Revista Brasileira de Milho e Sorgo

Brazilian Journal of Maize and Sorghum

ISSN 1980 - 6477

Journal homepage: www.abms.org.br/site/paginas

EFFECT OF EARWORM INJURIES ON FUMONISINS PRODUCTION IN Bt AND NON-Bt MAIZE

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Abstract – Tests were carried out to assess fumonisins production due to lepidopteran feeding on the ears of the hybrid DKB 390VTPRO (Cry1A105/Cry2Ab2) and in its non-Bt isogenic version. Maize ears were harvested and classified according to injury size, and fumonisin levels for each injury class were quantified. There were no significant differences between Bt maize and non-Bt maize in fumonisin production. However, the injury size due to earworm feeding was significant for fumonisin occurrence. The greater the injury, the higher the fumonisins level.

Keywords: *Spodoptera frugiperda*, *Helicoverpa zea*, mycotoxin, ear injury, transgenic maize, *Fusarium verticillioides*.

EFEITO DA INJÚRIA CAUSADA POR LAGARTAS DA ESPIGA NA PRODUÇÃO DE FUMONISINAS EM MILHO Bt E NÃO Bt

Resumo - Para avaliar a produção de fumonisinas causado pela alimentação de lepidopteros-praga nas espigas de milho Bt e não Bt, foram conduzidos testes, utlizando o híbrido de milho transgênico DKB 390VTPRO (Cry1A105/Cry2Ab2) e sua versão isogênica não-Bt (DKB 390). As espigas foram colhidas e divididas em quatro classes, quanto ao tamanho das injúrias e quantificados os teores de fumonisinas para cada classe. Não se verificou diferença significativa entre o milho Bt e sua versão não Bt, quanto ao níveis de ocorrência de fumonisinas. Contudo, houve efeito do tamanho da injuria na ocorrência de fumonisinas sendo que, quanto maior injúria, maior o nível de fumonisina.

Palavras-chave: *Spodoptera frugiperda*, *Helicoverpa zea*, micotoxina, dano espiga, milho transgênico, *Fusarium verticillioides*.

How to cite

MENDES, S. M.; PIMENTEL, M. A. G.; QUEIROZ, V. A. A.; SILVA, D. D.; COSTA, R. V. Effect of earworm injuries on fumonisins production in bt and non-bt maize hybrids. **Revista Brasileira de Milho e Sorgo**, v. 21, e1241, 2022.

DOI: https://doi.org/10.18512/rbms2022v21e1241

Spodoptera frugiperda (Smith) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) are the primary pests of maize ears in Brazil (Cruz, 1995; Bentivenha et al., 2017; Santos et al., 2016). Their injuries on maize ears facilitate fungal colonization and mycotoxin contamination on grains (Douwd, 2000; Bortolotto et al., 2016). In Brazil, fumonisins are the most common mycotoxins in maize. They are mainly produced by the fungus *Fusarium verticillioides*, which is detected in more than 90% of maize samples (Lanza et al., 2014). In Africa, the USA, Brazil, Italy, and China, the consumption of maize containing fumonisins has been associated with esophageal and liver cancer and neurotoxic effects (Terciolo et al., 2019).

Bt maize cultivation in Brazil has been widely adopted since its commercial release to manage caterpillars. Bt maize events are reported to have lower mycotoxins levels than non-Bt maize (Bordini et al., 2019; Bowers et al., 2014; Hammond et al., 2004). In the USA, there seems to be a consensus that the use of Bt maize reduces fungal infection and mycotoxins contamination on ears due to minor injury by larvae (Abbas et al., 2013; Bowers et al., 2013, 2014). However, Rocha et al. (2016) and Barroso et al. (2017) did not clarify the effect of the use of Bt maize in reducing fumonisins incidence. These authors postulated that there is a need for studies to determine the actual effect of the Bt technology in reducing the levels of fumonisins in maize. They also argue that Bt technology does not seem to reduce lepidopteran larvae on the ear effectively; the damage and injuries to the ears generate a gateway for fumonisins. They also suggest that studies should consider the interaction among abiotic factors related to the environment and the contamination degree by the mycotoxin. These factors may have significant effects on tropical crop conditions. Although injuries are essential for fungi to enter the ears, other infection routes allow the fungi to reach the grains, for example, the hairs of ears, systemic infections, and stems (Oldenburg et al., 2017).

In this context, we assessed the effects of the injury size caused by *S. frugiperda* and *H. zea* larvae on ears on the incidence of fumonisins levels in Bt and non-Bt maize.

Field experiments were conducted from December 2015 to June 2017 in the Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil (19°29′6″S, 44°10′ 46″W; 731 meters of altitude). Plants were grown in three crop seasons: season 1 from 12/14/2015 to 06/01/2016 (170 days); season 2 from 03/09/2016 to 08/19/2016 (163 days); and season 3 from 12/15/2016 to 06/08/2017 (175 days). Precipitation data, maximum, mean, and minimum temperatures were obtained daily in the climatological station

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of Embrapa. The experiment was conducted with four blocks containing twelve maize rows (0.7 m between and 10 m long). The experimental design was a randomized block factorial (3x2): three cropping seasons (1st, 2nd and 3rd seasons) and two hybrids (Bt and non-Bt). The injury size was classified into four levels (ears without injury, injuries with up to 2 cm, injuries from 2 to 4 cm, and injuries higher than 4 cm).

The hybrids used were DKB 390 (non-Bt) and DKB 390 VTPRO (MON89034 event, which expresses the Bt proteins Cry1A.105 and Cry2Ab2, Monsanto). The crop management was recommended for maize, without pesticide application, and mechanical weed control.

All ears of the four central rows of the plots were harvested. These ears were separated into four categories according to the injury size caused by larvae feeding, as mentioned above. All injuries in the ear were accounted for, not differentiating those caused by *S. frugiperda* or *H. zea*. After ears harvest, the grains were homogenized by quartering within each replicate. Grain samples (500 g) were sent to the mycotoxin analysis laboratory. The total fumonisins (B1 + B2) was extracted by a methanol/water solution (80/20) and purified by immunoaffinity columns (FumoniTest®), as recommended by the VICAM manual. The mycotoxin content quantification was performed using a series 4 Fluorimeter (VICAM).

Data were submitted to ANOVA, followed by F-test (α=0.05%). Total fumonisin levels and injury size data were subjected to analysis of variance ($P < 0.05$) and subsequent Tukey's HSD test. Fumonisin levels at crop seasons data were compared by *t*-test (α=0.05%).

The mean levels of total fumonisins (TF) were different among the crop seasons ($P<0.0001$; DF=3, F= 32.02) (Figure 1). In the first crop season, the mean TF contents were 2520μg kg^{-1}, twice higher than observed in the following seasons. The means were 1060 and 1090 μg kg^{-1} for the second and third crop seasons.

In the first crop season, there was rainfall of 638 mm (57% of it was in January 2016); precipitation in the second crop season was the lowest among the three years, with a total of 105.2 mm, and in the third crop season, it rained 436.4 mm. This factor must be considered since precipitation, and relative humidity directly interferes with the development of toxicogenic fungi and their ability to produce mycotoxins (Bhat et al., 2010). It is crucial to point out that TF levels were the highest during the first crop season, which correlates to the highest precipitation. This result demonstrates the effects of the climatic conditions on the formation of mycotoxins in grains. There was no effect of the interaction between the presence of the Bt toxin in

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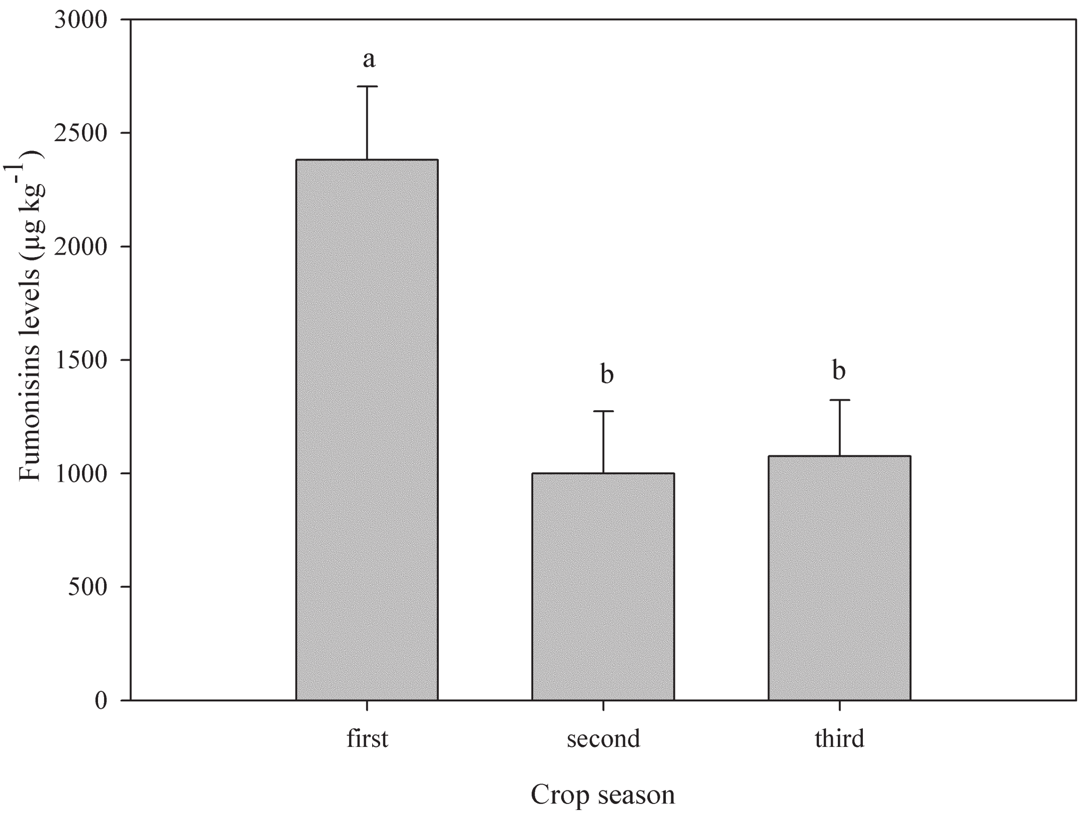

Figure 1. Total fumonisins levels (µg kg^{-1}) in maize ears at crop seasons. Means followed by the same letter do not differ by *t*-test (± CI, P=0.05).

the maize (Bt or its isogenic non-Bt) and the injury size (P=0.6750; DF=3, F=0.51). However, there was an effect of the injury size on the fumonisin incidence (P=0.0001; DF= 3, F=16.83). The fumonisin levels varied significantly within the classes of injury sizes. The higher the size of the injury caused by larvae feeding, the higher the incidence of TF in the grains (Figure 2).

Thus, regardless of whether the Bt maize (transgenic) or non-Bt maize, the higher the ear injury caused by larvae, the higher the possibility of contamination by fumonisins. Santos et al. (2016) argued that the expression of a Bt protein in grains is not yet a fully known factor. Grains can express the protein in segregation, in addition to the Xenia effect, which causes a different expression on endosperm and embryo. Besides, Balieiro Neto et al. (2013) found lower Bt protein levels in the ear. This result can be due to the higher accumulation of starch in the grains to the detriment of proteins, and, as the Bt toxin is a protein, it is found in lesser amounts.

Consequently, grains with lower Bt protein are more susceptible to larvae infestation on the

Figure 2. Total fumonisin levels (µg kg^{-1}) in function of the injury size caused by larvae (*Spodoptera frugiperda* and *Helicoverpa zea*) feeding in DKB 390 VTPRO maize and its non-Bt isogenic version.

ears. Our results are corroborated by Rocha et al. (2016), who evaluated fumonisin levels in Bt maize (MON 802 event, expressing Cry1Ab), and Barroso et al. (2017), who evaluated it on MON 802 and TC507 events (Cry1F expression). However, these authors did not find any correlation between fumonisins and Bt and non-Bt maize, indicating that grains contamination by fumonisins is not related to the presence of the Bt protein.

It is worth mentioning that *F. verticillioides*, which produces fumonisin, is the main fungus species associated with maize in tropical areas (Leslie & Summerell, 2006). Hence, the injury caused by larvae feeding on the ears opens the way for the fungus to spread and favors the grains contaminated by mycotoxins.

In conclusion, the main factor of higher fumonisins contamination on the ears is the injuries caused by larvae feeding. Therefore, the injury size is essential to the contamination level. The greater the injury caused by larvae, the higher the fumonisin incidence. In this context, researchers' effort is needed to find methods that improve the efficiency of caterpillar control on the ears and consequently mitigate this important contamination vehicle.

Acknowledgments

To FAPEMIG for the financial support.

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