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Susceptibility of Genetically Modified Maize Hybrids to Sugarcane Borer, *Diatraea saccharalis* (F.)[1], at Sinaloa, Mexico

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Abstract. Use of genetically modified hybrids of maize, *Zea mays* L., is a pest management tool with special significance when a pest such as sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), becomes less responsive to conventional control methods. Resistance of genetically modified maize hybrids 'Agrisure™ 3000 GT' and 'Agrisure® Viptera™ 3111' were evaluated against stalk borers and compared with conventional hybrids with or without insecticide. During evaluation, few borers were found, especially during the first year (2012), but, in most cases, stalk damage by larvae was less in Agrisure™ 3000 GT and Agrisure® Viptera™ 3111 than in their conventional isolines. Insecticide did not prevent damage by the pest and even treated conventional maize was damaged. Use of Agrisure™ 3000 GT and Agrisure® Viptera™ 3111 hybrids can be a management tool to prevent damage by sugarcane borer in maize.

Introduction

Genetically modified hybrids with genes that code *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae) proteins endow resistance against a wide range of mainly lepidopteran pests of maize, *Zea mays* L. (Bruck et al. 2006). Hybrids were developed with a Bt crystal protein to prevent damage by European corn borer, *Ostrinia nubilalis* (Hübner); southwestern corn borer, *Diatraea grandiosella* Dyar; and sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) (Abel et al. 2000, Castro et al. 2004); later, more than one gene (pyramided genes) was inserted to express more variants of the Bt toxins to improve hybrids and maximize pest control (Burkness et al. 2001, Niu et al. 2013, Yang et al. 2013).

Genetically modified hybrids are a pest management alternative to reduce crop damage and minimize use of insecticide in modern agriculture (Burkness et al. 2001, Duan et al. 2008, Ghimire et al. 2011, Hardke et al. 2011, Shelton 2012, Farias et al. 2013). In Mexico, genetically modified maize hybrids were evaluated in 2009 to control such key pests as corn earworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Piña and Solleiro 2013; Aguirre et al. 2015ab, 2016). Sugarcane borer is an important pest that tunnels stalks, kills growing points, or cuts central leaves that provide

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inoculation courts for entry by stalk pathogens into maize in some areas of Mexico. The second generation also can damage maize cobs to allow entry of fungi, resulting in cob rotting (Ortega 1987, CIMMYT 2016).

The economic threshold to initiate chemical control of the pest is when 10% of stalks are injured (CESAVEG 2015). However, adults are not trapped by a system that detects oviposition and egg hatching, and insecticide application could fail to control first and second-instar larvae before third instars enter the stalk where insecticide does not reach the larvae. Susceptibility of sugarcane borer to genetically modified maize hybrids expressing the Cry1Ab, Vip3Aa20, and mCry3A toxins of *B. thuringiensis* was evaluated at Sinaloa, Mexico.

Materials and Methods

Research was done at Oso Viejo, El Dorado, and El Camalote in Culiacan Country and at Navolato, both in the state of Sinaloa, Mexico, during the fall-winter growing seasons of 2012 and 2013. Biosafety conditions were used when plots were planted at least 500 m from commercial plots of maize and at least 21 days later than recommended to avoid cross-pollination with non-genetically modified hybrids in accordance with government regulations for field tests of genetically modified maize in Mexico (Halsey et al. 2005, LBOGM 2005).

Two Bt maize hybrids (Agrisure^{TM} 3000 GT with Cry1Ab and mCry3A and Agrisure® Viptera^{TM} 3111 with Cry1Ab, Vip3Aa20, and mCry3A proteins) were compared with the respective non-genetically modified isolines provided by Syngenta Agro S.A de C.V. de México (San Lorenzo 1009, Primer Piso, Colonia Del Valle, México). Cry1Ab and Vip3A20 proteins are considered resistant to Lepidoptera and mCry3A to Coleoptera.

A completely randomized block design with three replications per treatment was used on each date at each locality in 2012; Agrisure^{TM} 3000 GT and Agrisure® Viptera^{TM} 3111 and their isolines were planted on 15 February at Navolato, and only Agrisure® Viptera^{TM} 3111 was planted at El Dorado on 19 February. In addition, each hybrid had a corresponding treatment of only foliar insecticide (Table 1). In 2013, Agrisure® Viptera^{TM} 3111 was planted at Camalote and Oso Viejo on 14 and 15 March, respectively, with three treatments and four replications. Only Agrisure® Viptera^{TM} 3111 was used because it was the only hybrid authorized by the Mexican regulatory officials.

The designated experimental plots were treated twice with insecticide against fall armyworm; the first when plants reached the V4 stage (number of fully developed leaves) and were less than 20 cm tall, with 10% infestation, and the second at the V8 stage with 20% infestation (Table 1). Each experimental plot consisted of 10 rows 5 m long, with 0.8 m between rows with 40 to 50 seed planting density. The seedlings were later thinned to 34 plants per row. The experimental plot was surrounded by a buffer of six rows of conventional maize, and other buffer areas were planted between replications planted at the same time as the experimental maize as required by official regulation. Management of the plot followed the technical guide by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP 2010) for maize growers.

Natural infestation by sugarcane borer was evaluated just before harvest by randomly taking 10 plants from the four central rows. Each stalk was cut longitudinally, gallery length (cm) was measured, and the percentage of injured stalks was calculated.

Table 1. Treatments Used to Evaluate Susceptibility of *Diatraea saccharalis* to Genetically Modified Maize Hybrids during Fall-winter Growing Seasons of 2012 and 2013 at Sinaloa, Mexico

Year	Hybrid[a]	Locality	Insecticide[b]
2012	Agrisure® Viptera™ 3111	El Dorado	
	Isoline + ic		Lambda cyhalothrin—
		El Dorado	Emamectin benzoate
	Isoline	El Dorado	
	Agrisure® Viptera™ 3111	Navolato	
	Isoline + ic		Lambda cyhalothrin—
		Navolato	emamectin benzoate
	Isoline	Navolato	
	Agrisure™ 3000 GT	Navolato	
	Isoline + ic		Lambda cyhalothrin—
		Navolato	Emamectin benzoate
	Isoline	Navolato	
2013	Agrisure® Viptera™ 3111	El Camalote	
	Isoline + ic	El Camalote	Emamectin benzoate
	Isoline	El Camalote	
	Agrisure® Viptera™ 3111	Oso Viejo	
	Isoline + ic	Oso Viejo	Emamectin benzoate
	Isoline	Oso Viejo	

[a]ic = insecticidal control.
[b]Insecticides were applied at dosages: 500 ml/ha lambda cyhalothrin (Karate Zeon®5 CS), 200 ml/ha emamectin benzoate (Denim®19 CE)

PROC ANOVA was used to evaluate stalk damage and percentage of damaged plants. Means were compared by Fisher LSD ($P < 0.05$) (SAS 2002).

Results and Discussion

Few sugarcane borers were found in 2012, but both genetically modified hybrids were less damaged than their respective isolines including those treated with insecticide. At El Dorado, borer galleries in Agrisure® Viptera™ 3111 averaged 0.27 cm long, all were less than 8 cm, and 3.3% of stalks were damaged (Table 2). Insecticide-treated plants had galleries 1.27 cm long, and 16.7% of the plants were damaged. The non-treated isoline had galleries that averaged 0.87 cm long, but some galleries were 14 cm long, and 16.7% of plants were damaged.

No plants of Agrisure® Viptera™ 3111 event were damaged at Navolato, but the isoline treated with insecticide and the non-treated check had galleries that averaged 0.67 and 0.60 cm long, respectively (Table 2). Both hybrids had 6.7% of plants damaged.

Plants of Agrisure™ 3000 GT event in the same area were not damaged by the pest. Ten and 16.7% of isoline plants with or without insecticidal treatment were damaged, respectively, and galleries were 0.68 and 0.85 cm long.

Table 2. Average Gallery Size and Percentage of Stalks Damaged by Sugarcane Borer, *Diatraea saccharalis*, in Genetically Modified Maize Hybrids Agrisure® Viptera™ 3111 and Agrisure™ 3000 GT at El Dorado and Navolato, Sinaloa, 2012

Hybrid[a]	Locality	Gallery length (cm)	Damaged stalks (%)
Agrisure® Viptera™ 3111	El Dorado	0.27 a	3.33 a
Isoline + ic	El Dorado	1.27 a	16.67 b
Isoline	El Dorado	0.87 a	16.67 b
		F = 0.58 ns	F = 0.76*
Agrisure® Viptera™ 3111	Navolato	0.00 a	0.00 a
Isoline + ic	Navolato	0.67 a	6.67 a
Isoline	Navolato	0.60 a	6.67 a
		F = 0.72 ns	F = 0.80 ns
Agrisure™ 3000 GT	Navolato	0.00 a	0.00 a
Isoline + ic	Navolato	0.68 a	10.00 b
Isoline	Navolato	0.85 a	16.67 b
		F = 0.65 ns	F = 0.86*

Means followed by the same letter in a column are not statistically different (LSD, $P < 0.05$).

[a]ic = insecticidal control.

*Significant contrast F value at $P < 0.05$, ns = not significantly different, df = 2, 8.

In general, stalk feeding (gallery size) and number of stalks damaged by stalk borers were less on genetically modified hybrids Agrisure® Viptera™ 3111 and Agrisure™ 3000 GT than in their conventional isolines. However, treatments did not differ statistically ($P < 0.05$) at Navolato, Sinaloa, probably because of few pests, with many noninjured plants (0 values) in samples of treated maize.

Evaluation in 2013 at El Camalote showed no Agrisure® Viptera™ 3111 plants injured compared with non-genetically modified isolines with or without insecticidal treatment; 27.5 and 7.5% of stalks were damaged and galleries were 1.18 and 0.35 cm long, respectively, with lesions as long as 8 cm (Table 3).

Sugarcane borers were most abundant at Oso Viejo including in genetically modified Agrisure® Viptera™ 3111 with 22.5% damaged stalks with galleries averaging 1.55 cm long. But, compared with the isolines with or without insecticidal treatment, the hybrids had galleries averaging 10.23 and 6.95 cm long, respectively, and some galleries as long as 36 cm. Borers damaged 90 and 70% of stalks of the same isolines.

At both locations during 2013, stalk feeding (galleries) and number of plants damaged by sugarcane borer larvae were significantly less in genetically modified hybrid Agrisure® Viptera™ 3111 compared with its conventional isoline with or without insecticide. In 2013, stalk borers were abundant enough to accurately evaluate biological efficacy of the genetically modified technology against the pest.

Insecticide did not prevent damage by the pest, perhaps because the treatment did not coincide with pest occurrence. Insecticide was first applied when plants had four true leaves and later when plants had eight to 10 leaves, resulting in no difference between treated and nontreated isolines. At Navolato, growers did not apply insecticide to control the few borers present; however, genetically modified hybrids with Bt protein that effectively controls key pests of maize (Piña and Solleiro 2013; Aguirre et al. 2015ab, 2016) would also prevent damage if an outbreak of sugarcane borer occurred.

Table 3. Gallery Size and Percentage of Stalks Damaged by Sugarcane Borer, *Diatraea saccharalis*, in Genetically Modified Maize Hybrid Agrisure® Viptera™ 3111 at El Camalote and Oso Viejo, Sinaloa, 2013

Hybrid[a]	Location	Gallery length (cm)	Damaged stalks (%)
Agrisure® Viptera™ 3111	El Camalote	0.00 a	0.00 a
Isoline + ic	El Camalote	1.18 b	27.50 c
Isoline	El Camalote	0.35 a	7.50 b
		F = 6.27*	F = 9.70**
Agrisure® Viptera™ 3111	Oso Viejo	1.55 a	22.50 a
Isoline + ic	Oso Viejo	10.23 b	90.00 b
Isoline	Oso Viejo	6.95 b	70.00 b
		F = 7.18*	F = 29.34***

Means followed by the same letter in a column are not statically different (LSD, $P < 0.05$).

[a]ic = insecticidal control.

*, **, and *** indicate significant contrast F value at $P < 0.05$, < 0.01, and < 0.001, respectively, df = 2, 11.

Burkness et al. (2001) mentioned significantly less damage by European corn borer in Bt maize expressing Cry1Ab protein that obviated the need for insecticide. Farias et al. (2013), in a 2-year evaluation of Cry1Ab protein, found significant infestation and less damage by sugarcane borer where foliar insecticide failed to control the pest. Ghimire et al. (2011) showed pyramidal events better controlled maize stalk borer, but the scarce number of pests was insufficient to thoroughly evaluate Agrisure® Viptera™ 3111 and Agrisure™ 3000 GT.

In Mexico, offices that regulate genetically modified maize authorized experimental tests during only a 4-year period (Piña y Solleiro 2013; Aguirre et al. 2015ab, 2016). Storer et al. (2001) opined that to better evaluate the effect of hybrids against maize pests, larger plantings were needed because only moderate results were obtained in small research plots.

This is the first report of evaluating resistance of genetically modified maize hybrids against sugarcane borer in Mexico. More experiments in a wider range of locations and environmental conditions are needed to better understand this promising technology and support regulatory decisions in the matter.

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