Performance of transgenic maize genotypes against ear caterpillars in Argentina

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Abstract

Maize (*Zea mays* L.) is one of the most widely cultivated crops in the world and Argentina is a leading producer worldwide. Lepidopteran pests such as the fall army worm (*Spodoptera frugiperda* -J. E. Smith-) and the corn earworm (*Helicoverpazea* -Boddie-) cause ear damage, producing yield losses and facilitating the entry of pathogenic fungi such as *Aspergillus flavus*. Under conditions of high temperatures and drought stress,some *Aspergillus* strains can produce Aflatoxin B1 (AFB1), which is highly carcinogenic for humans and animals. The main aim of the current study was to determine the performance of PowerCore®, PowerCore® Ultra and Genuity® VT triple PRO®Bthybrid maize to corn ear caterpillar damage in Santiago del Estero, Argentina. Since 1990,the agricultural land area in this province has increased due to the forest clearance for agriculture. Climatic conditions in the region are favourable for aflatoxin. PowerCore® Ultra showed the highest yield and, PowerCore®registered the lowest severity of damage by corn ear caterpillar. The correlation between yield and insect damage severity was negative under the conditions evaluated.

Introduction

The geographical location of Argentina allows maize (*Zea mays* L.) to be grown over a vast area on a wide range of dates, positioning the country among the world leaders in production of this cereal, Santiago del Estero Province is responsible for 15 % to 20 % of the total country production (SIIA, 2018). The region is vulnerable to pathogenic fungi due to its highly variable climatic conditions and fragile edaphic structure, which, added to intensive farming processes, the availability of numerous new maize hybrids adapted to different agroclimatic environments and the effects of climate change on the precipitation regime (Cardini et al., 2018), among other factors, enable the prolongation of crop permanencein the area. These conditions facilitate an increased number of generations (multivoltines) of plague insects during the same crop growing season.

The main agents of damage in maize ears are the fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) and the corn earworm (*Helicoverpazea* (Boddie)). *S. frugiperda* larvae have cutting and defoliating habits and may cause direct damage when feeding on the grains. H. zea larvae cause damage to the stigmas, penetrate the ear and feed on the grains(Pogue, 2002). Both species cause economic losses due to the decrease in grain yield and in the commercial quality of the harvested grains. They facilitate the establishment of pathogens that cause ear rot and some also produce mycotoxins harmful to human and animal health, such as species of the genera *Aspergillus* and *Fusarium* (Boiça et al., 2001; Chulze, 2010; García et al., 2006; Munkvold et al., 1997; Santos et al., 2016; Wu, 2007).

Insect resistant transgenic or Btmaize are genetically engineered plants that express insecticide proteins obtained from the sporulating bacteria *Bacillus thuringiensis*, which produces two types of toxins: Cry proteins (Frankenhuyzen, 2009)and Vip proteins "*Vegetative Insecticidal Protein*" (Bravo et al., 2012).

More than 96 % of the maize sown in Argentina is transgenic (ArgenBio, 2019) and the continuous entry of Bt hybrids to the market, with new and more gene stacking expressing different toxins, increases the available tools to deal with insect attack. This technology needs to be accompanied by the creation of shelters by the farmers, with the purpose of delaying the emergence of resistant insects, of which there is already evidence (Trumper, 2014).

The experiment was conducted in the northwestern area of the Province, where since 1990 forest has been converted to agricultural land (Ginzo, 2015), allowing the development of large crop production centres and the increase of regional grinding facilities that add value to maize to achieve competitive advantages.

This study evaluatedthe performanceof PowerCore®, PowerCore® Ultra and Genuity® VT triple PRO® maize hybrids against corn ear caterpillar damage, their yields and aflatoxin levels.

Materials and Methods

Experimental plan

The experiment was performed during the years 2017 and 2018 in Otumpa, Santiago del Estero, Argentina (-27.2155°; -62.0627°; 156 mamsl) on a typical Haplustoll soil (INTA, 2019).The crop was planted by direct seeding in a field previously planted with soy. The plots were 546 m^2 consisting of seven rows 0.52 m apart and 150 m long with a density of 5.67 plants m^{-2}.

Commercial maize hybrids

Commercial temperate transgenic hybrids with different proteins introduced for lepidopteran insect control (ISAAA, 2019)and non-transgenic control were evaluated with5 treatments (Table 1).

Table 1 - Commercial hybrids evaluated in the years 2017 and 2018 and their corresponding introduced proteins for lepidopteran insect control.

Hybrids	Technology	Protein (inserted transgenes)
510 PW	**PowerCore®**	**Cry1F, Cry1A.105, Cry2Ab2**
510 PWU	PowerCore® Ultra	Cry1F, Cry1A.105, Cry2Ab2, Vip3Aa20
510 RR		Non - Bt
DK7210 VT3Pro	Genuity® VT triple PRO®	Cry1A105, Cry2Ab2, Cry3Bb1
DK 7210 RR		Non - Bt

Meteorological conditions

The meteorological data for the years 2017 and 2018 at the Otumpa experimental site in Santiago del Estero, Argentina (-27.2155°; -62.0627°; 156 mamsl) were recordedat agrometeorological station of the Instituto Nacional de TecnologíaAgropecuaria (INTA) and the Sociedad Rural of Quimilí, Santiago del Estero (Fig 1).

Sample collection and entomological evaluations

Once the crop reached physiological maturity, 300 ears were collected from the five central rows of each treatment.

The severity of the caterpillar ear damage was estimated as the percentage of damaged area by sampled ear. Since no decrease in yield was observed with low severity damage (Balbi and Flores, 2020), only the ears with damage severity over 3 % were analysed.

Every ear was threshed manually. The grains obtained were stove-dried until they reached 14 % humidity and the yield (gear-1) was determined.

Aflatoxin determination

Aflatoxins were detected and quantified in the different germplasms, following Trucksess et al. (1994). For this purpose, every sample was ground and homogenised to obtain 25 g of ground maize. An extraction solution of acetonitrile:water (84:16, V/V) was added and the supernatant was shaken and filtered. Four ml of raw extract were filtered through a MycoSep®224 AflaZon-cleanup column (Romer Laboratories, USA) and two ml of the purified extract were evaporated to dryness in gaseous nitrogen. The aflatoxins were detected and quantified by HPLC (high performance liquid chromatography) using the methodology described by Alaniz Zanón et al., (2018). Detection limits were 1 μg kg^{-1}.

Yield evaluation

To evaluate the yield differences, a mixed linear model was adjusted, and a generalised linear model was adjusted to determine the proportion of individuals with damage severity higher than 3 %(Stroup, 2012).

Statistical analysis

The means comparison was performed using the DGC test. For the statistical analysis, the InfoStatsoftware(Di Rienzo et al., 2019)was used with $p<0.05$.

The relations between the percentage of damaged ears, the yield for each treatment and the concentration of aflatoxinswere evaluated.

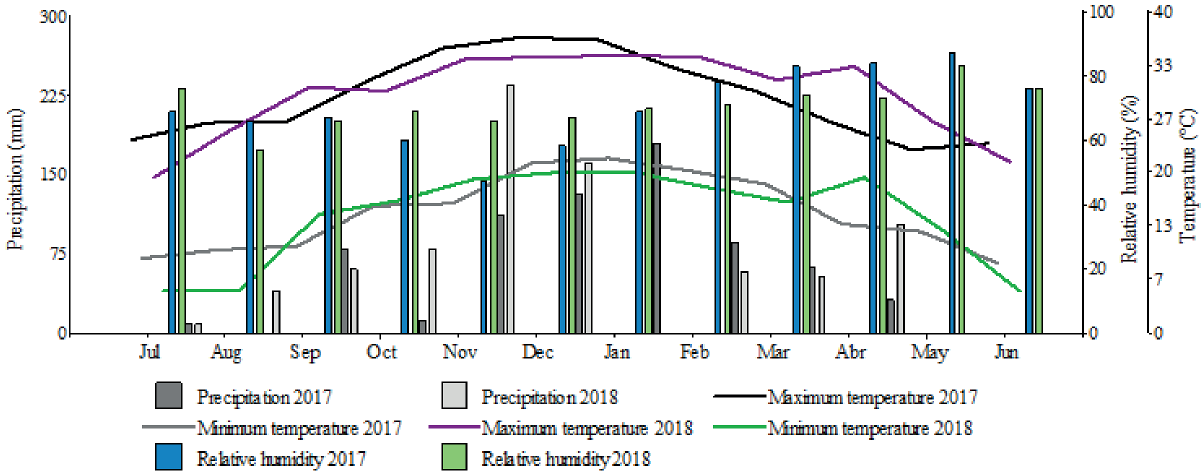

Fig. 1 - Ombrothermic diagram showing the evolution of the temperatures, precipitation and relative humidity of Otumpa, Santiago del Estero. Averages calculated withdata of temperature, precipitation and relative humidity collected from the INTA agrometeorological station and the Sociedad Rural of Quimilí, Santiago del Estero.

Results and discussion

Yield evaluation

The analysis of the differences among the meanyields for each treatment identified three groups in decreasing yield order: group I,the 510 PWU hybrid with 162.76 g ear-1, followed by group II, theDK 7210 VT3Pro hybrid with 155.87 g ear-1, and lastly groupIII, formed by 510 RR, DK 7210 RR and 510 PW, with 145.89 g ear-1, 143.06 g ear-1 and 139.55 g ear-1, respectively (Fig2).

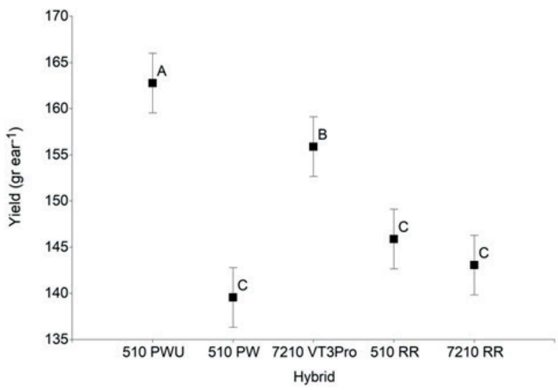

Fig. 2 - Means yields (g ear-1) for commercial Bt maize hybridsplanted in Santiago del Estero, Argentina, during the years 2017 and 2018. Yield groups identified:Group I, 510 PWU; Group II, DK 7210 VT3Pro; Group III,510 PW, 510 RR and DK 7210 RR.Mean values followed by the same letter are not significantly different (P<0.05; DGC test).

Ma and Subedi (2005)found that, under controlled conditions with no worm damage, there was no evidence of differences between Bt and non-Bt hybrids, which suggests that yield differences are due to the damage caused by caterpillars feeding on the ears (Betancourt and Scatoni, 1995; Bowen et al., 2014; Casmuz et al., 2010; Marques et al., 2019). In regard to this, Bernardi et al. (2016) found that S. frugiperda larvae survive less time in Bt than in non-Btmaize grains, indicating lower feeding time and consequently less ear damage.

Entomological evaluations

Significant differences were observed in the proportion of individuals with more than 3 % severity. The hybrid 510 RR had the highest caterpillar ear damage, followed by hybrids DK 7210 RR and DK 7210 VT3Pro, and lastly hybrids 510 PWU and 510 PW with the best performance showing the lowest proportion of ears damaged by caterpillars considering severity over 3 % (Fig 2). Waquil et al. (2013) observed differences in the damage to ears caused by *S. frugiperda*, being higher in hybrids with only one Cry protein than in hybrids with two stacked Cry proteins. Marques et al. (2019) determined that the Vip3Aa20 event and its stacking with different Cry proteins significantly reduced H. zeadamage to maize ears compared to the corresponding non-Bt hybrids. Balbi and Flores (2015)analysed the presence of H. zea caterpillars in ears from several Bt hybrids and observed that the hybrids with Viptera technology (Cry1Ab, Vip3Aa20, mcry3A) presented only 7.5 % of ears infected by caterpillars, followed by VT3Pro (Cry1A.105, Cry2Ab, Cry3Bb1) with 58 %, Powercore (Cry1A.105, Cry2Ab, Cry1Fa2) with 83 % and lastly TDMax hybrids (Cry1Ab) in which caterpillars were found in all the ears analysed.Yang et al. (2015)reported that Viptera 3111 technology (Vip3A, Cry1Ab) was highly effective against *H. Zea* damage compared to non-Bt hybrids. Similarly, Burkness et al. (2010) found that sweet corn with the Viptera trait was highly effective for the management of *H. zea* and *S. frugiperda*.

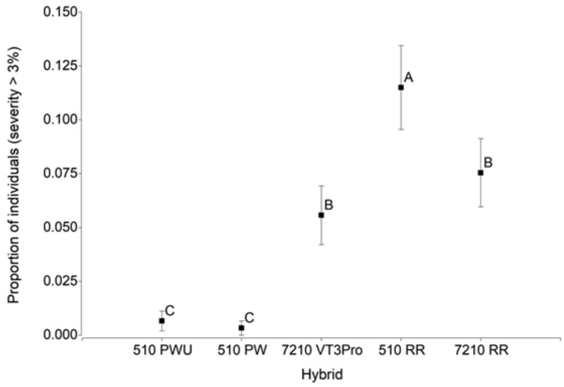

Fig. 3 - Proportion of individuals with severity higher than 3% for five commercial Bt maize hybrids (510 PWU, 510 PW and DK7210 VT3Pro) and non-Bt maize hybrids (510 RR and DK 7210 RR) planted in Santiago del Estero, Argentina, during the years 2017 and 2018.Mean values followed by the same letter are not significantly different (P<0.05; DGC test).

Effect of treatments on aflatoxin concentration

For the limit of detection of 1 µg kg^{-1}, the presence of aflatoxins was not detected in the different commercial hybridsevaluated.This can be linked to the accumulated precipitation duringthe years 2017 and 2018 being similarto the area´s mean precipitation of 600 mm to 700 mm annually (Fig1) (Galván et al., 2003). Probably, the crop did not undergo significant stress and the conditions were not favourable for aflatoxin contamination (Coppock et al., 2018; Darwish et al., 2014; Ni et al., 2011; Tola and Kebede, 2016; Villers, 2014).

Correlation analysis

A negative correlation was observed between insect damage severity and yield, for every hybrid (Spearman correlation of -0.25). Diener et al. (1987) andWicklow (1991)determined that every time the percentage of ear damage caused by lepidopterans doubled, the aflatoxin levels increased 20 times.Williams et al. (2005) and Wu (2007)studied the damage caused by caterpillars in maize ears in different Bt and non-Bt maize hybrids, reporting that, whenever the damage in ears increased, it also increased the aflatoxin concentration. Cardwell et al. (2000)reported that the level of infection of maize ears with *A. flavus* and *Fusarium verticillioides* was lower in Btmaize hybrids than in its corresponding non-Bt lines, thus indicating the direct relationship with caterpillar damage in maize ears. Similar results were obtained by Bakan et al. (2002) when studying the presence of fungi of the *Fusarium* genus in Bt and non-Btmaize hybrids. For aflatoxins,Hammond et al. (2004) andFolcher et al. (2010) reported that the fumonisin level, produced by fungi of the *Fusarium* genus, waslower in Bt than in non-Btmaize hybrids.

Conclusions

PowerCore® Ultra (510 PWU) technology showed the highest maizeyield.

PowerCore® andPowerCore® Ultra with their commercial hybrids 510 PW and 510 PWU, respectively,proved most resistant to ear caterpillars.

Yield and caterpillar damage severity levels, in the different hybrids, depended on the event technology or the stacking of Bt events employed, on the crop germplasm and on the environmental conditions, in which the crop, insects and pathogen interact.

Conflicts of Interest

Authors declare no conflict of interest.

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