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Research

Genetically modified maize resistant to corn earworm (Lepidoptera: Noctuidae) in Sinaloa, Mexico

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Abstract

Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae), the corn earworm, is a key pest causing damage to corn *Zea mays* L. (Poales: Poaceae). The development of hybrids expressing Cry1Ab, Vip3Aa20, and mCry3A protein of *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae) is an option to control this insect. Corn hybrids Agrisure™ 3000 GT, Agrisure® Viptera™ 3110, and Agrisure® Viptera™ 3111 were tested for corn earworm suppression in the agricultural region of Sinaloa during the 2011, 2012, and 2013 autumn–winter growing seasons, and compared with their respective isolines. Gallery length on the ear and the number of damaged ears were evaluated. The genetically modified hybrids demonstrated the effectiveness of inserted proteins to confer resistance to the corn earworm by killing the pest or reducing its growth and damage to the ear. Based on the results, Agrisure™ 3000 GT, Agrisure® Viptera™ 3110, and Agrisure® Viptera™ 3111 are useful elements for an integrated pest management program on corn in Sinaloa, Mexico.

Key Words: *Bacillus thuringiensis*; δ-endotoxin; *Helicoverpa zea*; transgenic

Resumen

El gusano elotero, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), es una de las principales plagas que causa daño al maíz *Zea mays* L. (Poales: Poaceae). El desarrollo de híbridos que expresan las proteínas Cry1Ab, Vip3Aa20 y mCry3A de *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae), son una opción de control para este insecto. Se evaluaron los híbridos de maíz Agrisure™ 3000 GT, Agrisure® Viptera™ 3110 y Agrisure® Viptera™ 3111 y se compararon con sus respectivas isolíneas para el control de gusano elotero, en la región agrícola de Sinaloa durante el ciclo otoño invierno 2011, 2012 y 2013. Se evaluó la longitud de galería en la mazorca y el número de mazorcas dañadas. Los híbridos genéticamente modificados demostraron la eficacia de las proteínas insertadas en el maíz sobre el gusano elotero, observándose que estos híbridos le confieren resistencia al ataque de esta plaga matándola o reduciendo su crecimiento causando menor daño al elote. Con base a los resultados, los eventos Agrisure™ 3000 GT, Agrisure® Viptera™ 3110 y Agrisure® Viptera™ 3111, son elementos útiles para ser incorporados en un programa de manejo integrado de plagas de maíz en Sinaloa, México.

Palabras Clave: *Bacillus thuringiensis*; δ-endotoxina; *Helicoverpa zea*; transgénico

The use of recombinant DNA technology offers new options in agricultural production strategies. The development of genetically modified (GM) hybrids includes using constructs for insect resistance to Lepidoptera and Coleoptera (Bruck et al. 2006) based on the δ-endotoxin produced by *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae) that provide a new tool for pest control in crop production (Fernandes et al. 2007).

Worldwide, corn is the crop in which more GM products have been developed than any other crop, with 133 hybrids produced, of which 108 provide insect resistance primarily to Lepidoptera and Coleoptera. In Mexico, from 2009 to date, 45 products resistant to insects have been approved, but only for research purposes or as pilot test releases (ISAAA 2014).

Zea mays L. (Poales: Poaceae) is autochthonous to Mexico (CONABIO 2006), and substantial agricultural domestication occurred well before the modern era. Subsequent breeding and selection of this corn germplasm worldwide resulted in Mexico now producing 22,663,953 tons in 2013 of which the states of Sinaloa and Jalisco account for 3,627,777 and 3,303,498 tons, respectively (SAGARPA-SIAP 2014).

Corn grown in Mexico is often affected by the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), which damages ear, reduces yield, and increases cob rot (Ortega 1987) by providing an inoculation court for establis`hment of fungal diseases (Ortega 1987; Wu 2006; Aguirre et al. 2014). The fungi produce mycotoxins harmful to human and animal health (Bakan et al. 2002), which are most important in growing areas with high relative humidity like Sinaloa where cob

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rot caused by *Fusarium* species (Hypocreales: Nectriaceae) has a direct effect on yield, causing losses over 30% (García et al. 2012).

Bt corn hybrids encoding Cry1Ab, Vip3Aa20, and mCry3A proteins have been approved for study under experimental conditions in Mexico, and the objective of this research was to evaluate the resistance of GM corn hybrids to corn earworm in Sinaloa, Mexico.

Materials and Methods

Research was carried out at Oso Viejo, El Dorado, and Camalote in the city of Culiacan and in the city of Navolato, both in the state of Sinaloa, Mexico, during the 2011–2013 autumn–winter growing seasons. Plots were planted under biosafety conditions, isolated by at least 500 m from commercial corn plantings, and planted at least 21 d later than recommended; delayed planting avoids cross-pollination with non-GM corn in accordance with government regulations for field tests with GM corn (Halsey et al. 2005; LBOGM 2005). Bt corn hybrids used in these tests were Agrisure™ 3000 GT with Cry1Ab and mCry3A proteins that provide resistance to Lepidoptera and Coleoptera, respectively; Agrisure® Viptera™ 3110 with Cry1Ab and Vip3A20 providing resistance to Lepidoptera, and Agrisure® Viptera™ 3111 with proteins Cry1Ab and Vip3Aa20 providing resistance to Lepidoptera and mCry3A to Coleoptera. These corn hybrids were compared with their respective non-GM isolines provided by Syngenta Agro SA de CV.

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 chemical 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, and Agrisure® Viptera™ 3111was planted at El Dorado on 19 Feb, also with and without insecticide applications. Only 3 replicates were planted in these areas. In 2013, Agrisure® Viptera™ 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).

All designated experimental plots during the 3 yr period received an insecticide treatment for *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) if plants less than 20 cm reached a 10% infestation level, or plants 20 cm or more reached a 20% infestation level. One application was made in 2011, and 2 applications in 2012 and 2013 (Table 1).

Each experimental plot consisted of 10 rows, each 5 m long, with 0.8 m between rows with 40 to 50 seeds per row. 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. Agricultural management of the plot followed the technical guide for corn growers developed by Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP-CIRNO 2010).

Ear damage evaluation under natural infestation by *H. zea* was performed just before harvest by taking 10 ears randomly from each of the 4 central rows. Length of gallery (cm) was measured starting from

Table 1. Treatments used to evaluate ear damage by corn earworm, *Helicoverpa zea*, in genetically modified corn grown for 3 yr in Sinaloa, Mexico.

Year	Genetic material[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.

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the ear tip, and the percentage of ears damaged by corn earworm was calculated.

Before the statistical analysis, percentage data of ears with damage were transformed by arcsine square root. A PROC ANOVA test was conducted to evaluate ear damage and number of ears with damage, and a mean comparison for treatments was done with a Fisher LSD test ($P < 0.05$) using SAS/STAT (SAS 2002; Version 9.0, SAS Institute, Cary, North Carolina, USA).

Results

Ear damage caused by corn earworm in each of the GM hybrids and isolines (Table 2) showed that GM hybrids suffered significantly less ($P < 0.05$) feeding damage by earworm than isolines (Fig. 1). Also, GM hybrids experienced a significantly lower ($P < 0.05$) proportion of ears with damage relative to the isoline controls (Figs. 1–3).

Agrisure™ 3000 GT and Agrisure® Viptera™ 3110 hybrids had significantly less ($P < 0.05$) corn earworm damage to ears than their respective isolines at Oso Viejo in 2011; the latter hybrid also had smaller galleries, < 1.0 cm, indicating better protection than the former hybrid to the pest (Table 2). Evaluation of the proportion of ears damaged showed difference between the GM hybrid Agrisure™ 3000 GT (62.7%) and the rest of the treatments, where the same hybrid with chemical control had 78.7% damaged ears and the isolines showed 80.8 and 71.6% damage with and without chemical treatment, respectively. However, percentage ear damage was lower in the GM corn line than the isolines ($F = 2.66$; df = 3, 15; $P = 0.0959$). Agrisure® Viptera™ 3110 with and without insect control, on the other hand, had less than 25% damaged ears and were statistically different ($P < 0.05$) from their respective isolines, which showed more than 71% ears with damage ($F = 19.39$; df = 3, 15; $P < 0.0001$) (Fig. 1).

Similar results were found at El Dorado and Navolato in 2012, where lesion size was significantly ($P < 0.05$) smaller in the GM hybrids than in the isolines. Galleries in GM hybrids were less than 1 cm long, as compared with their respective isolines, including the ones with insecticide treatment, showing galleries from 2 to 5 cm long (Table 2). The proportions of ears with damage were significantly less ($P < 0.05$). Agrisure® Viptera™ 3111 (El Dorado: $F = 5.25$; df = 3, 11; $P = 0.0270$; Navolato: $F = 37.90$; df = 3, 11; $P < 0.0001$) and Agrisure™ 3000 GT (Navolato: $F = 11.50$; df = 3, 11; $P = 0.0028$) had less than 50% ears with damage, whereas the isolines, including the ones with insecticide treatment, showed from 70 to 90% corn earworm–damaged ears (Fig. 2).

Table 2. Gallery length in ears from corn earworm, *Helicoverpa zea*, feeding in genetically modified corn at Sinaloa, Mexico.

Year	Genetic material[a]	Locality	Ear gallery length (cm)
2011	Agrisure™ 3000 GT	Oso Viejo	1.96 a
	Agrisure™ 3000 GT + ic	Oso Viejo	1.61 a
	isoline + ic	Oso Viejo	4.47 b
	isoline	Oso Viejo	5.02 b
			$F = 10.40$; df = 3, 15**
	Agrisure® Viptera™ 3110	Oso Viejo	0.16 a
	Agrisure® Viptera™ 3110 + ic	Oso Viejo	0.19 a
	isoline + ic	Oso Viejo	4.66 b
	isoline	Oso Viejo	5.02 b
2012	Agrisure® Viptera™ 3111	El Dorado	0.33 a
	Agrisure® Viptera™ 3111+ ic	El Dorado	0.45 a
	isoline + ic	El Dorado	2.20 b
	isoline	El Dorado	2.13 b
			$F = 12.22$; df = 3, 11**
	Agrisure® Viptera™ 3111	Navolato	0.37 a
	Agrisure® Viptera™ 3111 + ic	Navolato	0.47 a
	isoline + ic	Navolato	2.83 b
	isoline	Navolato	2.95 b
			$F = 11.07$; df = 3, 11**
	Agrisure™ 3000 GT	Navolato	0.45 a
	Agrisure™ 3000 GT + ic	Navolato	0.27 a
	isoline + ic	Navolato	4.37 b
	isoline	Navolato	5.50 b
			$F = 21.30$; df = 3, 11***
2013	Agrisure® Viptera™ 3111	Camalote	0.39 a
	isoline + ic	Camalote	2.31 b
	isoline	Camalote	3.11 b
			$F = 24.91$; df = 2, 11***
	Agrisure® Viptera™ 3111	Oso Viejo	0.20 a
	isoline + ic	Oso Viejo	2.72 b
	isoline	Oso Viejo	3.59 b
			$F = 23.94$; df = 2, 11***

[a]ic = insecticide control

[b]Genetically modified hybrids and their respective isolines followed by same letter do not differ significantly (ANOVA and LSD test; $P < 0.05$). **, *** indicate significant contrast F value at $P < 0.01$, $P < 0.001$, respectively.

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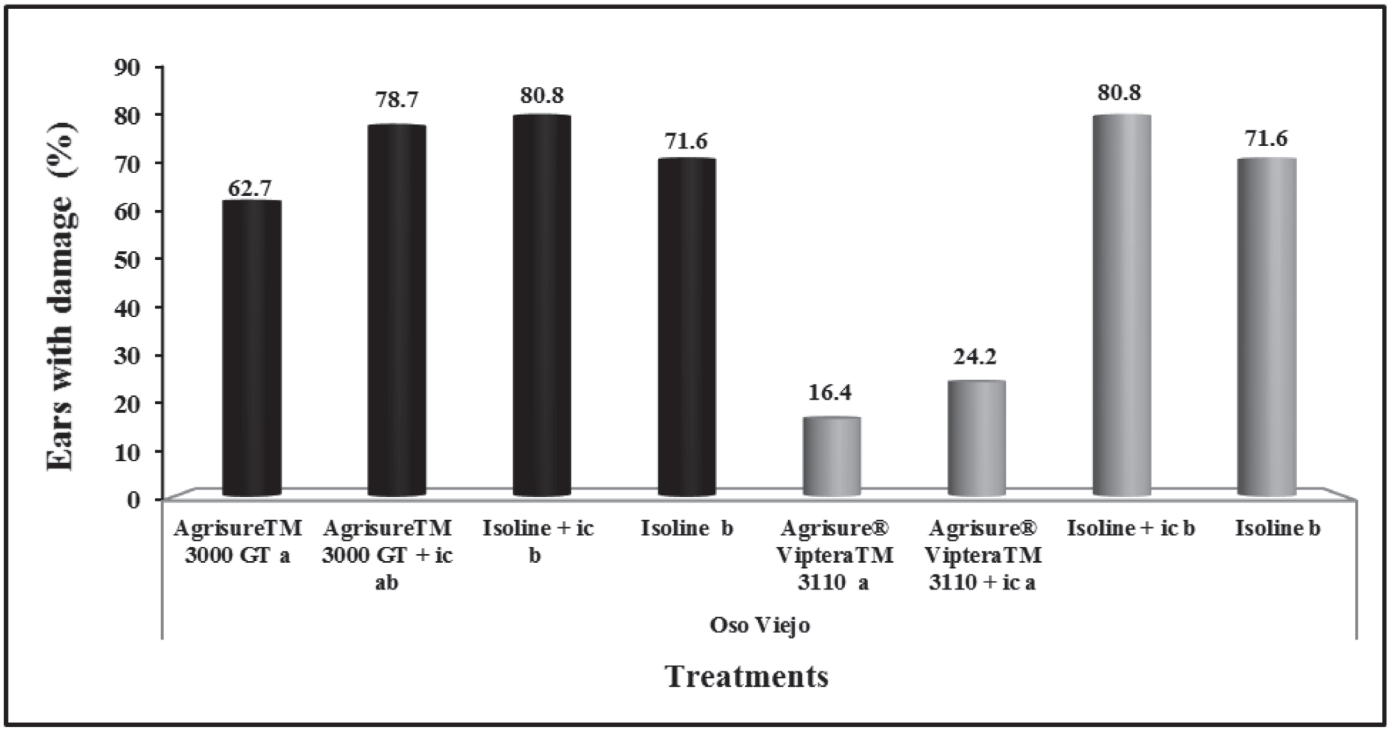


Fig. 1. Percentage of corn ears damaged by *Helicoverpa zea* in Agrisure™ 3000 GT, Agrisure® Viptera™ 3110, and their respective isolines at Oso Viejo, Culiacan (Sinaloa, Mexico) during 2011. Genetically modified hybrids and their respective isolines followed by the same letter do not differ significantly (LSD; $P > 0.05$). ic = insecticide control.

Agrisure® Viptera™ 3111 in 2013 at both Oso Viejo and Camalote had galleries less than 1 cm long and were statistically shorter ($P < 0.05$) than their respective isolines with galleries 2 to 4 cm long (Table 2). The proportion of ears damaged also was less in the GM hybrids relative to their isolines. At Camalote, less than 22% of the ears were damaged, which was significantly less ($F = 21.76$; df = 2, 11; $P = 0.0004$) than in the isolines. At Oso Viejo, only 9% were damaged, significantly ($F = 32.68$; df = 2, 11; $P < 0.0001$) less than in non-GM isolines (Fig. 3).

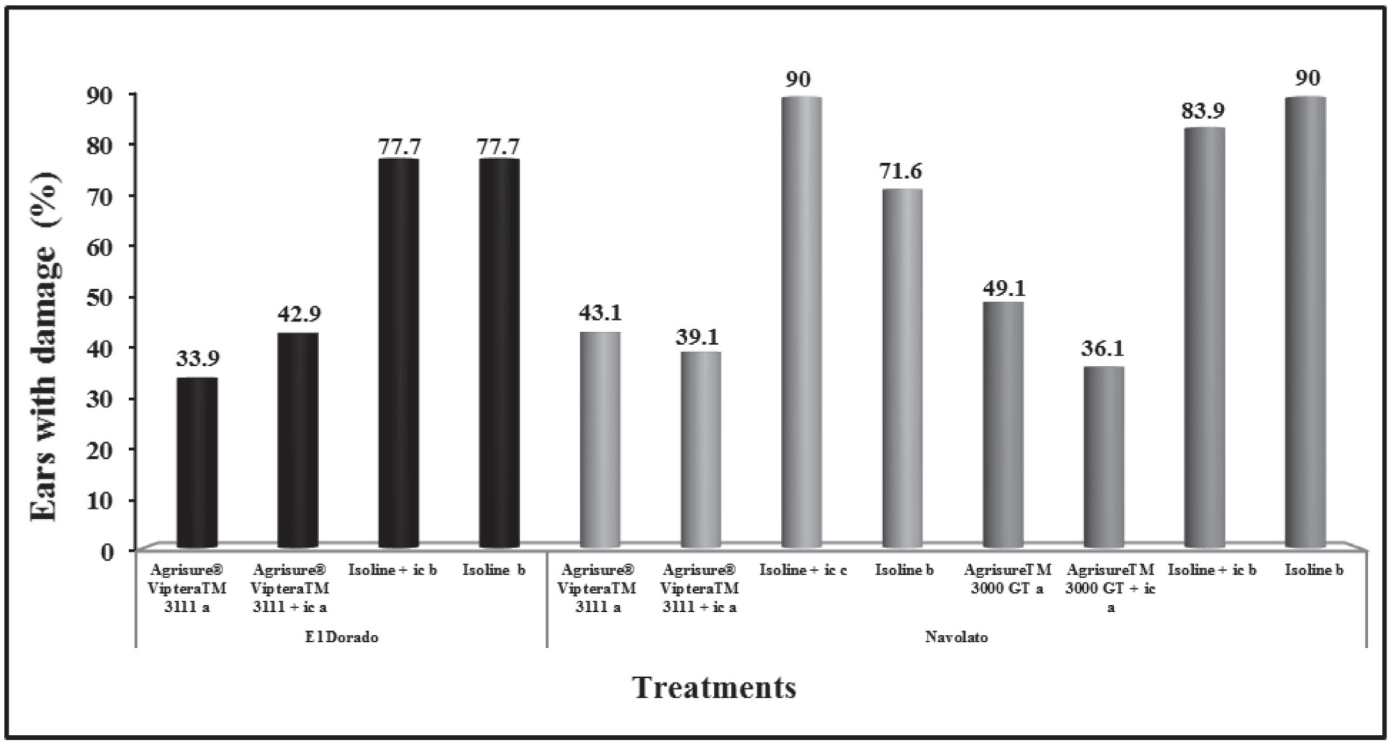


Fig. 2. Percentage of corn ears ear damaged by *Helicoverpa zea* in Agrisure® Viptera™ 3111, Agrisure™ 3000 GT, and their respective isolines at El Dorado, Culiacan, and Navolato (Sinaloa, Mexico). 2012. Genetically modified hybrids and their respective isolines followed by the same letter do not differ significantly (LSD; $P > 0.05$). ic = insecticide control

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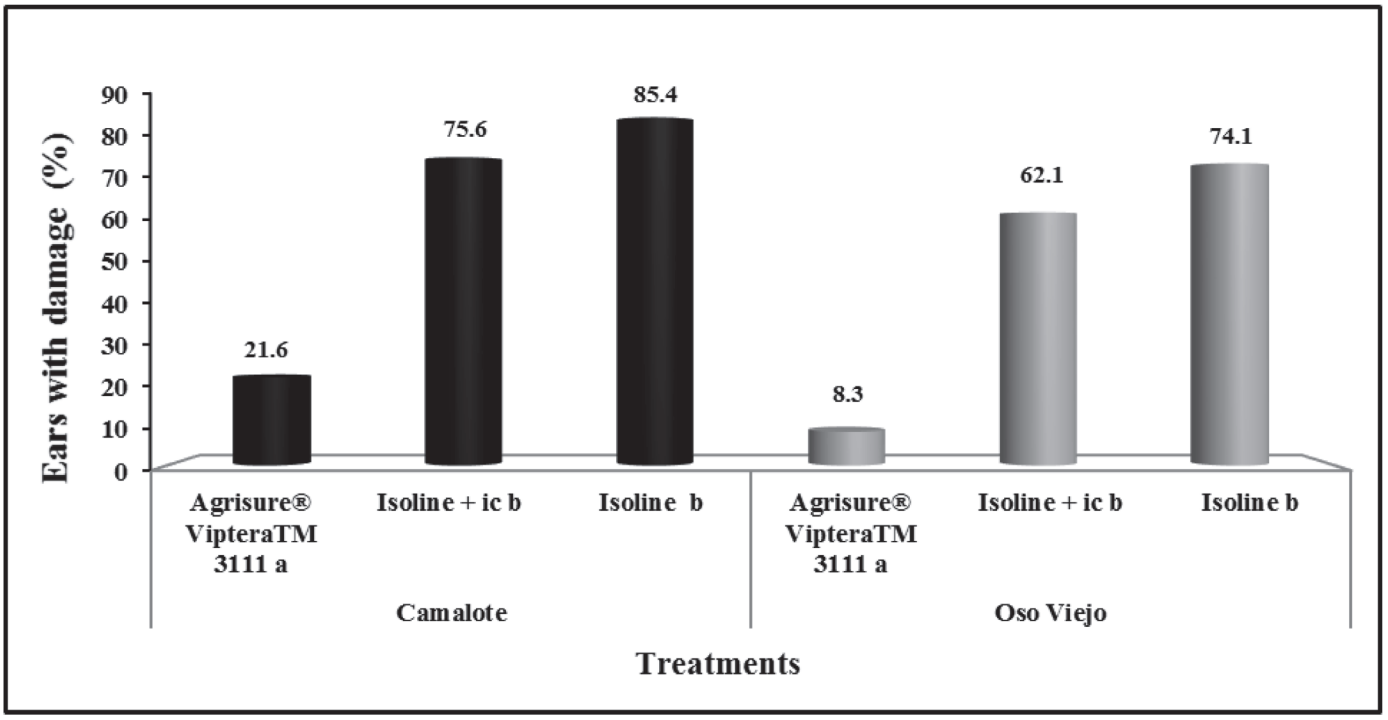


Fig. 3. Percentage of corn ears damaged by *Helicoverpa zea* in Agrisure® Viptera™ 3111 and its isoline at Camalote and Os`o Viejo, Culiacan (Sinaloa, Mexico). 2013. Genetically modified hybrid and isolines followed by the same letter do not differ significantly (LSD; $P > 0.05$). ic = insecticide control.`

Discussion

Agrisure™ 3000 GT, Agrisure® Viptera™ 3110, and Agrisure® Viptera™ 3111 hybrids showed resistance to corn earworm when compared with their isolines. The GM corn caused high mortality to the pest and reduced ear damage, showing that the Bt genes would be an excellent tool to prevent infestation by *H. zea*. Mummified 1st and 2nd instars were found on the GM hybrids, whereas fully developed larvae were routinely observed on the isolines lacking the Bt insertion (unpublished data).

These results are similar to those reported by Storer et al. (2001), who mentioned that Bt corn with the Cry1Ab (MON810 and Bt11) protein decreased corn earworm damage, reduced larval growth and development, and produced few adults due to mortality of larvae. They also suggested that for more effective reduction of pest density, Bt corn hybrids should be planted over wide areas. Buntin et al. (2004), in a 3 yr study with Bt corn in Georgia and Alabama, USA, found less ear infestation and damage in the GM hybrids than the non-GM corn. Similarly, Buntin (2008), reported that corn with the Cry1Ab (MON 810) protein displayed less infestation by the pests than non-GM corn. Reay-Jones et al. (2009) evaluated yield of several GM corn hybrids and noted that genetically modified hybrids had less damage than the non-GM isolines.

In these studies, chemical control did not prevent corn earworm damage because larvae that escape the insecticide and enter the ear are protected by the ear bracts from surface toxins. Insecticide in these areas does control *S. frugiperda* but not *H. zea*. The advantage of the GM hybrids is that they provide protection to both pests. Farias et al. (2013) noted that *H. zea* is not controlled by insecticides that target *S. frugiperda* and stated that insecticide applications targeting corn earworm should coincide with oviposition to kill hatching larvae before ear entry occurs.

All evaluated GM hybrids showed similar levels of protection from corn earworm under natural infestation; however, Agrisure™ 3000 GT experienced more damaged ears and bigger lesions than Agrisure® Viptera™ 3110 and Agrisure® Viptera™ 3111. This may be due to the latter having two Bt toxins inserted for Lepidoptera control (Cry1Ab δ-endotoxin and Vip3Aa20 vegetative insecticide protein), whereas the former has only the Cry1Ab for lepidopteran control.

Based on these results, Bt corn hybrids used in this research can be incorporated in an integrated pest management system for corn production at Sinaloa. However, they should be evaluated in other areas of Mexico because different environmental conditions may affect efficacy. Pest populations are variable, and susceptibility of different geographical populations to the Bt toxins may depend on biotic and abiotic factors (Zenner de Polanía et al. 2008). This variability can also be related to the hybrids used, due to the expression differences of the Cry proteins among hybrids (Farias et al. 2013). Also, co-occurring crops may affect pest pressure; agricultural areas that contain multiple Bt crops that target a shared pest (i.e., cotton and *H. zea*) warrant special attention due to increased risks that may occur from factors like cross-resistance that may arise to the Bt protein. This variability must be considered in developing and implementing insect control strategies with GM corn in different geographic regions (Monnerat et al. 2006).

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