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## Insecticide Resistance and Resistance Management

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

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### 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<sup>2</sup> (92.0–320) (Ahumada 2, Chihuahua), while the  $LC_{95}$  was between 207.1 (145–363) (Obregón, Sonora) and 1,217 ng/cm<sup>2</sup> (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  $\leq 6.45$  and  $\leq 5.05$ -fold, respectively. The 50% growth inhibition ( $GI_{50}$ ) ranged from 2.8 (0.008–9.3) (Obregón, Sonora) to 42.4 ng/cm<sup>2</sup> (3.6–147.0) (Cajeme 1, Sonora). The  $GI_{95}$  was between 75.4 (San Luis Río Colorado, Sonora) to 1,198 ng/cm<sup>2</sup> (Cajeme 1, Sonora). The relative inhibition at 50% of the growth, ( $RI_{50} = GI_{50}$  field /  $GI_{50}$  S-LAB) was  $\leq 3.5$  and at 95% ( $RI_{95}$ ) was  $\leq 1.91$ -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  $F_1$  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<sup>2</sup> (92.0–320) (Ahumada 2, Chihuahua); la  $LC_{95}$  estuvo entre 207.1 (145–363) (Obregón, Sonora) y 1,217 ng/cm<sup>2</sup> (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  $\leq 6.45$  y  $\leq 5.05$  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<sup>2</sup> (3.6–147.0) (Cajeme 1, Sonora). El  $GI_{95}$  estuvo entre 75.4 (San Luis Río Colorado, Sonora) y 1,198 ng/cm<sup>2</sup> (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<sup>2</sup> 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  $\geq 15$  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  $27 \pm 1^\circ\text{C}$  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  $4^\circ\text{C}$  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.

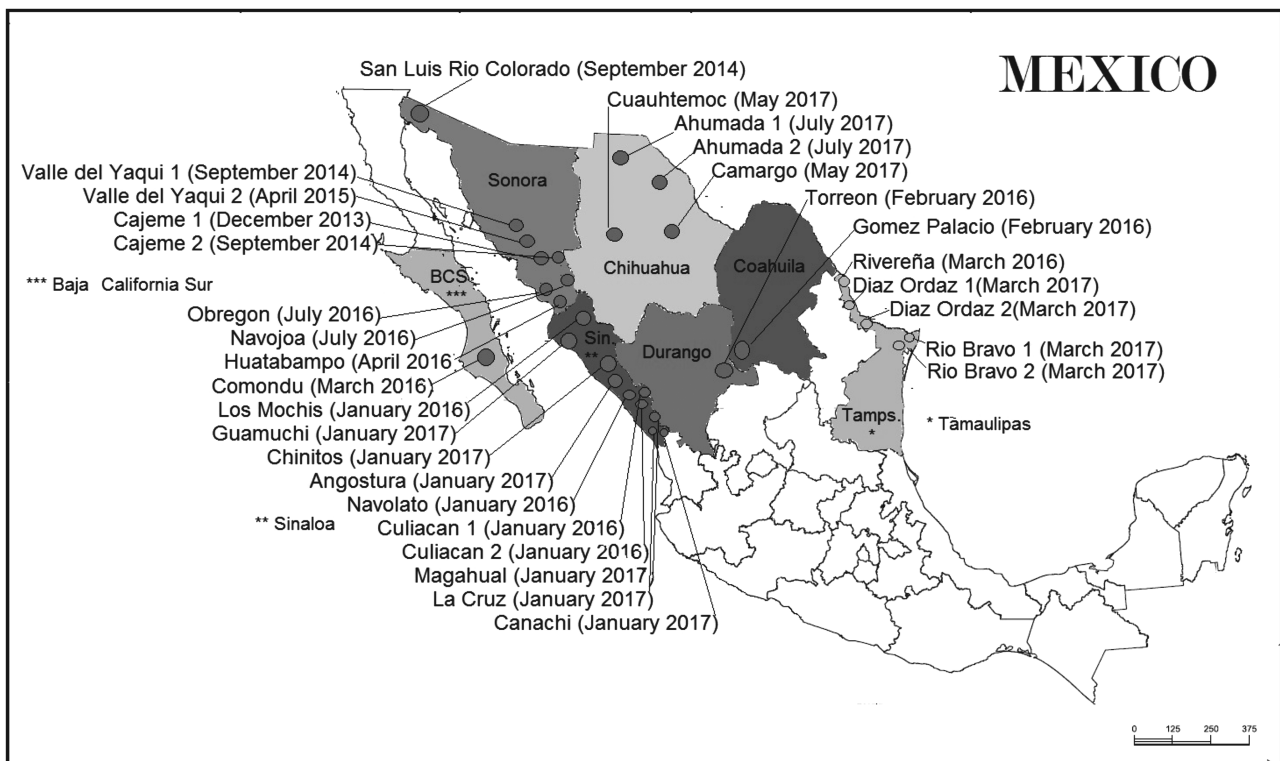


Fig. 1. Map of Mexico showing 28 geographical populations of *Spodoptera frugiperda* collected for the study

## 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  $\mu$ l 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<sup>2</sup>. Two hours later, each well was infested with an active neonate larva (*F*<sub>1</sub>, 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  $\mu$ l 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<sub>50</sub> and LC<sub>95</sub> 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<sub>50</sub> (SR<sub>50</sub>) and LC<sub>95</sub> (SR<sub>95</sub>) were calculated by dividing the LC<sub>50</sub> (LC<sub>95</sub>) values of the respective field population by the LC<sub>50</sub> (LC<sub>95</sub>) 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<sub>50</sub> and GI<sub>95</sub> 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<sub>50</sub> (RI<sub>50</sub>) and GI<sub>95</sub> (RI<sub>95</sub>) were calculated by dividing the GI<sub>50</sub> (GI<sub>95</sub>) values of the respective field population by the GI<sub>50</sub> (GI<sub>95</sub>) 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<sub>50</sub> and LC<sub>95</sub> 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<sub>50</sub> 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<sub>95</sub>, 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<sub>50</sub> and LC<sub>95</sub>, with SR<sub>95</sub> values of 3.23 and 7.1 $\times$ , 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 Río Colorado	Sept. 2014
Navojoa	Sonora	Navojoa	July 2016
Obregón	Sonora	Obregon	July 2016
Cuauhtémoc	Chihuahua	Cuauhtémoc	May 2017
Camargo	Chihuahua	Camargo	May 2017
Ahumada 1	Chihuahua	Ahumada	July 2017
Ahumada 2	Chihuahua	Ahumada	July 2017
Díaz Ordaz 1	Tamaulipas	Díaz Ordaz	Mar. 2017
Díaz Ordaz 2	Tamaulipas	Díaz Ordaz and Camargo	Mar. 2017
Río Bravo 1	Tamaulipas	Río Bravo	Mar. 2017
Río Bravo 2	Tamaulipas	Río Bravo	Mar. 2017
Rivera	Tamaulipas	Region Rivera	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 Guamúchil	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 $\times$ ) 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<sup>2</sup>, 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<sup>2</sup>, and the  $GI_{95}$  was 187.5 (66.8–132,841.0) ng/cm<sup>2</sup> (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<sup>2</sup> 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<sup>2</sup> (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<sup>2</sup>, and the  $GI_{95}$  from 98.9 (49.6–984.4; Cuauhtémoc) to 658.6 (448.3–1,097.0; Ahumada 2) ng/cm<sup>2</sup> (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<sup>2</sup>, and the  $LC_{95}$  was 214.6 (107.3–1,560.0) ng/cm<sup>2</sup> (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<sup>2</sup>, respectively (Table 3). The Torreón population had no survivors beyond 300 ng/cm<sup>2</sup>.

### 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<sup>2</sup> and the  $LC_{95}$  was 209.6 (168.0–276.0) ng/cm<sup>2</sup> (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<sup>2</sup> (Table 3). At 600 ng/cm<sup>2</sup>, 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<sup>2</sup>. 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<sup>2</sup> (Table 2). The lowest  $GI_{50}$  value was 1.5 (0.2–3.8; Chinitos) ng/cm<sup>2</sup> and the highest  $GI_{50}$  was 41.4 (16.0–85.5; Culiacán 2) ng/cm<sup>2</sup> (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<sup>2</sup> (Culiacán 1). All the populations evaluated had >92% mortality at the highest evaluated dose (600 ng/cm<sup>2</sup>).

### Sonora

The  $LC_{50}$  ranged from 14.4 (6.3–24.0; Cajeme 1) to 128.1 (110.0–149.0; Navojoa) ng/cm<sup>2</sup> and the  $LC_{95}$  was between 157.1 (85.3–571.0; Cajeme 1) and 778 (589–1,119.0; Navojoa) ng/cm<sup>2</sup> (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<sup>2</sup>, 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<sup>2</sup> (Table 3). At 600 ng/cm<sup>2</sup>, 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; Río Bravo 2) ng/cm<sup>2</sup> and the  $LC_{95}$  was between 228.3 (183.0–307.0; Díaz Ordaz 1) and 1,217 (510.8–7,390.0; Río Bravo 2) ng/cm<sup>2</sup> (Table 2). The  $GI_{50}$  was between 1.4 (0.2–3.7; Rivera) and 7.0 (0.3–16.7; Río Bravo 1) ng/cm<sup>2</sup> (Table 3). The  $GI_{95}$  ranged from 57.7 (39.9–116.2; Díaz Ordaz 1) to 656.8 (364.9–1,650.0; Río Bravo 2) ng/cm<sup>2</sup> (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	<i>n</i>	df	<i>b</i> ± SE	LC <sub>50</sub> <sup>a</sup> (95% FL) ng/cm <sup>2</sup>	LC <sub>95</sub> <sup>a</sup> (95% FL) ng/cm <sup>2</sup>	Pr > χ <sup>2</sup>	SR <sub>50</sub> <sup>b</sup>	SR <sub>95</sub> <sup>b</sup>
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
Rivera	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.

<sup>a</sup>LC<sub>50</sub> = estimated concentration that causes 50% mortality; CL<sub>95</sub> = estimated concentration that causes 95% mortality.

<sup>b</sup>Sensitive ratio (SR) = LC<sub>50 (95)</sub> field population/LC<sub>50 (95)</sub> 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/cm<sup>2</sup>). 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/cm<sup>2</sup>; 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<sub>95</sub> obtained from all six provinces in Mexico ranged between 209 and 1217 ng/cm<sup>2</sup> indicating *S. frugiperda* susceptibility to Cry1F in the geography. Additionally, a diagnostic concentration (LC<sub>95</sub>) 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	<i>n</i>	<i>df</i>	<i>b</i> ± SE	GI <sub>50</sub> <sup>a</sup> (95% FL) ng/cm <sup>2</sup>	GI <sub>95</sub> <sup>a</sup> (95% FL) ng/cm <sup>2</sup>	Pr > χ <sup>2</sup>	RI <sub>50</sub> <sup>b</sup>	RI <sub>95</sub> <sup>b</sup>
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								
Cauahémoc	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
Riverena	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

<sup>a</sup>GI<sub>50</sub> = estimated concentration that causes 50% growth inhibition in comparison to the untreated control; GI<sub>95</sub> = estimated concentration that causes 95% growth inhibition in comparison to the untreated control.

<sup>b</sup>Relative inhibition = GI<sub>50(95)</sub> field population/GI<sub>50(95)</sub> 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<sup>2</sup> as a discriminating dose (based on upper confidence limit of the highest LC<sub>99</sub>) to distinguish *S. frugiperda* 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.

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