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Abundance of non-target predators in genetically modified corn

Agustín Hernández-Juárez¹, Luis A. Aguirre^{1,*}, Ernesto Cerna¹, Mariano Flores¹, Gustavo A. Frías¹, Jerónimo Landeros¹, and Yisa M. Ochoa¹

Abstract

Genetically modified corn (maize) *Zea mays* (Poaceae) expressing *Bacillus thuringiensis* (Bt) Berliner (Bacillaceae) toxins is a controversial issue due to the risk they could pose to predators as non-target organisms. Thus it is important to evaluate that risk before Bt corn is released for commercial planting in Mexico. The effect of genetically modified corn hybrid Agrisure® Viptera™ 3111 on the abundance of non-target predators *Orius insidiosus* Say (Hemiptera: Anthocoridae), *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae), and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) was evaluated at Oso Viejo and El Camalote in Culiacan, Sinaloa, and compared with its non-genetically modified isolate with and without insecticide treatment in a randomized complete block design with 3 treatments and 4 replicates. Complete plant visual samplings were performed to determine predator abundance, frequency, and population fluctuation using the Kruskal-Wallis non-parametric statistical test. A total of 5,228 predators were collected in all hybrids in both localities: 2,431 at Oso Viejo and 2,797 at El Camalote with 2 peaks before and after pollination. In both locations, each predator population had a similar fluctuation in all hybrids. Although no statistical difference was found among treatments, in all cases, Agrisure® Viptera™ 3111 had higher abundance than the isolines with and without insecticide treatment. Results show that Agrisure® Viptera™ 3111 does not have a negative effect on predator abundance of *O. insidiosus*, *C. maculata*, and *C. carnea*.

Key Words: *Bacillus thuringiensis*; green lacewing; pirate bug; spotted pink lady beetle; transgenic corn

Resumen

El maíz genéticamente modificado (maíz) *Zea mays* (Poaceae) que expresa toxinas de *Bacillus thuringiensis* (Bt) Berliner (Bacillaceae) es un tema controversial debido al riesgo que podrían tener sobre los depredadores, debido a que los organismos no blanco son importantes para evaluar el riesgo ambiental antes de su liberación comercial en México. Se evaluó en Oso Viejo y El Camalote, Culiacán, Sinaloa, el efecto del maíz genéticamente modificado Agrisure® Viptera™ 3111 sobre la abundancia de depredadores no blanco, *Orius insidiosus* Say (Hemiptera: Anthocoridae), *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae), y *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), y se comparó con su híbrido convencional con y sin tratamiento con insecticida, bajo un diseño en bloques completamente al azar con 3 tratamientos y 4 repeticiones. Se realizaron inspecciones visuales de toda la planta para determinar la abundancia, frecuencia y fluctuación de los depredadores y analizado mediante estadística no paramétrica con la prueba de Kruskal-Wallis. Se registraron 5,228 depredadores en todos los híbridos, en ambas localidades: 2,431 en Oso Viejo y 2,797 en El Camalote, con dos picos poblacionales, antes y después de la polinización. En ambas localidades, cada población de depredadores presentó una fluctuación similar entre los híbridos. Aunque no se encontró diferencias estadísticas entre los tratamientos, en todos los casos en el Agrisure® Viptera™ 3111 tuvo mayor abundancia que los convencionales con y sin tratamiento con insecticida. Los resultados reflejan que el maíz Agrisure® Viptera™ 3111 no tienen un efecto negativo sobre la abundancia de *O. insidiosus*, *C. maculata*, y *C. carnea*.

Palabras Clave: *Bacillus thuringiensis*; crisopa verde; chinche pirata; catarina rosa manchada; maíz transgénico

Genetically modified corn, *Zea mays* L. (Poaceae), hybrids contain *Bacillus thuringiensis* (Bt) Berliner (Bacillaceae) genes that express the crystal (Cry) toxins with insecticide properties, to control lepidopteran insects (Bruck et al. 2006), such as European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae); corn and sugar cane borers, *Diatraea grandiosella* Dyar and *D. sacharalis* (F.) (Lepidoptera: Crambidae); corn earworm, *Helicoverpa zea* (Boddie); and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Abel et al. 2000; Burkness et al. 2001; Castro et al. 2004; Niu et al. 2013; Yang et al. 2013).

In an agroecosystem, other insects in the trophic chain that are not target pests can be affected. Such is the case with entomophagous in-

sects that play an important role in pest regulation (Dutton et al. 2003), such as *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae), and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), that prey on a number of different arthropods (Muma 1959; Canard et al. 1984; Ulhaq 2006; Bahena 2008). These predators can be exposed indirectly to the Cry protein when consuming their prey that are feeding on Bt crops, despite the specificity of the protein to the target insects (Groot & Dicke 2002; Bruck et al. 2006).

Genetically modified hybrids offer an effective method of pest management by reducing insecticide treatments (Duan et al. 2008; Ghimire et al. 2011; Hardke et al. 2011; Shelton 2012; Farias et al.

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2013), reducing environmental damage, and reducing the exposure of growers to chemicals (Soberón & Bravo 2008); however, there are concerns about the negative effects on biological diversity, especially over beneficial and non-target insect herbivores (Bruck et al. 2006). Although no scientific evidence has been shown proving that Bt corn has negative effects on them (Bakhsh et al. 2015), there is a hypothesis that some non-target arthropods may be affected by the protein exposure (Higgins et al. 2009).

Considering the above-mentioned hypothesis, it is important to conduct research in all field-released genetically modified events to determine the effect on non-target species, especially in Mexico. The objective of this research was to evaluate the effect of the Agrisure® Viptera™ 3111 corn hybrid on the abundance of 3 predators in Sinaloa, Mexico.

Materials and Methods

Research was carried out at Oso Viejo (24.406633°N, 107.165650°W) and Camalote (24.372316°N, 107.314550°W) in the city of Culiacan in Sinaloa State, Mexico, during the 2013 autumn-winter growing season. Plots were planted using biosafety conditions, isolated at least 500 m from commercial corn plantings, and planted at least 21 d later than recommended. This delayed planting avoids cross-pollination with non-genetically modified corn, in accordance with government regulations for field tests with genetically modified corn in Mexico (Halsey et al. 2005; LBOGM 2005).

The Bt corn hybrid used in these tests was Agrisure® Viptera™ 3111 with the stacked proteins Cry1Ab and Vip3Aa20 providing resistance to Lepidoptera and mCry3A to Coleoptera. These corn hybrids were compared with their respective non-genetically modified isolines provided by Syngenta Agro SA de CV (Ciudad de México, México).

Agrisure® Viptera™ 3111 was planted at Camalote and Oso Viejo on 14 and 15 Mar 2013, respectively. A randomized complete block design was used with 3 treatments (genetically modified hybrid, isoline, and isoline plus insecticide) and 4 replicates (Table 1).

The isoline was treated twice with emamectin benzoate (Denim®19 CE, 200 mL active ingredient per ha; Syngenta Agro) to control fall armyworm. The first treatment was done when plants reached the V4 stage (number of fully developed leaves) and were less than 20 cm tall with 10% infestation; the second treatment was done at the V8 stage when 20% infestation was reached (Table 1).

Each experimental plot consisted of 10 rows, each 5 m long, with 0.8 m between rows, and with a 40 to 50 seed planting density. The seedlings were adjusted later 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 at 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).

Visual inspections were carried out for 3 of the more abundant predator species in the corn agroecosystem of Sinaloa: *Orius insidiosus*, *Chrysoperla carnea*, and *Coleomegilla maculata*. The inspections started 30 d after sowing and up to 1 wk before harvest (1.4 m plants). This activity was carried out every 2 wk, sampling 10 plants randomly, and checking them carefully from the base to the youngest leaf or spike. The identified species were recorded for analysis, and to determine the population abundance, frequency, and fluctuation. Abundance refers to the number of insects found in each sampling date, and frequency refers to the proportion (expressed as %) of samples in which the predators were found.

The total abundance data of each predator in each material evaluated were analyzed by the non-parametric Kruskal-Wallis test, applied to 3 or more groups using the Minitab 18 statistical software (Minitab, Inc., State College, Pennsylvania, USA). This test uses ranges of data from independent samples to test the hypothesis that it has come from populations with equal medians, with the objective of detecting differences among the predator species collected from Agrisure® Viptera™ 3111 corn and its conventional isolines, with and without insecticide application.

Results

A total of 5,228 predators of the 3 studied species were found on the genetically modified hybrid Agrisure® Viptera™ 3111 and the conventional isolines, with and without insecticide treatment: 2,431 at Oso Viejo and 2,797 at El Camalote.

Although no statistical difference was found among treatments, the mean abundance of *O. insidiosus* was greater in the Agrisure® Viptera™ 3111 hybrid than in the isolines in both locations, with a total of 369 individuals at Oso Viejo, and 217 at El Camalote. The isolines had 218 and 286 at Oso Viejo, and 177 and 192 at El Camalote, with and without insecticide treatment, respectively (Table 1). Frequency of the predators in samples at Oso Viejo was 80% (the number of times collected from total sampling dates), in the Agrisure® Viptera™ 3111 and the untreated isoline, and 100% in the isoline without insecticide, with 2 population peaks before and after pollination, decreasing with crop maturation (Fig. 1a).

At El Camalote, the predator had a 100% frequency in all hybrids during crop development, with 1 population increase before pollination (18–20 May), and another small one after pollination (Fig.1b).

Chrysoperla carnea at Oso Viejo had a total abundance of 409 individuals; of those, 179 were on Agrisure® Viptera™ 3111, 120 on the isoline with insecticide treatment, and 110 on the hybrid without treatment. Abundance of the lacewing at El Camalote was higher with a total of 607 individuals, 230 on the genetically modified hybrid, and 216 and 161 in the

Table 1. Total and mean abundance of 3 predator species on a genetically modified corn hybrid Agrisure® Viptera™ 3111, and their conventional hybrid (with and without insecticide) at Oso Viejo and El Camalote, Sinaloa, Mexico.

Locality	Predators	Hybrids of maize						Kruskal-Wallis Test
		Agrisure® Viptera™3111	Mean	Isoline + i	Mean	Isoline	Mean	
Oso Viejo	<i>Orius insidiosus</i>	369	42.3	218	24.9	286	32.8	df = 2; P = 0.820
	<i>Chrysoperla carnea</i>	179	43.8	120	29.3	110	26.9	df = 2; P = 0.460
	<i>Coleomegilla maculata</i>	536	46.6	257	22.4	356	31.0	df = 2; P = 0.610
El Camalote	<i>Orius insidiosus</i>	217	37.0	177	30.2	192	32.8	df = 2; P = 0.911
	<i>Chrysoperla carnea</i>	230	37.9	216	35.6	161	26.5	df = 2; P = 0.845
	<i>Coleomegilla maculata</i>	638	39.8	588	36.6	378	23.6	df = 2; P = 0.932

+ i = insecticide treatment used on fall armyworm.

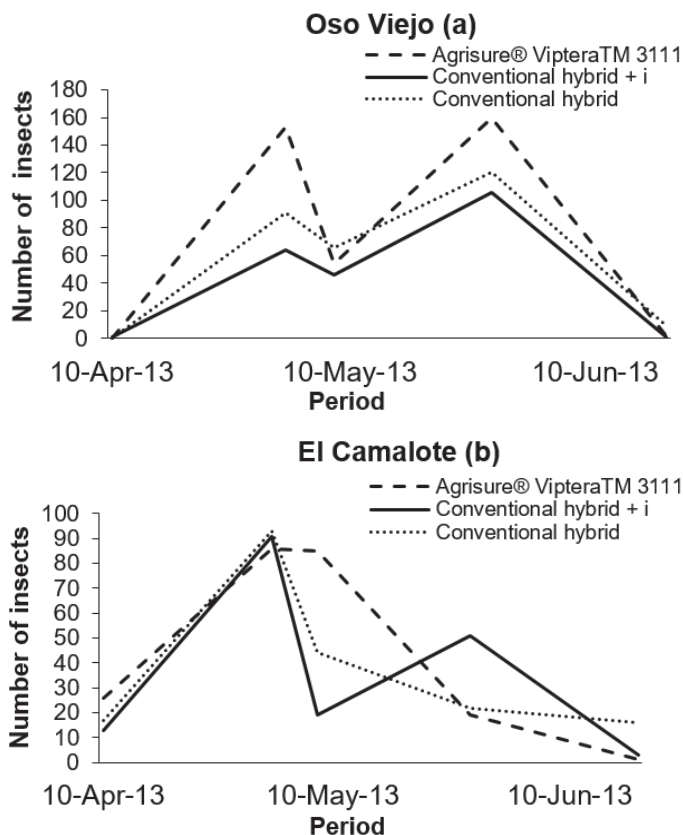


Fig. 1. Population fluctuation (total number of insects) of *Orius insidiosus* on Agrisure® Viptera™ 3111 maize and its conventional hybrids with and without insecticide control at Oso Viejo (a) and El Camalote (b) on each sampling date. + i = insecticide.

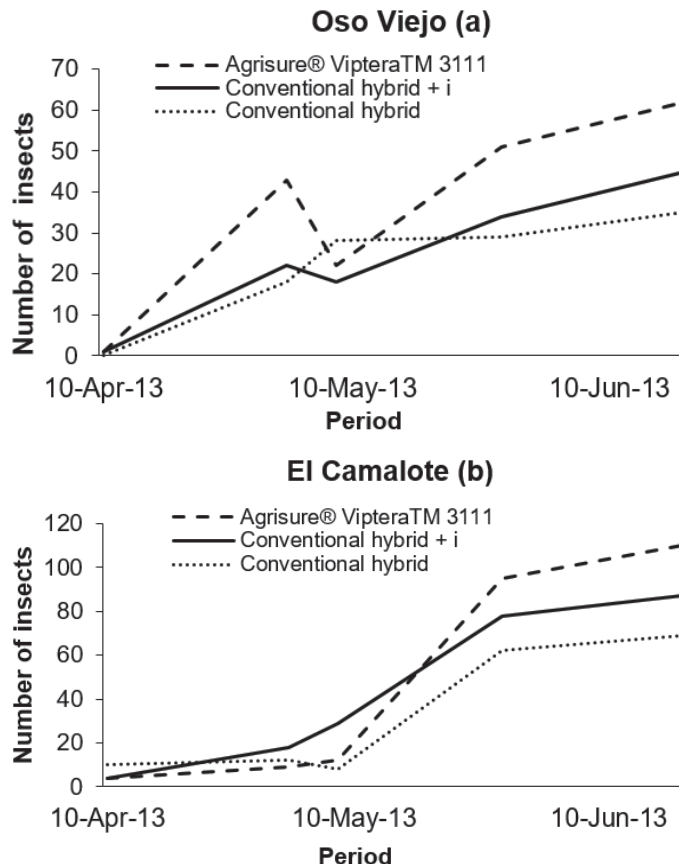


Fig. 2. Population fluctuation (total number of insects) of *Chrysoperla carnea* on Agrisure® Viptera™ 3111 maize and its conventional hybrids with and without insecticide control at Oso Viejo (a) and El Camalote (b) on each sampling date. + i = insecticide.

hybrids with and without insecticide treatment, respectively (Table 1). Again, in both locations Agrisure® Viptera™ 3111 had the largest number of insects, followed by the insecticide-treated hybrid. Frequency of the predator at Oso Viejo was 100% on Agrisure® Viptera™ 3111 and the insecticide-treated hybrid, and 80% on the untreated one, with population increasing after pollination until harvest (Fig. 2a), whereas at El Camalote all hybrids had a 100% frequency and a similar population pattern as Oso Viejo, with an increase after pollination (Fig. 2b).

The spotted pink lady beetle, *C. maculata*, was the most abundant species of the studied predators in both locations. At Oso Viejo, *C. maculata* abundance had a total of 1,149 in all hybrids, 536 in Agrisure® Viptera™ 3111, 257 in the insecticide treated hybrid, and 356 in the untreated hybrid. At El Camalote Agrisure® Viptera™ showed the higher population density (638) again, followed by the treated hybrid (588), and the untreated hybrid with 378 insects (Table 1).

All hybrids at both localities, *C. maculata* had an 80% frequency during crop development and a similar population fluctuation, increasing in density before pollination. At Oso Viejo, Agrisure® Viptera™ population increased until harvest, whereas the conventional hybrids population decreased (Fig. 3a). At El Camalote, only the conventional hybrid decreased its population at the end of development (Fig. 3b).

Discussion

The 3 predator species evaluated by visual sampling showed similar population densities in both locations with no statistical difference found

among hybrids in any of the predator species (Table 1). The population started with less than 10 insects in each sample, except for *O. insidiosus* at El Camalote that started with a higher density (26) per sampling date. In all cases, the population increased with crop development, and this can be considered normal due to the fact that their prey (phytophagous insects) increased during crop development as well (Figs. 1–3) with 2 increases before and after pollen shed. *Coleomegilla maculata* may include pollen or nectar in its diet, which causes a population growth, principally when the crop is flowering (Hoffmann & Frodsham 1993).

The higher population density on the Agrisure® Viptera™ 3111 is due to the fact that the genetically modified hybrid, that is resistant to *S. frugiperda*, provides more feeding resources to secondary or non-target arthropods, thereby attracting their natural enemies (Pons et al. 2005; Rose & Dively 2007).

Agrisure® Viptera™ 3111 had a higher mean population density than the isolate treated with insecticide, and that was due to the effect of emamectin benzoate over the natural enemies. However, when comparing the conventional isolines with and without chemical treatment, the insecticide effect was observed only with *O. insidiosus* in both locations and *C. maculata* at Oso Viejo. However, it did not occur in *C. carnea* in either location, probably due to the fact that this predator has an ability to avoid insecticide contact, whereas the other 2 tend to stay on the plant for longer periods of time (Bahena 2008), as observed by their greater abundance, especially of *C. maculata*.

The lower abundance in the hybrids without treatment might be due to the foliar damage done by the fall armyworm, providing less food for other plant feeders, and thereby lowering hosts' abundance

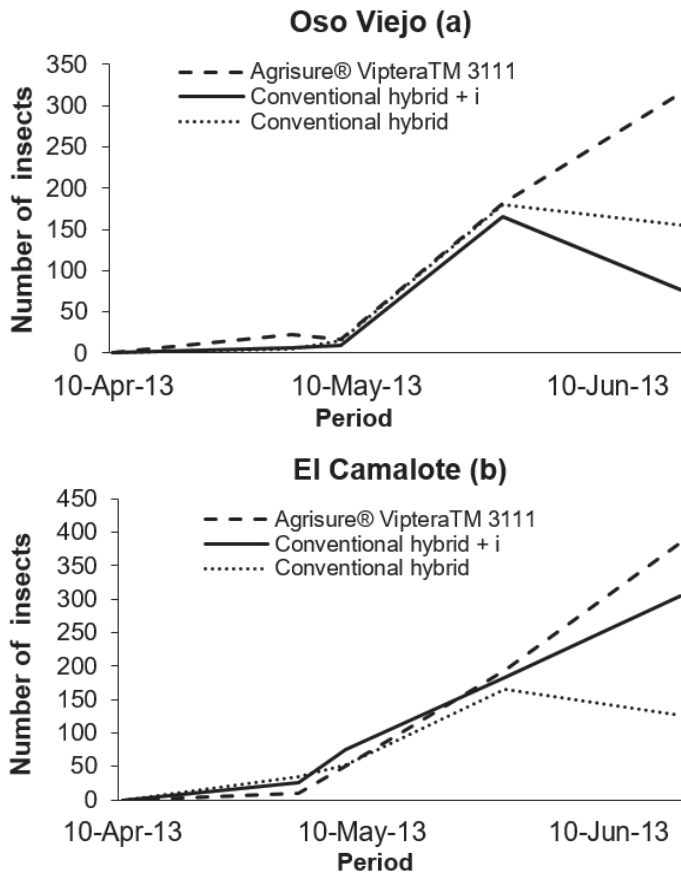


Fig. 3. Population fluctuation (total number of insects) of *Coleomegilla maculata* on Agrisure® Viptera™ 3111 maize and its conventional hybrids with and without insecticide control at Oso Viejo (a) and El Camalote (b) on each sampling date. + i = insecticide.

to natural enemies; on the other hand, foliar damage in this hybrid caused poor growth and pollen production and was less attractive to pink lady beetles.

Our results show that genetically modified corn expressing the Cry toxins of *B. thuringiensis* does not have a negative effect on abundance, frequency, or change in population density of the studied predators. Furthermore, to date there are no known mechanisms by which the Bt protein could affect non-target species (Daly & Buntin 2005).

In Brazil, Fernandes et al. (2007) did not find any negative effect of the Cry1Ab and VIP3A proteins on predator populations in Bt corn, stating that the Bt technology does not have a negative effect on predator populations in corn. Al-Deeb and Wilde (2003), using the Cry3Bb1 protein in Kansas, did not find reduction in *O. insidiosus* or *C. maculata* populations, concluding that Bt corn does not affect beneficial arthropods. Daly and Butin (2005) mention no significant difference in *C. maculata* populations between Bt and conventional corn.

De la Poza et al. (2005) evaluated Bt corn (Cry1Ac) over predators' abundance during a period of 3 yr, finding no adverse effect of the technology over them, thereby suggesting that this corn is compatible with those predators in the agroecosystem.

In Iowa, Pilcher et al. (2005) found few differences in abundance in *O. insidiosus*, *C. maculata*, and *C. carnea* between Bt Cry1Ab corn and its conventional hybrid, mentioning that the results were as expected due to their feeding and searching behavior. Ahmad et al. (2006), using the Cry3Bb1 protein, also found no statistically significant differences in abundance of *O. insidiosus* and *C. maculata* between Bt and conventional corn. Pilcher et al. (1997), Orr and Landis (1997), and Candolfi et

al. (2004) mention that Bt (Cry1Ab) corn did not have a negative effect on *C. carnea* under field conditions. On the other hand, Jasinski et al. (2003), found the same abundance pattern on non-target arthropods except for *C. carnea*, Staphylinidae, and soil mites, where populations were higher on the conventional hybrid. The authors stated that there were very few negative impacts associated with transgenic corn.

Concerns have risen that genetically modified plants expressing the Bt toxin could present a risk to non-target arthropods; however, research performed at Sinaloa, Mexico, with Agrisure® Viptera™ 3111 did not provide results indicating a negative effect of the genetically modified hybrid on the frequency or abundance of the studied predators. Further evaluations of this technology are recommended, and this could result in a reduction in the use of pesticides, preserve biodiversity, and be useful as a pest management tool (Yu et al. 2011).

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