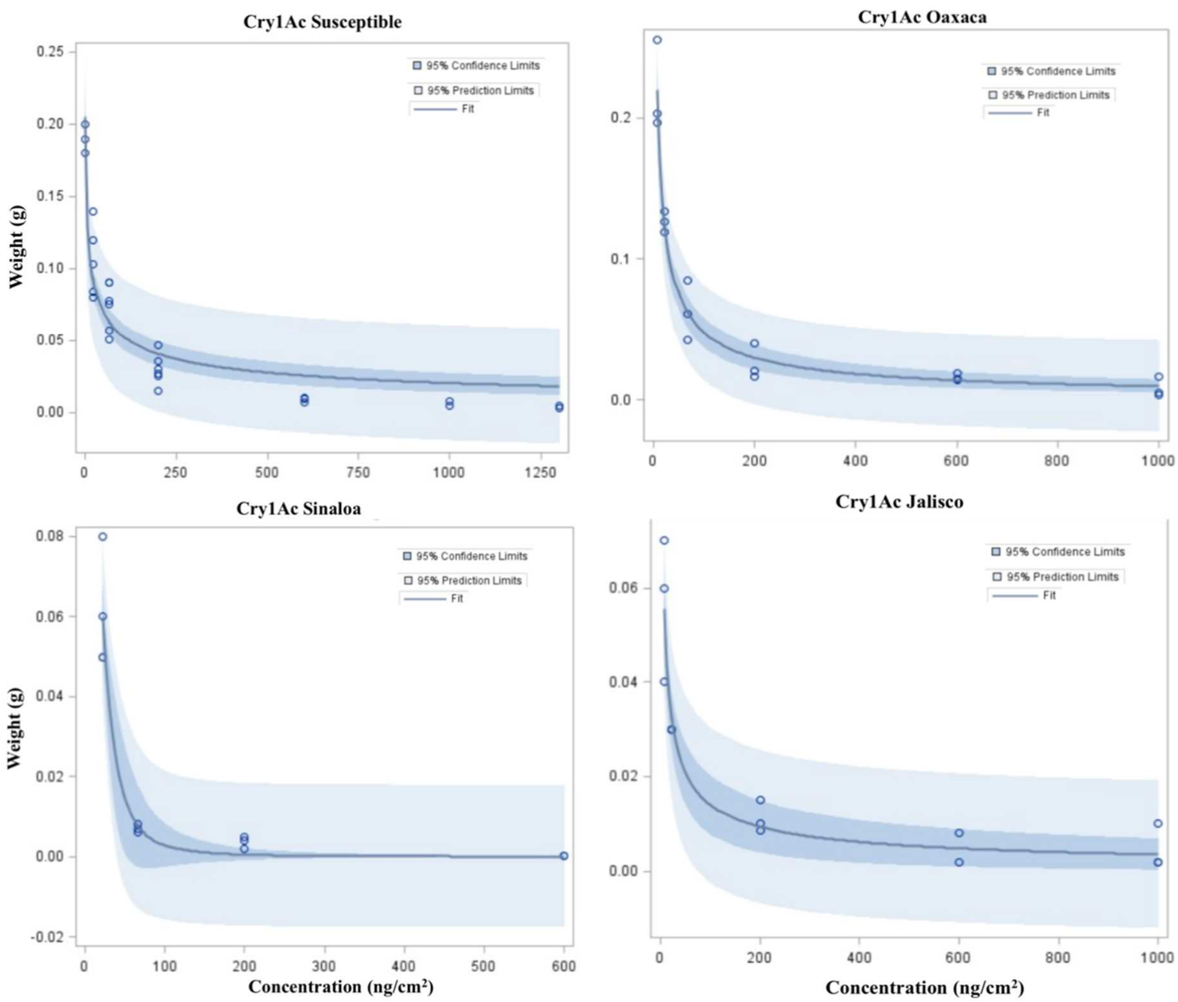


**Figure 4.** Concentration – larval weight reduction response to Cry1Ac of fall armyworm from Mexico and the SUS reference population.

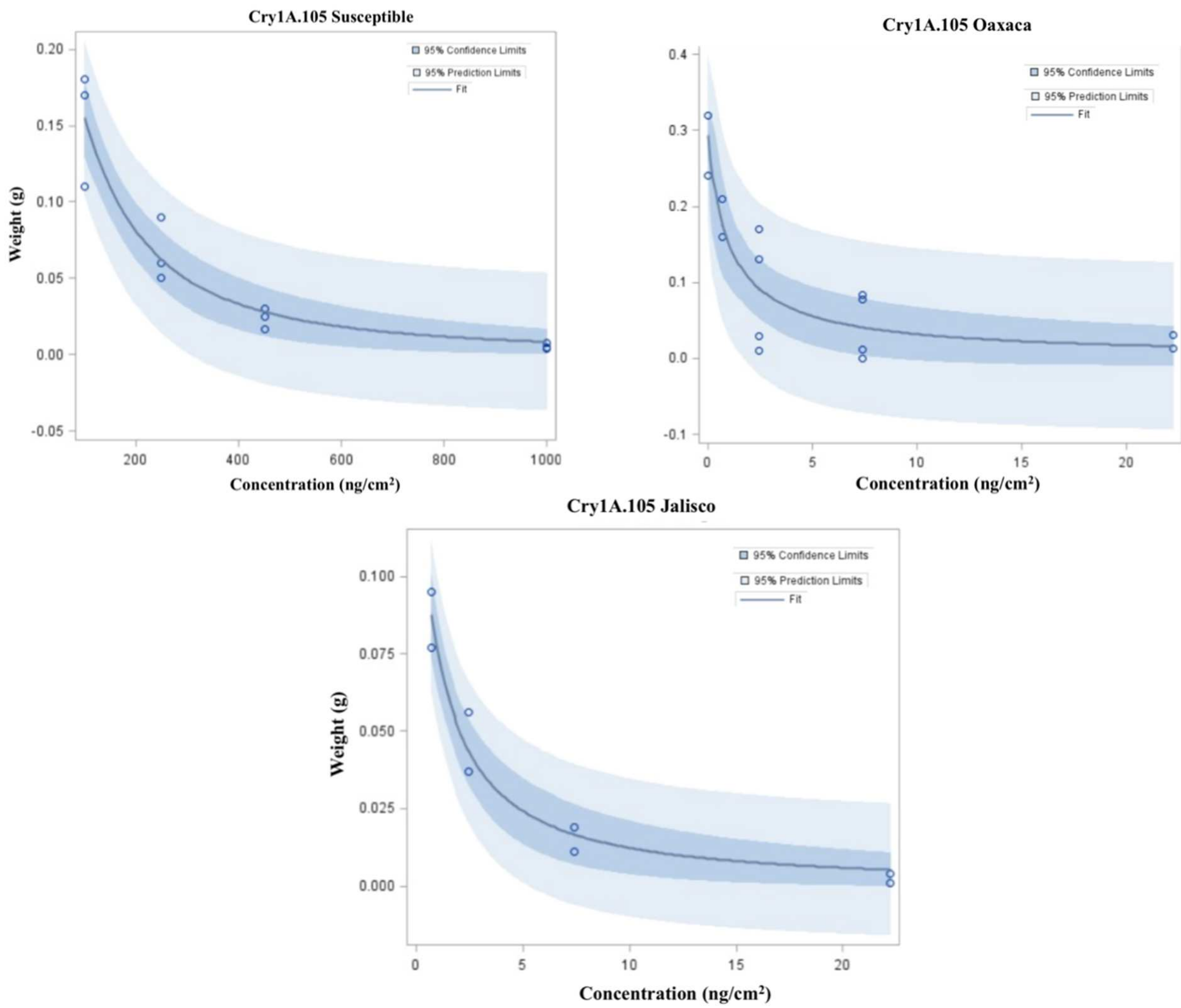


**Figure 5.** Concentration – larval weight reduction response to Cry1A.105 of fall armyworm from Mexico and the SUS reference population.

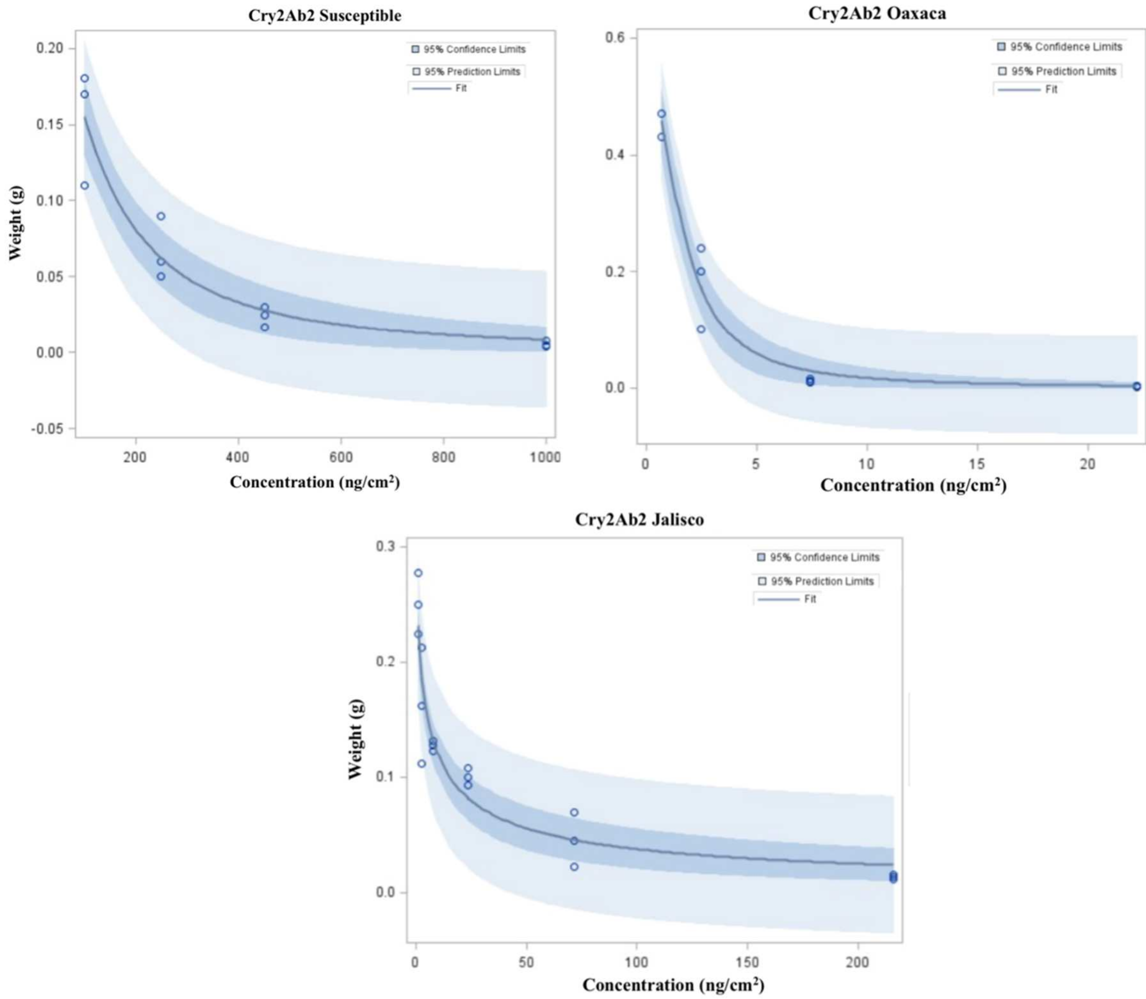


**Figure 6.** Concentration – larval weight reduction response to Cry2Ab2 of fall armyworm from Mexico and the SUS reference population.

Fall armyworm from Puerto Rico

The fall armyworm PR population showed the highest $LC_{50}$ values to the four Bt proteins among all the populations tested (Table 5). However, $LC_{50}$ and $LC_{90}$ were not estimated for Cry1F because the highest dose used (10,000 ng/cm$^2$) did not cause significant mortality. Also, Cry1F didn't cause significant weight differences to estimate the $EC_{50}$. Results with Cry1Ac showed a $RR_{50}$ value of 12.2-fold relative to the $LC_{50}$ of the SUS population. However, we were unable to reach 100% mortality with the highest Cry1Ac concentration used (6,000 ng/cm$^2$) and mortality achieved with the highest concentration was only 62%. In addition, the slope value obtained was the lowest from all Bt proteins (0.5±0.08). For Cry2Ab2 and Cry1A.105, resistance ratios relative to the $LC_{50}$ of the SUS population were less than 2-fold; slope values for these two proteins were similar with those obtained with the fall armyworm Mexican populations.

$EC_{50}$ values estimated for fall armyworm PR with Cry1Ac and Cry1A.105 were significantly higher than the values obtained with fall armyworm SUS and fall armyworm from Mexico. For Cry2Ab2, fall armyworm PR displayed an $EC_{50}$ value significantly lower than that of the susceptible population, but was still higher than the obtained for the fall armyworm populations from Mexico (Table 5). Overall, fall armyworm PR showed higher susceptibility to Cry2Ab2, since both $LC_{50}$ and $EC_{50}$ obtained with this Bt protein were the lowest ($LC_{50}$= 119.2, $EC_{50}$= 24.7±5.8) of the four Cry proteins tested.

Figure 7 shows the weight reduction caused by Cry1Ac in the fall armyworm PR population in comparison with the SUS reference population. A 100% weight reduction was obtained with the highest Cry1Ac concentration in the SUS population. However, in the fall armyworm PR population, only 70% weight reduction was achieved at 6,000 ng/cm$^2$, which was the highest concentration we could achieve with our stock solution.

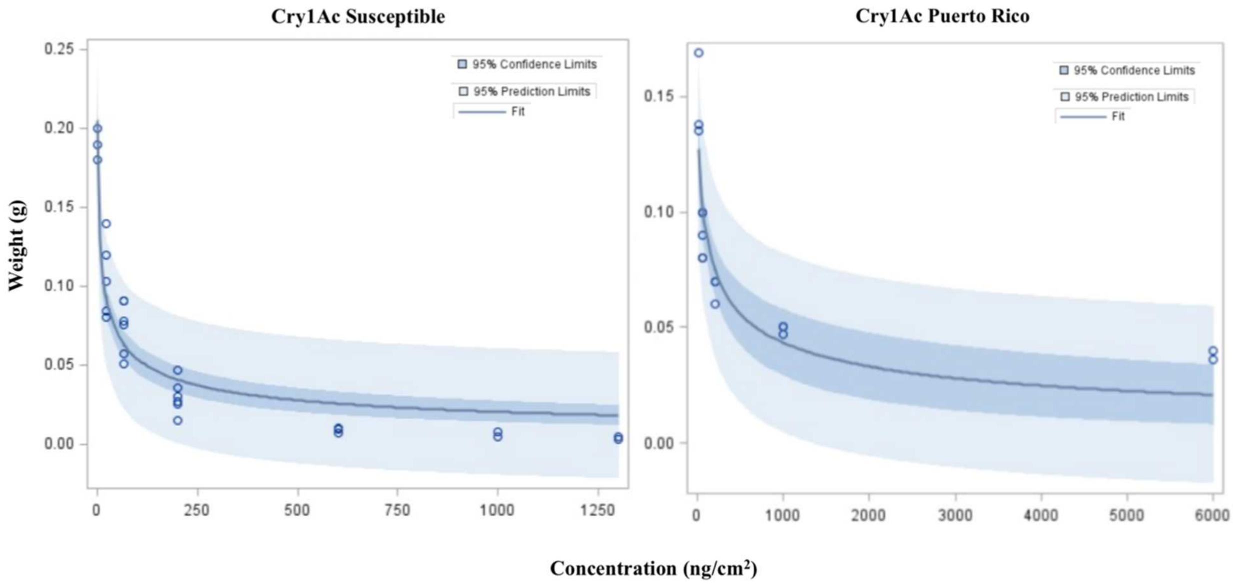


**Figure 7.** Concentration – larval weight reduction response to Cry1Ac of *Spodoptera frugiperda* from Puerto Rico and the SUS reference population.

Figures 8 and 9 show the weight reduction results with Cry1A.105 and Cry2Ab2. Fall armyworm PR response was similar to that of the SUS population, with the main decreases in weight occurring between 7.41 – 1,000 ng/cm$^2$.

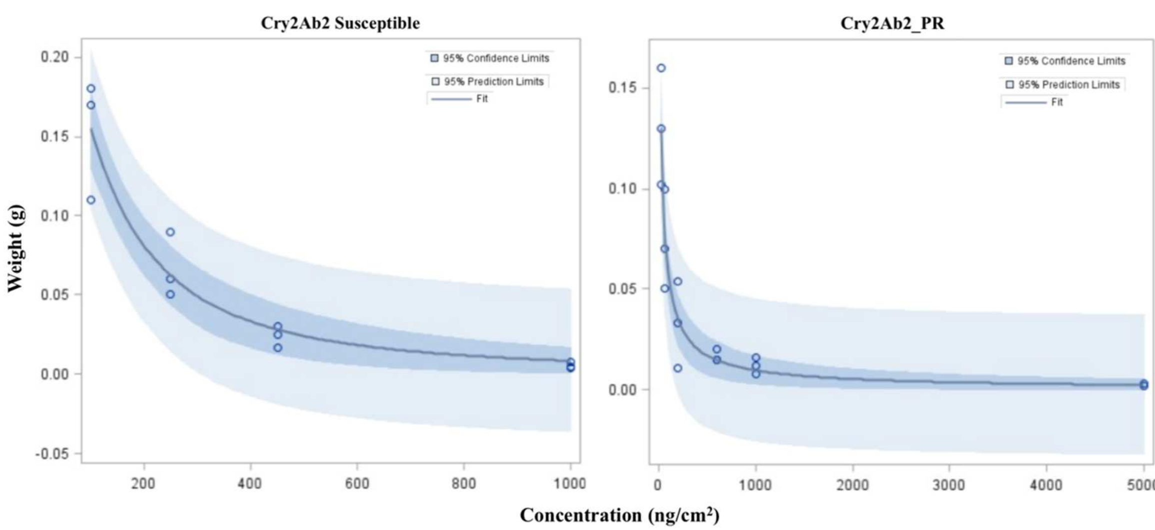


**Figure 8.** Concentration – larval weight reduction response to Cry1A.105 of fall armyworm from Puerto Rico and the SUS reference population.

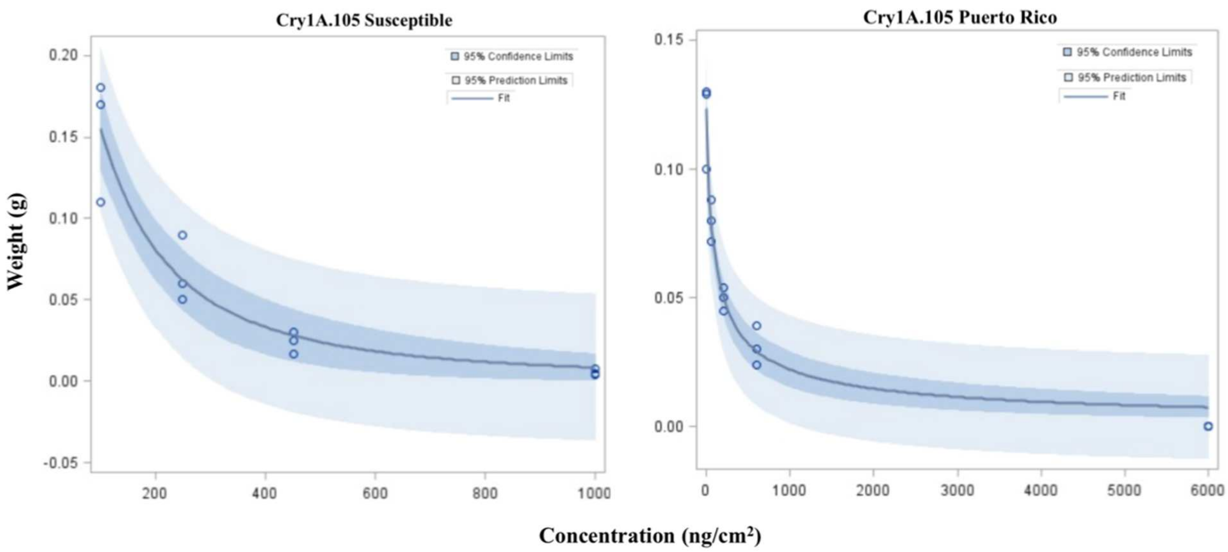


**Figure 9.** Concentration – larval weight reduction response to Cry2Ab2 of fall armyworm from Puerto Rico and the SUS reference population.

## Discussion

Previous analysis of fall armyworm susceptibility to Cry1Fa and Cry1Ac in Mexico, with populations collected from the states of Tamaulipas and Estado de Mexico, located in the north east and central regions of the country respectively, showed that fall armyworm is highly susceptible to these two proteins (Blanco et al. 2010). On more recent field trials, fall armyworm from Tamaulipas showed susceptibility to the Bt corn hybrids, Agrisure 3110®, expressing Cry1Ab and Vip3Aa20; Agrisure Viptera 3111® producing Cry1Ab, Vip3Aa20 and mCry3A; and Agrisure 3000 GT® expressing Cry1Ab and mCry3A (Aguirre et al. 2015). Our results confirm that fall armyworm populations from Mexico are susceptible to other Bt toxins.

Fall armyworm is also the main corn pest in Puerto Rico, where it is now been controlled with pyramided Bt hybrids and synthetic insecticides (Storer et al. 2012, Niu et al. 2013 and Blanco et al. 2016). Because corn in this island is mainly grown for hybrid seed research, pest tolerance is very low, which has led to a high number of insecticide applications to control fall armyworm (Belay et al. 2012). Our data indicates that fall armyworm PR remains highly resistant to Cry1F and Cry1Ac. These results are consistent with the first report by Storer et al. (2010), where fall armyworm populations were collected in 2007 and 2008, meaning that fall armyworm resistance to Cry1F in Puerto Rico has been fixed, even after 10 years since TC1507 maize was removed from the field. This also coincides with the results obtained by Vélez et al. (2013), in which bioassays conducted from 2010 to 2013 showed high levels of resistance to Cry1F in fall armyworm from Puerto Rico.

Cry1F resistance in Puerto Rico has been described as recessive, conferred by a single locus, autosomal and with no maternal effects (Storer et al. 2010, Velez et al. 2013). Additionally, the molecular mechanism of Cry1F resistance in fall armyworm from Puerto Rico and Brazil was characterized as a reduction or complete lack of Cry1F binding to the brush border membrane vesicles (Vélez et al. 2016 and Monnerat et al. 2015). It has been proposed that this mechanism of resistance is behind the high levels of cross-resistance to Cry1Aa proteins in Cry1F field-evolved resistant populations, but low cross-resistance has been found to Cry1Ac, Cry1Ab and Cry2A proteins (Storer et al. 2010, Vélez et al. 2013, Jakka et al. 2014 and Monnerat et al. 2015).

Cry1Ac has been reported to have low biological activity against different populations of fall armyworm, to the point that it is deemed ineffective to control this pest (Yang et al. 2016, Yang et al. 2017). The low value of the log dose-probit regression slope from our experiments (Table 5) indicates that fall armyworm PR exhibits high variability in its response to Cry1Ac. Previous experiments with fall armyworm from different locations in Puerto Rico have demonstrated that resistance to

Cry1Ac is widespread in the corn producing areas (Blanco et al. 2010, Storer et al. 2010). The $EC_{50}$ obtained in our experiments (68 ng/cm$^2$) is 6.5-fold higher than the $EC_{50}$ value (10.4 ng/cm$^2$) obtained by Blanco et al. (2010), indicating that resistance to this Bt protein has increased in the past six years. Furthermore, the fact that we were unable to obtain 100% mortality with the highest Cry1Ac concentration, tells us that this protein displays a marginal effect against fall armyworm PR.

Cry1A.105 is a chimeric Bt protein constituted by domain exchange from the following Bt proteins: Cry1Ab (domain I), Cry1Ac1 (domain II), Cry1F (domain III) and Cry1Ac (C-terminal) (Biosafety Clearing-House 2014). Resistance to Cry1A.105 has been characterized using laboratory-selected resistant fall armyworm populations, showing high levels of cross-resistance between Cry1 proteins (Niu et al. 2016a). Similarly, field-collected fall armyworm in Brazil was selected for resistance to Cry1F and later showed cross-resistance to Cry1A.105 and Cry1Ab, but only low levels of resistance to Cry2Ab2 (Bernardi et al. 2015). Our results showed that although fall armyworm PR is highly resistant to Cry1F ($LC_{50}$ >10,000 ng/cm$^2$) it remains susceptible to Cry1A.105 ($RR_{50}$= 1.4-fold). Cry1F resistant fall armyworm from Puerto Rico has previously been described as susceptible to both Cry1A.105 and Cry2Ab2, so that they have been included in the next generation pyramided Bt corn, event MON89034 expressing Cry1F, Cry1A.105 and Cry2Ab2 (Storer et al. 2012). In addition, Niu et al. (2016a) indicated that the level of dominance of the Cry1A.105 resistance in fall armyworm might vary depending on the following factors: fall armyworm population, bioassay method and the maize hybrid.

Cry2Ab2 was first commercialized in 2010 as a component of the pyramided Bt maize containing the event MON 89034, in combination with Cry1A.105 (Hernández-

Rodríguez et al. 2013 and Niu et al. 2016b). Pyramided Bt hybrids with this same composition have been grown in Puerto Rico after the detection of the field-evolved resistance to Cry1F (Storer et al. 2012). This means that fall armyworm in Puerto Rico has been exposed to this protein for at least seven years. Our results show that fall armyworm PR remains highly susceptible to Cry2Ab2 ($RR_{50}$= 0.5-fold). Comparing FLs of the $LC_{50}$ and $LC_{90}$ between fall armyworm PR, fall armyworm Jal and Oax, we conclude that there is no significant difference in their susceptibility to Cry2Ab2, even though the Mexican fall armyworm populations have never been exposed to this Bt protein.

Fall armyworm has shown high susceptibility to Cry2Ab2 in previous reports (Hernández-Rodríguez et al. 2013, Bortolotto et al. 2016, Niu et al. 2016b). Midgut binding experiments with this protein in different Lepidopteran species: *Heliothis virescens*, *Helicoverpa zea*, *Helicoverpa armigera* (Gouffon et al. 2011); *Helicoverpa armigera* and *Helicoverpa puntigera* (Caccia et al. 2010), have demonstrated that Cry2Ab proteins don't share binding sites with Cry1A and Cry1F proteins. This could mean that if cross-resistance were to appear in fall armyworm from Puerto Rico to Cry2Ab2, a more generalized resistance mechanism might be involved.

The different susceptibility levels to Bt proteins of fall armyworm from different regions in Latin America (Monnerat et al. 2006), show the importance of regional monitoring to test changes in the control levels that these proteins exert over this pest. A study analyzing mitochondrial haplotype ratios between fall armyworm from Puerto Rico, Florida, Texas and Brazil, concluded that fall armyworm from Puerto Rico showed more similarities with fall armyworm from Florida, than with fall armyworm from Texas and Brazil, suggesting interactions between fall armyworm from those two regions

(Rodney N. Nagoshi, Meagher, and Jenkins 2010). The later detection of fall armyworm resistant to Cry1F in Florida seems to confirm this interaction and raises concerns over the potential spread of Bt resistance alleles in fall armyworm that can affect regions where this pest is already a great management challenge (F. Huang et al. 2014).

## Conclusion

GE corn producing Bt proteins has high potential for fall armyworm control in Mexico. This country may benefit from all the research that has been generated regarding resistance management to Bt technology, and from the lessons learned from regions where fall armyworm has developed field-evolved resistance to Bt crops.

Fall armyworm management in Puerto Rico has successfully preserved susceptibility to Cry2Ab and Cry1A.105 proteins to date. Unfortunately, Cry1F and Cry1Ac resistance seem to be established in fall armyworm from this island.

## CHAPTER 4: RECOVERY POTENTIAL OF THE FALL ARMYWORM *Spodoptera frugiperda* (JE SMITH) (LEPIDOPTERA: NOCTUIDAE) AFTER LONG-TERM HIGH-DOSE EXPOSURE OF BT PROTEINS


### Abstract

Fall armyworm has shown low susceptibility to certain Bt proteins and is one of the Lepidopteran pests with the highest number of field-evolved resistance cases to Bt corn. Fitness cost studies have shown that Bt proteins increase the developmental time and decrease pupal weight of the resistant. Other studies on short-term Bt exposure indicate that there is potential for recovery when larvae pass the third instar threshold.

We carried out experiments to determine fall armyworm neonates' potential for recovery after long-term exposure to high doses of the Bt proteins Cry1F and Cry1Ac. Survival, weight, developmental stage and adult emergence were evaluated using fall armyworm from Puerto Rico and the susceptible population. Long-term Cry1F exposure in the fall armyworm susceptible population showed reduced survival with the highest concentration (31%), and both Cry1F and Cry1Ac caused later adult emergence compared to controls. No detrimental effect resulted from long-term exposure to Cry1F and Cry1Ac in fall armyworm from Puerto Rico. Susceptible fall armyworm have the potential to recover after it has been exposed to 600 ng/cm$^2$ of Cry1F and 1,000 ng/cm$^2$ of Cry1Ac for seven days under laboratory conditions. Therefore, various implications for IRM strategies are discussed.

## Introduction

Genetically engineered (GE) crops with the ability to produce insecticidal proteins from the bacteria *Bacillus thuringiensis* (Bt), have become fundamental in agriculture in many developed and developing countries (Smyth et al. 2015). In some of these regions, this technology has helped decrease the amount of synthetic insecticides deployed in the field, which benefited non-target organisms such as natural enemies and wildlife, plus decreased pollution and human health hazards (Huang et al. 2002, Qaim and Zilberman 2003, Bennett et al. 2006, Qaim 2010).

The evolution of resistance to Bt technology is the main threat to the sustainability of this management strategy. There has been extensive theoretical and experimental analysis of the current Insecticide Resistance Management (IRM) strategies designed to delay the evolution of resistance to GE Bt crops (Gould 1994, 1998, 2000, Tabashnik 2008a, 2008b, Huang, Andow, and Buschman 2011, Tabashnik et al. 2013, Yang et al. 2014 and Garcia et al. 2016). The most popular strategy worldwide is the “high-dose/refuge”, in which farmers are required to plant a percentage of non-Bt acreage to serve as refuge for susceptible insects. This strategy is predicated on a low frequency of resistance alleles in the population, the few resistant survivors mate with susceptible insects from the refuge, their heterozygous offspring would be killed by the high-dose Bt protein produced by the GE plants, delaying population-level resistance (Andow and Hutchinson, 1998 and Huang et al. 2011).

The survival of heterozygote insects is the main factor that can change the frequency of resistance alleles in a given population (Horikoshi et al. 2016). Therefore, to develop a successful IRM program it is necessary to consider all the factors that may favor the survival of heterozygotes. For instance, Fall armyworm larvae have been described to move between plants and have considerable dispersion across rows in crop fields (Pannuti et al. 2016). Larval movement in the field between Bt and non-Bt plants may expose heterozygotes to sublethal doses of Bt proteins and increase their chance for survival (Binning et al. 2014, Garcia et al. 2016). This is particularly relevant since there is evidence that Bt corn plants may not produce homogenous concentrations of Bt proteins in every tissue, which can vary depending on the hybrid genetic background and stress factors (Trtikova et al. 2015).

The development of second generation pyramided GE crops producing multiple toxins, allowed the re-configuration of the refuge from separate blocks or rows, to an integrated design. Now Bt and non-Bt seeds are included in the seed mix, in a strategy known as “refuge-in-the-bag” (RIB). Since RIB simplifies agronomic practices, the most important advantage is ensuring farmer’s compliance with the refuge component of IRM programs (Rule et al. 2014, Fei et al. 2015). The key conditions for the efficacy of RIB are: 1) no cross resistance between Bt proteins produced by the GE hybrid, 2) no single toxin crops are grown in proximity to the pyramided Bt field and 3) limited movement of the target pest’s larval stages from plant to plant (Fei et al. 2015).

Another important assumption for the success of current IRM strategies is that the targeted pests will exhibit considerable fitness costs associated with the resistance to Bt proteins. Experiments conducted with fall armyworm have shown that this insect displays negligible fitness costs for the resistance to Cry1F (Jakka et al. 2014, Vélez et al. 2014). Yet, there is evidence that some fall armyworm populations show significant fitness costs for the resistance to this Bt protein, suggesting that the genetic basis of Cry1F resistance in this pest may be more diverse than previously considered (Dangal and Huang 2015). Therefore, the objective of this study is to determine and compare the recovery potential between fall armyworm from Puerto Rico and a susceptible population after it has been exposed to high doses of Cry1F and Cry1Ac for a seven-day period.

## Materials and Methods

Insect populations

Insects from Puerto Rico (PR) were collected in corn from June to November 2016. Monsanto provided a fall armyworm laboratory susceptible (SUS) population. Fall armyworm field populations were collected during larval development directly from affected corn plants. Insects were placed in 10 ml plastic cups with 4-6 ml of artificial diet (Southland Products Incorporated: fall armyworm diet). Larvae were sent to our laboratory at the Food Safety and Toxicology building, Michigan State University, East Lansing, Michigan, United States.

Insect rearing

Larvae were held at 27±1 ºC and a photoperiod of 16:8 hours light: dark. Insect development was supervised every other day, when pupae turned from orange-red to dark red, they were placed in groups of 40 inside collapsible insect cages (Bioquip, Rancho Dominguez, CA) to provide room for emerging adults to fly and mate, a sponge with a solution of 10% sugar in a petri dish was provided as food source. After three days of adult emergence, adults from each cage were placed in 1L paper brown bags and a wet sponge with a solution of 10% sugar in a petri dish was added. Every other day moths were transferred to a new paper bag; eggs masses laid in the brown paper bag were separated and placed in a plastic container with moist paper towels. Neonate larvae were transferred to bioassay trays (Frontier Agricultural Sciences, Newark, DE) with 1 ml of artificial diet per well. Neonates ±24h old were used in the bioassays.

Bt proteins

DOW Agrosciences (Indianapolis, IN) provided lyophilized Cry1F and Cry1Ac and 10 mM CAPS buffer (3-(cyclohexylamino)-1-propane sulfonic acid), diluted in deionized water, pH 10.5. Proteins were kept in a desiccator (Fisher Scientific rectangular Desi-Vac Container™), both proteins and buffer were placed in a refrigerator at 4°C.

Bioassays

These bioassays were conducted with Cry1F and Cry1Ac proteins using the fall armyworm SUS and PR populations. One or two concentrations that caused above 85% mortality in the diet overlay bioassays in Chapter 3 were used. Table 6 shows the Bt protein concentrations used per population and total number of insects treated. Since there was no significant mortality with Cry1F in the PR population, we used the highest dose included in the diet overlay bioassay (10,000 ng/cm$^2$). Control treatments consisted of diet plus CAPS buffer. Fall armyworm neonates were exposed to the Bt proteins using the procedure described for the diet overlay bioassay (pp. 50-51). After seven days of exposure, only larvae weighing ≤0.1 mg or that failed to molt to second instar but that still moved when prodded with a small paintbrush, were transferred to individual plastic 10 ml soufflé cups (Gordon® Food Service) with 6 ml of fresh diet.

The larvae from the PR population was treated with Cry1F, since there was no distinguishable detrimental effect of the Bt protein on treated larvae, we conducted the experiment with larvae that was exposed to 10,000 ng/cm$^2$ of Cry1F. Controls were also transferred to cups with 6 ml of fresh diet after the 7d period. Three to four replications were conducted per Bt protein for each population. Recovery and development was evaluated every three days, the parameters recorded for each insect were: 1) survival (live/dead), 2) weight (g), 3) life stage, and 4) adult emergence.

**Table 7**. Bt protein concentrations and total number of insects used in recovery bioassays.

| **Population** | **Bt protein** | **Concentration (ng/cm$^2$)** | **n[a]** |
|---|---|---|---|
| Susceptible | Cry1F | 600 | 256 |
| | | 1,000 | |
| | Cry1Ac | 1,000 | 256 |
| | | 1,300 | |
| Puerto Rico | Cry1F | 10,000 | 128 |
| | Cry1Ac | 3,000 | 160 |
| | | 6,000 | |

[a]Total number of insects used.

<u>Statistical analysis</u>

For the Bt recovery bioassays, weight was analyzed using a randomized block design in a repeated measures model, using proc glimmix in SAS version 9.3 (SAS Institute 2011), with “repetition” as the random factor. Survival and adult emergence were analyzed using a random block designs in a repeated measures model with a binary distribution using proc glimmix in SAS and “repetition” as the random factor. For the stage parameter, we analyzed the recorded weight for each developmental stage by treatment for each fall armyworm population, using one-way ANOVA in proc glm, with treatment as the main effect in SAS version 9.3 (SAS Institute 2011). Treatment means were separated using Tukey-Kramer adjustment for multiple comparisons at α=0.05.

## Results

To analyze the results obtained in the recovery bioassay, we divided this section with the parameters evaluated: survival, adult emergence, weight and life stage. We compared the effects of high concentrations of Bt proteins Cry1F and Cry1Ac in the development of the treated insects between the two populations: fall armyworm SUS and PR.

<u>Survival</u>

Cry1F. Figure 10A reveals the average survival percentages obtained with the fall armyworm SUS population. High survival for all treatments was recorded on the third day after the larvae were placed in the non-treated diet (control= 100%, 600 ng/cm$^2$=92% and 1,000 ng/cm$^2$= 83%), and no statistical differences between the means were found on this day: control vs 600 ng/cm$^2$ $P$=0.9600, control vs 1,000ng/cm$^2$ $P$=0.9572 and 600ng/cm$^2$ vs 1,000 ng/cm$^2$ $P$=0.1881. In the following evaluation days, survival in the lowest Cry1F concentration (600 ng/cm$^2$= 600 ng/cm$^2$) was not statistically different than the control, but a clear difference was observed between the control and the highest Cry1F concentration (1,000 ng/cm$^2$) throughout the evaluation period. On the last evaluation (day 26) 1,000 ng/cm$^2$, with an average survival of 31%, differed significantly from both 600 ng/cm$^2$ ($P$=0.0003) and the control ($P$<0.0001).

Figure 10B shows that survival in the fall armyworm PR population after treatment with Cry1F (10,000 ng/cm$^2$) decreased from 98% three days after transferred to non-treated diet, to 60% on the last evaluation date (day 26). However, these results were not statistically different from the control ($P$=0.5646).

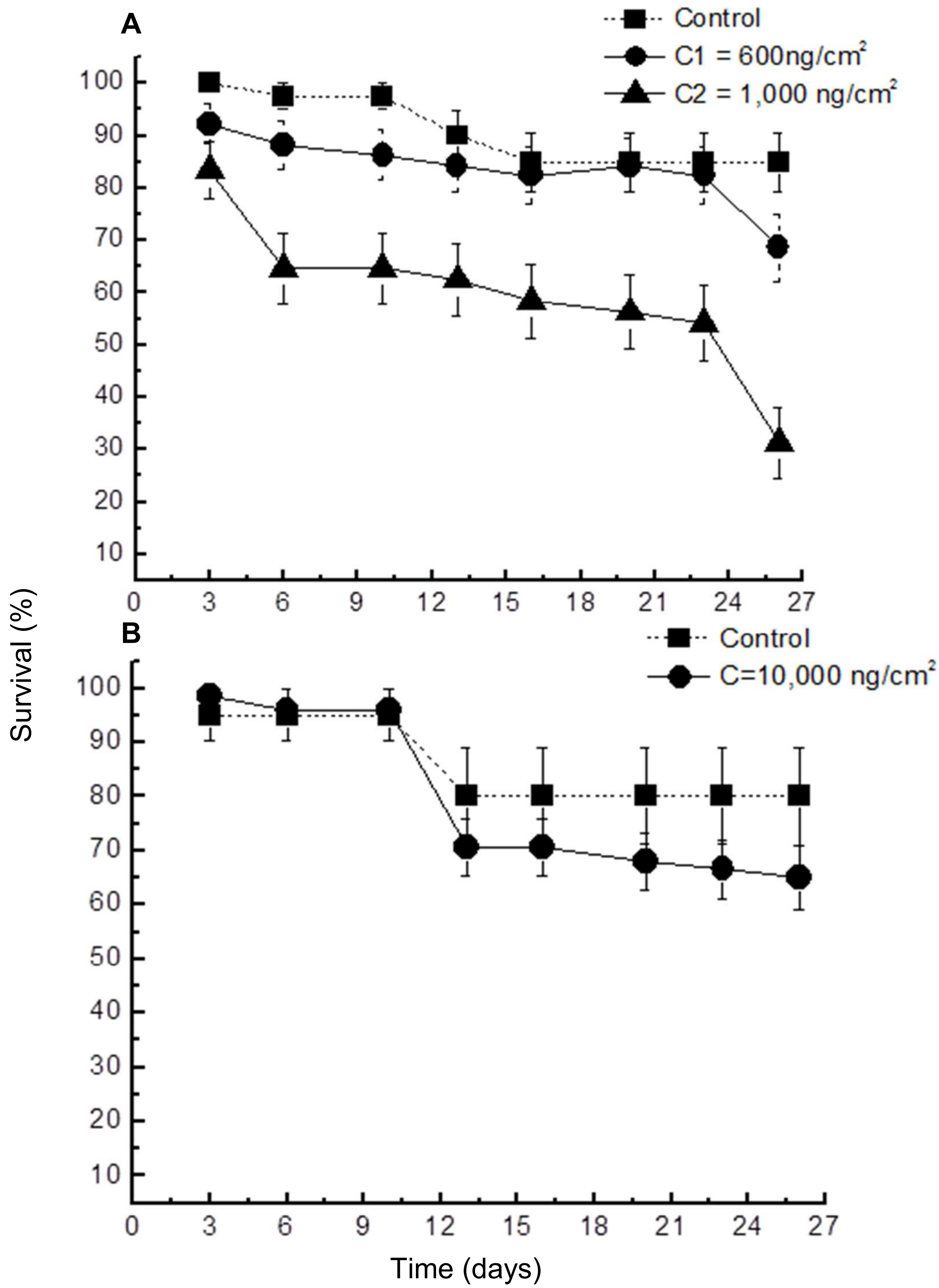


**Figure 10.** Fall armyworm survival percentages throughout development after exposure to high concentrations of Cry1F: A) susceptible reference and B) Puerto Rico population.

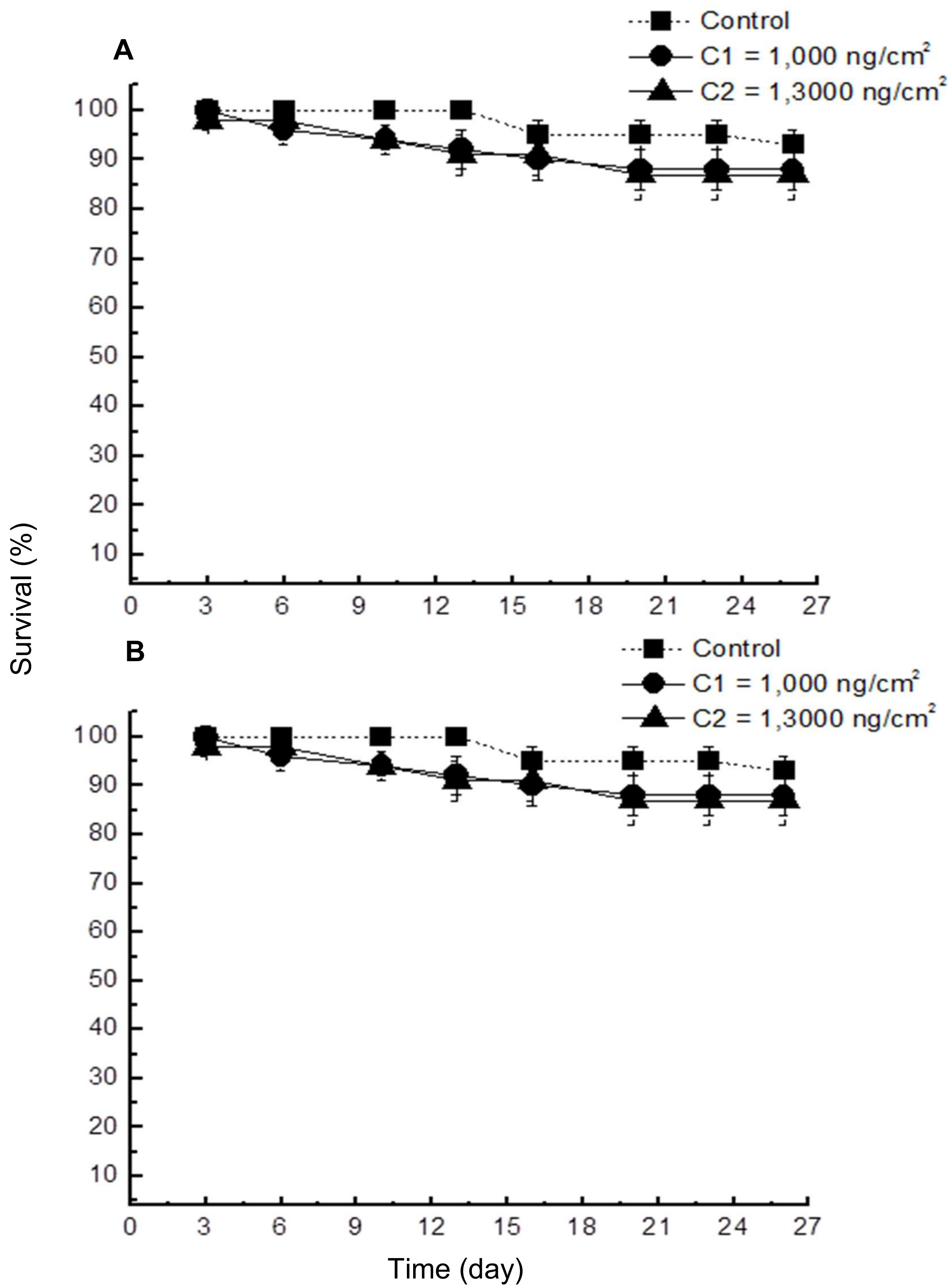


**Figure 11.** Fall armyworm survival percentages throughout development after exposure to high concentrations of Cry1Ac: A) susceptible reference and B) Puerto Rico population.

Cry1Ac. As observed in Figure 11A, all treatments in the fall armyworm SUS recorded high survival rates. In general, mortality in this population remained low throughout the evaluation period with an average survival percentage of 93% for the control and 88% and 87% for 1,000 ng/cm2 and 1,300 ng/cm2 respectively. Nevertheless, they were not statistically different: control vs 1,000 ng/cm2 (P=0.3588), control vs 1,300 ng/cm2 (P=0.2391) and 1,000 ng/cm2 vs 1,300 ng/cm2 (P=0.7952). Yet, these data did suggest a trend associated with the 1,000 ng/cm2 concentration (above).

No statistical differences in survival were found between treatments and control in fall armyworm PR (Figure 11B). Ten days after transfer to non-treated diet, all treatments had an average survival above 90%. In the last evaluation day ($26^{th}$ day) the control treatment recorded the lowest survival percentage (75%) compared with the two Bt treatments: 3,000 ng/cm$^2$= 81% and 6,000 ng/cm$^2$= 79%, but they were not statistically different: control vs 3,000 ng/cm$^2$ ($P$=0.3915), Control vs 1,300 ng/cm$^2$ ($P$=0.4659) and 3,000 ng/cm$^2$ vs 6,000 ng/cm$^2$ ($P$=0.7877).

Adult emergence

In fall armyworm SUS (Figure 12A), the two Cry1F concentrations had different effects in adult emergence in comparison with the control ($P$=<0.0001). Adult emergence in the lowest Bt concentration (600 ng/cm$^2$) was registered at the same time than the emergence in the control, but the percentage was low (9%), while the control had an initial percentage of 72%. Initial adult emergence in the highest Bt concentration (1,000 ng/cm$^2$) was on the $23^{rd}$ day, with a very low percentage (4%). On this same day, adult emergence had increased in both control and 600 ng/cm$^2$ treatment to 85% and

40% respectively. Yet, on the last day, emergence in the control remained 85% as in the previous evaluation, but both Bt treatments increased their emergence rate significantly: 600 ng/cm$^2$= 62% and 1,000 ng/cm$^2$=31%.

In fall armyworm PR (Figure 12B), adult emergence was significantly different between control and Bt protein treatment (10,000 ng/cm$^2$) ($P$=<0.0001). Initial adult emergence was registered on the same day (23$^{rd}$) for both control and 10,000 ng/cm$^2$. However, there were significant differences in the emergence rate between them: on day 23, 75% of control adults had emerged, while for the 10,000 ng/cm$^2$ treatment only 33%. On the last day (26$^{th}$), 85% of adults had emerged in the control, while 64% was the total adult emergence percentage for the 10,000 ng/cm$^2$ treatment.

Cry1Ac. In Figure 13A, we can see in the fall armyworm SUS population adult emergence was first registered for the control on day 20, while emergence in the Bt treatments was first registered on day 23. Although the overall emergence percentage was higher in the control (93%), than in the treatments, 84% for both 1,000 ng/cm$^2$ and 1,300 ng/cm$^2$, they were not statistically different: control vs 1,000 ng/cm$^2$ ($P$=0.1248), control vs 1,300 ng/cm$^2$ ($P$=0.1373), and 1,000 ng/cm$^2$ vs 1,300 ng/cm$^2$ ($P$=0.9511).

In fall armyworm PR (Figure 13B), emergence in both control and Bt treatments 3,000 ng/cm$^2$ and 6,000 ng/cm$^2$ was registered until day 23. Emergence percentages for control (23%) and 3,000 ng/cm$^2$ (20%) were statistically equivalent ($P$=0.8340) on the 23$^{rd}$ day, 6,000 ng/cm$^2$ had a higher initial emergence rate of 50%. Unexpectedly, on the 26$^{th}$ day, both control and 6,000 ng/cm$^2$ showed above 75% emergence, while 3,000 ng/cm$^2$ emergence was 57%. Nonetheless, both treatments were not statistically different from the control: control vs 3,000 ng/cm$^2$ ($P$=0.4249), and control vs 6,000 ng/cm$^2$ $P$=0.3667.

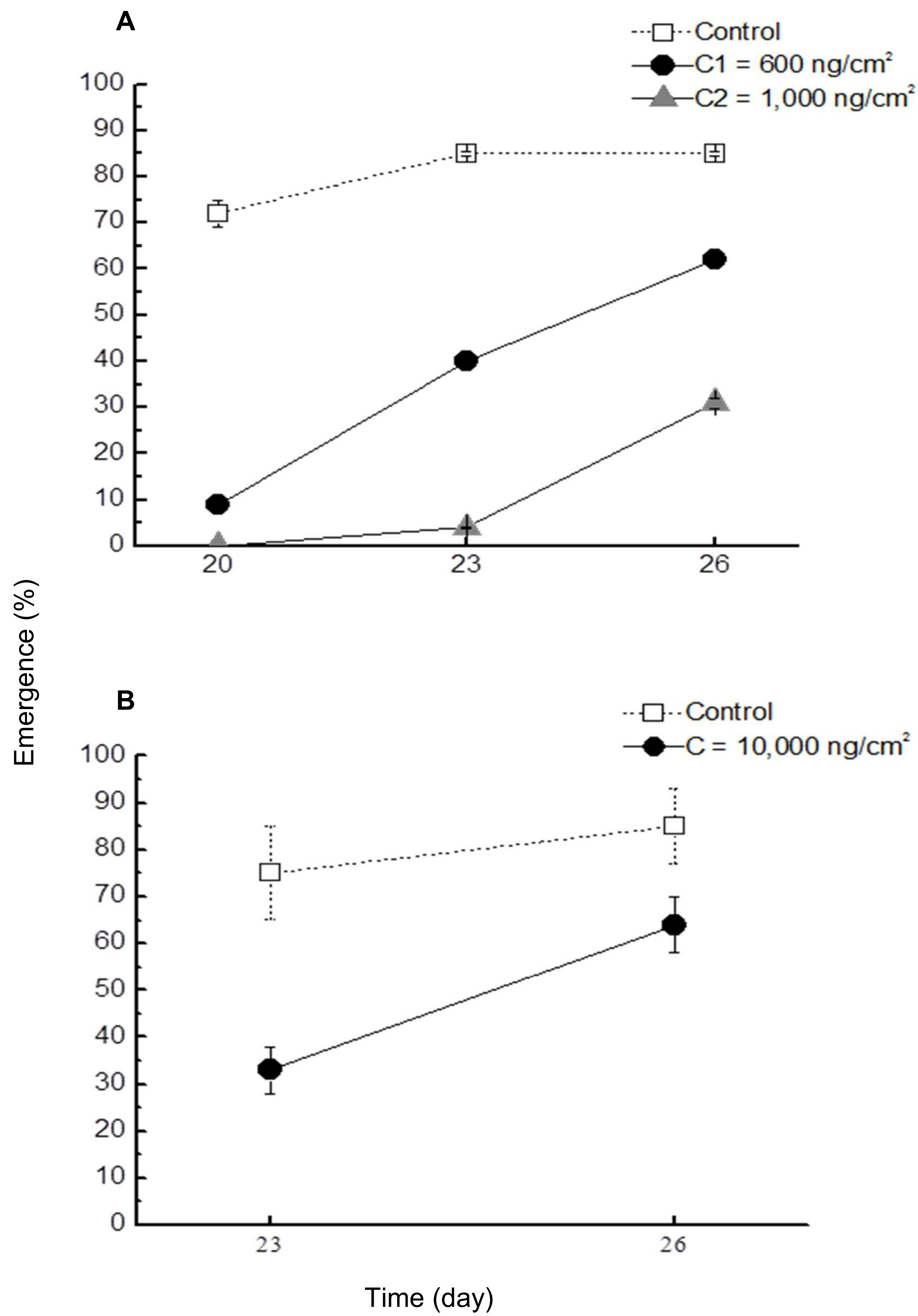


**Figure 12.** Cumulative adult emergence after exposure to high concentrations of Cry1F: A) susceptible reference and B) Puerto Rico population.

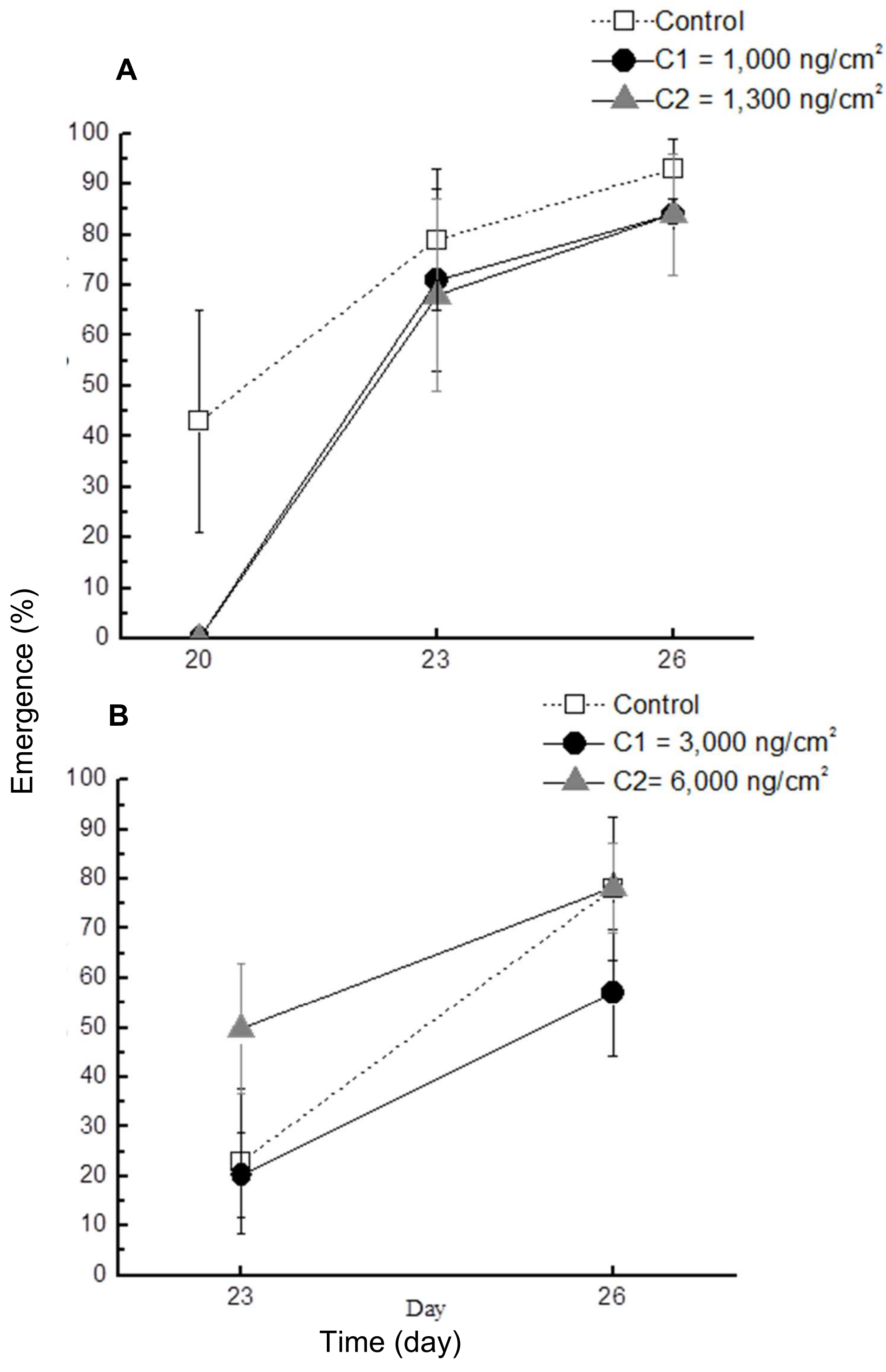


**Figure 13.** Cumulative adult emergence after exposure to high concentrations of Cry1F: A) susceptible reference and B) Puerto Rico populations.

Weight and stage

Cry1F. In Figure 14A and B show the effect of Cry1F in the weight of treated fall armyworm SUS and PR respectively. In fall armyworm SUS, there was a dramatic effect in the weight of the Bt treatments, 600 ng/cm$^2$ and 1,000 ng/cm$^2$ development was completely out of phase in comparison with control. Although both 600 ng/cm$^2$ and 1,000 ng/cm$^2$ treatments started with similar weight (below ≤0.1 mg), after the second evaluation day (6), there was a significant differentiation between them 600 ng/cm$^2$=0.18g and 1,000 ng/cm$^2$=0.06g ($P$<0.0001). Yet, in the last three evaluation dates, there were no statistical differences between treatments and control weights: control vs 600 ng/cm$^2$ ($P$=0.9020), control vs 1,000 ng/cm$^2$ ($P$=0.9617). Figure 14 further shows that the weight of each developmental stage for 600 ng/cm$^2$ and 1,000 ng/cm$^2$ with respect to the control started differentiating after sixth instar.

Weight variations between control and 10,000 ng/cm$^2$ in fall armyworm PR were less striking and there was no out of phase development in comparison with fall armyworm SUS (Figure 14B). Means were significantly different on the following evaluation days: 13 ($P$=0.0472), 16 ($P$=0.0209) and 20 ($P$=0.0091). However, on the last two days, mean weights were not statistically different: 23 ($P$=0.7680) and 26 ($P$=0.1639)

Cry1Ac. The most obvious effect when comparing the two populations SUS and PR was the altered development in the Bt treated larvae from the SUS population, but no significant effect was observed in the fall armyworm PR population.

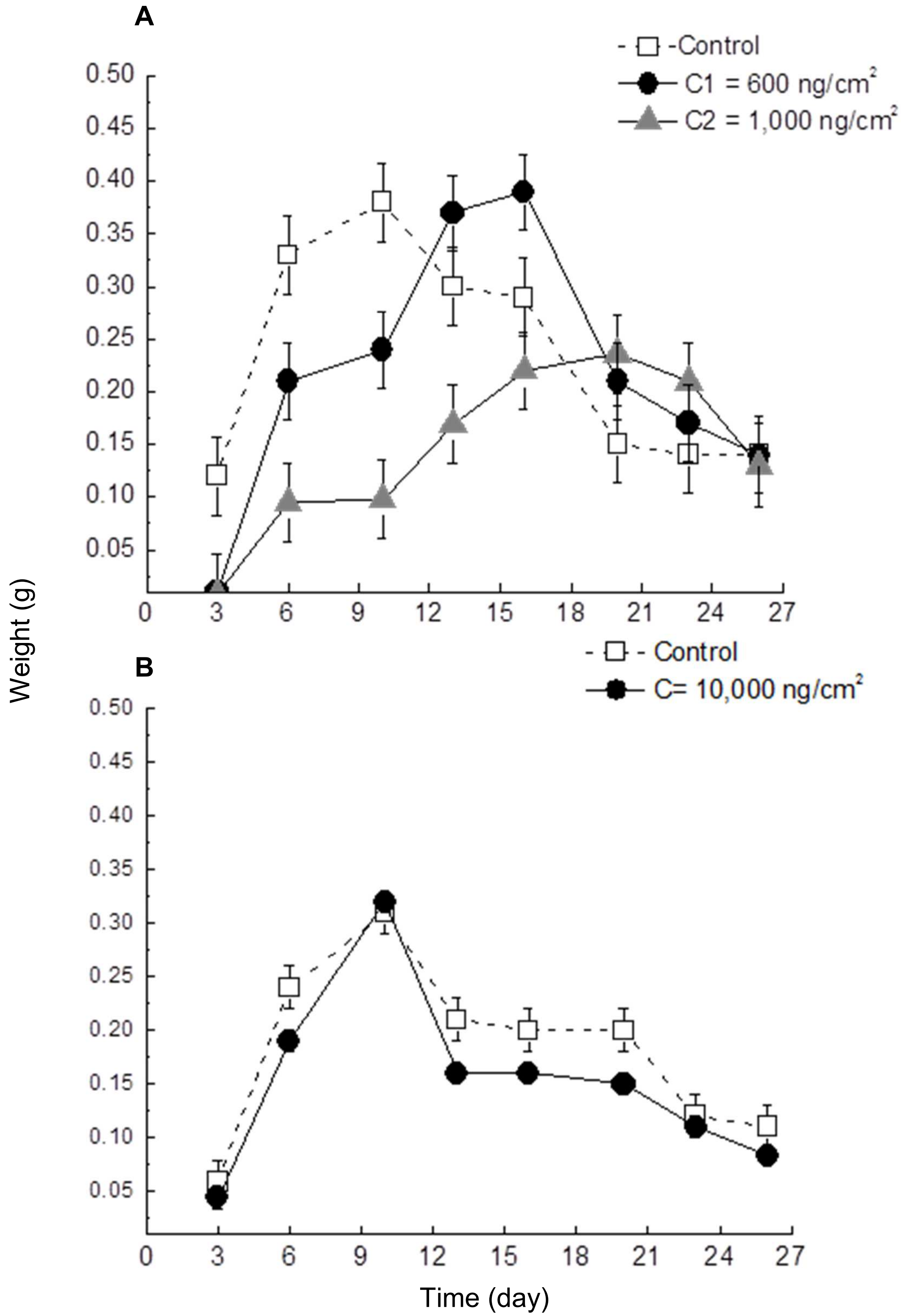


**Figure 14.** Fall armyworm weight after exposure to high concentrations of Cry1F: A) susceptible reference and B) Puerto Rico population.

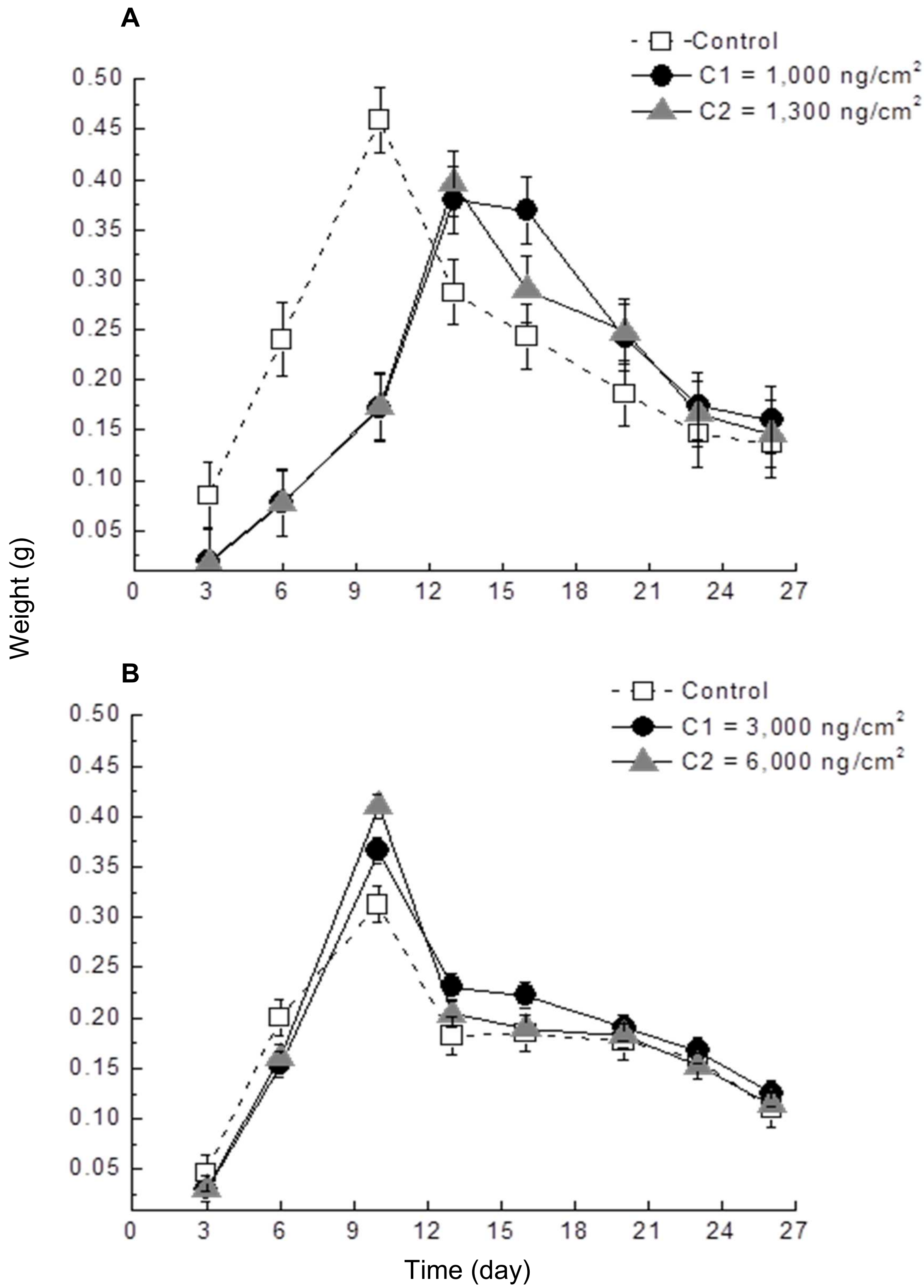


**Figure 15.** Fall armyworm weight after exposure to high concentrations of Cry1Ac: A) susceptible reference and B) Puerto Rico population.

There were no significant weight differences between the two Bt concentrations in fall armyworm SUS and although development was not synchronized with the control, by day 26 all treatment means were statistically similar: control vs 1,000 ng/cm$^2$ ($P$=0.0675), control vs 1,300 ng/cm$^2$ ($P$=0.1906) and 1,000 ng/cm$^2$ vs 1,300 ng/cm$^2$ ($P$=0.4309).

In the PR population, significant differences between treatment means were found on day 10: control vs 3,000 ng/cm$^2$ ($P$=0.0158), control vs 6,000 ng/cm$^2$ ($P$<0.0001) and 3,000 ng/cm$^2$ vs 6,000 ng/cm$^2$ ($P$=0.0158). The highest weight mean was registered on the highest Bt concentration 6,000 ng/cm$^2$=0.41g, followed by 3,000 ng/cm$^2$=36.6g and control=31.2g. Nonetheless, weight means were not statistically different in the following evaluation dates.

## Discussion

One of the main pillars of an effective IRM programs in Bt crops is the compliance of the refuge component (Tabashnik et al. 2013). The RIB strategy was design to counter act the lack of compliance of the refuge, placing the responsibility on the industry rather than the farmers. However, there has been serious criticism from expert scientists that claim this strategy poses high risks for resistance development (Yang et al. 2014, Fei et al. 2015). One of the strongest arguments is that if Bt plants and non-Bt plants are in close proximity in corn, this would facilitate the survival of potential heterozygote larvae, increasing the frequency of resistance alleles. Previous work has proven that fall armyworm has the potential to recover from Bt protein damage when exposed as second or third instars (Hardke et al. 2011, Binning et al. 2014). Still, it is important to determine whether fall armyworm can stand long-term exposure as earlier instars and complete development.

In general, there was a clear difference between survival means obtained with the two concentrations of Cry1F in the fall armyworm SUS population (Figure 10). Throughout the evaluation period, a clear decrease in survival % was observed in the C2 treatment (1,000 ng/cm$^2$). In contrast, both CT and C treatments in fall armyworm PR followed a similar trend, with survival % dropping on the 13$^{th}$ day and remaining relatively stable thereafter. The lower survival percentage in fall armyworm PR on the last evaluation date, in comparison with the fall armyworm SUS population, could be attributed to the fact that the fall armyworm SUS population has adapted to laboratory rearing conditions, rather than to the effect of the Bt protein treatment, since the difference in % survival between CT and C was not significantly different in fall armyworm PR.

For Cry1Ac, survival trends were similar between fall armyworm populations. As with Cry1F, a relatively lower survival percentage was observed in fall armyworm PR in comparison with the fall armyworm SUS. Yet, since the survival means in fall armyworm PR were significantly equivalent between treatments, we can also assume that the differences with the fall armyworm SUS were due to fall armyworm PR not being adapted to laboratory conditions. These results further confirm the reports of Cry1Ac displaying low activity against fall armyworm (Yang et al. 2016, Yang et al. 2017).

Our results show that there was asynchronous adult emergence in both Cry1F and Cry1Ac treatments between fall armyworm SUS and PR, but also within treatments (Figure 11). Former experiments to determine potential fitness costs associated with field-evolved resistance to Cry1F in fall armyworm from Puerto Rico concluded that resistant fall armyworm shows longer larvae developmental time, which then results in different adult emergence days between resistant (RR and RS) and susceptible (SS) fall armyworm populations (Jakka et al. 2014). The authors pointed out that this may have costly consequences in the field, as the main purpose of the refuge strategy would be violated, because SS moths wouldn't be in contact with RR and RS individuals (Jakka et al. 2014).

Long-term exposure to Cry1F in fall armyworm SUS had a clear detrimental effect in the weight of the treated insects (Figure 12A and Figure 13A), but this effect was less significant in the Cry1Ac treatments (Figure 3). Fall armyworm PR displayed lower mean weight values than the SUS for all stages, regardless of the Bt treatment (Figure 12 and Figure 13). These results agree with the fitness costs experiments conducted by Dangal and Huang (2015), where they found 61.1% weight reduction in larvae in fall armyworm from Puerto Rico. Hence, the lower weight values in our experiments with fall armyworm PR could be due to the overall fitness cost of the field-evolved resistance to Cry1F and Cry1Ac and not to the effect of the Bt protein treatments.

These results further illustrate fall armyworm's resilience to the effect of Bt proteins. Since, it has been suggested that first instar fall armyworm larvae are the ones to determine the feeding site (Pannuti et al. 2015), it was necessary to determine the potential recovery of this pest to midgut damage from early stages. In addition, fall armyworm movement in field conditions has been determined to occur within neighboring plants in the same row from where the egg mass were located (Pannuti et al. 2016). In the context of RIB, this means that fall armyworm may be in contact to sublethal doses of Bt proteins; if the heterozygous Bt exposed larvae moves to a non-Bt plant nearby and recovers, this can favor the survival of those heterozygotes and increase the frequency of resistance alleles in the population (Binning et al. 2014). This can also happen if older instars developing in non-Bt plants later move to a Bt-plant, when they are less susceptible to midgut damage by the Bt proteins (Binning et al. 2014 and Garcia et al. 2016). This is especially relevant in RIB fields, where cross-pollination increases the risk of refuge corn to express single or a mixture of Bt proteins in the kernels (Burkness and Hutchison 2012, Yang et al. 2014).

In addition, feeding preference of non-Bt versus Bt plants has been characterized in another Noctuid, the cabagge looper (CL) *Trichoplusia ni* (Hübner) with Bt and non-Bt cotton, first instar larvae were able to detect Bt cotton and move to non-Bt cotton leaves (Li, Greenberg and Liu 2006). Likewise, Binning et al. (2014) described that early instar fall armyworm larvae show aversion to Bt expressing plants and may move to a non-Bt host. Fall armyworm neonates would face more than one Bt protein in each Bt plant, drastically reducing neonate survival chances, unless the population exhibits cross-resistance to more than one of the pyramided traits and a high capacity to recover after damage caused by the Bt proteins.

## Conclusion

High levels of fall armyworm recovery after been exposed to high doses of Cry1Ac and Cry1F for seven days suggest that this specie has an outstanding capacity to recover from to severe weight loss and perhaps midgut damage caused by Bt proteins. However, additional recovery experiments with plant tissue and whole plants should be designed to determine additional factors that may play a significant role in the potential for recovery after Bt exposure in fall armyworm. This chapter provides evidence that fall armyworm has the potential to recover from severe midgut damage caused by Bt proteins, and sets the base for further analysis on the molecular mechanism of midgut damage and restoration.

# **CHAPTER 5**: CONCLUSION

Fall armyworm is the most important corn pest in Latin America (Blanco et al. 2016), and over the last two decades its relevance has increased in southern US, where it used to be considered a secondary pest (Sparks 1979). With the advent of new molecular and biochemical tools, we now understand more about this species, migration patterns (Johnson 1987, Nagoshi and Meagher 2008, Nagoshi et al. 2012, Westbrook et al. 2016), host preferences, strain separation (Pashley 1986, Pashley and Martin 1987, Pashley 1988, Pashley et al. 1992, Nagoshi and Meagher 2004) and mechanisms of resistance to insecticides  (Yu 1983, 1991, 1992, Adamczyk et al. 1997, Yu et al. 2003, Tavares et al. 2010, Belay et al. 2012). Yet, fall armyworm has demonstrated significant complexity that challenges making general assumptions on its response to control technologies.

## **Status of synthetic insecticide susceptibility in fall armyworm**

In the second chapter, of synthetic insecticide susceptibility was updated in fall armyworm collected from different regions with different management backgrounds in Mexico and Puerto Rico. Corn production in these two territories has significantly different purposes, which translates into different levels of selection pressure on fall armyworm populations. Traditionally, the general idea is that the higher the selection pressure with insecticides, the higher resistance levels evolve in field populations.

### Puerto Rico

In Puerto Rico, IRAC designed a very strict control program against fall armyworm ,that considers the rotation of nine modes of action during the three corn production seasons in the Island (Head 2016). But because of the low pest tolerance in this production system, up to 29 sprays are needed to achieve the desired pest control levels. This represents an increase of four sprays from the 25 reported by Belay et al (2012).

Our study results show that fall armyworm in Puerto Rico perhaps is the most resistant population to synthetic insecticides on the American continent. These results display the highest resistance levels to older modes of action like organophosphates, carbamates and pyrethroids, as well as to newer chemistries including diamides. Yet, this study shows that fall armyworm remains relatively susceptible to the nAChR allosteric modulators: specifically spinosad and spinetoram. In addition, we included a modes of action that is not currently used for fall armyworm control on this island, GLUCL allosteric modulators: emamectin benzoate and abamectin, have demonstrated the highest levels of toxicity against fall armyworm.

To preserve the thriving seed industry in Puerto Rico which is facing the continuous threat of fall armyworm, it will be necessary to design new strategies. Although there are alternative synthetic insecticides to control this pest, the need to integrate other control measures is critical. In its report from 2016, IRAC remarks the need to consider alternative strategies to control fall armyworm, including an established a fallow period between production seasons, or alternatively, introduce crop rotation (Head 2016). Yet, fall armyworm has a wide host range; therefore, continuous fall armyworm monitoring and finding a suitable crop that fall armyworm does not utilize could be another challenge.

Another alternative for fall armyworm control mentioned by IRAC, is the preservation of beneficial insects (Head 2016). Blanco et al (2010) reported about 50% of parasitism in fall armyworm samples collected from Santa Isabel, Puerto Rico; yet, during our study 6-7 years later, the proportion of parasitized insects failed to reach 5%. Our samples were collected in Ponce, Puerto Rico 34 km (driving distance) between the two locations. Unfortunately, this could mean that the populations of beneficial insects are declining. An in depth analysis of monitoring the presence of non-target beneficial arthropods is essential throughout the several growing seasons in order to determine each season's status.

Mexico

In Mexico, corn is the most important staple food and the crop that is produced in every state. For this reason, pest control in corn-fields varies widely between and within regions (Blanco et al. 2014). Blanco et al. (2014) also noted that this heterogeneity is the main reason behind the lack of sound region-wide IPM programs. Fall armyworm is ranked as the most important pest in most of the corn growing regions in this country; farmers report the need to spray at least 2-3 times per crop cycle, but this may vary in different growing systems (Blanco et al. 2016). We expected to detect resistance cases to commonly used insecticides in fall armyworm from regions where there is high dependency to this control tool. But, fall armyworm's susceptibility to insecticides varied between regions and collection years. Our data was not correlated with the production system's from which the samples were collected. This outcome suggests the interplay of other factors that may have significant influence on the pest response to insecticide selection pressure. In addition, a more comprehensive sampling site of fall armyworm populations will be important to determine with more precision the pesticide resistance status.

There are many aspects of fall armyworm habits that should be analyzed for the Mexican context including migration patterns within the country. For example, fall armyworm from Oaxaca was collected from corn fields with traditional agricultural practices and zero sprays of synthetic insecticides. Nonetheless, in the population from 2013 we found a significant resistant ratio for permethrin ($RR_{50}$=19-fold), one of the most common insecticides used in Mexico. One potential explanation is that on that year there was an influx of resistant fall armyworm individuals from neighboring regions where synthetic insecticides are sprayed. This seems to be confirmed by the bioassays conducted in 2016 with fall armyworm from the same region in Oaxaca, where we obtained a six-fold lower resistant ratio ($RR_{50}$=3-fold) than the previously obtained in 2013.

Understanding the potential interactions of fall armyworm populations in Mexico could yield an array of IPM programs that could be adopted for different production systems. Although resistance is not widespread, nor is it consistent throughout the years, yet there are still reports of the need to conduct as high as 12 sprays in a single production cycle (Unpublished data, personal communication with Mota-Sanchez). This number of sprays per season is perhaps bigger than the number of sprays in Puerto Rico. However, there is in general only one corn production season per year in Mexico. In addition, the continued pesticide overuse can lead to pest resistance or emergence of other pest species which could cause significant economic and health issues. Thus, there is a clear need for sound and responsible use of synthetic insecticides to protect human health and the environment. Climate change might result in an increase of the density and number of generations of fall armyworm since FAW survival is higher during dry seasons. Therefore, an increase in pesticide selection pressure will result in higher resistance evolution to deployed pesticides.

## Status of Bt susceptibility in fall armyworm

The third chapter provided information on the status of fall armyworm susceptibility to Bt proteins in Mexico and Puerto Rico. Currently, this technology is not available commercially in Mexico, while in Puerto Rico pyramided Bt corn is grown for parent seed production.

The high levels of Cry1F and Cry1Ac fall armyworm resistance in the population from Ponce, Puerto Rico (after more than ten years since the detection of resistance in this island) suggests that the resistance alleles in these populations are now fixed. Vélez et al. (2014) indicated that the lack of significant fitness costs was an indicator of the frequency of resistance alleles in fall armyworm from Puerto Rico, which was higher than previously thought. In contrast, Cry2Ab2 and Cry1A.105 remain highly active against fall armyworm from Puerto Rico, which means that Integrated Resistance Management (IRM) programs in the island should focus on strategies that keep the effectiveness of these proteins.

In Mexico, fall armyworm populations have demonstrated high susceptibility to all Bt proteins tested, Cry1A.105, Cry2Ab2, Cry1F and Cry1Ac. $LC_{50}$ and $LC_{90}$ values were lower than those obtained for the susceptible reference population. Therefore, this technology could be a suitable alternative for Mexican large-scale corn growers; taking into consideration the lessons learned from other regions where fall armyworm developed resistance to Bt proteins.

One of the pillars of IPM practice is the continuous monitoring of susceptibility of targeted pests to the control tools applied in the field. Moreover, would be wise to determine the baseline susceptibility and human health risks to an insecticide before it is introduced in a new region. Without this key information, local farmers will not be able to predict the production potential and the active ingredient's lifespan.

### Fall armyworm recovery after long-term Bt exposure

Chapter four provides evidence that fall armyworm has the potential to recover from severe weight loss caused by long-term exposure to Bt proteins. There was a clear detrimental effect in the survival of the susceptible reference population with the highest dose of Cry1F. In this same population, both Cry1F and Cry1Ac treated larvae showed asynchronous development and adult emergence rates. Particularly, adult emergence rates in Cry1F treated insects were significantly lower than the control; however, this effect was not observed with Cry1Ac. These results appear to confirm that Cry1F display higher toxicity against fall armyworm than Cry1Ac.

Fall armyworm from Puerto Rico was not affected by the highest doses of Cry1F and Cry1Ac, even after feeding for seven continuous days since first instar. Interestingly, the only significant detrimental effect was the rate of adult emergence with Cry1F, but not with Cry1Ac. This suggests that there could be a hidden long-term negative effect after long-term exposure in this resistant population.

### Future work

There are many routs to go from here. Fall armyworm from Puerto Rico presents a special opportunity to study insect adaptations to different types of insecticides, since populations from this island have developed field-evolved resistance to at least six different mode of action of synthetic insecticides and two Bt proteins: Cry1F and Cry1Ac. One explanation is that fall armyworm has developed one major generalized resistance mechanism, like an over expression of detoxification enzymes, that would allow insects to break down and excrete toxic molecules. So a logical next step should be to conduct biochemical and molecular studies to explore whether resistance in fall armyworm from Puerto Rico is do to a metabolic mechanism.

Fitness cost studies have concluded that larval development and growth are not affected by high doses of Cry1F and Cry1Ac in fall armyworm from Puerto Rico. Our results with the susceptible population, on the potential of recovery after long-term Bt protein exposure suggests that fall armyworm has the capacity of recovery after severe midgut damage. Next research steps with this resistant population should include: 1) measure the extent of gut damage after long-term Cry protein exposure and 2) examine the potential recovery mechanisms. These studies would provide important insights into an additional mechanism through which this population is coping with selection pressure by Bt technology.

RAGM

December 2017

**APPENDIX**

## RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2016-05

Rebeca Adriana Gutierrez Moreno

"Susceptibility of the fall armyworm, S*podoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) to Bt proteins and synthetic insecticides from different corn production systems in Mexico and Puerto Rico"

Museum where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

**Table 8.** List of voucher specimens:

| Family | Genus/Species | Life stage | Quantity | Preservation method |
|---|---|---|---|---|
| **Noctuidae** | *Spodoptera frugiperda* | Adult | 20 | Pinned |

## REFERENCES

## REFERENCES

Abbott, W. S. 1925. A Method of Computing the Effectiveness of an Insecticide. J. Econ. Entomol 18 (2): 265–67.

Adamczyk, J., W. Holloway, B. R., Leonard, and J.B. Graves. 1997. Susceptibility of fall armyworm collected from different plant hosts to selected insecticides and transgenic Bt cotton. J. Cotton Sci. 1 (1): 21.

Adams, A., J. Gore, A. Catchot, F. Musser, D. Cook, N. Krishnan, and T. Irby. 2016. Susceptibility of *Helicoverpa zea* (Lepidoptera: Noctuidae) neonates to diamide insecticides in the midsouthern and southeastern United States. J. Econ. Entomol. 109 (5): 2205–2209.

Aguirre, Luis A., A. Hernández, M. Flores, R. Pérez-Zubiri, E. Cerna, J. Landeros, and G. A. Frías. 2015. Comparison of the level of damage by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on genetically-modified and conventional maize plants in northern Mexico. Southwest. Entomol. 40 (1): 171–78.

Ahmad, M., M. S. Ahmed, and H. Sayyed. 2009. Efficacy of insecticide mixtures against pyrethroid- and organophosphate-resistant populations of *Spodoptera litura* (Lepidoptera: Noctuidae). Pest Manag Sci. 65 (3): 266.

Ahmad, M., I. Denholm, and R. H. Bromilow. 2006. Delayed cuticular penetration and enhanced metabolism of deltamethrin in pyrethroid-resistant strains of *Helicoverpa armigera* from China and Pakistan. Pest Manag Sci. 62 (9): 805–805.

Ahmad, M., and M. I. Arif. 2009. Resistance of Pakistani field populations of spotted bollworm *Earias vittella* (Lepidoptera: Noctuidae) to pyrethroid, organophosphorus and new chemical insecticides. Pest Manag Sci. 65 (4): 433–439.

Alava, D. and A. Lagunes Tejeda. 1976. Resistencia cruzada a varios tipos de insecticidas después de producir resistencia a paratión metílico en *Spodoptera exigua* (Hubner) [jitomate, *Lycopersicum esculentum*]. In 11. Congreso Nacional de Entomología. México, DF., 7-10 Mar 1976.

Albert, L. A. 2006. Panorama de los plaguicidas en México. RETEL Revista de Tóxicología En Línea 8: 1–17.

Al-Sarar, A., F. R. Hall and R. A. Downer. 2006. Impact of spray application methodology on the development of resistance to cypermethrin and spinosad by fall armyworm *Spodoptera frugiperda* (JE Smith). Pest Manag Sci. 62 (11): 1023–31.

Alyokhin, A., M. Baker, D. Mota-Sanchez, G. Dively, and E. Grafius. 2008. Colorado potato beetle resistance to insecticides. American Journal of Potato Research 85 (6): 395–413.

Alyokhin, A., D. Mota-Sanchez, M. Baker, W. E. Snyder, S. Menasha, M. Whalon, G. Dively, and W. F. Moarsi. 2015. The red queen in a potato field: integrated pest management versus chemical dependency in colorado potato beetle control. Pest Manag. Sci. 71 (3): 343–356.

Andow, D. A. 1998. Now or never: serious new plans to save a natural pest control. Union of Concerned Scientists. Cambridge, Massachusetts. http://www.ucsusa.org/sites/default/files/legacy/assets/documents/food_and_agriculture/now-front.pdf

Andrews, K. L. 1988. Latin American research on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Fla. Entomol. 71 (4): 630–53.

Ashley, T. R., B. R. Wiseman, F. M. Davis, and K. L. Andrews. 1989. The fall armyworm: a bibliography. The Florida Entomologist 72 (1): 152–202.

Balasubramani, V., A. H. Sayyed, and N. Crickmore. 2008. Genetic characterization of resistance to deltamethrin in *Plutella xylostella* (Lepidoptera: Plutellidae) from India. J. Econ. Entomol. 101 (6): 1911–1918.

Baltazar, B. M., L. Castro-Espinoza, A. Espinoza-Banda, J. M. de la Fuente-Martinez, J. A. Garzon-Tiznado, J. Gonzalez-Garcia, M. A. Gutierrez. 2015. Pollen-mediated gene flow in maize: implications for isolation requirements and coexistence in Mexico, the center of origin of maize. Plos One 10 (7).

Barros, E. M., J. B. Torres, J. R. Ruberson, and M. D. Oliveira. 2010. Development of *Spodoptera frugiperda* on different hosts and damage to reproductive structures in cotton. Entomol Exp Appl. 137 (3): 237–45.

Baxter, S. W., J.-Z. Zhao, L. J. Gahan, A. M. Shelton, B. E. Tabashnik, and D. G. Heckel. 2005. Novel genetic basis of field-evolved resistance to bt toxins in *Plutella xylostella*. Insect Molecular Biology 14 (3): 327–334.

Belay, D. K., R. M. Huckaba, and J. E. Foster. 2012. Susceptibility of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), at Santa Isabel, Puerto Rico, to different insecticides. Fla. Entomol. 95 (2): 476–478.

Bennett, R., U. Kambhampati, S. Morse, and Y. Ismael. 2006. Farm-level economic performance of genetically modified cotton in Maharashtra, India. Rev. Agr. Econ. 28 (1): 59–71.

Bernardi, O., R. J. Sorgatto, A. D. Barbosa, F. A. Domingues, P. M. Dourado, , R. A. Carvalho, S. Martinelli, G. P. Head, and C. Omoto. 2014. Low susceptibility of *Spodoptera cosmioides*, *Spodoptera eridania* and *Spodoptera frugiperda* (lepidoptera: noctuidae) to genetically-modified soybean expressing Cry1ac protein. Crop Prot. 58: 33–40.

Binning, R. R., J. Coats, X. Kong, and R. L. Hellmich. 2014. Susceptibility and aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt maize and considerations for insect resistance management. Journal of Economic Entomology 107 (1): 368–374.

Biosafety Clearing-House. 2014. Gene and DNA sequence. Database. February 24.

Blanco, C. A., W. Chiaravalle, M. Dalla-Rizza, J. R. Farias, M. F. García-Degano, G. Gastaminza, D. Mota-Sánchez, G. G. Murúa, C. Omoto, and B. K. Pieralisi. 2016. Current situation of pests targeted by Bt crops in Latin America. Curr Opin Insect Sci. 15: 131–138.

Blanco, C. A., J. G. Pellegaud, U. Nava-Camberos, D. Lugo-Barrera, P. Vega-Aquino, J. Coello, A. P. Terán-Vargas, and J. Vargas-Camplis. 2014. Maize pests in Mexico and challenges for the adoption of integrated pest management programs. J Integr Pest Manag. 5(4): 1–9.

Blanco, C. A., M. Portilla, J. L. Jurat-Fuentes, J. F. Sánchez, D. Viteri, P. Vega-Aquino, A. P. Terán-Vargas. 2010. Susceptibility of Isofamilies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1Ac and Cry1Fa proteins of *Bacillus thuringiensis*. Southwest Entomol. 35 (3): 409–415.

Bloomquist, J. R., and T. A. Miller. 1985. Carbofuran triggers flight motor output in pyrethroid-blocked reflex pathways of the house fly. Pest Biochem Physiol. 23 (2):247-55.

Borlaug, Norman E. 1983. Contributions of conventional plant breeding to food production. Science 219 (4585): 689–93.

Bortolotto, O. C., A. de Freitas-Bueno, A. P. de Queiroz, and G. Vieira-Silva. 2016. Larval development of *Spodoptera eridania* and *Spodoptera frugiperda* fed on fresh ear of field corn expressing the Bt proteins (Cry1F and Cry1F+ Cry1A.105+ Cry2Ab2). Cienc Rural, no. AHEAD: 0–0.

Bottrell, D. R. 1979. Integrated Pest Management.

Bourguet, D., M. Desquilbet, and S. Lemarié. 2005. Regulating insect resistance management: the case of non-Bt corn refuges in the US. Journal of Environmental Management 76 (3): 210–20.

Bravo, Alejandra, S. Jansens, and M. Peferoen. 1992. Immunocytochemical localization of *Bacillus thuringiensis* insecticidal crystal proteins in intoxicated insects. J. Invertebr. Pathol. 60 (3): 237–246.

Brewer, M. J., and J. T. Trumble. 1989. Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entom. 82 (6): 1520–1526.

Brookes, G., and P. Barfoot. 2017. Environmental impacts of genetically modified (GM) crop use 1996–2015: impacts on pesticide use and carbon emissions. GM Crops & Food 8 (2): 117–47.

Burkness, E. C., and W. D. Hutchison. 2012. Bt pollen dispersal and Bt kernel mosaics: integrity of non-Bt refugia for lepidopteran resistance management in maize. J. Econ. Entom. 105 (5): 1773–1780.

Busato, G. R., A. D. Grützmacher, M. Silveira-Garcia, F. Pinheiro-Giolo, M. João-Zotti, and J. de Magalhães-Bandeira. 2005. Exigências térmicas e estimativa do número de gerações dos biótipos 'milho' e 'arroz' de *Spodoptera frugiperda*. Pesqui. Agropecu. Bras. 40: 329–35.

CABI and EPPO. 1997. Data Sheets on Quarantine Pests Spodoptera Frugiperda. CAB International. https://www.eppo.int/QUARANTINE/data_sheets/insects/LAPHFR_ds.pdf.

Caccia, S., C. S. Hernández-Rodríguez, R. J. Mahon, S. Downes, W. James, N. Bautsoens, J. Van Rie, and J. Ferre. 2010. Binding site alteration is responsible for field-isolated resistance to *Bacillus thuringiensis* Cry2A insecticidal proteins in two *Helicoverpa species*. PloS One 5 (4).

Capinera, J. L. 2000. Fall armyworm, *Spodoptera frugiperda* (JE Smith)(Insecta: Lepidoptera: Noctuidae). University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS. Gainsville, Florida, United States.

Carroll, M. W., G. Head, M. Caprio, and L. Stork. 2013. Theoretical and empirical assessment of a seed mix refuge in corn for southwestern corn borer. Crop Protection 49: 58–65.

Carson, HL.1963. Silent spring. Coll. Univ. 38 (3): 294–96.

Carvalho, R. A., C. Omoto, L. M. Field, M. S. Williamson, and C. Bass. 2013. Investigating the Molecular Mechanisms of Organophosphate and Pyrethroid Resistance in the Fall Armyworm Spodoptera Frugiperda. PLoS One 8 (4).

Casmuz, A., M. L. Juárez, M. G. Socías, M. G. Murúa, S. Prieto, S. Medina, E. Willink, and G. Gastaminza. 2010. Revisión de los hospederos del gusano cogollero del maíz, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Rev. Soc. Entomol. Argent. 69 (3–4): 209–231.

Chandler, L.D., S. D. Pair, and J. R. Raulston. 1992. Effects of selected insect growth regulators on longevity and mortality of corn earworm and fall armyworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 85 (5): 1972–1978.

Che, W., T. Shi, Y. Wu, and Y. Yang. 2013. Insecticide resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. J. Econ. Entomol. 106 (4): 1855–62.

Chilcutt, C. F., and B. Tabashnik. 1995. Evolution of pesticide resistance and slope of the concentration-mortality line: are they related? J. Econ. Entomol. 88 (1): 11–20.

Chilcutt, C. F., and B. E. Tabashnik. 2004. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. Proceedings of the National Academy of Sciences of the United States of America 101 (20): 7526–29.

Conner, J. K., and D. L. Hartl. 2004. A Primer of Ecological Genetics. Sinauer Associates Incorporated.

Conway, G. R., and E. B. Barbier. 2013. After the Green Revolution: Sustainable Agriculture for Development. Routledge.

Cortez-Mondaca, E. and Rodriguez-Cota, F. G. 2012. Manejo integrado de gusano cogollero basado en el aprovechamiento de enemigos naturales en maíz, en Sinaloa, México. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Folleto técnico No. 36.

Dangal, Vikash, and Fangneng Huang. 2015. Fitness costs of Cry1F Resistance in two populations of fall armyworm, *Spodoptera frugiperda* (JE Smith), collected from Puerto Rico and Florida. J. Invertebr. Pathol. 127: 81–86.

Dhurua, S., and Govind T. Gujar. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders)(Lepidoptera: Gelechiidae), from India. Pest Manag. Sci. 67 (8): 898–903.

Díaz-Gomez, O., J. C. Rodriguez, A. M. Shelton, A. Lagunes-T, and R. Bujanos-M. 2000. Susceptibility of *Plutella xylostella* (L.)(Lepidoptera: Plutellidae) populations in Mexico to commercial formulations of *Bacillus thuringiensis*. J. Econ. Entom. 93 (3): 963–70.

Diez-Rodríguez, G. I., and C. Omoto. 2001. Inheritance of lambda-cyhalothrin resistance in *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). Neotrop Entomol. 30 (2): 311–16.

Dively, G. P., P. D. Venugopal, and C. Finkenbinder. 2016. Field-evolved resistance in corn earworm to Cry proteins expressed by transgenic sweet corn. Plos One 11 (12).

Dulmage, H. T., H. M. Graham, and E. Martinez. 1978. Interactions between the tobacco budworm, *Heliothis virescen*s, and the δ-endotoxin produced by the HD-1 isolate of *Bacillus thuringiensis* var. kurstaki: relationship between length of exposure to the toxin and survival. J. Invertebr. Pathol. 32 (1): 40–50.

Dulmage, H. T., and E. Martinez. 1973. The effects of continuous exposure to low concentrations of the δ-endotoxin of B*acillus thuringiensi*s on the development of the tobacco budworm, *Heliothis virescens*. J. Invertebr. Pathol. 22 (1): 14–22.

FAO. 2017. Staple foods: what do people eat? FAO Corporate Document Repository. Accessed August 10. http://www.fao.org/docrep/u8480e/u8480e07.htm.

Fao, Ifad. 2015. WFP (2014) The State of Food Insecurity in the World 2014: Strengthening the Enabling Environment for Food Security and Nutrition, Rome: FAO.

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. dos Santos, and C. Omoto. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Prot. 64: 150–58.

Federación, Diario Oficial de la. 2005. Ley de Bioseguridad de Organismos Genéticamente Modificados. México, Ciudad de México, 1–44.

Fei, Y., D. Kerns, and F. Huang. 2015. Refuge-in-the-bag strategy for managing insect resistance to Bt maize. Outlooks on Pest Management 26 (5): 226–228.

Fernandez-Cornejo J. 2004. The seed industry in US agriculture: an exploration of data and information on crop seed markets, regulation, industry structure, and research and development. Agriculture Information Bulletin No. (AIB-786), 81. https://www.ers.usda.gov/webdocs/publications/42517/13616_aib786_1_.pdf?v=41055.

Ferré, J., M. D. Real, J. Van Rie, S. Jansens, and M. Peferoen. 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. P. Ntl Acad Sci. 88 (12): 5119–5123.

Finney, D. J. 1971. Probit Analysis: 3d Ed. Cambridge University Press.

Frazier, M. 2017. A short history of pest management. Pennsylvania Integrated Pest Management. Accessed August 10. http://extension.psu.edu/pests/ipm/schools-childcare/schools/educators/curriculum/contents/shorthistory.

Garcia, A. G., C. P. Ferreira, F. L. Cônsoli, and W. AC Godoy. 2016. Predicting evolution of insect resistance to transgenic crops in within-field refuge configurations, based on larval movement. Ecol Complex 28: 94–103.

Gassmann, A.J., J.L. Petzold-Maxwell, E.H.Clifton, M.W. Dunbar, A.M. Hoffmann, D.A. Ingber and R.S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. P. Ntl Acad Sci. 111(14): 5141-5146.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS One 6 (7).

Giraudo, M., F. Hilliou, T. Fricaux, P. Audant, R. Feyereisen, and G. Le Goff. 2015. Cytochrome P450s from the fall armyworm (*Spodoptera frugiperda*): responses to plant allelochemicals and pesticides. Insect Mol. Biol. 24 (1): 115–28.

Goergen, G., P. Lava-Kumar, S. B. Sankung, A. Togola, and M. Tamò. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith)(Lepidoptera, Noctuidae), a new alien invasive pest in west and central Africa. PLoS One 11 (10).

Gould, F.. 1988. Genetic engineering, integrated pest management and the evolution of pests. Trends Biotechnol. 6 (4): S15–18.

Gould, F. 1994. Potential and Problems with High□dose Strategies for Pesticidal Engineered Crops. Biocontrol Sci. Tech. 4 (4): 451–61.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Ann Rev Entom. 43 (1): 701–26.

Gould, F. 2000. Testing Bt refuge strategies in the field. Nat. Biotechnol. 18 (3): 266–67.

Groot, A. T., M. Unbehend, S. Hänniger, M. L. Juárez, S. Kost, and D. G. Heckel. 2016. Evolution of reproductive isolation of *Spodoptera frugiperda*. Pheromone Communication in Moths: Evolution, Behavior, and Application, 291.

Gryspeirt, A., and J.C. Grégoire. 2012. Effectiveness of the high dose/refuge strategy for managing pest resistance to *Bacillus thuringiensis* (Bt) plants expressing one or two toxins. Toxins 4 (10): 810–35.

Gunning, R. V., H. T. Dang, F. C. Kemp, I. C. Nicholson, and G. D. Moores. 2005. New resistance mechanism in helicoverpa armigera threatens transgenic crops expressing *Bacillus thuringiensis* Cry1Ac toxin. Appl Environ Microb 71 (5): 2558–2563.

Gunning, R. V., G. D. Moores, and A. L. Devonshire. 1999. Esterase inhibitors synergise the toxicity of pyrethroids in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pestic. Biochem. Phys. 63 (1): 50–62.

Hardke, J. T., B. R. Leonard, F. Huang, and R. E. Jackson. 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins. Crop Protection 30 (2): 168–72.

Head, G. 2016. IRAC-US efforts in Puerto Rico. http://www.irac-online.org/documents/puerto-rico-task-team/.

Hernández, E., Y. C. Pérez, D. Jiménez-García, J. Cinco-Patrón, and H. Bernal. 2017. Management and health of three corn farming systems in the Region of Llanos de San Juan, Puebla, Mexico. Agroecol. Sust. Food. 41 (1): 76–97.

Hernández-Rodríguez, C. S., P. Hernández-Martínez, J. Van Rie, B. Escriche, and J. Ferré. 2013. Shared midgut binding sites for Cry1A. 105, Cry1Aa, Cry1Ab, Cry1Ac and Cry1Fa proteins from *Bacillus thuringiensis* in two important corn pests, *Ostrinia nubilalis* and *Spodoptera frugiperda*. PloS One 8 (7): e68164.

Higley, L. G., and L. P. Pedigo. 1996. Economic Thresholds for Integrated Pest Management. Vol. 9. U of Nebraska Press.

Höfte, H., and H. R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol Rev. 53 (2): 242–55.

Horikoshi, R. J., D. Bernardi, O. Bernardi, J. B. Malaquias, D. M. Okuma, L. L. Miraldo, F. S. de A. Amaral, and C. Omoto. 2016. Effective dominance of resistance of *Spodoptera frugiperda* to Bt maize and cotton varieties: implications for resistance management. Scientific Reports 6 (October): 34864.

Hruska, A. J., and S. M. Gladstone. 1988. Effect of period and level of infestation of the fall armyworm, *Spodoptera frugiperda*, on irrigated maize yield. Fla. Entomol. 249–54.

Huang, F., D. A. Andow, and L. L. Buschman. 2011. Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. Entomol. Exp. Appl. 140 (1): 1–16.

Huang, F., J. A. Qureshi, G. P. Head, P. A. Price, R. Levy, F. Yang, and Y. Niu. 2016. Frequency of *Bacillus thuringiensis* Cry1A. 105 resistance alleles in field populations of the fall armyworm, *Spodoptera frugiperda*, in Louisiana and Florida. Crop Prot. 83: 83–89.

Huang, F., J. A. Qureshi, R. L. Meagher Jr, D. D. Reisig, G. P. Head, D. A. Andow, X. Ni, D. Kerns, G. D. Buntin, and Y. Niu. 2014. Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt Maize. PloS One 9 (11).

Huang, J., R. Hu, S. Rozelle, and C. Pray. 2005. Insect-resistant GM Rice in farmers' fields: assessing productivity and health effects in China. Science 308 (5722): 688–90.

Huang, J., S. Rozelle, C. Pray, and Q. Wang. 2002. Plant biotechnology in China. Science 295 (5555): 674–676.

IRAC. 2017. The IRAC Mode of Action classification. Insecticide Resistance Action Committee. Accessed February 18.

(ISAAA) International Service for the Aquisition of Agri-Biotech Applications. 2017. Pocket K No. 16: Biotech Crop Highlights in 2016. May. http://www.isaaa.org/resources/publications/pocketk/16/.

Jakka, S. R. K., V. R. Knight, and J. L. Jurat-Fuentes. 2014a. Fitness costs associated with field-evolved resistance to Bt maize in Spodoptera frugiperda (Lepidoptera: Noctuidae). J. Econ. Entom. 107 (1): 342–51.

Jakka, S. R. K., V. R. Knight, and J. L. Jurat-Fuentes. 2014b. *Spodoptera frugiperda* (JE Smith) with field-evolved resistance to Bt maize are susceptible to Bt pesticides. J. Invertebr. Pathol. 122: 52–54.

Johnson, S. J. 1987. Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the Western Hemisphere. Insect Sci. Appl. 8 (4): 543–49.

Kato, T., C. Mapes, L. Mera, J. Serratos, and R. Bye. 2009. Orígen y diversificación del maíz. Genes. Mexico, D.F: Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. (http://www.biodiversidad.gob.mx/genes/origenDiv.html).

Keleman, A., J. Hellin, and M. R. Bellon. 2009. Maize diversity, rural development policy, and farmers' practices: lessons from Chiapas, Mexico. The Geographical Journal 175 (1): 52–70.

Knipling, E. F. 1980. Regional management of the fall armyworm: a realistic approach? Fla. Entomol. 63 (4): 468–80.

Leon-Garcia, I., E. Rodriguez-Leyva, L. D. Ortega-Arenas, and J. F. Solis-Aguilar. 2012. Insecticide susceptibility of *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae) associated with turfgrass at Quintana Roo, México. Agrociencia-Mexico. 46 (3): 279–287.

Li, G., D. Reisig, J. Miao, F. Gould, F. Huang, and H. Feng. 2016. Frequency of Cry1F non-recessive resistance alleles in north carolina field populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). PloS One 11 (4).

Li, Y. X., S. M. Greenberg, and T. X. Liu. 2006. Effects of Bt cotton expressing Cry1Ac and Cry2Ab and non-Bt cotton on Behavior, Survival and Development of Trichoplusia Ni (Lepidoptera: Noctuidae). Crop Protec. 25 (9): 940–948.

Liu, Fengyi, Zhiping Xu, Yu Cheng Zhu, Fangneng Huang, Yanhua Wang, Huiling Li, Hua Li, Congfen Gao, Weijun Zhou, and Jinliang Shen. 2010. Evidence of field-evolved resistance to Cry1Ac-expressing Bt cotton in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in northern China. Pest Manag. Sci. 66 (2): 155–161.

López Montesinos, O. A., E. Franco-Pérez, E. E. Santos-Fuentes, I. Luna-Espinoza, and F. Aragón-Cuevas. 2016. Perceptions and attitudes of the Mexican urban population towards genetically modified organisms. Brit. Food J. 118 (12): 2873–92.

López-Edwards, M., J. L. Hernández-Mendoza, A. Pescador-Rubio, J. Molina-Ochoa, R. Lezama-Gutiérrez, J. J. Hamm, and B. R. Wiseman. 1999. Biological differences between five populations of fall armyworm (Lepidoptera: Noctuidae) collected from corn in Mexico. Fla. Entomol, 254–62.

Luo, K., D. Banks, and M. J. Adang. 1999. Toxicity, binding, and permeability analyses of four *Bacillus thuringiensis* Cry1 δ-endotoxins using brush border membrane vesicles of *Spodoptera exigua* and *Spodoptera frugiperda*. Appl. Environ. Microb. 65 (2): 457–64.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92 (1): 21–32.

Martin, T., O. G. Ochou, M. Vaissayre, and D. Fournier. 2003. Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Hübner)(Lepidoptera: Noctuidae) from West Africa. J. Econ. Entomol. 96 (2): 468–74.

Matsuoka, Y., Y. Vigouroux, M. M. Goodman, J. Sanchez, E. Buckler, and J. Doebley. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. P. Natl. Acad. Sci. 99 (9): 6080–6084.

McCaffery, A. R., A. J. Walker, and C. P. Topper. 1991. Insecticide resistance in the bollworm, *Helicoverpa armigera* from Indonesia. Pestic. Sci. 32 (1): 85–90.

Meagher, R. L., R. N. Nagoshi, C. Stuhl, and E. R. Mitchell. 2004. Larval development of fall armyworm (Lepidoptera: Noctuidae) on different cover crop plants. Fla. Entomol. 87 (4): 454–460.

Melander, Ai L. 1914. Can insects become resistant to sprays. J. Econ. Entomol 7 (1): 167.

Monnerat, R., E. Martins, C. Macedo, P. Queiroz, L. Praça, C. M. Soares, H. Moreira, I. Grisi, J. Silva, and M. Soberon. 2015. Evidence of field-evolved resistance of *Spodoptera frugiperda* to Bt corn expressing Cry1F in Brazil that is still sensitive to modified Bt toxins. PLoS One 10 (4).

Monnerat, R., E. Martins, P. Queiroz, S. Ordúz, G. Jaramillo, G. Benintende, J. Cozzi, M. D. Real, A. Martinez-Ramirez, and C Rausell. 2006. Genetic variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) populations from Latin America is associated with variations in susceptibility to *Bacillus thuringiensis* Cry toxins. Appl. Environ. Microb. 72 (11): 7029–7035.

Morillo, F., and A. Notz. 2007. Resistencia de *Spodoptera frugiperda* (Smith)(Lepidoptera: Noctuidae) a lambdacihalotrina y metomil. Entomotropica 16 (2): 79–87.

Mota-Sanchez, D. and Wise, J. 2017. Arthropod Pesticide Resistance Database. Accessed June 21. https://www.pesticideresistance.org/display.php?page=species&arId=200.

Mota-Sanchez, D., R. M. Hollingworth, E. J. Grafius, and D. D. Moyer. 2006. Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera : Chrysomelidae). Pest Manag. Sci. 62 (1): 30–37.

Nagoshi, R. N., and R. L. Meagher. 2004. Behavior and distribution of the two fall armyworm host strains in Florida. Fla. Entomol. 87 (4): 440–49.

Nagoshi, R. N, and R. L Meagher. 2008. Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. Fla. Entomol. 91 (4): 546–54.

Nagoshi, R. N., R. L. Meagher, and M. Hay-Roe. 2012. Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. Ecol Evol 2 (7): 1458–1467.

Nagoshi, R. N., R. L. Meagher, and M. Hay-Roe. 2014. Assessing the resolution of haplotype distributions to delineate fall armyworm (Lepidoptera: Noctuidae) migratory behaviors. J. Econ. Entomol. 107 (4): 1462–70.

Nagoshi, R. N., R. L. Meagher, and D. A. Jenkins. 2010. Puerto Rico fall armyworm has only limited interactions with those from Brazil or Texas but could have substantial exchanges with Florida populations. J. Econ. Entomol. 103 (2): 360–67.

Nascimento, A. R. B. D, J. R. Farias, D. Bernardi, R. J. Horikoshi, and C. Omoto. 2015. Genetic basis of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to the chitin synthesis inhibitor lufenuron. Pest Manag. Sci. 72 (4): 810-815.

Nascimento, A. R. B. D., P. Fresia, F. L. Cônsoli, and C. Omoto. 2015. Comparative transcriptome analysis of lufenuron-resistant and susceptible strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). BMC Genomics 16 (980).

Niu, Y., G. P. Head, P. A. Price, and F. Huang. 2016. Performance of Cry1A. 105-selected fall armyworm (Lepidoptera: Noctuidae) on transgenic maize plants containing single or pyramided Bt genes. Crop Protec. 88: 79–87.

Niu, Y., R. L. Meagher Jr, F. Yang, and F. Huang. 2013. Susceptibility of field populations of the fall armyworm (Lepidoptera: Noctuidae) from Florida and Puerto Rico to purified Cry1F protein and corn leaf tissue containing single and pyramided Bt genes. Fla. Entomol. 96 (3): 701–13.

Niu, Y., J. A. Qureshi, X. Ni, G. P. Head, P. A. Price, R. L. Meagher, D. Kerns, R. Levy, X. Yang, and F. Huang. 2016. F2 screen for resistance to *Bacillus thuringiensis* Cry2Ab2-maize in field populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) from the southern United States. J. Invertebr Pathol. 138: 66–72.

Omoto, C., O. Bernardi, E. Salmeron, R. J. Sorgatto, P. M. Dourado, A. Crivellari, R. A. Carvalho, A. Willse, S. Martinelli, and G. P. Head. 2016. Field-evolved Resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag. Sci. 72 (9): 1727-1736.

Onstad, D. W. 2014. Chapter 1 - Major Issues in Insect Resistance Management. In Insect Resistance Management (Second Edition), edited by David W. Onstad, 1–23. San Diego: Academic Press.

OriginLab. 2007. Origin 8 User Guide. OriginLab Corporation.

Otero A. 2015. Agricultural biotechnology annual Mexico. USDA Foreign Agricultural Service. https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Agricultural%20Biotechnology%20Annual_Mexico%20City_Mexico_7-15-2015.pdf.

Pannuti, L. E. R., E. L. L. Baldin, T. E. Hunt, and S. V. Paula-Moraes. 2015. On-plant larval movement and feeding behavior of fall armyworm (Lepidoptera: Noctuidae) on reproductive corn stages. Environ. Entomol. 45 (1): 192–200.

Pannuti, L. E. R., S. V. Paula-Moraes, T. E. Hunt, E. L. L. Baldin, L. Dana, and J. V. Malaquias. 2016. Plant-to-plant movement of Striacosta albicosta (Lepidoptera: Noctuidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize (*Zea mays*). J. Econ. Entomol. 109 (3): 1125-1131.

Pardo□López, L., M. Soberón, and A. Bravo. 2012. *Bacillus thuringiensis* insecticidal three domain cry toxins: mode of action, insect resistance and consequences for crop protection. FEMS Microbiology Reviews. 37 (1): 3-22.

Pashley, D. P. 1986. Host-Associated Genetic Differentiation in fall Armyworm (Lepidoptera: Noctuidae): a sibling species complex? Ann. Entomol. Soc. Am. 79 (6): 898–904.

Pashley, D. P. 1988. Current Status of Fall Armyworm Host Strains. Fla. Entomol. 227–34.

Pashley, Dorothy P., Abner M. Hammond, and Tad N. Hardy. 1992. Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 85 (4): 400–405.

Pashley, D. P., and J. A. Martin. 1987. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 80 (6): 731–33.

Perez-Zubiri, J. R., E. Cerna-Chavez, L. A. Aguirre-Uribe, J. Landeros-Flores, M K. Harris, and R. Rodriguez-Herrera. 2016. Population variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize (Poales: Poaceae) associated with the use of chemical insecticides. Fla. Entomol. 99 (2): 329–31.

Pitre, H. N. 1988. Relationship of fall armyworm (Lepidoptera: Noctuidae) from Florida, Honduras, Jamaica, and Mississippi: susceptibility to insecticides with reference to migration. Fla. Entomol. 56–61.

(PRABIA) Puerto Rico Agricultural Biotechnology Industry Association. 2017. Agricultural biotechnology in Puerto Rico. PRABIA. Accessed August 15. https://www.prabia.org/eng-agbio-pr.

Prowell, D. P., M. McMichael, and J. F. Silvain. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 97 (5): 1034–44.

Qaim, M.. 2010. Benefits of genetically modified crops for the poor: household income, nutrition, and health. New Biotechnol. 27 (5): 552–57.

Qaim, M., and D. Zilberman. 2003. Yield effects of genetically modified crops in developing countries. Science 299 (5608): 900–902.

Ramos-Madrigal, J., B. D. Smith, J. V. Moreno-Mayar, S. Gopalakrishnan, J. Ross-Ibarra, M. T. P. Gilbert, and N. Wales. 2016. Genome sequence of a 5,310-year-old maize cob provides insights into the early stages of maize domestication. Curr. Biol. 26 (23): 3195–3201.

Rule, D. M., S. P. Nolting, P. L. Prasifka, N. P. Storer, B. W. Hopkins, E. F. Scherder, M. W. Siebert, and W. H. Hendrix. 2014. Efficacy of pyramided Bt proteins Cry1F, Cry1A. 105, and Cry2Ab2 expressed in SmartStax corn hybrids against Lepidopteran insect pests in the northern United States. J. Econ. Entomol. 107 (1): 403–9.

Saleem, M. A., M. Ahmad, M. Ahmad, M. Aslam, and A. H. Sayyed. 2008. Resistance to selected organochlorin, organophosphate, carbamate and pyrethroid, in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. J. Econ. Entomol. 101 (5): 1667–75.

Salgado, V. L. 1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pestic. Biochem. Phys. 60 (2): 91–102.

Santos, L. M. D., L. Rodrigues-Redaelli, L. M. G. Diefenbach, and C. F. Stoffel-Efrom. 2003. Larval and pupal stage of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in sweet and field corn genotypes. Braz. J. Biol. 63 (4): 627–33.

SAS Institute. 2011. SAS user’s guide version 9.3. SAS Institute Inc., Cary, NC, USA. http://support.sas.com/index.html.

Sattelle, D. B., D. Cordova, and T. R. Cheek. 2008. Insect ryanodine receptors: molecular targets for novel pest control chemicals. Invertebr. Neurosci. 8 (3): 107.

(SAGARPA) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. 2015a. Agenda Técnica Agrícola de Chihuahua.

http://www.inifap.gob.mx/Documents/inicio/Agendas_Tec/2017/Agenda%20T%C3%A9cnica%20Chihuahua%20OK.pdf

(SAGARPA) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. 2015b. Agenda Técnica Agrícola de Coahuila. http://www.inifap.gob.mx/Documents/agendas_tecnologicas/07_Coahuila_2015_SIN.pdf

(SAGARPA) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. 2015c. Agenda Técnica Agrícola de Jalisco. http://www.inifap.gob.mx/Documents/agendas_tecnologicas/14_Jalisco_2015_SIN.pdf

(SAGARPA) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. 2015d. Agenda Técnica Agrícola de Oaxaca. http://www.inifap.gob.mx/Documents/agendas_tecnologicas/20_Oaxaca_2015_SIN.pdf

(SAGARPA) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. 2015e. Agenda Técnica Agrícola de Sinaloa. www.inifap.gob.mx/Documents/agendas_tecnologicas/25_Sinaloa_2015_SIN.pdf

(SAGARPA) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. 2015f. Agenda Técnica Agrícola de Sonora. http://www.inifap.gob.mx/Documents/agendas_tecnologicas/26_Sonora_2015_SIN.pdf

(SAGARPA) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. 2015g. Agenda Técnica Agrícola de Tamaulipas. www.inifap.gob.mx/Documents/.../28_Tamaulipas_2015_SIN.pdf

(SE) Secretaría de Economía. 2012. Análisis de la cadena de valor maíz-tortilla: sotuación actual y factores de competencia local. http://www.2006-2012.economia.gob.mx/files/comunidad_negocios/industria_comercio/informacionSectorial/20120411_analisis_cadena_valor_maiz-tortilla.pdf.

Servicio Nacional de Información Agroalimentaria y Pesquera. 2016. Anuario estadístico de la producción agrícola. Accessed November 2. http://infosiap.siap.gob.mx/aagricola_siap_gb/icultivo/index.jsp.

Sims, S. B., John T. Greenplate, T. B. Stone, M. A. Caprio, and F. L. Gould. 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins. In ACS Symposium Series, 645:229–42. ACS Publications.

Smith, J.L., M.D. Lepping, D.M. Rule, Y. Farhan, and A.W. Schaafsma. 2017. Evidence for field-evolved resistance of *Striacosta albicosta* (Lepidoptera: Noctuidae) to Cry1F *Bacillus thuringiensis* protein and transgenic corn hybrids in Ontario, Canada. J. Econ. Entomol. 110(5): 2217-2228.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 82–87.

Stern, V. M. R. F., R. Smith, R. Van den Bosch, and K. Hagen. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Calif. Agr. 29 (2): 81–101.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. D. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J. Econ. Entomol. 103 (4): 1031–38.

Storer, N. P., M. E. Kubiszak, J. E. King, G. D. Thompson, and A. C. Santos. 2012. Status of resistance to Bt maize in *Spodoptera frugiperda*: lessons from Puerto Rico. J. Invertebr. Pathol. 110 (3): 294–300.

Strassburg, B., A. E. Latawiec, L. G. Barioni, C. A. Nobre, V. P. Da Silva, J. F. Valentim, M. Vianna, and E. D. Assad. 2014. When enough should be enough: improving the use of current agricultural lands could meet production demands and spare natural habitats in Brazil. Sci Tech. 28: 84–97.

Su, J., T. Lai, and J. Li. 2012. Susceptibility of field populations of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. Crop Protec. 42: 217–22.

Tabashnik, B. E. 2008. Delaying insect resistance to transgenic crops. P. Natl. Acad Sci. 105 (49): 19029–30.

Tabashnik, B. E., T. Brevault, and Y. Carriere. 2013. Insect resistance to Bt crops: lessons from the first billion acres. Nat. Biotechnol. 31 (6): 510–21.

Tabashnik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83 (5): 1671–76.

Tabashnik, B. E., A. J. Gassmann, D. W. Crowder, and Y. Carriére. 2008. Insect resistance to Bt crops: evidence versus theory. Nat. Biotechnol. 26 (2): 199–202.

Tabashnik, B. E., Y. B. Liu, N. Finson, L. Masson, and D. G. Heckel. 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. P. Natl. Acad Sci. 94 (5): 1640–44.

Tabashnik, B. E., J. B. J. Van Rensburg, and Y. Carrière. 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. J. Econ. Entomol. 102 (6): 2011–25.

Tavares, W. S, M.A Costa, I. Cruz, R. D Silveira, J. E. Serrao, and J. C. Zanuncio. 2010. Selective effects of natural and synthetic insecticides on mortality of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its predator eriopis connexa (Coleoptera: Coccinellidae). J. Environ. Sci. Healt. B: Pesticides, Food Contaminants and Agricultural Wastes 45 (6): 557–61.

Tohnishi, M., H. Nakao, T. Furuya, A. Seo, H. Kodama, K. Tsubata, S. Fujioka, H. Kodama, T. Hirooka, and T. Nishimatsu. 2005. Flubendiamide, a novel insecticide highly active against Lepidopterous insect pests. J. Pestic. Sci. 30 (4): 354–60.

Traxler, G., S. Godoy-Avila, J. Falck-Zepeda, and J. J. Espinoza-Arellano. 2003. Transgenic cotton in Mexico: a case study of the Comarca Lagunera. In The Economic and Environmental Impacts of Agbiotech, 183–202. Springer.

Troczka, Bartlomiej J., Martin S. Williamson, Linda M. Field, and TG Emyr Davies. 2016. Rapid selection for resistance to diamide insecticides in plutella xylostella via specific amino acid polymorphisms in the ryanodine receptor. Neurotoxicology.

Trtikova, M., O. G. Wikmark, N. Zemp, A. Widmer, and A. Hilbeck. 2015. Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PloS One 10 (4).

UN DESA. 2017. World Population Prospects. https://esa.un.org/unpd/wpp/Publications/Files/WPP2017_KeyFindings.pdf.

USDA ERS. 2017a. Corn and Other Feed Grains: Background. Accessed August 12. https://www.ers.usda.gov/topics/crops/corn/background.aspx.

USDA ERS. 2017b. Corn and Other Feed Grans: Trade. Accessed August 10. https://www.ers.usda.gov/topics/crops/corn/trade/.

USDA ERS. 2017c. Recent Trends in GE Adoption. Accessed August 9. https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx.

USDA FAS. 2017. World Agricultural Production. https://apps.fas.usda.gov/psdonline/circulars/production.pdf.

Vacher, C., D. Bourguet, F. Rousset, C. Chevillon, and M. E. Hochberg. 2003. Modelling the spatial configuration of refuges for a sustainable control of pests: a case study of Bt cotton. J. Evolution. Biol. 16 (3): 378–87.

Van Rensburg, J. B. J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic Maize. South African Journal of Plant and Soil 24 (3): 147–51.

Vargas-Parada, L. 2014. GM maize splits Mexico: legal challenge to transgenic crops has created a rift in the Country's Scientific Community. Nature 511 (7507): 16–18.

Vasquez, B. L. 1995. Resistant to most insecticides. Book of Insects Records University of Florida Gainesville, USA.

Vélez, A. M., T. A. Spencer, A. P. Alves, A. L. B. Crespo, and B. D. Siegfried. 2014. Fitness costs of Cry1F resistance in fall armyworm, *Spodoptera frugiperda*. J. Appl. Entomol. 138 (5): 315–25.

Vélez, A. M., T. A. Spencer, A. P. Alves, D. Moellenbeck, R. L. Meagher, H. Chirakkal, and B. D. Siegfried. 2013. "Inheritance of Cry1F resistance, cross-resistance and frequency of resistant alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). B. Entom. Res. 103 (06): 700–713.

Vélez, Ana M., N. N. Vellichirammal, J. L. Jurat-Fuentes, and B. D. Siegfried. 2016. Cry1F Resistance among Lepidopteran pests: a model for improved resistance management? Curr. Opin. Insect Sci. 15: 116–24.

Westbrook, J. K., R. N. Nagoshi, R. L. Meagher, S. J. Fleischer, and Siddarta Jairam. 2016. Modeling seasonal migration of fall armyworm moths. Int. J. Biometeorol. 60 (2): 255–67.

Williams, T., H. C. Arredondo-Bernal, and L. A. Rodríguez-del-Bosque. 2013. Biological pest control in Mexico. Ann. Rev. Entomol. 58 (1): 119.

Yang, F., D. L. Kerns, S. Brown, R. Kurtz, T. Dennehy, B. Braxton, G. Head, and F. Huang. 2016. Performance and cross-crop resistance of Cry1F-maize selected *Spodoptera frugiperda* on transgenic Bt cotton: Implications for Resistance Management. Scientific Reports 6.

Yang, F., D. L. Kerns, G. Head, S. Brown, and F. Huang. 2017. Susceptibility of Cry1F-maize resistant, heterozygous, and susceptible *Spodoptera frugiperda* to Bt proteins used in the transgenic cotton. Crop Protec. 98: 128–35.

Yang, F., D. L. Kerns, G. P. Head, B. R. Leonard, R. Levy, Y. Niu, and F. Huang. 2014. A challenge for the seed mixture refuge strategy in Bt maize: impact of cross-pollination on an ear-feeding pest, corn earworm. Plos One 9 (11).

Young, J. R. 1979. Fall armyworm: control with insecticides. Fla. Entomol. 62 (2): 130–33.

Yu, S. J. 1983. Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) Larva. J. Econ. Entomol. 76 (2): 219–22.

Yu, S. J.. 1991. Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (JE Smith). Pestic. Biochem. Phys. 39 (1): 84–91.

Yu, S. J.. 1992. Detection and biochemical characterization of insecticide resistance in fall armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 85 (3): 675–82.

Yu, S. J., S. N. Nguyen, and G. E. Abo-Elghar. 2003. Biochemical characteristics of insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (JE Smith). Pestic. Biochem. Phys. 77 (1): 1–11.

Zalk, R., O. B. Clarke, A. D. Georges, R. A. Grassucci, S. Reiken, F. Mancia, W. A. Hendrickson, J. Frank, and A. R. Marks. 2015. Structure of a mammalian ryanodine receptor. Nature 517 (7532): 44–49.

Zhang, H., W. Yin, J. Zhao, L. Jin, Y. Yang, S. Wu, B. E. Tabashnik, and Y. Wu. 2011. Early warning of cotton bollworm resistance associated with intensive planting of Bt Cotton in China. PLoS One 6 (8).

Zhao, J. Z., H. L. Collins, Y. X. Li, R. F. L. Mau, G. D. Thompson, M. Hertlein, J. T. Andaloro, R. Boykin, and A. M. Shelton. 2006. Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb, and emamectin benzoate. J. Econ. Entomol. 99 (1): 176–81.