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Evaluation of foliar damage by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically modified corn (Poales: Poaceae) in Mexico

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Abstract

The fall armyworm, *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), is a key pest of corn, *Zea mays* L. (Poales: Poaceae), in Mexico. The development of genetically modified (GM) corn hybrids for resistance to this insect, with the inclusion of several genes coding for proteins Cry1Ab, Vip3Aa20, and mCry3A of *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (*Bt*), offer an alternative to conventional insecticides to control this pest. Resistance to fall armyworms of the GM corn hybrids Agrisure 3000 GT, Agrisure Viptera 3110, and Agrisure Viptera 3111 was evaluated in 4 locations at Sinaloa for a 3 yr period. Damage evaluation showed that the maize hybrids with the *Bt* gene insertion were not affected by the fall armyworm as compared with their respective isolines, which were seriously damaged. The results reaffirm the insect control benefits provided by this technology and provide a baseline for resistance management.

Key Words: *Bacillus thuringiensis*; δ-endotoxin; fall armyworm; leaf damage

Resumen

El gusano cogollero *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), es la plaga de mayor importancia económica del maíz *Zea mays* L. (Poales: Poaceae) en México. El desarrollo de híbridos de maíz genéticamente modificados para resistencia a este insecto, con la inserción de diversos genes que codifican para las proteínas Cry1Ab, Vip3Aa20 y mCry3A de *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (*Bt*), ofrecen una alternativa a los insecticidas convencionales de control de esta plaga. Se evaluó durante tres años, el daño foliar del gusano cogollero en maíz GM con los híbridos Agrisure 3000 GT, Agrisure Viptera 3110 y Agrisure Viptera 3111 en cuatro localidades del estado de Sinaloa. La evaluación del daño demostró que el maíz con la inserción de genes de Bt son eficaces para contrarrestar o no ser afectado por el daño provocado por la plaga, en comparación con sus respectivas líneas convencionales que fueron seriamente dañadas. Los resultados reafirman los beneficios del control de insectos que ofrece esta tecnología y proporciona una línea base para el manejo de la resistencia.

Palabras Clave: *Bacillus thuringiensis*; δ-endotoxina; gusano cogollero; daño foliar

The fall armyworm, *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), is indigenous to the American continent (Sena et al. 2003) and has been reported to infest 186 host plant species in North and Central America (Casmuz et al. 2010). Corn, *Zea mays* L. (Poales: Poaceae), is the primary host of economic importance wherever it is grown in Mexico (Sena et al. 2003). Tropical and subtropical areas are most seriously affected (Ortega 1987; Rodríguez & Marín 2008) with losses incurred from post-emergence to maturity (Ortega 1987). Yield losses over 30% are common (Herrera 1979; García-Gutiérrez et al. 2012), and in some cases total crop loss occurs (Silva-Aguayo et al. 2010).

A reliance on chemical control to manage pest populations has become increasingly ineffective as *S. frugiperda* now expresses resistance to several toxicological groups of insecticides (Georghiou & Mellon 1983; Yu 1991; Pacheco-Covarrubias 1993; Morillo & Notz 2001; Yu et al. 2003).

The development of new control techniques led to the elaboration of genetically modified corn hybrids expressing a *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (*Bt*) crystal protein that, when consumed by lepidopterous larvae, proved fatal to the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), the southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae), the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), the corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), and *S. frugiperda* (Abel et al. 2000; Castro et al. 2004). A pyramided strategy that combines 2 or more *Bt* genes deployed in the same corn plant is now used to conserve insecticidal efficacy (Burkness et al. 2010; Niu et al. 2013; Yang et al. 2013). Genetically modified corn (GM) hybrids with *Bt* genes have also been developed to resist a wider range of pests within Lepidoptera and Coleoptera (Buntin et al. 2004a,b; Buntin 2008; Duan et al. 2008; Hardke et al. 2011). These hybrids support a

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pest management strategy in modern agriculture (Fernandes et al. 2007), although concerns from an economic, scientific, and social standpoint remain in Mexico.

Mexico is the center of origin of over 61 native races of corn (Reyes 1990; Matsuoka 2005; CONABIO 2006; Kato et al. 2009), and there is concern that GM corn could jeopardize those races (Kato-Yamakake 2004; Serratos-Hernández et al. 2004; Turrent et al. 2010); however, Baltazar et al. (2015) suggest that measures such as spatial isolation could minimize contamination risks. More information is needed in Mexico to validate if the *Bt* Cry proteins of GM corn are effective in controlling the crop pests under various environmental conditions beyond those reported by Aguirre et al. (2015a) to control corn earworm in the state of Sinaloa and by Aguirre et al. (2015b) to control *S. frugiperda* in the state of Tamaulipas. Thus, the objective of this study was to evaluate foliar damage in corn hybrids with Cry1Ab, Vip3Aa20, and mCry3A toxins from *B. thuringiensis* to control *S. frugiperda* larvae in Sinaloa, Mexico, during 3 growing seasons.

Materials and Methods

Research was conducted at Oso Viejo, El Dorado, and Camalote in the city of Culiacan and the city of Navolato, both in the state of Sinaloa, during the 2011–2013 fall–winter growing seasons. Plots were planted under biosafety conditions, isolated at least 500 m from commercial corn plots and planted at least 21 d later than recommended to avoid cross-pollination with non-GM hybrids in accordance with government regulations for field tests with GM corn (Halsey et al. 2005; LBOGM 2005).

Three *Bt* corn hybrids (Agrisure™ 3000 GT with Cry1Ab and mCry3A proteins; Agrisure® Viptera™ 3111 with Cry1Ab, Vip3Aa20, and mCry3A; and Agrisure® Viptera™ 3110 with Cry1Ab and Vip3Aa20) were used in this research and compared with their respective non-GM isolines provided by Syngenta Agro S.A de C.V. de México (San Lorenzo 1009, Primer Piso, Colonia Del Valle, 03100, México, D.F.). The first two hybrids are resistant to Lepidoptera and Coleoptera and the last one is resistant to Lepidoptera.

A randomized complete block design was used in each locality and date. In 2011, Agrisure 3000 GT and Agrisure Viptera 3110, plus their isolines, were planted at Oso Viejo. In addition, each variety had a corresponding treatment that included a foliar insecticide control (see Table 1). There were 4 replicate blocks per treatment, and they were planted on 28 Jan. In 2012, Agrisure Viptera 3111 and Agrisure 3000 GT hybrids, with and without insecticide treatments, were planted on 15 Feb at Navolato. Agrisure Viptera 3111 was planted at El Dorado on 19 Feb, also with and without insecticide applications (see Table 1). Only 3 replicates were planted in these areas. In 2013, Agrisure Viptera 3111 was planted at Camalote and Oso Viejo on 14 and 15 March, respectively, with 3 treatments (GM hybrid, isoline, isoline plus insecticide) and 4 replicates (see Table 1). In addition, experimental plots during the 3 yr period received an insecticide treatment for *S. frugiperda* if plants less than 20 cm tall reached a 10% infestation level, or plants 20

Table 1. Insecticide treatments used to evaluate leaf damage by fall armyworms in genetically modified (GM) corn during 2011–2013 fall–winter growing seasons in localities of Culiacan and Navolato, Sinaloa, Mexico.

Year	Hybrid[a]	Locality	Insecticide[b,c]
2011	Agrisure 3000 GT	Oso Viejo	without insecticide application
	Agrisure 3000 GT + ic	Oso Viejo	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline + ic	Oso Viejo	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline	Oso Viejo	check
	Agrisure Viptera 3110	Oso Viejo	without insecticide application
	Agrisure Viptera 3110 + ic	Oso Viejo	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline + ic	Oso Viejo	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline	Oso Viejo	check
2012	Agrisure Viptera 3111	El Dorado	without insecticide application
	Agrisure Viptera 3111+ ic	El Dorado	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline + ic	El Dorado	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline	El Dorado	check
	Agrisure Viptera 3111	Navolato	without insecticide application
	Agrisure Viptera 3111 + ic	Navolato	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline + ic	Navolato	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline	Navolato	check
	Agrisure 3000 GT	Navolato	without insecticide application
	Agrisure 3000 GT + ic	Navolato	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline + ic	Navolato	permethrin—lambda cyhalothrin—emamectin benzoate
	Isoline	Navolato	check
2013	Agrisure Viptera 3111	Camalote	without insecticide application
	Isoline + ic	Camalote	emamectin benzoate
	Isoline	Camalote	check
	Agrisure Viptera 3111	Oso Viejo	without insecticide application
	Isoline + ic	Oso Viejo	emamectin benzoate
	Isoline	Oso Viejo	check

[a]ic = insecticide control
[b]check = isoline without insecticide application
[c]Insecticides were applied at the following rates: permethrin, 400 mL/ha; lambda cyhalothrin, 500 mL/ha; emamectin benzoate, 200 mL/ha.

cm or taller reached a 20% infestation level, at the rate of 2 applications per year (Table 1).

Each experimental plot consisted of 10 rows, each 5 m long, with 0.8 m between rows with a 40 to 50 seed planting density. The seedlings were later thinned to 34 plants per row. The experimental plot was surrounded with a buffer area of 6 rows of conventional corn, and other buffer areas were planted between replicates, which were planted the same time as the experimental material as required by official regulations. Agricultural management of the plot followed the technical guide for corn growers developed by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP 2010).

Foliar damage under natural infestation by fall armyworms was evaluated by sampling 10 plants randomly in the 4 central rows at V6–V8 (2011), and V2–V4, V6–V8, and V10–V12 (2012 and 2013) phenological stages of the plant. A numerical scale (1–9), also known as the Davis scale, was used to evaluate foliar feeding damage (Davis et al. 1989, 1992; Mihm 1983) ranging from 1 = no foliar damage (highly resistant) to 9 = severe foliar damage (totally susceptible).

PROC ANOVA and Tukey's multiple rank test ($P < 0.05$) were used to compare among treatments. SAS/STAT (SAS version 9.0; SAS Institute, Cary, North Carolina) software was used to analyze the percentage of injury to plants injured and the damage ratings (Davis scale).

Results

Genetically modified hybrids at Oso Viejo in 2011 did not show significant ($P > 0.05$) damage by fall armyworms. The isolines with insecticide treatment also had low damage scores, 1.85 and 1.22 (Davis scale) for Agrisure 3000 GT and Agrisure Viptera 3110, respectively, with only some pinhole feeding marks on leaves and no significant differences between treatments ($P > 0.05$). In contrast, isolines without chemical treatment had an average of damage score of 4.20 in both hybrids, which possessed large holes and long lesions on leaves. *Spodoptera frugiperda* did not damage Agrisure Viptera 3110 and Agrisure 3000 GT with insecticide treatment, and 5.0% of plants were damaged in the latter without treatment. The non-GM hybrids displayed damaged plants from 15.0 to 90.0%, including those in which chemical control was used (Table 2).

At El Dorado and Navolato in 2012, results were similar to those in 2011. Agrisure Viptera 3111 and Agrisure 3000 GT, including those with insecticide treatment, showed little foliar damage (<1.4 on the Davis scale) and few injured plants, with some plants with pinholes and only 13.3% of plants injured in the Agrisure 3000 GT treatment. The isolines of the GM hybrids were significantly different, with 52.2 to 81.1% of plants injured and plant damage that varied from 2.89 to 4.97 on the Davis scale, including large holes and long leaf lesions (Table 3).

In 2013, Agrisure Viptera 3111 did not show feeding signs by *S. frugiperda* at any of the test sites, whereas the isolines were heavily infested including those in which chemical control was applied. Foliar injury was 1.72 to 3.22 on the Davis scale and 35.8 to 74.2% of plants injured, including large lesions (Table 4).

Table 2. Foliar damage and percentage of plants injured by fall armyworms on the genetically modified hybrids Agrisure 3000 GT and Agrisure Viptera 3110 and their respective isolines at Oso Viejo, Culiacan, Sinaloa, in 2011.

Hybrid[a]	Leaf damage[b,c]	Plants injured (%)[c]
Agrisure 3000 GT	1.10 a	5.0 a
Agrisure 3000 GT + ic	1.00 a	0.0 a
Isoline + ic	1.85 a	30.0 b
Isoline	4.20 b	82.5 c
	F = 17.33***	F = 38.58***
Agrisure Viptera 3110	1.00 a	0.0 a
Agrisure Viptera 3110 + ic	1.00 a	0.0 a
Isoline + ic	1.22 a	15.0 b
Isoline	4.20 b	90.0 c
	F = 13.22***	F = 297.00***

[a]ic = insecticide control
[b]Mean numerical scale
[c]Genetically modified hybrids and their respective isolines followed by the same letter do not differ significantly (ANOVA and Tukey's test; $P > 0.05$). *** Indicates significant F value at $P < 0.001$, df = 3,15.

Discussion

Agrisure 3000 GT, Agrisure Viptera 3110, and Agrisure Viptera 3111 hybrids were resistant to *S. frugiperda* as compared with their severely injured respective isolines during the 3 yr research study in the Sinaloa corn-growing areas. These results are similar to those found by Aguirre et al. (2015b), testing the same *Bt* hybrids in the state of Tamaulipas. Also, Piña & Solleiro (2013) indicated that experimental tests of GM corn in various areas of Mexico are consistently efficacious in controlling key pests.

This research showed that use of GM *Bt* corn hybrids provides season-long protection from *S. frugiperda*. In contrast, chemical control only protects the plant when the insecticide residue is present, and a failure in timing of application(s) represents a risk in control efficacy. Piña & Solleiro (2013) reported that protecting non-*Bt* corn in several areas of Mexico from infestations of corn earworms and fall armyworms required from 3 to 5 insecticide applications per season, and from 720 g to 3.6 kg of active ingredient per ha. In addition, researchers in other countries reported similar results of *Bt* corn with the Cry1Ab toxin for controlling the fall armyworm with respect to conventional hybrids with and without insecticide control (Buntin et al. 2001, 2004a,b; Buntin 2008; Hardke et al. 2011; Huang et al. 2011; Ríos-Díez et al. 2012).

Hybrids with the *Bt* toxin used in this research consistently demonstrated reduction in foliar damage. However, in all areas tested, Agrisure 3000 GT displayed more injured plants and larger lesion size than Agrisure Viptera 3110 and Agrisure Viptera 3111. The higher level of damage is thought to be due to Agrisure 3000 GT having only 1 *Bt* toxin (Cry1Ab) for Lepidoptera control, whereas the other tested hybrids have 2 pyramided *Bt* genes. Multiple genes for resistance are thought to provide better resistance to the pest. Agrisure Viptera 3110 and Agrisure Viptera 3111 have 2 toxins (pyramid events) for Lepidoptera control, the δ-endotoxin Cry1Ab and the vegetative insecticide protein Vip3Aa20, which provide excellent protection to the crop, not only from *S. frugiperda* but also from other Lepidoptera (Burkness et al. 2010; Niu et al. 2013; Yang et al. 2013).

Planting dates of experimental plots were at least 21 d later than the recommended date in Sinaloa. This requirement was imposed by regulatory authorities in order to avoid cross pollination with conventional corn in the area. This condition put the experimental plots in this research under high pest pressure, which came from surrounding corn fields and sorghum fields. Despite this high level of pest pressure, the *Bt* toxin in the crop reduced the infestation level and damage. If these GM hybrids were planted on the recommended planting date under optimal conditions to the crop, these *Bt* hybrids could be expected to perform well. Such a corn pest management program would reduce the use of chemical insecticides, allow the crop to better express its genetic potential, and conserve yield and grain quality by decreasing foliar damage.

Table 3. Foliar damage and percentage of plants injured by fall armyworms on the genetically modified corn hybrids Agrisure Viptera 3111 and Agrisure 3000 GT and their respective isolines at El Dorado and Navolato, Sinaloa, in 2012.

Hybrid[a]	Locality	Leaf damage[b,c]	Plants injured (%)[c]
Agrisure Viptera 3111	El Dorado	1.00 a	0.0 a
Agrisure Viptera 3111 + ic	El Dorado	1.00 a	0.0 a
Isoline + ic	El Dorado	3.53 b	64.4 b
Isoline	El Dorado	2.89 b	56.7 b
		$F = 10.35$***	$F = 16.54$***
Agrisure Viptera 3111	Navolato	1.07 a	1.1 a
Agrisure Viptera 3111 + ic	Navolato	1.07 a	1.1 a
Isoline + ic	Navolato	3.20 b	52.2 b
Isoline	Navolato	4.90 c	81.1 c
		$F = 11.52$***	$F = 14.99$***
Agrisure 3000 GT	Navolato	1.34 a	13.3 a
Agrisure 3000 GT + ic	Navolato	1.13 a	7.8 a
Isoline + ic	Navolato	3.87 b	66.7 b
Isoline	Navolato	4.97 b	77.8 b
		$F = 9.65$***	$F = 10.39$***

[a]ic = insecticide control
[b]Mean numerical scale
[c]Genetically modified hybrids and their respective isolines followed by the same letter do not differ significantly (ANOVA and Tukey's test; $P > 0.05$). *** Indicates significant F value at $P < 0.001$, df = 11,35.

Table 4. Foliar damage and percentage of plants injured by fall armyworms on the genetically modified corn hybrid Agrisure Viptera 3111 and its respective isoline at Camalote and Oso Viejo, Culiacan, Sinaloa, in 2013.

Hybrid[a]	Locality	Leaf damage[b,c]	Plants injured (%)[c]
Agrisure Viptera 3111	Camalote	1.00 a	0.0 a
Isoline + ic	Camalote	1.72 b	35.8 b
Isoline	Camalote	3.22 c	74.2 c
		$F = 48.92$***	$F = 58.44$***
Agrisure Viptera 3111	Oso Viejo	1.00 a	0.0 a
Isoline + ic	Oso Viejo	1.80 b	39.2 b
Isoline	Oso Viejo	2.63 c	62.5 c
		$F = 35.46$***	$F = 27.38$***

[a]ic = insecticide control
[b]Mean numerical scale
[c]Genetically modified hybrid and the respective isoline treatments followed by the same letter do not differ significantly (ANOVA and Tukey's test; $P > 0.05$). *** Indicates significant F value at $P < 0.001$, df = 8,35.

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