Baseline of Susceptibility to the Cry1F Protein in Mexican Populations of Fall Armyworm

Authors: Rivero-Borja, Maribel, Rodríguez-Maciel, J. C., Urzúa Gutiérrez, J. A., Silva-Aguayo, G., Chandrasena, Desmi I., et al.

Source: Journal of Economic Entomology, 113(1) : 390-398

Published By: Entomological Society of America

URL: https://doi.org/10.1093/jee/toz280

Journal of Economic Entomology, 113(1), 2020, 390–398
doi: 10.1093/jee/toz280
Advance Access Publication Date: 6 November 2019
Research

Baseline of Susceptibility to the Cry1F Protein in Mexican Populations of Fall Armyworm

Maribel Rivero-Borja,[1] J. C. Rodríguez-Maciel,[1,5] J. A. Urzúa Gutiérrez,[2] G. Silva-Aguayo,[3] Desmi I. Chandrasena,[4] Ninfa C. Felix-Bermudez,[4] and Nicholas P. Storer[4]

[1]Posgrado en Entomología y Acarología, Colegio de Postgraduados, Montecillo, Mexico, [2]Departmento de Parasitología Agrícola, Universidad Autónoma Chapingo, Texcoco, Mexico, [3]Facultad de Agronomía, Universidad de Concepción, Campus Chillán, Chillán, Chile, [4]Corteva Agriscience™, Johnston, IA and [5]Corresponding author, e-mail: concho@colpos.mx

Subject Editor: Aaron Gassmann

Received 2 July 2019; Editorial decision 24 September 2019

Abstract

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is currently the most important maize pest in Mexico. Its control is mainly based on the use of conventional insecticides. Additionally, Bt-maize expressing Cry1F protein represents an alternative to control this pest. We estimated the baseline susceptibility in Mexican populations of *S. frugiperda* to Cry1F protein. Twenty-eight geographical populations were field collected from Baja California Sur, Chihuahua, Coahuila, Durango, Sinaloa, Sonora, and Tamaulipas states. The F_1 neonate larvae of each population were subjected to diet-overlay bioassay. After 7 d of Cry1F exposure, the percent mortality and the percent growth inhibition with respect to the untreated control were recorded (S-LAB). The LC_{50} ranged from 14.4 (6.3–24.0) (Cajeme 1, Sonora) to 161.8 ng/cm^2 (92.0–320) (Ahumada 2, Chihuahua), while the LC_{95} was between 207.1 (145–363) (Obregón, Sonora) and 1,217 ng/cm^2 (510.8–7,390.0) (Río Bravo 2, Tamaulipas). The sensitivity ratios at 50% mortality, (LC_{50} field/LC_{50} S-Lab) and 95% mortality were ≤6.45 and ≤5.05-fold, respectively. The 50% growth inhibition (GI_{50}) ranged from 2.8 (0.008–9.3) (Obregón, Sonora) to 42.4 ng/cm^2 (3.6–147.0) (Cajeme 1, Sonora). The GI_{95} was between 75.4 (San Luis Río Colorado, Sonora) to 1,198 ng/cm^2 (Cajeme 1, Sonora). The relative inhibition at 50% of the growth, (RI50 = GI50 field /GI_{50} S-LAB) was ≤3.5 and at 95% (RI_{95}) was ≤1.91-fold. These results indicated susceptibility to Cry1F protein in the evaluated populations of *S. frugiperda*.

Key words: *Spodoptera frugiperda*, Bt protein, maize, baseline, Bt corn

RESUMEN

Spodoptera frugiperda (J. E. Smith), es la plaga de maíz más importante en México. Su control se basa en insecticidas convencionales y la proteína Cry1F del maíz Bt, es alternativa para controlar esta plaga. Se estimó la línea base de respuesta en poblaciones mexicanas de *S. frugiperda* a la proteína Cry1F. Veintiocho poblaciones se recolectaron de Baja California Sur, Chihuahua, Coahuila, Durango, Sinaloa, Sonora y Tamaulipas. Las larvas de neonatas de F1 de cada población se sometieron a bioensayo de capa. A los siete días de exposición, el porcentaje de mortalidad y el porcentaje de inhibición del crecimiento se registraron. La LC_{50} varió de 14.4 (6.3–24.0) (Cajeme 1, Sonora) a 161.8 ng/cm^2 (92.0–320) (Ahumada 2, Chihuahua); la LC_{95} estuvo entre 207.1 (145–363) (Obregón, Sonora) y 1,217 ng/cm^2 (510.8–7,390.0) (Río Bravo 2, Tamaulipas). La relación de sensibilidad al 50% de mortalidad, (campo LC_{50} / LC_{50} S-Lab) y 95% de mortalidad fueron de ≤45 y ≤5.05 veces, respectivamente. El 50% de inhibición del crecimiento (GI_{50}) varió de 2.8 (0.008–9.3) (Obregón, Sonora) a 42.4 ng/cm^2 (3.6–147.0) (Cajeme 1, Sonora). El GI_{95} estuvo entre 75.4 (San Luis Río Colorado, Sonora) y 1,198 ng/cm^2 (Cajeme 1, Sonora). La relación de sensibilidad al 50% de la inhibición del crecimiento (RI_{50} = campo GI_{50} / GI_{50} S-LAB) fue aproximadamente 3.5 y al 95% (RI_{95}) fue 1.91 veces. Existe amplio rango de susceptibilidad a la proteína Cry1F en las poblaciones evaluadas.

Palabras clave: *S. frugiperda*, Bt protein, maize, baseline, Bt corn

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the most important pests of maize (Torres and Cotes 2005). This species originated in the Americas and spread to the tropical and subtropical regions of the world (Andrews 1988, Clavijo and Perez Greiner 2000). It has currently dispersed in the Western hemisphere (Storer et al. 2012; Velez et al. 2013, 2016; Bernardi et al. 2015) and more recently in Africa (Bateman et al. 2018), India (CGIAR 2018), and China (Jing et al. 2019, USDA-FAS 2019). This species has been observed feeding on 186 hosts located in 42 botanical families (Pashley 1986). Moreover, *S. frugiperda* can cause damage to late-planted crops in subtropical regions and throughout the cropping season in the tropical regions (Chandrasena et al. 2018).

In Mexico, *S. frugiperda* is widely distributed (Pacheco 1985) and considered the most economically important pest of maize. It can reduce yield from 45% (Hruska and Gladstone 1988) to 100% (Cruz et al. 1999). The application of organo-synthetic insecticides has been the most-used strategy to control this pest (Pacheco 1994). However, insecticide resistance in *S. frugiperda* is currently reported in Americas to 29 active ingredients belonging to six insecticide groups (Mota-Sanchez and Wise 2017). It is estimated that up to 1,152 tons of pyrethroid are used in México against *S. frugiperda* (Terán-Vargas 2008) and annually, around 3,000 tons of organo-synthetic insecticides are sprayed against this pest (Blanco et al. 2014). Despite the abundant use of chemical compounds, the fall armyworm still causes significant yield loss. As an alternative approach, developers of Bt technology have been generating transgenic maize plants that express genes from the bacterium *Bacillus thuringiensis* Berliner that confer resistance to economically important pests such as the fall armyworm.

Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. developed the GM-maize event TC1507 (Herculex I Insect Protection), which expresses the Cry1F protein derived from *Bacillus thuringiensis* var. *aizawai* (Bt). TC1507 provides control of different foliar, ear-feeding and stalk boring lepidopteran including *S. frugiperda* and has been adopted in major maize production countries such as the United States, Argentina, and Brazil (Storer et al. 2012, Chandrasena et al. 2018).

Bt maize has potential to effectively control the fall armyworm and significantly reduce the use of insecticides in almost 4 million acres in Mexico. Since October 2013, a moratorium on the use of Bt maize was established limiting its cultivation, including field experiments. However, farmers anticipate a positive resolution to this matter, given the lack of scientific evidence related to adverse effects derived from the use of this technology (NAS 2017). Meanwhile, the efforts have been centered on conducting the studies supporting commercialization such as establishing baselines for susceptibility of the target pest populations, to enable future development of resistance management programs in Mexico.

To date, insect pests have demonstrated the ability to develop resistance to Bt crops around the globe (Tabashnik et al. 2003). For example, the level of resistance to the Cry1F protein was so high in Puerto Rico that no fall armyworm larvae exposed to 10,000 ng/cm^2 were killed. Field resistance is influenced heavily by the strength of the implemented resistance management program (Siegfried et al. 2007), importance of nonstructured refuge (Shelton and Zhao 2009), number of maize growing seasons per year (Storer 2010), and even the risk of resistance allele immigration mediated by wind movement across wide areas (Drake and Farrow 1988) or commercial trade of infested agricultural products (Nagoshi et al. 2018).

Deployment of a sound resistance management program is of importance to extend the durability of this technology. Before a Bt crop is deployed in the field, it is helpful to determine the baseline susceptibility of primary pests to estimate the natural geographical variation in susceptibility to specific Bt proteins expressed by the genetically modified crop. This information constitutes a reference that enables the measurement of potential changes in population susceptibility once these technologies are used in the field and selection for resistance occurs. Therefore, the objective of this study was to determine the baseline response to the Cry1F protein derived from *B. thuringiensis* in neonate larvae of *S. frugiperda* populations from the main maize-producing Mexican States of Baja California Sur, Chihuahua, Coahuila, Durango, Sonora, and Tamaulipas.

Materials and Methods

Populations

In total, 28 geographical populations of *S. frugiperda* were field-collected from non-Bt commercial field maize in Baja California Sur, Chihuahua, Coahuila, Durango, Sinaloa, Sonora, and Tamaulipas (Fig. 1) between 2013 and 2017 (Table 1). For each population, five maize fields with a distance of approximately ≥15 km between them were selected. At least five sampling points were randomly selected per field, and the maize plants were visually inspected to detect those with new damage. Then, a single more than or equal to third instar was field-collected per infested plant. In total, 280–560 larvae were field-collected per population and placed individually in cups containing 8.0 ml of meridic diet (Southland products, Lake Village, AR). A population which has been maintained approximately for 10 yr under pesticide-free laboratory conditions, with occasional input of larvae collected from maize in Montecillo, State of Mexico, where this species has not been subjected to insecticide applications, was used as a reference for comparison.

Insect Rearing

Once in the laboratory, the field-collected larvae were quarantined for 1 wk. Then, the dead, diseased, and parasitized larvae were discarded. The healthy larvae were individually transferred to a glass vial containing 10 ml of artificial diet prepared according to manufacturer's instructions (Insect Media for *Spodoptera frugiperda*, Southland Products, Inc., Lake Village, AR). In all cases, at least 90% of the field-collected larvae were healthy and reared to the adult stage to obtain at least 1,000 F_1 healthy neonate larvae for each bioassay. Once the adults emerged, they were visually inspected to confirm species identity (Capinera 2000). Groups of 20 pairs of adults were placed in a 16-size brown paper bag, and 15% sucrose water was provided as a food source. Females laid their eggs on the inner surface of the bag, which were collected every other day and incubated to obtain neonate larvae. The rearing of this species was at 27 ± 1°C with a photoperiod of 14:10 (L:D) h and a relative humidity of 70–80%.

Protein

The insecticidal Cry1F protein of *B. thuringiensis* (90% a.i, Lot TSN304065) was provided by Dow AgroSciences de México S.A. de C.V. The lyophilized protein was stored under refrigeration at 4°C in a vacuum-sealed container that contained a desiccant. CAPS (*N*-cyclohexyl-3-aminopropanesulfonic acid) (Sigma–Aldrich, St. Louis, MO) buffer was used to dilute the protein to prepare the required concentrations.

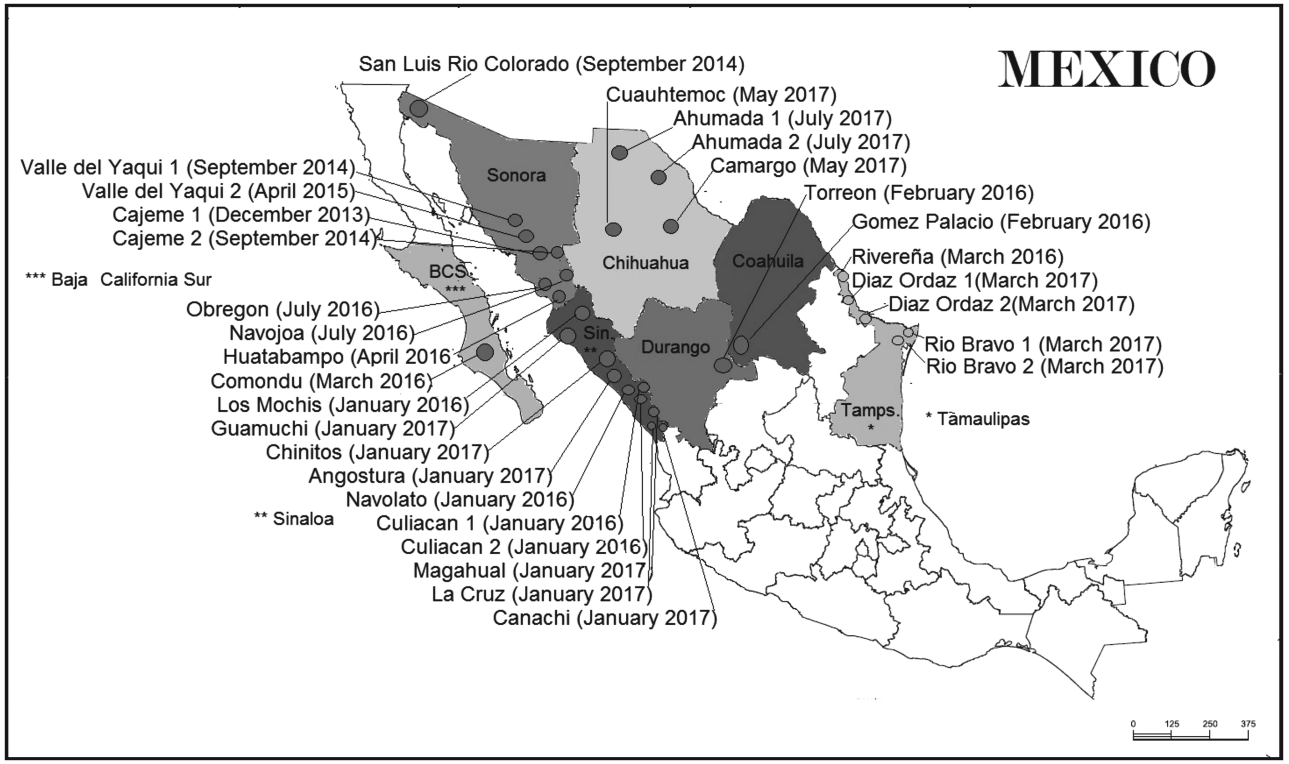

Fig. 1. Map of Mexico showing 28 geographical populations of *Spodoptera frugiperda* collected for the study

Bioassay

In each well of the bioassay trays (Bio-Ba-128, C-D International, Pitman, NJ), 1.5 ml of meridic diet (Southland Products, Lake Village, AR) was deposited. After 2 h, once the diet solidified, 50 µl of the required concentration of the Cry1F protein was deposited using overlay method in each well to form a layer on the diet surface of 1.5 cm^2. Two hours later, each well was infested with an active neonate larva (F_1, 0 to 24 h of age and without previous exposure to meridic diet). At least seven concentrations that covered the range from 0 to 100% mortality determined by preliminary assays were used. For each concentration, 16 larvae were used per dose, with at least five replications. Each replication included an untreated control to which only 50 µl of the CAPS buffer was applied.

After 7 d of exposure to this protein, the following variables were determined: percentage mortality, percent inhibition of growth measured by comparing weight of surviving larvae in a given treatment with the untreated control. Those that did not respond to the stimulus when prodded with a dissecting needle or failed to develop to second instar (L2 stage) were counted as dead. The maximum accepted mortality in the control was 20% and treatment mortality was corrected for control mortality using Abbott's formula (Abbott 1925). For calculating the percentage of growth inhibition, as indicated by weight, all the surviving larvae in the control, as well as those in the respective concentrations, were separately grouped and weighed. Then, the average weight of the larvae was measured. Based on the comparison of the average weights, the relative weight of the larvae surviving on each concentration compared to the weight of the larvae surviving on control treatment was used to calculate the growth inhibition. Dead insects and larvae that did not develop to second instar (L2) were combined for calculating percent effective mortality with the argument that any instar failing to reach second instar by the duration indicate that they are unfit to survive further.

Statistical Analysis

The variable, percentage of effective mortality was analyzed using the Probit model (Finney 1971) to estimate LC_{50} and LC_{95} values, as well as 95% Fiducial confidence intervals (CI) by using the Proc Probit procedure of the SAS statistical package (SAS Institute 2008). The sensitivity ratios (SR) at the level of LC_{50} (SR_{50}) and LC_{95} (SR_{95}) were calculated by dividing the LC_{50} (LC_{95}) values of the respective field population by the LC_{50} (LC_{95}) of the susceptible population (S-LAB) as suggested by Storer et al. 2010. Growth inhibition was calculated as percent weight reduction between the treatment and control. GI_{50} and GI_{95} values were estimated by the Proc Probit procedure using the SAS statistical package (SAS Institute 2008). The relative inhibition ratios (RI) at the level of GI_{50} (RI_{50}) and GI_{95} (RI_{95}) were calculated by dividing the GI_{50} (GI_{95}) values of the respective field population by the GI_{50} (GI_{95}) of the susceptible population (S-LAB).

Results

The results by each state from which *S. frugiperda* were collected are presented below. The range within a parenthesis indicate 95% Fiducial CIs for the corresponding LC or EC value.

We detected differences, both at LC_{50} and LC_{95} level, between some field-collected populations and the susceptible laboratory population (S-LAB) (Tables 2 and 3). These differences were more common at the LC_{50} level, where 18 out of 28 (64.2%) evaluated field populations required higher dosage to reach the same level of mortality (50%). At the CL_{95}, only two field population (Canachi-Sinaloa and Navojoa-Sonora) displayed differences with S-LAB; these two population showed differences with the susceptible population at both LC_{50} and LC_{95}, with SR_{95} values of 3.23 and 7.1×, respectively (Table 2). We observed fewer differences regarding

Table 1. Origin of the populations of *Spodoptera frugiperda* field-collected in four states of Mexico

Population	State	Place of collection	Date of collection
Huatabampo	Sonora	Huatabampo	April 2013
Cajeme 1	Sonora	Cajeme	Dec. 2013
Cajeme 2	Sonora	Cajeme	Sept. 2014
Valle del Yaqui 1	Sonora	The Yaqui Valley	April 2014
Valle del Yaqui 2	Sonora	The Yaqui Valley	April 2015
San Luis Río Colorado	Sonora	San Luis Rio Colorado	Sept. 2014
Navojoa	Sonora	Navojoa	July 2016
Obregón	Sonora	Obregon	July 2016
Cuauhtémoc	Chihuahua	Cuauhtemoc	May 2017
Camargo	Chihuahua	Camargo	May 2017
Ahumada 1	Chihuahua	Ahumada	July 2017
Ahumada 2	Chihuahua	Ahumada	July 2017
Díaz Ordaz 1	Tamaulipas	Diaz Ordaz	Mar. 2017
Díaz Ordaz 2	Tamaulipas	Diaz Ordaz and Camargo	Mar. 2017
Río Bravo 1	Tamaulipas	Rio Bravo	Mar. 2017
Río Bravo 2	Tamaulipas	Rio Bravo	Mar. 2017
Rivereña	Tamaulipas	Region Rivereña	Mar. 2017
Culiacán 1	Sinaloa	Culiacan	Jan. 2017
Culiacán 2	Sinaloa	Culiacan	Jan. 2017
Los Mochis	Sinaloa	Los Mochis	Jan. 2017
Navolato	Sinaloa	Navolato	Jan. 2017
Chinitos	Sinaloa	Chinitos, Angostura	Jan. 2017
Angostura	Sinaloa	Angostura	Jan. 2017
Guamúchil	Sinaloa	Salvador Alvarado, a Guamuchil	Jan. 2017
Magahual	Sinaloa	Area of Magahual, Elota	Jan. 2017
La Cruz	Sinaloa	Area of La Cruz, Elota	Jan. 2017
Canachi	Sinaloa	Area of Canachi, Elota	Jan. 2017

States where only a single population was made was excluded from the table.

the growth inhibition values; only one field-collected population (Ahumada 1- Chihuahua) displayed differences at the GI_{50} level (2.42×) with the S-LAB reference (Table 3).

Baja California Sur

The LC_{50} and the LC_{95} were 28.9 (23.9–34.3) and 245.4 (180–370.2) ng/cm^2, respectively (Table 2). The estimated concentration that inhibited 50% of growth in comparison to the untreated control (GI_{50}) was 8.6 (0.02–23.0) ng/cm^2, and the GI_{95} was 187.5 (66.8–132,841.0) ng/cm^2 (Table 3).

Chihuahua

The LC_{50} ranged from 27.3 (21.0–34.1; Cuauhtémoc) to 161.8 (92.0–320.0; Ahumada 2) ng/cm^2 and the LC_{95} was between 390.9 (317.9–507.4; Camargo) and 1,065 (273.4–158,954,188; Ahumada 1) ng/cm^2 (Table 2) as shown with wide Fiducial limits. When compared with the S-LAB, these field-collected populations showed wide variation in the sensitive ratio at 50% mortality (SR_{50}) from 1.09- to 6.45-fold, and the SR_{95} was between 2.09- and 4.42-fold (Table 2). The GI_{50} ranged from 12.0 (2.8–21.3; Cuauhtémoc) to 29.3 (23.1–36.2; Ahumada 2) ng/cm^2, and the GI_{95} from 98.9 (49.6–984.4; Cuauhtémoc) to 658.6 (448.3–1,097.0; Ahumada 2) ng/cm^2 (Table 3).

Coahuila

Only a single population was tested from this region. The LC_{50} for Torreón population was 39.9 (23.6–68.2) ng/cm^2, and the LC_{95} was 214.6 (107.3–1,560.0) ng/cm^2 (Table 2). When compared with the susceptible population, the SR_{50} of the population was 1.59-fold and the SR_{95} was 0.89-fold (Table 2). The GI_{50} and the GI_{95} values were 30.4 (3.9–77.4) and 661.9 (184.8–550,300.0) ng/cm^2, respectively (Table 3). The Torreón population had no survivors beyond 300 ng/cm^2.

Durango

A single population (Gómez Palacio; collected February 2016) was assayed from this region. The LC_{50} of Gómez Palacio population was 50.3 (44.0–57.0) ng/cm^2 and the LC_{95} was 209.6 (168.0–276.0) ng/cm^2 (Table 2). The GI_{50} was 4.6 (0.0007–16.2) and the GI_{95}, 376.3 (110.8–1,804,182) ng/cm^2 (Table 3). At 600 ng/cm^2, none of the treated larvae survived.

Sinaloa

Ten field-collected populations were evaluated from the state of Sinaloa. The LC_{50} ranged from 29.4 (22.9–37.0; Culiacán 2) to 107.2 (74.0–157.0; Culiacán 1) ng/cm^2. The LC_{95} was between 261.5 (117.3–3,757.0; Los Mochis) and 702 (396.0–1,934.0; Culiacán 1) ng/cm^2 (Table 2). The lowest GI_{50} value was 1.5 (0.2–3.8; Chinitos) ng/cm^2 and the highest GI_{50} was 41.4 (16.0–85.5; Culiacán 2) ng/cm^2 (Table 3). The GI_{95} ranged from 205.5 (82.7–10,380.0; Los Mochis) to 788.2 (221.8–166,846.0; Culiacán 1) ng/cm^2 (Culiacán 1). All the populations evaluated had >92% mortality at the highest evaluated dose (600 ng/cm^2).

Sonora

The LC_{50} ranged from 14.4 (6.3–24.0; Cajeme 1) to 128.1 (110.0–149.0; Navojoa) ng/cm^2 and the LC_{95} was between 157.1 (85.3–571.0; Cajeme 1) and 778 (589–1,119.0; Navojoa) ng/cm^2 (Table 2). The GI_{50} was between 2.8 (0.008–9.3; Obregón) to 42.4 (3.6–147.0; Cajeme 1) ng/cm^2, and the GI_{95} varied from 75.4 (52.4–139.5; San Luis Río Colorado) to 1,198 (256.8–195,915,619.0; Cajeme 1) ng/cm^{-2} (Table 3). At 600 ng/cm^2, only three populations had live larvae (Cajeme 1, Obregón, and Navojoa). The SR_{50} varied from 0.23 (San Obregón population, Sonora) to 3.5 (Cajeme 1 population, Sonora). The SR_{95} ranged from 0.12 (San Luis Río Colorado, Sonora) to 1.91 (Cajeme 1, Sonora). In both cases, the state of Sonora had the populations with the highest variation in $RI_{50(95)}$.

Tamaulipas

The LC_{50} ranged from 37.8 (16.0–96.0; Díaz Ordaz 2) to 79.7(48.3–134.2; Rio Bravo 2) ng/cm^2 and the LC_{95} was between 228.3 (183.0–307.0; Díaz Ordaz 1) and 1,217 (510.8–7,390.0; Rio Bravo 2) ng/cm^2 (Table 2). The GI_{50} was between 1.4 (0.2–3.7; Rivereña) and 7.0 (0.3–16.7; Rio Bravo 1) ng/cm^2 (Table 3). The GI_{95} ranged from 57.7 (39.9–116.2; Díaz Ordaz 1) to 656.8 (364.9–1,650.0; Rio Bravo 2) ng/cm^2 (Table 3).

At the (SR_{50}–SR_{95}) level, the highest variation in all of the field populations respect to the susceptible one (S-LAB) were as follows: Baja California (1.15–1.02), Chihuahua (6.45–4.42), Coahuila (1.59–0.89), Durango (2.0–0.87), Sinaloa (4.27–2.91), Sonora (5.1–3.23), and Tamaulipas (3.18–5.05) (Table 2)

Discussion

Susceptibility to Cry1F across the various regions was indicated by mortality and growth parameters as discussed. To date, the

Table 2. Mortality of neonate larvae of *Spodoptera frugiperda* from several Mexican States, exposed for seven days to the Cry1F protein of *Bacillus thuringiensis*

Population	*n*	df	*b* ± SE	LC_{50}[a] (95% FL) ng/cm²	LC_{95} *a* (95% FL) ng/cm²	Pr > χ^2	SR_{50}[b]	SR_{95}[b]
State of Baja California Sur								
Comondú	480	4	1.77 ± 0.2	28.9 (23.9–34.3)	245.4 (180.6–370.2)	0.61	1.15	1.02
State of Chihuahua								
Cuauhtémoc	480	4	1.3 ± 0.1	27.3 (21.0–34.1)	502.3 (319.2–967.1)	0.5	1.09	2.09
Camargo	560	5	2.7 ± 0.2	97.6 (**86.0–110.8**)	390.9 (317.9–507.4)	0.4	3.89	1.62
Ahumada 1	480	4	1.7 ± 0.5	121.7 (**47.7–2,113**)	1,065 (273.4–158,954,188)	<0.0001	4.85	4.42
Ahumada 2	560	5	2. 5 ± 0.5	161.8 (**92.0–320.0**)	711.9 (348.0–5,708.0)	<0.0001	6.45	2.96
State of Coahuila								
Torreón	400	3	2.3 ± 0.37	39.9 (23.6–68.2)	214.6 (107.3–1,560.0)	0.01	1.59	0.89
State of Durango								
Gómez Palacio	480	4	2.7 ± 0.19	50.3 (**44.0–57.0**)	209.6 (168.0–276.0)	0.5	2.00	0.87
State of Sinaloa								
Culiacán 1	560	5	2.01 ± 0.24	107.2 (**74.0–157.0**)	702.0 (396.0–1,934.0)	0.01	4.27	2.91
Culiacán 2	400	3	1.33 ± 0.16	29.4 (22.9–37.0)	504.1 (290.5–1,209.0)	0.5	1.17	2.09
Los Mochis	400	3	2.01 ± 0.36	39.8 (21.6–74.0)	261.5 (117.3–3,757.0)	0.006	1.59	1.09
Navolato	480	4	1.8 ± 0.2	54.0 (**35.3–83.9**)	444.1 (223.2–1,849.0)	0.01	2.15	1.84
Chinitos	480	4	2.5 ± 0.3	67.7 (**50.3–92.5**)	307.5 (194.5–691.9)	0.05	2.70	1.28
Angostura	480	4	2.3 ± 0.2	94.6 (**82.0–110.0**)	478.6 (364.0–685.0)	0.4	3.77	1.99
Guamúchil	560	5	2.1 ± 0.2	54.1 (**39.0–73.0**)	317.7(202.0–663.0)	0.05	2.16	1.32
Magahual	480	4	2.1 ± 0.5	72.4 (**32.0–222.0**)	434.3 (164.0–35,856.0)	<0.0001	2.88	1.80
La Cruz	400	3	3.3 ± 0.6	101.8 (**63.0–176.0**)	321.5 (182.0–1,899.0)	0.01	4.06	1.33
Canachi	480	4	2.0 ± 0.5	73.6 (**32.0–250.0**)	469.7 (**1,670.0–65,916.0**)	<0.0001	2.93	1.95
State of Sonora								
Cajeme 1	560	5	1.6 ± 0.3	14.4 (6.3–24.0)	157.1 (85.3–571.0)	0.03	0.57	0.65
Cajeme 2	400	3	2.0 ± 0.3	33.2 (19.7–54.7)	213.2 (104.7–1,504.0)	0.01	1.32	0.89
Valle del Yaqui	320	2	1.5 ± 0.2	32.5 (26.2–41.7)	387.1 (207.6—16–1,168.0)	0.94	1.29	1.61
San Luis Río Colorado	400	3	2.3 ± 0.5	40.0 (20.0–86.0)	212.8 (95.0–5,496.0)	2.25	1.59	0.88
Navojoa	480	4	2.9 ± 0.5	128.1 (**110.0–149.0**)	778.0 (**589.0–1,119.0**)	0.15	5.10	3.23
Obregón	560	5	2.7 ± 0.3	49.9 (**39.0–64.0**)	207.1 (145.0–363.0)	0.17	1.99	0.86
State of Tamaulipas								
Díaz Ordaz 1	400	3	2.7 ± 0.2	55.0 (**48.0–63.0**)	228.3 (183.0–307.0)	0.25	2.19	0.95
Díaz Ordaz 2	400	3	2.0 ± 0.5	37.8 (16.0–96.0)	244.1 (96.0–29,978.0)	0.0001	1.51	1.01
Río Bravo 1	400	3	2.1 ± 0.2	40.7 (**35.0–47.8**)	257.1 (186.2–402.2)	0.42	1.62	1.07
Río Bravo 2	560	5	1.4 ± 0.2	79.7 (**48.3–134.2**)	1,217 (510.8–7,390.0)	0.05	3.18	5.05
Rivereña	480	4	1.9 ± 0.3	64.5 (**41.5–104.6**)	487.1 (239.5–2,256.0)	0.01	2.57	2.02
Susceptible								
S-LAB	320	2	1.67 ± 0.2	25.1 (20.3–30.8)	240.9 (145.7–562.02)	0.5		

n = total larvae, *b* = slope ± standard error of the slope, FL = 95% Fiducial limit.

[a]LC_{50} = estimated concentration that causes 50% mortality; CL_{95} = estimated concentration that causes 95% mortality.

[b]Sensitive ratio (SR) = $LC_{50\ (95)}$ field population/$LC_{50\ (95)}$ susceptible population (S-LAB).CLs in bold indicate that the Fiducial limits (95%) of the Susceptible (S-LAB) and the respective field-collected populations did not overlap.

established baseline is the first record of regional susceptibility of *S. frugiperda* to Cry1F in Mexico. RR value ≥10 could be indicating insect resistant development to insecticides as defined by Tabashnik et al. (2009, 2014) and Mota-Sanchez et al. (2002). Therefore, these populations appeared susceptible to the Cry1F protein because none of them reached that status.

In several regions of Latin America such as Argentina, Brazil, Colombia, Puerto Rico, Uruguay and Mexico, *S. frugiperda* is still a devastating pest in conventional and Bt maize (Blanco et al. 2014). Resistance to Cry1F (TC1507) in this pest was first documented in Puerto Rico during 2006 (Storer et al. 2010). Recently, Cry1F resistance and reduced susceptibility in *S. frugiperda* have been identified in Florida (Huang et al. 2014) and North Carolina (Li et al. 2016). Storer et al. (2012) found that resistant fall armyworm population from Puerto Rico showed no mortality when exposed to the Cry1F protein, even at the highest dosage evaluated (10,000 ng/cm²). In some areas of South America, resistance to Cry1F was detected after several years of commercialization, such as Brazil (>5,000-fold; Farias et al. 2014a,b) and Argentina (survival of 16.6–97% to the diagnostic concentration of 2,000 ng/cm²; Chandrasena et al. 2018). In Puerto Rico, resistance was characterized as autosomal and recessive (Storer et al. 2010), similar to the inheritance of resistance found in Florida (Huang et al. 2014), Brazil (Farias et al. 2014a,b), and Argentina (Chandrasena et al. 2018). Although flights are unlikely to happen between more distant geographies (Nagoshi et al. 2007a,b; 2018), migratory nature of this pest may also enable spread of resistant individuals to Mexico from nearby geographies, such as Brazil and Argentina, where resistance has been confirmed and where environmental conditions are not a limitation to their dispersal.

The highest LC_{95} obtained from all six provinces in Mexico ranged between 209 and 1217 ng/cm² indicating *S. frugiperda* susceptibility to Cry1F in the geography. Additionally, a diagnostic concentration (LC_{99}) derived from such a field-collected population

Table 3. Percentage of weight inhibition compared to the untreated control of *Spodoptera frugiperda* neonate larvae from several states of Mexico, exposed for 7 d to the Cry1F protein of *Bacillus thuringiensis*

Population	*n*	*df*	*b* ± SE	GI_{50}[a] (95% FL) ng/cm^2	GI_{95}[a] (95% FL) ng/cm^2	Pr > χ^2	RI_{50}*b*	RI_{95}[b]
State of Baja California Sur								
Comondú	560	5	1.22 ± 0.37	8.6 (0.02–23.0)	187.5 (66.8–132,841.0)	<0.0001	0.71	0.30
State of Chihuahua								
Cuauhtémoc	480	4	1.80 ± 0.39	12.0 (2.8–21.3)	98.9 (49.6–984.4)	0.0002	0.99	0.16
Camargo	560	5	1.61 ± 0.24	17.0 (8.4–26.7)	177.3 (98.5–568.1)	0.0089	1.40	0.28
Ahumada 1	480	4	1.39 ± 0.12	19.1 (14.9–23.5)	287.2 (201.7–467.0)	0.95	1.58	0.46
Ahumada 2	560	5	1.21 ± 0.09	29.3 (**23.1–36.2**)	658.6 (448.3–1,097.0)	0.67	2.42	1.05
State of Coahuila								
Torreón	560	5	1.22 ± 0.34	30.4 (3.9–77.4)	661.9 (184.8–550,300.0)	<0.0001	2.51	1.05
State of Durango								
Gómez Palacio	560	5	0.86 ± 0.29	4.6 (0.0007–16.2)	376.3 (110.8–1,804,182)	<0.0001	0.38	0.60
State of Sinaloa								
Culiacán 1	560	5	1.02 ± 0.26	19.8 (2.1–45.5)	788.2 (221.8–166,846.0)	<0.0001	1.64	1.26
Culiacán 2	560	5	1.49 ± 0.32	41.4 (16.0–85.5)	522.7 (193.7–11,781.0)	<0.0001	3.42	0.83
Los Mochis	480	4	1.57 ± 0.38	18.4 (3.5–36.4)	205.6 (82.7–10,380.0)	<0.0001	1.52	0.33
Navolato	480	4	1.44 ± 0.21	15.4 (7.3–23.9)	212.6 (111.9–835.0)	0.03	1.27	0.34
Chinitos	480	4	0.68 ± 0.12	1.5 (0.2–3.8)	360.4 (178.5–1,459.0)	0.83	0.12	0.57
Angostura	480	4	0.80 ± 0.12	3.02 (0.9–5.9)	332.9 (182.3–965.2)	0.82	0.25	0.53
Guamúchil	560	5	0.78 ± 0.10	3.5 (1.33–6.52)	434.7 (250.7–1,038.0)	0.002	0.29	0.69
Magahual	480	4	0.85 ± 0.12	4.6 (1.9–8.0)	384.8 (215.6–1,016).0	0.17	0.38	0.61
La Cruz	480	4	1.16 ± 0.12	14.2 (10.0–18.7)	367.0 (237.7–690.4)	0.55	1.17	0.58
Canachi	480	4	1.23 ± 0.12	14.9 (10.8–19.2)	318.5 (213.8–561.2)	0.31	1.23	0.51
State of Sonora								
Cajeme 1	560	5	1.13 ± 0.35	42.4 (3.6–147.0)	1,198 (256.8–195,915,619)	<0.0001	3.50	1.91
Cajeme 2	480	4	1.45 ± 0.41	14.4 (0.4–32.0)	193.3 (70.21–270,752.0)	<0.0001	1.19	0.31
Valle del Yaqui	480	4	1.66 ± 0.48	22.6 (2.1–55.6)	220.6 (77.8–534,292.0)	<0.0001	1.87	0.35
San Luis Río Colorado	400	3	1.27 ± 0.20	3.8 (1.6–6.3)	75.4 (52.4–139.5)	0.18	0.31	0.12
Navojoa	560	5	0.89 ± 0.11	3.9 (1.7–6.8)	265.8 (168.2–530.1)	0.69	0.32	0.42
Obregón	480	4	0.96 ± 0.24	2.8 (0.008–9.3)	145.6 (60.0–6,566.0)	0.03	0.23	0.23
State of Tamaulipas								
Díaz Ordaz 1	320	2	1.69 ± 0.29	6.1 (3.4–8.5)	57.7 (39.9–116.2)	0.81	0.50	0.09
Díaz Ordaz 2	400	3	0.89 ± 0.17	2.1 (0.4–4.6)	147.8 (84.5–463.2)	0.72	0.17	0.24
Rio Bravo 1	560	5	1.19 ± 0.30	7.0 (0.3–16.7)	166.4 (70.1–4,094.0)	<0.0001	0.58	0.26
Rio Bravo 2	560	5	0.78 ± 0.09	5.1 (2.3–8.8)	656.8 (364.9–1,650.0)	0.25	0.42	1.03
Rivereña	480	4	0.72 ± 0.13	1.4 (0.2–3.7)	270.4 (143.0–931.5)	0.76	0.12	0.43
Susceptible								
S-LAB	320	2	0.96 ± 0.19	12.1 (6.6–17.1)	628.0 (240.9–5,612.0)	0.22		

n = total larvae, *b* = slope ± standard error of the slope, FL = 95% Fiducial limit

[a]GI_{50} = estimated concentration that causes 50% growth inhibition in comparison to the untreated control; GI_{95} = estimated concentration that causes 95% growth inhibition in comparison to the untreated control.

[b]Relative inhibition = $GI_{50\,(95)}$ field population/$GI_{50\,(95)}$ susceptible population (S-LAB).

GIs in bold indicate that the Fiducial limits (95%) of the Susceptible (S-LAB) and the respective field-collected population did not overlap, indicating that the field population required higher dosage to reach the same effect.

can be considered as a field-relevant concentration to detect field evolved resistance. Brazil and Argentina reported a concentration of 2,000 ng/cm^2 as a discriminating dose (based on upper confidence limit of the highest LC_{99}) to distinguish *S. frugiperd*a populations developing resistance to Cry1F (Farias et al. 2014a,b; Chandrasena et al. 2018) in the field. Despite the observed variability, our results did not indicate resistance to Cry1F protein in Mexico as several nearby geographies.

The sustainability of a Bt crop depends in part on the effectiveness of the implemented insecticide resistance management program (Tabashnik et al. 2003) and involves baseline susceptibility estimation, implementation of resistance monitoring plan, monitoring, and remediation. The characterization of susceptibility in a population is a complex issue given the fact that the variation in response to the Bt protein depends on the complex interaction of the genes with its environment. In this case, we considered that larval response is not be mediated by the expression of the alleles for resistance since they are assumed to be at very low frequency, and generally are recessive. Also, the bioassays are carried out with a specific meridic diet to provide uniform conditions, thus minimizing variation in susceptibility imposed by factors such as differences in nutritional source where the target pest feeds under field conditions (Carrie et al. 2016, Deans et al. 2016). Other factors such as bioassay method, mortality criteria, type of parameter evaluated (mortality or growth inhibition) and characteristics of toxicological lot(s) should remain similar in order to minimize the variation in susceptibility due to external factors (Saeglitz et al. 2006, Bird and Akhurst 2007, Blanco et al. 2008b, Storer et al. 2010). Immigration of resistant alleles might be another source of variation in the response to Bt proteins since

the fall armyworm displays long distance flights assisted by wind (Nagoshi et al. (2017). Under laboratory conditions, baseline susceptibility is determined by phenotypic bioassays (Blanco et al. 2008b) or by estimating the frequency of alleles for resistance (Blanco et al. 2008a, Farias et al. 2016). The phenotypic bioassays consist of either depositing a layer of Bt protein (diet-overlay) on the surface of the diet (Farias et al. 2014a,b) or mixing the protein with the diet (diet-incorporated). The overlay method requires less amount of protein, nevertheless irregularities in the layer deposition on the diet could result in inconsistent results derived from nonuniform exposure of the larvae (Siegfried et al. 2007) and this method may not be appropriate when dealing with larvae that typically burrow into the diet to feed, such as the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) (Patin et al. 1999). Phenotypic bioassays are not expected to detect small changes in the allele frequency (Andow and Alstad 1998) but are cheaper, use less protein, is less time-consuming and can provide information meaningful to assess changes in population susceptibility. The methodologies such as the F_2 screen to estimate the frequency of alleles for resistance provide reliable information about the proportion of this type of allele in comparison to the susceptible counterpart, even though its partially recessive (Andow and Ives 2002), but the use of this option is expensive and time-consuming. However, regardless of the methodology deployed, laboratory research itself is not sufficient to detect resistance (Natália et al. 2016) but generates alerts that must be confirmed with field observations. It is recommended to conduct susceptibility evaluations at least annually in both, the laboratory and field (Shelton and Zhao 2009), in order to detect resistant individuals as early as possible (Dennehy 1987). Under field conditions, the performance of the pest in both, Bt and the conventional crop, should be evaluated in several locations before large-scale deployment. Phenotypic resistance is adequately detected in the field, rather than in the laboratory, when the larva survives on Bt plants, reaches the adult stage and transmits its genes to the next generation (Andow and Ives 2002). In order to provide a clearer concept, Tabashnik and Carrière (2017) defined field-evolved resistance as a 'genetically based decreased in susceptibility of an insect population to a Bt toxin caused by selection in the field'.

Before Bt crop deployment in the field, it is considered that the alleles conferring resistance to those Bt genes are rare in the population, with a frequency in the order of 10^{-3} (Tabashnik 1994, Gould et al. 1997, Burd et al. 2003) making the homozygous resistant individuals rare and recessive with a high proportion of homozygous and heterozygous susceptible insects. Even though Bt maize is not grown in Mexico, we consider that the frequency of alleles for resistance is above the indicated value; wide-area transgenic crops in Texas are grown and the dispersal of individuals to host crops in the studied areas is possible. Additionally, illegal cultivation of Bt maize in Mexico is not discarded and may contribute to selection pressure.

Several elements of resistance management have to be implemented in addition to monitoring susceptibility to Cry1F in target pest populations with bioassays, to address the complexity of introducing Bt crops in countries such as Mexico. Resistance must be managed by a combination of tactics such as application of Integrated Pest Management practices, continuing to promote the use of refuge by outreach education programs for growers, introduction of pyramided Bt crops, and also involving proactive engagement with stakeholders, such as the academia, industry, regulatory authorities, and grower organizations.

Conclusion

The susceptibility to Cry1F across various regions were indicative of mortality and growth parameters discussed. The results indicated that the evaluated populations of *S. frugiperda* are susceptible to the Cry1F protein. The established baseline is the first record of regional susceptibility of *S. frugiperda* to Cry1F in Mexico to date. This multi-region assessment completes the investigation of baseline susceptibility to Cry1F in *S. frugiperda* in Mexico indicating potential of Cry1F containing maize to be an effective tool for the control of this pest.

Acknowledgements

We like to extend our sincere thanks to academic research and technical laboratory staff who participated in various activities; Chad Boeckman (Corteva Agriscience) and Mike Connelly (Pioneer Hi-Bred) for their support to coordinate this project.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Andow, D. A., and D. N. Alstad. 1998. F2 screen for rare resistance alleles. J. Econ. Entomol. 91: 572–578.

Andow, D. A., and A. R. Ives. 2002. Monitoring and adaptive resistance management. Ecol. Appl. 12: 1378–1390.

Andrews, K. L. 1988. Latin American research on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Fla. Entomol. 71: 630–653.

Bateman, M. L., R. K. Day, B. Luke, S. Edgington, U. Kuhlman, and M. J. W. Cock. 2018. Assessment of potential biopesticide options for managing fall armyworm (*Spodoptera frugiperda*) in Africa. J. Appl. Entomol. 142: 805–819.

Bernardi, D., E. Salmeron, R. J. Horikoshi, O. Bernardi, P. M. Dourado, R. A. Carvalho, S. Martinelli, G. P. Head, and C. Omoto. 2015. Cross-resistance between Cry1 proteins in fall armyworm (*Spodoptera frugiperda*) may affect the durability of current pyramided Bt maize hybrids in Brazil. PLoS One 10: e0140130.

Bird, L. J., and R. J. Akhurst. 2007. Variation in susceptibility of *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae) in Australia to two *Bacillus thuringiensis* toxins. J. Invertebr. Pathol. 94: 84–94.

Blanco, C. A., O. P. Perera, F. Gould, D. V. Sumerford, G. Hernández, C. A. Abel, and D. A. Andow. 2008a. An empirical test of the F2 screen for detection of *Bacillus thuringiensis*-resistance alleles in tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 101: 1406–1414.

Blanco, C. A., N. P. Storer, C. A. Abel, R. Jackson, R. Leonard, J. D. Lopez, Jr, G. Payne, B. D. Siegfried, T. Spencer, and A. P. Terán-Vargas. 2008b. Baseline susceptibility of tobacco budworm (Lepidoptera: Noctuidae) to Cry1F toxin from *Bacillus thuringiensis*. J. Econ. Entomol. 101: 168–173.

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: 1–9.

Burd, A. D., F. Gould, J. R. Bradley, J. W. Van Duyn, and W. J. Moar. 2003. Estimated frequency of nonrecessive Bt resistance genes in bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in eastern North Carolina. J. Econ. Entomol. 96: 137–142.

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, Gainesville, FL.

Carrie, A. D., S. T. Spencer, A. Tessnow, P. Tamez-Guerra, M. Pusztai-Carey, and G. A. Sword. 2016. Nutrition affects insect susceptibility to *Bt* toxins. Sci. Rep. 7: 39705.

CGIAR. 2018. Fall armyworm reported in India: battle against the pest extends now to Asia. Available from https://maize.org/fall-armyworm-reported-in-india-battle-against-the-pest-extends-now-to-asia/. Accessed October 23, 2019.

Chandrasena, D. I., A. M. Signorini, G. Abratti, N. P. Storer, M. L. Olaciregui, A. P. Alves, and C. D. Pilcher. 2018. Characterization of field-evolved resistance to *Bacillus thuringiensis*-derived Cry1F δ-endotoxin in *Spodoptera frugiperda* populations from Argentina. Pest Manag. Sci. 74: 746–754.

Clavijo, S., and Y. G. Perez Greiner. 2000. Protección y Sanidad Vegetal (Capítulo 6), pp. 345–361. *In* F. H. Nieves and C. G. Narváez (eds.), Insectos plagas del maíz (Sección 2). Fundación Polar, Caracas, Venezuela.

Cruz I., M. L. C., Figueiredo, A. C., Oliveira and C. A., Vasconcelos. 1999. Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil under three levels of aluminum saturation. Int. J. Pest Manage. 45: 293–296.

Jing, D., J. Guo, Y. Jiang, J. Zhao, A. Sethi, K. He and Z. Wang. 2019. Initial detections and spread of invasive *Spodoptera frugiperda* in China and comparisons with other noctuid larvae in corn fields using molecular techniques. Insect Sci. 1–11. doi:10.1111/1744-7917.12700

Deans, C. A., G. A. Sword, and S. T. Behmer. 2016. Nutrition as a neglected factor in insect herbivore susceptibility to Bt toxins. Curr. Opin. Insect Sci. 15: 97–103.

Dennehy, T. J. 1987. Decision-making for managing pest resistance to pesticides, pp. 118–126. *In* M. G. Ford, D. W. Holloman, B. P. S. Khanbay, and R. M. Sawicki (eds.), Combating resistance to xenobiotics: biological and chemical approaches. Ellis Horwood, Chichester, England.

Drake, V. A., and R. A. Farrow. 1988. The influence of atmospheric structure and motions on insect migration. Annu. Rev. Entomol. 33: 183–210.

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. dos Santos, and C. Omoto. 2014a. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Prot. 64: 150–158.

Farias, J. R., R. J. Horikoshi, A. C. Santos, and C. Omoto. 2014b. Geographical and temporal variability in susceptibility to Cry1F toxin from *Bacillus thuringiensis* in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations in Brazil. J. Econ. Entomol. 107: 2182–2189.

Farias, J. R., D. A. Andow, R. J. Horikoshi, D. Bernardi, R. D. Ribeiro, A. R. Nascimento, A. C. Santos, and C. Omoto. 2016. Frequency of Cry1F resistance alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Pest Manag. Sci. 72: 2295–2302.

Finney, D. J. 1971. Probit analysis. Cambridge University Press, Cambridge, United Kingdom.

Gould, F., A. Anderson, A. Jones, D. Sumerford, D. G. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. Proc. Natl. Acad. Sci. USA. 94: 3519–3523.

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. 71: 249–254.

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, Y. Niu, *et al*. 2014. Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt maize. PLoS One 9: e112958.

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): e0154492.

Mota-Sanchez, D., and J. Wise. 2017. Arthropod pesticide resistance database. Michigan State University, East Lansing, MI. Available from https://www.pesticideresistance.org/. Accessed October 22, 2019.

Mota-Sanchez, D., S. P. Bills, and M. E. Whalon. 2002. Arthropod resistance to pesticides: status and overview, pp. 241–272. *In* W. Wheeler and B. Gainesville (eds.), Pesticides in agriculture and the environment. Marcel Decker, New York.

Nagoshi, R. D., P. Silvie, R. L. Meagher, L. López, and V. Machado. 2007a. Identification and comparison of fall armyworm (Lepidoptera: Noctuidae) host strains in Brazil, Texas, and Florida. Ann. Entomol. Soc. Am. 100: 394–402.

Nagoshi, R. N., P. Silvie, and R. L. Meagher. 2007b. Comparison of haplotype frequencies differentiate fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Florida and Brazil. J. Econ. Entomol. 100: 954–961.

Nagoshi, R. N., S. Fleischer, R. L. Meagher, M. Hay-Roe, A. Khan, M. G. Muru, P. Silvie, C. Vergara, and J. Westbrook. 2017. Fall armyworm migration across the Lesser Antilles and the potential for genetic exchanges between North and South American populations. PLoS One 12(2): e0171743.

Nagoshi, R. N., G. Goergen, H. D. Plessis, J. van den Berg, and R. Meagher Jr. 2018. Genetic comparisons of fall armyworm populations from 11 countries spanning sub-saharan Africa provide insights into strain composition and migratory behaviors. Sci. Rep. 9: 8311.

NAS. 2017.Genetically engineered crops. National Academies of Sciences, Engineering, Medicine, Washington, DC. Available from https://nas-sites.org/ge-crops/2016/05/17/report/

Natália, A. L., S. M. Mendes, O. F. Santos-Amaya, C. A. Santos, T. P. M. Teixeira, R. N. C. Guedes, and E. J. G. Pereira. 2016. Rapid selection and characterization of Cry1F resistance in a Brazilian strain of fall armyworm. Entomol. Exp. Appl. 158: 236–247.

Pacheco, M. F. 1985. Plagas de los cultivos agrícolas en Sonora y Baja California. Libro Técnico No. 1. SARH-CIANO, Ciudad Obregón, Sonora. pp. 414.

Pacheco, M. F. 1994. Plagas de los cultivos oleaginosos en México. Libro Técnico. No. 3. SARH-CIANO, Ciudad Obregón, Sonora. México.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae) a sibling species complex? Ann. Entomol. Soc. Am. 79: 898–904.

Patin, A. L., T. J. Dennehy, M. A. Sims, B. E. Tabashnik, Y. B. Liu, L. Antilla, D. Gouge, T. J. Henneberry and R. Staten. 1999. Status of pink bollworm susceptibility to B.t. in Arizona, pp. 991–996. *In* Proceeding of Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Saeglitz, C., D. Bartsch, S. Eber, A. Gathmann, K. U. Priesnitz, and I. Schuphan. 2006. Monitoring the Cry1Ab susceptibility of European corn borer in Germany. J. Econ. Entomol. 99: 1768–1773.

SAS Institute. 2008. PROC user's manual, version, 6th ed. SAS Institute, Cary, NC.

Shelton, A. M., and J. Zhao. 2009. Resistance management to transgenic insecticidal plants, pp. 247–259. *In* E. B. Radcliffe, W. D. Hutchison, and R. E. Cancelado RE (eds.), Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press, Cambridge, MA.

Siegfried, B. D., T. Spencer, A. L. Crespo, N. P. Storer, G. P. Head, E. D. Owens, and D. Guyer. 2007. Ten years of Bt resistance monitoring in the European corn borer: what we know, what we don't know, and what we can do better. Am. Entomol. 53: 208–214.

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: 1031–1038.

Storer, N. P., M. E. Kubiszak, J. Ed 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: 294–300.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39: 47–79.

Tabashnik, B. E., and Y. Carrière. 2017. Surge in insect resistance to transgenic crops and prospects for sustainability. Nat. Biotechnol. 35: 926–935.

Tabashnik, B. E., Y. Carrière, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton, and J. Z. Zhao. 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. J. Econ. Entomol. 96: 1031–1038.

Tabashnik, B. E., D. Mota-Sanchez, M. E. Whalon, R. M. Hollingworth, and Y. Carrière. 2014. Defining terms for proactive management of resistance to Bt crops and pesticides. J. Econ. Entomol. 107: 496–507.

Terán-Vargas, A. P. 2008. Evaluación del nivel de infestación de las plagas y malezas más comunes del maíz en el sur de Tamaulipas en el ciclo otoño-invierno 2006–2007. Informe final. Campo Experimental Las Huastecas INIFAP, Tamaulipas, México.

Torres, L., and A. M. Cotes. 2005. Efecto de la crioconservación sobre la viabilidad y actividad biocontroladora de *Nomuraea rileyi* contra

Spodoptera frugiperda (Lepidoptera: Noctuidae. Rev. Colomb. Entomol. 31: 133–138.

[USDA-FAS] United States Department of Agriculture-ForeingAgricultural Service. 2019. Update: fall armyworm now in 15 of China´s provinces. GAIN Report number: CH19033. Available from https://www.fas.usda.gov/data/china-update-fall-armyworm-now-15-china-s-provinces#targetText=The%20Fall%20Armyworm%20(FAW%3B%20Spodoptera,million%20mu)%20of%20grain%20production. Accessed October 22, 2019.

Velez, 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). Bull. Entomol. Res. 103: 700–713.

Velez, A. M., A. P. Alves, E. E. Blankenship, and B. D. Siegfried. 2016. Effect of Cry1F maize on the behavior of susceptible and resistant *Spodoptera frugiperda* and *Ostrinia nubilalis*. Entomol. Exp. Appl. 159: 37–45.