Concentration–Mortality Response of Mexican Populations Fall Armyworm (Lepidoptera: Noctuidae) to Commercial Formulations of *Bacillus thuringiensis*[1]

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Abstract The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is the most economically important pest of maize (*Zea mays* L.) grown in Mexico. In order to identify biologically based management tactics for this pest, we determined the concentration–mortality response of 16 *S. frugiperda* populations collected in Mexico to two commercial formulations of *Bacillus thuringiensis* Berliner (*Bt*) (XenTari® [*Bt* subsp. *aizawai*] and DiPel® [*Bt* subsp. *kurstaki*]; Valent de México SA De CV, Jalisco, México). Laboratory bioassays established median lethal concentrations (LC_{50}s) for each *Bt* formulation × population combination. We also determined mean larval weight 7 d after exposure and the number of larvae that reached the third instar of development. The populations were susceptible to both *Bt* formulations but they were, overall, more susceptible to *Bt* subsp. *aizawai* (XenTari) than to *Bt* subsp. *kurstaki* (DiPel). These results can serve as a reference to detect changes in *S. frugiperda* response to these *Bt* commercial products over time.

Key Words fall armyworm, bioinsecticides, bioassay, resistance, corn

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is the most economically important pest of maize (*Zea mays* L.) grown in Mexico (Aguirre et al. 2016, Blanco et al. 2014). Conventional chemical insecticides are the main method of controlling this pest in the field, with an estimated 30,000 kg of active ingredients applied each cropping cycle in Mexico (Blanco et al. 2014). However, because the pest has developed resistance to these insecticides, which are also economically costly and environmentally adverse, there is an ongoing search for alternatives of management of pest populations (Roh et al. 2007, Sauka and Benintende 2008). One alternative are bioinsecticides based on proteins derived from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) (Herrero et al. 2016, Silva et al. 2015). *Bt* occurs naturally in soil, produces a parasporal crystal body during its sporulation phase, and contains one or more δ-endoproteins with

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insecticidal activity (Sanahuja et al. 2011) against a broad variety of pest species of the orders Lepidoptera (including *Spodoptera* spp.), Coleoptera, Diptera, Hymenoptera, Hemiptera, and Blattaria (Herrero et al. 2016).

Bt-based products are the most consistent and significant bioinsecticides on the global market (Sauka and Benintende 2008, Whalon and Wingerd 2003), supplying approximately 95% of the bioinsecticide market (Devi et al. 2019). Regulatory policies have led to the cancellation or restricted use of more than half of the registered conventional insecticides (e.g., organophosphates, carbamates, chlorinated hydrocarbons), thus, accounting for the growth of biopesticides in the market. The Organization for Economic Cooperation and Development predicts that the market of biopesticides could grow to occupy 20% of the world pesticide market by the end of 2020 (Whalon and Wingerd 2003).

In Mexico, commercial formulations of *Bt* are not routinely used to manage the fall armyworm, but it is expected to increase over time. However, risk of the development of resistance also may increase. For this reason, the objective of this study was to determine the current mortality response of *S. frugiperda* larval populations in the states of Chihuahua, Coahuila, Durango, Sinaloa, Sonora, and Tamaulipas to two commercial formulations of *Bt*-based insecticides. One is a formulation for *Bt* subsp. *aizawai* (XenTari®; Valent de México SA De CV, Jalisco, México); the other is a formulation of *Bt* subsp. *kurstaki* (DiPel®; Valent de México SA De CV).

Materials and Methods

Fall armyworm populations. Larvae were collected from maize in the states of Chihuahua, Coahuila, Durango, Sinaloa, Sonora, and Tamaulipas, and from sorghum (*Sorghum bicolor* (L.) Moench) in Sinaloa. At each site, five commercial plantings, separated by at least 15 km, were selected for collection of larvae. Larger larvae ($\geq$3rd instar) were collected from each planting, exercising care to collect one larva per plant, with the plants being more than 2 m apart to reduce the probability of individual larvae having the same parents. A total of 340 larvae were collected from the plantings at each site and were designated as a population and identified as to location (Table 1). Each population was transported to the laboratory where they were maintained for 1 week. Dead, diseased, or parasitized larvae were then discarded, with at least 85% of the larvae in each population reaching adulthood.

A source of susceptible larvae was a colony of *S. frugiperda* provided by the Insecticide Toxicology laboratory of the graduate program in Entomology and Acarology of the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico, Mexico. This colony had been maintained under laboratory conditions and free of insecticide selection pressure for more than 10 yr. Occasionally, larvae collected from maize fields of Montecillo, State of Mexico, where no chemical or other control method has been used, had been incorporated into the colony to maintain colony vigor. Larvae from this colony served as a susceptible reference population in these tests.

Healthy larvae from each of these populations were placed individually in plastic cups containing 8 ml of meridic diet (Fall Armyworm Diet; Southland Products

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Table 1. *Spodoptera frugiperda* populations evaluated.

Population	Collection Place	Date	Geographical Coordinates
CHI-AHU1	Ahumada, Chihuahua	July 2016	30.083806°N, −106.292028°W
CHI-AHU2	Ahumada, Chihuahua	July 2016	29.970639°N, −106.163361°W
CHI-CUA1	Cuauhtémoc, Chihuahua	July 2016	28.725972°N, −107.035111°W
CHI-CUA2	Cuauhtémoc, Chihuahua	July 2016	28.468444°N, −106.883861°W
CHI-DEL1	Ciudad Delicias, Chihuahua	July 2016	28.158389°N, −105.446111°W
COA-FIM1	Francisco I. Madero, Coahuila	May 2016	25.760333°N, −103.247472°W
COA-MAT1	Matamoros, Coahuila	May 2016	25.604611°N, −103.218639°W
DUR-GPA1	Gómez Palacio, Durango	May 2016	25.754889°N, −103.374667°W
SIN-CUL1	Culiacán, Sinaloa	September 2016	24.688917°N, −107.229028°W
SON-NAV1	Navojoa, Sonora	July 2016	27.116667°N, −109.839222°W
SON-OBR1	Obregón, Sonora	July 2016	27.409722°N, −110.139861°W
TAM-DIO1	Gustavo Díaz Ordaz, Tamaulipas	March 2016	26.183806°N, −98.465889°W
TAM-DIO2	Gustavo Díaz Ordaz, Tamaulipas	April 2016	26.291722°N, −98.816750°W
TAM-DIO3	Gustavo Díaz Ordaz, Tamaulipas	April 2016	26.224569°N, −98.733100°W
TAM-RIO1	Rio Bravo, Tamaulipas	March 2016	25.864528°N, −97.969361°W
TAM-VAH1	Valle Hermoso, Tamaulipas	April 2016	25.668833°N, −97.910361°W

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Incorporated, Lake Village, AR) until they pupated. Groups of 30–40 pupae were placed in brown paper bags to await adult emergence. Adults were provided with a solution of 10% honey in distilled water. Bags were changed every 3 d, and the masses of eggs on the inner surfaces of the bags were collected and placed in a plastic container with larval diet. Larvae, pupae, and adults were maintained in a rearing chamber at 27 ± 1°C, 70 ± 10% relative humidity (RH), and on a 12:12 h photoperiod.

***Bt* formulations.** Two commercial formulations of *Bt* were included in these assays. DiPel is a formulation of *Bt* subsp. *kurstaki* that contains the insecticidal proteins Cry1Aa, Cry1Ab, Cry1Ac, and Cry2A and has a potency of 32,000 international units of potency/mg (UIT/mg). XenTari is a formulation of *Bt* subsp. *aizawai* that contains the proteins Cry1Aa, Cry1Ab, Cry1C, and Cry1D and has a potency of 15,000 UIT/mg. Both are formulated as a dry flowable at a concentration equivalent to 103 g active ingredient/kg (Valent de México SA De CV).

Bioassays. Initially, larvae were exposed to several concentrations of each formulation to determine those concentrations that yielded 0% and 100% mortality (i.e., biological response window). Then, seven equidistant intermediate logarithmic concentrations that covered the range for each population were tested in concentration–mortality response bioassays. The bioassay procedure followed the methods described by Marçon et al. (1999). One milliliter of the meridic diet was deposited into each well of a bioassay tray (Bio-BA-128; C-D International Inc., Pitman, NJ) and allowed to solidify. A 50-μl droplet of each *Bt* concentration (dissolved in distilled water with 0.1% Triton X-100 [Sigma-Aldrich, St. Louis, MO]) was deposited on the diet in the appropriate wells. After 1 h, neonate larvae that had emerged 24 h previously and had not been fed the meridic diet were placed individually in the wells. Trays were then covered with plastic cling film (Pull N' Peel Tab Bio-Cv-16; C-D International Inc.) and perforated to permit gas exchange. Sixteen larvae per concentration/replication were used, with 3–5 replications performed on different days. Each replication included a control to which 50 μl of distilled water only was added to the diet in each well. Trays were placed in a rearing chamber maintained at 27 ± 1°C, 70 ± 10% RH, and on a 12:12 h photoperiod

Seven days later, larval mortality, larval weight, the number of larvae that had reached third instar were recorded for each treatment. Flaccid larvae or those that moved abnormally when stimulated with a paintbrush were considered dead. The third instar of development was determined visually by comparing with those in normal rearing conditions, that is, larva measured ≥1 cm long and weighed ≥10 mg. Surviving larvae of each replication were weighed together to obtain an average weight, and this value was used to calculate the percentage of weight reduction relative to untreated larvae.

Statistical analysis. Mortality data were subjected to probit analysis by means of the SAS statistical software, version 9.4 (SAS Institute 2016), to estimate values of lethal concentration (LC) causing 50% and 95% mortality of a population (LC_{50} and LC_{95}), the slopes, and standard errors. The populations were not considered statistically different if the 95% confidence limits at a given level of mortality overlapped (Robertson and Preisler 1992). The maximum accepted level for the control was 10%, and mortality in treatments was corrected using the Abbott (1925) formula. Once LC_{50} and LC_{95} were determined for the field populations and the

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susceptible reference population, relative response was obtained by dividing the LC_{50} or LC_{95} of each of the field populations by the LC_{50} or LC_{95} of the susceptible population, respectively (Dhingra and Sarup 1990). Before analysis, to achieve normality, the percentage of larvae that reached third instar and the percentage of weight inhibition relative to the control were transformed by the arcsine function of the square root of the response percentage/100. Later, an analysis of variance and comparison of means (Tukey, $P = 0.05$) were performed (SAS Institute 2016) to determine whether there were significant differences within each population.

Results

Mortality response. Variation in response was greater with DiPel than with XenTari (Tables 2, 3). The estimated LC_{50} values of the 14 field populations of *S. frugiperda* exposed to XenTari for 7 d varied from 82.5 (SIN-CUL1) to 246.1 ng/cm^2 (TAM-DIO1), while LC_{95} values varied from 975.4 (SON-OBR1) to 4.024 ng/cm^2 (COA-MAT1) (Table 2). With LC_{50}, the largest variation was 3.1× and, with LC_{95}, it was 1.9× (Table 2). Estimated values of LC_{50} of the 16 *S. frugiperda* field populations exposed to DiPel varied from 50.8 (CHI-DEL1) to 857.2 ng/cm^2 (TAM-DIO2) and LC_{95} from 2.973 (SON-OBR1) to 31.944 ng/cm^2 (TAM-DIO3) (Table 3). With LC_{50}, we detected a variation of 4.7× and, with LC_{95}, a variation of 5.0× (Table 3). The LC_{50} of seven field populations (CHI-AHU1, CHI-DEL1, COA-FIM1, COA-MAT1, DUR-GPA1, SIN-CUL1, and SON-NAV1) was lower than the reference population (Table 3).

Weight inhibition. The percentage of weight inhibition of neonate larvae treated with XenTari varied from 9.4% to 99.9%, while DiPel was from 14.6% to 99.9% (Tables 4, 5). At the highest dose of DiPel (24,873.73 ng/cm^2), there were surviving larvae in seven populations (CHI-AHU2, CHI-CUA2, CHI-DEL1, COA-FIM1, TAM-DIO1, TAM-DIO2, and TAM-RIO1), although weight inhibition was nearly 100% (Table 5).

Percentage of larvae that reached third instar. With XenTari, at 2.487.37 to 0.25 ng/cm^2, the percentage of larvae that reached third instar varied from 0% to 100% (Table 6). With the control, 76.6% to 100% of the larvae reached third instar (Table 6). With the highest dose of DiPel (24,873.73 ng/cm^2), there were third-instar larvae in three populations (CHI-AHU2, TAM-DIO2, and TAM-RIO1) (Table 7). With the dose of 2,487.37 to 0.25 ng/cm^2, the percentage of third-instar larvae varied from 0% to 100%. In the control, this percentage was 78.13% to 100% (Table 7).

Discussion

Spodoptera frugiperda affects maize production in Mexico. The main tool for its control has been the application of conventional chemical insecticides (Blanco et al. 2014). However, in Mexico, a change in susceptibility to groups of insecticides routinely applied in nearby regions for its control has been reported in *S. frugiperda* (Gutiérrez-Moreno et al. 2019). In addition, *Bt* technology is not yet available in Mexico due to restrictions to it use in maize, thus, resulting in continuing dependence on synthetic insecticides. Commercial *Bt* products available on the market express the joint action of diverse proteins, which individually have shown

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Table 2. Concentration–mortality response of *S. frugiperda* larvae exposed for 7 d to XenTari.

Population	*n**	Slope ± SE	LC_{50} (FL)*	LC_{95} (FL)*	RR_{50}**	RR_{95}**
SUS-LAB	512	1.16 ± 0.16	80.3 (52.5–113.2)	2,125 (1,104–6,260)	1.0	1.0
CHI-AHU1	510	1.79 ± 0.27	192.5 (92.9–378.4)	1,598 (690.7–12,467)	2.4	0.8
CHI-AHU2	510	1.99 ± 0.22	211.5 (169.0–266.5)	1,412 (943.0–2,563)	2.6	0.7
CHI-CUA1	512	1.30 ± 0.23	91.1 (63.4–129.1)	1,693 (757.3–8,636)	1.1	0.8
CHI-CUA2	512	1.71 ± 0.17	143.2 (111.2–181.7)	1,308 (887.4–2,239)	1.8	0.6
CHI-DEL1	512	1.28 ± 0.23	138.6 (47.7–305.4)	2,699 (923.6–45,071)	1.7	1.3
COA-FIM1	512	1.46 ± 0.17	230.4 (174.6–310.2)	3,067 (1,729–7,377)	2.9	1.4
COA-MAT1	512	1.00 ± 0.22	92.0 (13.2–300.5)	4,024 (934.4–332,633)	1.1	1.9
DUR-GPA1	512	1.61 ± 0.16	133.1 (101.5–170.8)	1,396 (935.8–2,427)	1.7	0.7
SIN-CUL1	640	1.36 ± 0.35	82.5 (11.4–234.7)	1,338 (402.1–154,141)	1.0	0.6
SON-NAV1	512	2.20 ± 0.25	183.4 (148.7- 226.9)	1,026 (710.8–1,767)	2.3	0.5
SON-OBR1	512	2.22 ± 0.35	177.6 (143.0–231.5)	975.4 (595.3–2,397)	2.2	0.5
TAM-DIO1	378	2.36 ± 0.42	246.1 (124.9–517.4)	1,222 (563.5–13,137)	3.1	0.6
TAM-DIO2	512	1.65 ± 0.19	141.0 (108.6–182.0)	1,398 (879.6–2,790)	1.8	0.7
TAM-DIO3	512	1.68 ± 0.16	179.7 (139.6–228.2)	1,706 (1,159–2,891)	2.2	0.8

* *n*, number of treated insects; LC, lethal concentration (ng/cm^2); FL, confidence limits at 95%.
** RR, relative response = LC_{50} or LC_{95} of the field populations compared with the LC_{50} or LC_{95} of the susceptible population.

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Table 3. Concentration–mortality response of *S. frugiperda* larvae exposed for 7 d to DiPel.

Population	*n**	Slope ± SE	LC_{50} (FL)*	LC_{95} (FL)*	RR_{50}**	RR_{95}**
SUS-LAB	512	1.06 ± 0.19	182.7 (60.2–434.6)	6,428 (1,912–113,279)	1.0	1.0
CHI-AHU1	512	0.97 ± 0.20	66.8 (9.8–214.2)	3,280 (739.7–349,290)	0.4	0.5
CHI-AHU2	511	1.07 ± 0.10	298.8 (213.8–411.3)	10.183 (5,649–23,040)	1.6	1.6
CHI-CUA1	512	1.02 ± 0.12	189.8 (130.5–267.0)	7,719 (3,870–21,916)	1.0	1.2
CHI-CUA2	512	1.16 ± 0.11	220.5 (159.0–298.7)	5,761 (3,362–12,179)	1.2	0.9
CHI-DEL1	512	0.77 ± 0.07	50.8 (30.2–79.6)	6,797 (3,417–17,131)	0.3	1.1
COA-FIM1	512	0.91 ± 0.11	145.7 (64.2–284.5)	9,567 (3,354–62,411)	0.8	1.5
COA-MAT1	384	0.84 ± 0.10	91.5 (53.0–146.5)	8,303 (3,595–29,599)	0.5	1.3
DUR-GPA1	509	1.21 ± 0.13	141.6 (100.6–191.8)	3,216 (1,885–6,947)	0.8	0.5
SIN-CUL1	640	1.06 ± 0.18	125.1 (43.0–273.6)	4,412 (1,378–74,388)	0.7	0.7
SON-NAV1	512	0.88 ± 0.12	80.8 (46.3–123.2)	5,848 (2,723–19,997)	0.4	0.9
SON-OBR1	512	1.55 ± 0.14	259.3 (200.7–332.9)	2,973 (1,937–5,357)	1.4	0.5
TAM-DIO1	381	1.15 ± 0.12	421.8 (294.3–600.1)	11,296 (5,957–28,730)	2.3	1.8
TAM-DIO2	512	1.18 ± 0.11	857.2 (636.5–1.172)	20,977 (11,739–46,501)	4.7	3.3
TAM-DIO3	512	0.90 ± 0.11	470.9 (322.0–716.9)	31,944 (12,266–149,071)	2.6	5.0
TAM-RIO1	384	1.02 ± 0.11	569.5 (387.9–843.4)	22,985 (10,960–68,005)	3.1	3.6
TAM-VAH1	456	1.33 ± 0.23	292.4 (106.3–739.9)	5,095 (1,568–160,451)	1.6	0.8

* *n*, number of treated insects; LC, lethal concentration (ng/cm^2); FL, confidence limits at 95%.
** RR, relative response = LC_{50} or LC_{95} of the field populations between the LC_{50} or LC_{95} of the susceptible population.

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Table 4. Percentage of weight gain inhibition of *S. frugiperda* larvae exposed for 7 d to XenTari.

	Concentration (ng/cm^2)*					
Population	**2,487.37**	**1,243.69**	**248.74**	**124.37**	**24.87**	**0.25**
SUS-LAB	—	99.4 ± 0.0 a	93.5 ± 1.4 a	92.9 ± 1.7 a	63.9 ± 7.0 ab	29.9 ± 10.9 b
CHI-AHU1	99.9 ± 0.0 a	99.7 ± 0.1 a	95.1 ± 2.2 ab	91.2 ± 1.5 ab	66.9 ± 8.6 b	26.3 ± 11.3 c
CHI-AHU2	—	99.7 ± 0.2 a	95.9 ± 0.3 a	91.0 ± 2.4 ab	75.8 ± 1.7 b	18.1 ± 5.9 c
CHI-CUA1	—	—	97.5 ± 0.9 a	96.3 ± 0.8 a	86.6 ± 2.5 a	37.1 ± 7.5 b
CHI-CUA2	99.8 ± 0.0 a	99.6 ± 0.0 a	95.6 ± 1.4 a	93.5 ± 2.6 a	74.9 ± 2.9 b	15.0 ± 1.5 c
CHI-DEL1	—	99.6 ± 0.1 a	97.3 ± 0.7 a	89.3 ± 2.7 ab	72.5 ± 5.8 b	27.4 ± 9.1 c
COA-FIM1	—	99.2 ± 0.3 a	91.5 ± 3.3 a	89.9 ± 2.0 a	62.1 ± 7.2 b	34.9 ± 14.9 c
COA-MAT1	—	99.7 ± 0.0 a	98.3 ± 0.6 a	89.8 ±1.2 ab	74.2 ± 5.3 b	17.3 ± 2.1 c
DUR-GPA1	99.8 ± 0.0 a	98.2 ± 0.0 a	97.9 ± 0.9 a	94.1 ± 1.5 a	71.6 ± 4.1 b	12.5 ± 2.4 c
SIN-CUL1	—	—	96.9 ± 1.4 a	92.0 ± 1.7 a	70.2 ± 5.5 b	18.0 ± 6.4 c
SON-NAV1	—	99.5 ± 0.0 a	97.3 ± 0.9 a	94.1 ± 1.0 a	69.9 ± 4.6 b	33.0 ± 13.1 c
SON-OBR1	—	—	98.6 ± 0.5 a	96.4 ± 0.4 a	80.5 ± 2.0 b	24.0 ± 5.1 c
TAM-DIO1	—	99.6 ± 0.0 a	95.2 ± 1.2 a	92.1 ± 2.6 a	76.9 ± 0.4 b	37.6 ± 3.0 c
TAM-DIO2	—	98.9 ± 0.0 a	96.4 ± 0.8 a	89.2 ± 1.7 ab	59.0 ± 10.4 b	16.8 ± 21.6 c
TAM-DIO3	99.8 ± 0.0 a	99.5 ± 0.2 a	96.7 ± 1.5 a	89.4 ± 4.0 ab	66.8 ± 7.6 b	9.4 ± 5.1 c

* Means in the same row followed by the same letter are not significantly different ($P = 0.05$, Tukey test). Data were transformed to the arcsine function of the square root of the response percentage/100; nontransformed means in the original units are presented.

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Table 5. Percentage of weight gain inhibition of *S. frugiperda* larvae exposed for 7 d to DiPel.

	Concentration (ng/cm^2)*						
Population	**24,873.73**	**2,487.37**	**1,243.69**	**248.74**	**124.37**	**24.87**	**0.25**
SUS-LAB	—	99.1 ± 0.2 a	99.3 ± 0.1 a	93.2 ± 3.4 a	89.6 ± 2.7 ab	70.3 ± 4.9 b	29.7 ± 10.3 c
CHI-AHU1	—	99.5 ± 0.0 a	99.9 ± 0.0 a	97.8 ± 0.7 a	95.3 ± 1.5 a	86.3 ± 5.1 a	25.9 ± 7.5 b
CHI-AHU2	99.5 ± 0.0 a	98.8 ± 0.5 a	98.6 ± 0.7 a	94.7 ± 1.6 a	92.0 ± 1.5 ab	80.7 ± 3.8 b	34.8 ± 9.2 c
CHI-CUA1	—	99.3 ± 0.5 a	99.4 ± 0.2 a	96.8 ± 0.8 a	94.6 ± 1.3 a	82.2 ± 1.9 b	14.6 ± 10.9 c
CHI-CUA2	99.7 ± 0.0 ab	99.8 ± 0.0 a	99.2 ± 0.3 ab	97.3 ± 0.7 ab	95.8 ± 1.0 ab	88.5 ± 2.7 b	35.9 ± 3.3 c
CHI-DEL1	99.5 ± 0. 0a	99.2 ± 0.1 a	99.6 ± 0.2 a	95.9 ± 2.1 a	95.0 ± 1.6 a	88.1 ± 2.5 a	32.7 ± 10.6 b
COA-FIM1	99.5 ± 0.0 a	99.5 ± 0.2 a	99.0 ± 0.2 a	96.9 ± 0.7 a	94.8 ± 2.4 a	65.5 ±11.8 ab	47.0 ± 35.3 b
COA-MAT1	—	99.3 ± 0.2 a	99.6 ± 0.1 a	98.3 ± 0.7 a	98.4 ± 0.6 a	83.2 ± 3.5 a	33.5 ± 18.6 b
DUR-GPA1	—	99.8 ± 0.1 a	99.7 ± 0.1 a	98.7 ± 0.5 a	93.8 ± 0.6 a	88.0 ± 2.1 a	29.1 ± 6.8 b
SIN-CUL1	—	99.5 ± 0.1 a	98.7 ± 0.5 a	97.9 ± 0.5 a	95.7 ± 1.1 a	80.4 ± 2.7 b	25.4 ± 4.8 c
SON-NAV1	—	99.8 ± 0.0 a	99.3 ± 0.3 a	98.9 ± 0.3 a	97.5 ± 0.4 a	92.6 ± 0.7 a	30.9 ± 5.0 b
SON-OBR1	—	99.5 ± 0.0 a	99.6 ± 0.0 a	97.5 ± 0.5 ab	94.2 ± 1.7 ab	86.5 ± 2.0 b	35.4 ± 4.2 c
TAM-DIO1	99.8 ± 0.0 a	99.4 ± 0.2 a	98.8 ± 0.4 a	98.2 ± 0.2 a	96.4 ± 1.4 a	82.4 ± 2.4 b	46.0 ± 1.9 c
TAM-DIO2	99.6 ± 0.0 a	99.4 ± 0.3 a	98.8 ± 0.3 a	96.4 ± 0.5 a	92.1 ± 1.9 a	80.9 ± 3.6 a	28.0 ± 11.5 b
TAM-DIO3	—	98.9 ± 0.7 a	99.0 ± 0.2 a	95.8 ± 0.5 a	94.0 ± 2.6 a	74.4 ± 2.7 b	19.9 ± 8.2 c
TAM-RIO1	99.9 ± 0.0 a	99.4 ± 0.4 a	98.9 ± 0.5 a	96.8 ± 0.7 a	91.8 ± 0.9 a	77.9 ± 7.3 a	45.8 ± 18.2 b
TAM-VAH1	—	99.3 ± 0.3 ab	99.4 ± 0.2 a	96.8 ± 0.8 ab	93.2 ± 2.0 ab	84.5 ± 3.7 b	30.7 ± 9.7 c

* Means in the same row followed by the same letter are not significantly different ($P = 0.05$, Tukey test). Data were transformed to the arcsine function of the square root of the response percentage/100; nontransformed means in the original units are presented.

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Table 6. Percentage of *S. frugiperda* larvae that reached third instar following exposure for 7 d to XenTari.

	Concentration (ng/cm^2)*						
Population	**2,487.37**	**1,243.69**	**248.74**	**124.37**	**24.87**	**0.25**	**Control**
SUS-LAB	—	0.00 ± 0.0 a	17.19 ± 3.9 ab	20.31 ± 3.9 ab	46.88 ± 10.7 bc	70.31 ± 6.4 c	76.6 ± 1.6 c
CHI-AHU1	0.00 ± 0.0 a	2.08 ± 2.1 a	27.23 ± 6.7 b	39.06 ± 3.0 b	81.25 ± 6.8 c	92.19 ± 1.6 c	98.44 ± 1.6 c
CHI-AHU2	—	3.13 ± 3.1 a	33.33 ± 3.9 b	44.06 ± 10.3 b	87.50 ± 5.7 c	98.44 ± 1.6 c	98.44 ± 1.6 c
CHI-CUA1	—	—	17.19 ± 7.8 a	25.00 ± 2.6 a	53.13 ± 5.4 b	92.19 ± 3.0 c	98.44 ± 1.6 c
CHI-CUA2	0.00 ± 0.0 a	6.25 ± 0.0 a	28.13 ± 6.0 a	37.50 ± 10.8 a	82.81 ± 5.3 b	100.00 ± 0.0 b	100.00 ± 0.0 b
CHI-DEL1	—	0.00 ± 0.0 a	9.38 ± 1.8 ab	29.69 ± 5.3 b	54.69 ± 7.8 c	95.31 ± 3.0 d	96.88 ± 1.8 d
COA-FIM1	—	0.00 ± 0.0 a	34.38 ± 7.4 b	42.19 ± 7.4 b	75.00 ± 3.6 c	87.50 ± 4.4 c	90.63 ± 6.0 c
COA-MAT1	—	0.00 ± 0.0 a	10.94 ± 3.9 ab	35.94 ± 3.9 bc	56.25 ± 5.7 cd	78.13 ± 5.4 de	84.38 ± 4.0 e
DUR-GPA1	0.00 ± 0.0 a	6.25 ± 0.0 a	7.81 ± 3.9 a	21.88 ± 6.0 a	71.88 ± 6.0 b	98.44 ± 1.6 b	95.31 ± 3.0 b
SIN-CUL1	—	—	6.25 ± 3.4 a	16.25 ± 1.5 a	50.00 ± 7.1 b	95.00 ± 3.6 c	97.50 ± 2.5 c
SON-NAV1	—	0.00 ± 0.0 a	6.25 ± 2.6 a	14.06 ± 3.0 a	79.69 ± 9.0 b	98.44 ± 1.6 b	96.88 ± 3.1 b
SON-OBR1	—	—	9.38 ± 4.0 a	31.25 ± 2.6 b	87.50 ± 8.8 c	100.00 ± 0.0 c	96.88 ± 1.8 c
TAM-DIO1	—	6.25 ± 0.0 a	35.42 ± 8.3 ab	54.17 ± 11.6 bc	91.67 ± 5.5 cd	100.00 ± 0.0 d	100.00 ± 0.0 d
TAM-DIO2	—	6.25 ± 0.0 a	25.00 ± 4.4 ab	46.88 ± 8.3 b	76.56 ± 1.6 c	89.06 ± 3.0 c	95.31 ± 3.0 c
TAM-DIO3	6.25 ± 0.0 a	9.38 ± 3.1 a	29.69 ± 11.8 a	50.00 ± 12.2 ab	85.94 ± 3.9 b	93.75 ± 2.6 b	93.75 ± 2.6 b

* Means in the same row followed by the same letter are not significantly different ($P = 0.05$, Tukey test). Data were transformed to the arcsine function of the square root of the response percentage/100; nontransformed means in the original units are presented.

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Table 7. Percentage of *S. frugiperda* larvae that reached third instar following exposure for 7 d to DiPel.

	Concentration (ng/cm^2)*			
Population	**24,873.73**	**2,487.37**	**1,243.69**	**248.74**
SUS-LAB	—	0.00 ± 0.0 a	0.00 ± 0.0 a	9.38 ± 4.0 a
CHI-AHU1	—	0.00 ± 0.0 a	0.00 ± 0.0 a	12.50 ± 3.6 ab
CHI-AHU2	6.25 ± 0.0 a	4.17 ± 2.1 a	8.33 ± 5.5 a	26.56 ± 7.8 ab
CHI-CUA1	—	8.33 ± 8.3 a	6.25 ± 0.0 a	17.19 ± 5.3 a
CHI-CUA2	0.00 ± 0.0 a	3.13 ± 3.1 ab	7.81 ± 1.6 ab	20.31 ± 3.0 bc
CHI-DEL1	0.00 ± 0.0 a	0.00 ± 0.0 a	0.00 ± 0.0 a	14.06 ± 5.9 ab
COA-FIM1	0.00 ± 0.0 a	3.13 ± 3.1 a	0.00 ± 0.0 a	21.88 ± 5.4 a
COA-MAT1	—	3.13 ± 3.1 a	3.13 ± 3.1 a	10.42 ± 5.5 a
DUR-GPA1	—	0.00 ± 0.0 a	0.00 ± 0.0 a	9.38 ± 5.4 ab
SIN-CUL1	—	0.00 ± 0.0 a	0.00 ± 0.0 a	1.25 ± 1.3 a
SON-NAV1	—	0.00 ± 0.0 a	6.25 ± 6.3 a	9.38 ± 4.0 a
SON-OBR1	—	3.13 ± 3.1 a	2.08 ± 2.1 a	17.19 ± 6.9 ab
TAM-DIO1	0.00 ± 0.0 a	16.67 ± 5.5 ab	25.00 ± 3.6 ab	25.00 ± 7.2 ab
TAM-DIO2	6.25 ± 0.0 a	15.63 ± 8.3 a	26.56 ± 5.3 ab	56.25 ± 6.8 bc
TAM-DIO3	—	12.50 ± 3.6 a	12.50 ± 3.6 a	39.06 ± 6.9 a
TAM-RIO1	6.25 ± 0.0 a	29.17 ± 8.3 a	27.08 ± 5.5 a	68.75 ± 6.3 b
TAM-VAH1	—	8.93 ± 2.7 a	14.84 ± 3.3 a	41.90 ± 4.8 b

* Means in the same row followed by the same letter are not significantly different ($P = 0.05$, Tukey test). Data were transformed to the arcsine function of the square root of the response percentage/100; nontransformed means in the original units are presented.

varied effect against *S. frugiperda*. Our results reported herein demonstrate that the populations we tested have a similar response to both formulations and that the variability exhibited is due to natural variation of the populations (Tables 2, 3).

With XenTari at the LC_{50} level, we identified six populations that were different from the susceptible population (CHI-AHU2, COA-FIM1, SON-NAV1, SON-OBR1, TAM-DIO1, TAM-DIO3). However, at the LC_{95} level, none of the populations were statistically different (Table 2). With DiPel, only one population (TAM-DIO2) was significantly different at the LC_{50} level from the susceptible population while, at the LC_{95} level, response was similar to that of the susceptible population (Table 3).

These results are highly relevant to establishment of monitoring programs for management of resistance induced by repeated use of insecticides of this type, as well as for possible adoption of *Bt* technologies for management of this insect. An

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Table 7. Extended.

Concentration (ng/cm^2)*			
124.37	**24.87**	**0.25**	**Control**
18.75 ± 4.4 a	54.69 ± 3.9 b	79.69 ± 1.6 c	84.38 ± 6.5 c
20.31 ± 5.3 ab	53.13 ± 14.1 bc	87.50 ± 4.4 c	96.88 ± 1.8 c
40.63 ± 7.9 ab	62.50 ± 9.2 bc	87.50 ± 4.4 c	95.31 ± 3.0 c
31.25 ± 8.5 a	65.63 ± 11.6 b	96.88 ± 1.8 b	95.31 ± 1.6 b
34.38 ± 4.0 c	64.06 ± 6.4 d	100.00 ± 0.0 e	98.44 ± 1.6 e
15.63 ± 4.0 ab	31.25 ± 9.2 b	79.69 ± 3.0 c	93.75 ± 2.6 c
28.13 ± 6.0 a	64.06 ± 4.7 b	82.81 ± 5.3 b	78.13 ± 7.4 b
20.83 ± 7.5 ab	47.92 ± 4.2 bc	81.25 ± 12.5 c	83.33 ± 2.1 c
29.69 ± 3.9 bc	50.00 ± 9.2 c	96.88 ± 1.8 d	98.44 ± 1.6 d
2.50 ± 1.5 a	43.75 ± 8.4 b	91.25 ± 3.2 c	96.25 ± 2.5 c
28.13 ± 4.0 b	48.44 ± 3.0 c	98.44 ± 1.6 d	100.00 ± 0.0 d
39.06 ± 10.3 bc	67.19 ± 4.7 c	98.44 ± 1.6 d	100.00 ± 0.0 d
44.03 ± 16.3 b	93.75 ± 0.0 c	100.00 ± 0.0 c	100.00 ± 0.0 c
67.19 ± 10.3 cd	89.06 ± 6.9 cd	95.31 ± 1.6 d	93.75 ± 2.6 d
39.06 ± 6.9 a	70.31 ± 9.3 b	92.19 ± 4.7 b	93.75 ± 2.6 b
75.00 ± 7.2 b	83.33 ± 4.2 b	89.58 ± 2.1 b	97.92 ± 2.1 b
80.33 ± 3.6 c	80.82 ± 6.3 cd	98.44 ± 1.6 de	100.00 ± 0.0 e

interesting observation of our results is that the populations in which we obtained the highest LC_{50} levels were in the region bordering the United States, where *Bt* technology is frequently used. However, these levels do not suggest development of resistance to the evaluated products, but they do point to the need to study susceptibility of populations of this region to individual proteins used in the formulations.

Worldwide, the strains *Bt* subsp. *aizawai* and *Bt* subsp. *kurstaki*, present in the commercial formulations of XenTari and DiPel, respectively, are used for management of lepidopteran insects. These serovars differ in the presence of proteins that have shown variable toxic effect to the fall armyworm. The *Bt* subsp. *aizawai* strain in XenTari contains a mixture of the proteins Cry1Aa, Cry1Ab, Cry1C, and Cry1D, while the strain *Bt* subsp. *kurstaki* of DiPel contains the proteins Cry1Aa, Cry1Ab, Cry1Ac, and Cry2A (Valent Biosciences, https://www.valentbiosciences.com/cropprotection/wp-content/uploads/sites/2/2017/06/xentari-protoxin-blend-technical-bulletin-ag-5398.pdf; last accessed 10 January 2020). Studies

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on the spectrum of Cry proteins on *S. frugiperda* have shown that the proteins Cry1Bb, Cry1Be, Cry1Ca, Cry1Da, Cry1Fa, and Cry2Ae are active against this lepidopteran, while the proteins Cry1Aa and Cry1Ab have shown low activity (Van Frankenhuyzen 2009). Likewise, Aranda et al. (1996) found that the proteins Cry1C and Cry1D were more active in the fall armyworm than the proteins Cry1Aa, Cry1Ab, Cry1Ac, Cry1B, and Cry1E. These results show that the commercial formulation XenTari (*Bt* subsp. *aizawai*) contains proteins that are more active against *S. frugiperda* than DiPel (*Bt* subsp. *kurstaki*), showing that XenTari is more toxic than DiPel at the levels of LC_{50} and LC_{95} in the susceptible population, as well as having higher slope values observed in the bioassays of the evaluated populations (Tables 2, 3). Percentage of weight inhibition and the number of larvae reaching the third instar also show a response that is directly proportional to the increase in concentration of both formulations. We observed that a higher dosage of *Bt* subsp. *kurstaki* than of *Bt* subsp. *aizawai* was required for both evaluated parameters (Tables 4, 5). The parameter larval growth inhibition was used as a response criterion in addition to mortality because it is an important variable to measure susceptibility of insects to *Bt* (Ali and Luttrell 2009). Studies on *S. frugiperda* susceptibility to commercial *Bt* formulations (XenTari and DiPel) are limited, and most have been conducted with the individual proteins contained in these formulations. Our results show that XenTari is more toxic than DiPel against *S. frugiperda*, coinciding with other laboratory studies (Jakka et al. 2014) and field observations (dos Santos et al. 2009). Moreover, our data show variation in susceptibility to the products XenTari and DiPel of different fall armyworm populations in Mexico, which we consider to be natural variation because of the low selection pressure of these insecticides. These results can serve as a reference for detecting changes in the response of *S. frugiperda* to these *Bt*-based commercial products.

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References Cited

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

Aguirre, L.A., A. Hernández-Juárez, M. Flores, E. Cerna, J. Landeros, G.A. Frías and M.K. Harris. 2016. Evaluation of foliar damage by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically modified corn (Poales: Poaceae) in Mexico. Fla. Entomol. 99: 276–280.

Ali, M.I. and R.G. Luttrell. 2009. Response estimates for assessing heliothine susceptibility to *Bt* toxins. J. Econ. Entomol. 102: 1935–1947.

Aranda, E., J. Sanchez, M. Peferoen, L. Güereca and A. Bravo. 1996. Interactions of *Bacillus thuringiensis* crystal proteins with the midgut epithelial cells of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 68: 203–212.

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

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challenges for the adoption of integrated pest management programs. J. Integr. Pest Manag. 5: E1–E9.

Devi, P.V., P. Duraimurugan and K.S.V.P. Chandrika. 2019. *Bacillus thuringiensis*-based nanopesticides for crop protection, Pp. 249–260. *In* Koul, O. (ed.), Nano-Biopesticides Today and Future Perspectives. Academic Press, New York. 485 pp.

Dhingra, S. and P. Sarup. 1990. Development of techniques for detecting resistance in crop pests to insecticides. J. Entomol. Res. 14: 156–163.

dos Santos, K.B., P. Neves, A.M. Meneguim, R.B. dos Santos, W.J. dos Santos, G.V. Boas, V. Dumas, E. Martins, L.B. Praca, P. Queiroz, C. Berry and R. Monnerat. 2009. Selection and characterization of the *Bacillus thuringiensis* strains toxic to *Spodoptera eridania* (Cramer), *Spodoptera cosmioides* (Walker) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). Biol. Control 50: 157–163.

Gutiérrez-Moreno, R., D. Mota-Sánchez, C.A. Blanco, M.E. Whalon, H. Terán-Santofimio, J.C. Rodríguez-Maciel and C. DiFonzo. 2019. Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. J. Econ. Entomol. 112: 792–802.

Herrero, S., Y. Bel, P. Hernández-Martínez and J. Ferré. 2016. Susceptibility, mechanisms of response and resistance to *Bacillus thuringiensis* toxins in *Spodoptera* spp. Curr. Opin. Insect Sci. 15: 89–96.

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

Marçon, P.C., L.J. Young, K.L. Steffey and B.D. Siegfried. 1999. Baseline susceptibility of European corn borer (Lepidoptera: Crambidae) to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 92: 279–285.

Robertson, J.L. and H.K. Preisler. 1992. Pesticide Bioassays with Arthropods. CRC, Boca Raton, FL.

Roh, J.Y., J.Y. Choi, M.S. Li, B.R. Jin and Y.H. Je. 2007. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J. Microbiol. Biotechnol. 17: 547–559.

Sanahuja, G., R. Banakar, R.M. Twyman, T. Capell and P. Christou. 2011. *Bacillus thuringiensis*: A century of research, development and commercial applications. Plant Biotechnol. J. 9: 283–300.

SAS Institute. **2016.** SAS User's Manual 9.4. SAS Institute Inc., Cary, NC.

Sauka, D.H. and G.B. Benintende. 2008. *Bacillus thuringiensis*: Generalidades. Un acercamiento a su empleo en el biocontrol de insectos lepidópteros que son plagas agrícolas. Rev. Argent. Microbiol. 40: 124–140.

Silva, M.C., H.A.A. Siqueira, L.M. Silva, E.J. Marques and R. Barros. 2015. Cry proteins from *Bacillus thuringiensis* active against diamondback moth and fall armyworm. Neotrop. Entomol. 44: 392–401.

Van Frankenhuyzen, K. 2009. Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J. Invertebr. Pathol. 101: 1–16.

Whalon, M.E. and B.A. Wingerd. 2003. *Bt*: Mode of action and use. Arch. Insect Biochem. Physiol. 54: 200–211.

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