April 2014, Vol. 8, No. 4, pp. 329-334
Journal of Life Sciences, ISSN 1934-7391, USA

Interaction Between *Helicoverpa zea* Damage with Corncob Diseases on Genetically Modified Corn in Sinaloa, México

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Received: February 06, 2014 / Accepted: March 13, 2014 / Published: April 30, 2014.

Abstract: GM (genetically modified) corn with the CrylAb, mCry3A and Vip3Aa20 protein of Bt (*Bacillus thuringiensis*) and its respective isoline as control with and without chemical control for corn earworm were used in this research. *Fusarium* corncob damage was lower on GM corn with (12.5%) and without (25.7%) insecticide treatment as compared with the isoline that had 48.3% and 83.1% of damaged corncobs with and without chemical control, respectively. Corn smut cob damage was also lower on GM corn with (3.2%) and without (6.3%) insect control compared with 15.5% and 49.7% damage with and without insecticide treatment, respectively. *Fusarium* sp. corncob rot was also lower on GM corn with 5.7% and 9.5% whereas a 24.6% and 63% rot was observed on the isoline with and without insecticide control, respectively. *Ustilago maydis* severity was also lower on Bt corn finding 0.07% and 0.25% damage on treatments with and without insect control as compared with the isoline that showed an 11.6% and a 41.4% smut rot with and without insecticide treatment, respectively. The authors conclude that GM corn resistant to *Helicoverpa zea* prevents damage by the pest, eliminating the entrance pathway for *Fusarium* sp. and *Ustilago maydis*.

Key words: *Bacillus thuringiensis*, transgenic, *Fusarium*, *Ustilago maydis*, mycotoxins, corn smut.

1. Introduction

Corn planting in Sinaloa has increased from 156,015.00 ha in 1980 to 409,402.91 ha in 2012 as the economic importance in the region was realized through double cropping (spring-summer and autumn-winter) [1]. These year round planting conditions favor the development of pests and diseases which were not of much economic importance in the past.

Diseases of major importance in Sinaloa are those causing cob rot that have a direct effect on yield and also exert an indirect effect on grain quality due to mycotoxins (aflatoxins, fumonisins, etc.). Disease incidence is higher in humid areas especially during the rainy season from flowering to harvest when insect and bird feeding expose inoculation courts to infection by pathogens. Pathogens found on corn grain in that area under laboratory conditions are: *Fusarium* spp., *Penicillium* spp., *Alternaria* spp., *Aspergillus* spp. and *Nigrospora oryzae*, with *Fusarium* spp. (45.70%) and *Penicillium* spp. (68.67%), the most commonly found. Another pathogen found in corn fields is corn smut (*Ustilago maydis*), which grows on grains forming galls filled by the fungus spores [2].

Bt corn is recommended to reduce cob rot and the attendant mycotoxin contamination resulting from infection by *Fusarium* spp. [3]. Insect damage to corn cobs is one of the most important factors for mycotoxin contamination because they make lesions in grains that provide a pathway for fungal colonization; insects are also direct vectors of fungal spores [4]. Thus, Bt corn resistant to Lepidoptera

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virtually eliminates infection pathways and thereby prevent entrance of pathogenic fungi.

Bt corn purportedly has less corncob rot and fumosine contamination when compared with conventional corn, indicating, that under certain circumstances, Bt corn can improve human and animal safety by avoiding risks from mycotoxin consumption [5, 6].

Considering that Bt corn has been approved to grow in México under experimental conditions and its efficacy has not been tested, this research had the objective to compare the Bt corn hybrids Agrisure 3000 GT and Agrisure 3110 with their respective conventional isolines to establish the relationship between corn earworm damage and the incidence of corncob diseases in Sinaloa, México.

2. Materials and Methods

2.1 Localization

Research was conducted at "El Temporal" farm, (24°22'42" N and 107°09'57" W) in Culiacán Sinaloa. Soil is sandy-loam, 2.1% organic matter, pH 6.10. Planting was done on January 28th, 2011.The experimental plot was 500 m distant from any other corn field and planted 21 days later than the recommended date in the area to avoid cross pollination with commercial corn; this protocol met the regulation requirement for GM corn field experimentation in México.

2.2 Genetic Materials

Genetically modified hybrids were provided by Syngenta Agro S.A de C.V.: Agrisure 3000 GT with the proteins Cry1Ab (*Bacillus thuringiensis* var. *Kurstaki* strain HD1), mCry3A (*Bacillus thuringiensis* subspecies *tenebrionis*), which confers resistance to Lepidoptera: *Spodoptera frugiperda*, *Spodoptera exigua*, *Heliothis virescens* and *Helicoverpa zea* and Coleoptera: *Diabrotica* spp., and the mutate enzyme 5-enolpyruvylshikimate-3-phorphate-synthase (mEPSPS), that makes it tolerant to glyphosate and glufosinate ammonium. The other hybrid used was Agrisure 3110 that has the proteins Cry1Ab (*Bacillus thuringiensis* var. *Kurstaki* strain HD1), Vip3Aa20 (*Bacillus thuringiensis* strain AB88) effective against lepidopteran species: *Spodoptera frugiperda*, *Spodoptera exigua*, *Heliothis virescens*, *Helicoverpa zea* and the mutate enzyme 5-enolpyruvylshikimate-3-phosphate-syntase (mEPSPS) that makes it tolerant to Glyphosate and Glufosinate ammonium herbicides. These hybrids were compared with their respective conventional isolines that lack the Bt proteins.

2.3 Experimental Design

A random block design was used with eight treatments, including controls, and four replicates; each treatment had ten yellow corn rows 5 m long separated by 0.8 m between rows. Additional insect management treatments were applied to both GM corn and isolines using Permethrin, Lamda-cyhalthrin and Emamectin benzoate two times during the crop cycle further targeting lepidopteran pests (Table 1).

Forty seeds were planted in each row and later adjusted to 34 plants per row. The entire experimental plot was surrounded by 5 m long rows of a local white corn hybrid. Agronomic management of the experimental area followed the cultural practices used by the commercial growers in the region.

2.4 Cob Damage Estimation

The number of diseased corncobs (incidence) was evaluated taking all of the cobs in the two central rows of each replicate in each treatment and calculating the percentage (%) of cobs damaged by *Fusarium* sp. and *Ustilago maydis*. Severity evaluation by *Fusarium* was evaluated by taking the total surface of the cob as 100 % and estimating the proportion of the cob area damaged by the fungus.

2.5 Statistical Analysis

A complete randomized block design statistical

Table 1 Treatments used to evaluate corncob rot incidence and severity by *Fusarium* sp. and incidence by *Ustilago maydis* in GM corn hybrids Agrisure 3000 GT and Agrisure 3110 at Culiacán, Sinaloa, 2011.

Treatment	Genotype	Insecticide
1	Isoline	Check
2	Isoline + IC	Permethrin-Lambda-cyhalothrin-Emamectin benzoate
3	Agrisure 3000 GT + IC	Permethrin-Lambda-cyhalothrin-Emamectin benzoate
4	Agrisure 3000 GT	No insecticide application
5	Isoline	Check
6	Isoline + IC	Permethrin-Lambda-cyhalothrin-Emamectin benzoate
7	Agrisure 3110 + IC	Permethrin-Lambda-cyhalothrin-Emamectin benzoate
8	Agrisure 3110	No insecticide application

IC = Insecticide control (insecticide application). Check = Isoline without insecticide application.

analysis was conducted using the SAS statistic software [7] and a Scheffé ($P \leq 0.05$) multiple range tests.

3. Results and Discussion

Percentage of corncobs affected by *Fusarium* sp. and *Ustilago maydis* were significantly lower in Agrisure 3000 GT technology hybrids when compared with their respective conventional isolines. The same significant difference was observed between the Agrisure 3000 GT and the conventional isoline with insect control presumably because the insecticide does not give permanent protection to the plant whereas the GM corn does give that kind of protection; however, damage to the conventional isolines by *Fusarium* sp. and *U. maydis* was also significantly lower in those treated with insecticide than in those without chemical control (Table 2). Corncob rot ascribed to *Fusarium* sp. varied between Agrisure 3000 GT with (25.7%) and without (12.5%) chemical control and their respective isolines that showed cob rot of 48.3% and 83.1% with and without insect control, respectively. The same results were observed for *U. maydis* in which the Agrisure 3000 GT technology showed a 6.3% and 3.2% disease incidence, whereas the conventional treatments had 15.5% and 49.7% damage with and without insect control in each case respectively (Table 2).

Severity analisis of corn cob rot caused by *Fusarium* sp., showed that genetically modified corn

Table 2 Percent cob rot caused by *Fusarium* sp. and *Ustilago maydis* on genetically modified corn hybrid Agrisure 3000 GT and its conventional isoline.

Treatment	Percent cobs with rot	
	Fusarium sp	*Ustilago maydis*
Isoline	83.1a	49.7a
Isoline + IC	48.3b	15.5b
Agrisure 3000 GT + IC	25.7c	6.3b
Agrisure 3000 GT	12.5c	3.2b

IC = Insecticide control. Means (a, b, c) followed by the same letter were not significantly different (Scheffé $\alpha = 0.05$).

Agrisure 3000 GT, was significantly lower than their conventional controls. GM corn had a 5.72% and 9.54% cob rot with and without insecticide treatment respectively; on the other hand, the isoline presented cob rot percentages of 24.6 when treated with insecticide and 63.0 without chemical control (Fig. 1).

Incidences of *Fusarium* sp. and *U. maydis* in the GM corn Agrisure 3110 hybrid are shown in Table 3. The number of corn cobs damaged were significantly lower on this hybrid than in the treatments with conventional corn. Agrisure 3110 incurred very little corn cob rot caused by *Fusarium* sp. when treated (1.8%) and not treated (0.3%) for insect control, whereas the conventional hybrid, had 29.7% of cobs infected with the pathogen when treated with insecticide and 60.8% damaged cobs without chemical insect control. Corn smut incidence was also significantly lower on the Agrisure 3110 hybrid showing cob damage from 0% to 0.7% with and without insecticide control, respectively, compared

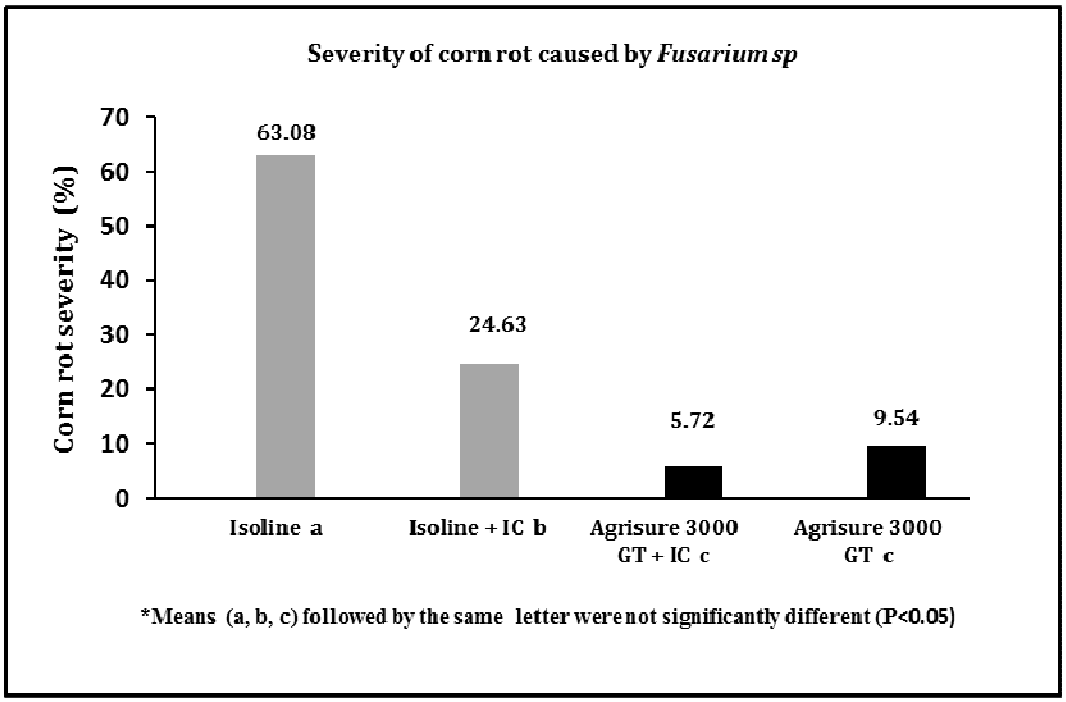

Fig. 1 Cob rot severity caused by *Fusarium* sp. on genetically modified corn Agrisure 3000 GT hybrid and its isoline at Culiacán, Sinaloa. IC = Insecticide control.

Table 3 Percent corn cob rot caused by *Fusarium* sp. and *Ustilago maydis* on genetically modified corn hybrid Agrisure 3110 and its conventional isoline.

Treatment	Percent cobs with rot	
	Fusarium sp.	*Ustilago maydis*
Isoline	60.8a	27.7a
Isoline + IC	29.7b	6.1b
Agrisure 3110 + IC	1.8c	0.7b
Agrisure 3110	0.3c	0.0b

IC = Insecticide control. Means (a, b, c) followed by the same letter were not significantly different (Scheffé = 0.05).

with the conventional hybrid that presented with 6.1% and 27.7% of diseased cobs when treated and not treated with insecticide, respectively.

Evaluation of the affected area of the cob (severity) by *Fusarium* sp. showed that the GM corn hybrid Agrisure 3110 had significantly lower damage than conventional corn. Agrisure 3110 severity measured 0.25% when treated with insecticide and 0.07% without treatment, whereas the conventional hybrid presented severity percentages of 11.62% and 41.44% with and without insecticide treatment, respectively (Fig. 2).

These results show that Agrisure 3000 GT and Agrisure 3110 efficacy is superior to treating isoline with insecticide; the Bt technology provides prophylactic protection to the plant that affects corn earworm and armyworm (when attacking the cob) larvae at first entry, whereas the insecticide application (s) provide only the temporal protection that the product residual permits, which results in some larval entry between sprays under conditions of heavy infestations. Additionally, insecticide sprays do not control larvae that achieve first entry because thereafter they are protected by the cob bracts.

Although Agrisure 3000 GT and Agrisure 3110 technologies were not developed for disease management, they provide an indirect additional beneficial production factor that significantly affects yield. The prevention of damage by lepidopteran pests also prevents cob rot fungi from gaining egress to susceptible tissue. Grain infection and disease caused by *Ustilago maydis* and *Fusarium* sp. are common in the Sinaloa region. The latter can be vectored by corn earworm [4] and is also known to produce mycotoxins that are dangerous to humans and animals [3].

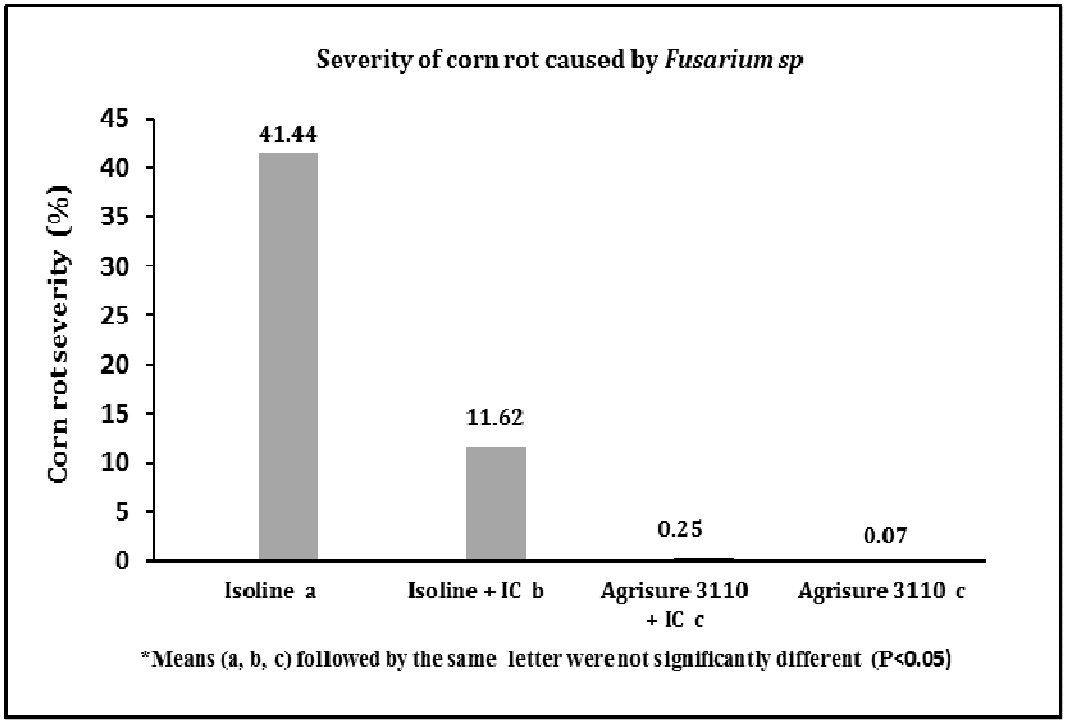

Fig. 2 Cob rot severity caused by Fusarium sp on genetically modified corn Agrisure 3110 hybrid and its isoline at Culiacán, Sinaloa. IC = Insecticide control.

Results found in this research probe the interaction between corn earworm and *Fusarium* sp. and *U. maydis* increasing their rot incidence and severity on corn as stated by authors of Refs. [5, 8-10] that reported also 9 to 10 times lower fumonisine content in Bt corn than in their isolines. This indicates that the use of these technologies not only prevent yield loss by the pests, but also prevents plant pathogen damage to the crop and minimizes risks of mycotoxin consumption.

4. Conclusions

Bt corn Agrisure 3000 GT and Agrisure 3110 technologies provide protection from diseases caused by *Fusarium* sp. and *Ustilago maydis* by being resistant to *Helicoverpa zea* and *Spodoptera frugiperda* that eliminate entrance pathways for the fungi.

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