EVALUATING THE IMPACT OF BT CORN HYBRIDS ON MYCOTOXINS IN TENNESSEE

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Abstract

Corn (*Zea mays*) is susceptible to ear rots caused by fungal pathogens including *Aspergillus* and *Fusarium* spp., which can contribute to yield loss. Some species within these genera may also produce secondary metabolites known as mycotoxins (*Aspergillus* » aflatoxin (AF) and *Fusarium* » fumonisin (FUM) or vomitoxin (DON)). Mycotoxins pose a significant risk to human and/or animal health and contamination of corn with mycotoxins can decrease crop value due to restricted use and prompt rejection if mycotoxins exceed threshold levels. Mycotoxins have been managed in two ways: 1. Management of the toxigenic fungi; and 2. Management of ear-feeding insects that provide entry points for toxigenic fungi. The latter methods, such as the selection of Bt hybrids, have been at the forefront of mycotoxin management. Since the first introduction of crops containing the Bt protein Cry1s in 1996, the use of Bt corn hybrids and their effect on mycotoxins have been extensively studied. With the introduction of the Bt protein VIP into corn and cotton, the use of this specific protein to impact mycotoxins has not been thoroughly investigated compared to the Cry proteins. Corn hybrids containing no Bt proteins, 2 Bt proteins (Crys), and 3 Bt proteins (Crys+VIP) were planted in 2020-2021 and evaluated for the presence of mycotoxin-producing fungi and mycotoxin levels. In this paper, the results of these trials will be discussed for hybrid comparison and insect damage assessment.

Introduction

Corn (*Zea mays*) is the most widely produced crop in the United States, accounting for more than 91 million acres planted (USDA-NASS, 2021) and it contributes significantly to both the U.S. economy and food security. However, corn is susceptible to late season, post-harvest fungal pathogens such as *Aspergillus* or *Fusarium* spp., which can result in the production of secondary metabolites known as mycotoxins, *Aspergillus* » aflatoxin (AF) and *Fusarium* » fumonisin (FUM) and vomitoxins (DON) (Masiello et al., 2019; Logrieco et al., 2021). Mycotoxins pose a significant risk to human and/or animal health, and contamination of corn with mycotoxins can decrease crop value due to the restricted use and prompt rejection if mycotoxins exceed threshold levels set by the United States Food and Drug Administration (FDA) (Dohlman E, 2008; Peivasteh-Roudsari et al., 2021). Mycotoxins have been managed in two ways: 1. Management of the toxigenic fungi; and 2. Management of the ear-feeding insects that provide entry points that help the toxigenic fungi colonize more readily (Munkvold et al., 2018). Due to the difficulty of controlling the mycotoxin-producing fungi, the latter has been the predominant control method for mitigation of mycotoxins, such as the utilization of Bt corn hybrids. Since the release of the first Bt protein in corn in 1996, the use of Bt corn hybrids has been reported to decrease FUM production (Cappelle, 2018); however, claims on AF reduction due to the use of Bt corn hybrids are contradictory and are still being studied extensively in corn-producing states across the United States. One of the primary problems of using Bt corn hybrids for the secondary benefit of reducing mycotoxins, is the overuse of Bt in crops and the potential risk of resistance development. The main concern being that Corn earworms, *Helicoverpa zea* (CEW – same species as Cotton bollworm in cotton) has developed resistance to the Bt Cry proteins. CEW is not a yield reducing pest in corn but causes significant yield loss in cotton, and both crops have the latest VIP protein. VIP has good control of CEW but there are concerns of early development of resistance. In regard to this concern, the primary objective of this research will be to evaluate the impact Bt and Non-Bt corn hybrids have on aflatoxin and fumonisin levels in Tennessee.

Materials and Methods

Planting and Sample Collection

Six commercial corn hybrids, including Bt and non-Bt corn hybrids, were tested at two locations (Jackson and Milan, TN) with two different planting dates (early-April and late-May) for each location. The Jackson location was irrigated while the Milan location was dry land. Non-Bt and Bt hybrids (Two Bt traits, or Three Bt Traits) were planted in 2020 and 2021, hybrids changed each year however they always consisted of (Non-Bt), (Cry1A.105 and Cry2Ab2), (Cry1A.105, Cry2Ab2 and Vip3Aa20) (Bayer Crop Science), or (Cry1F and Cry1Ab), (Cry1F, Cry1Ab, and Vip3Aa20) (Corteva). A no-till production system was used, and all management procedures followed the University

of Tennessee Extension Service's recommendations for field corn. Additionally, from one trial, ears were hand harvested from the non-Bt and examined for insect damage then placed into categories (No damage, Corn Earworm (CEW) damage, and CEW + Southwestern corn borer) to investigate damage in relation to mycotoxins and mycotoxigenic fungi. Trials were harvested by a combine at maturity and a grain sample was taken for each plot then stored in dry paper bags in a chest freezer (0ºF) until processing. A representative sample was taken from each plot and replicated twice using the guidelines explained in (Canadian Grain Commission, 2019). Two analyses were run using SAS (SAS Institute, Cary, NC) PROC GLIMMIX to determine 1. Presence of *Aspergillus* or *Fusarium* 2. Levels of mycotoxin (AF and FUM).

Evaluation of *Aspergillus* or *Fusarium* Presence

Whole kernels from grain samples were surface sterilized by using a 10% bleach sterilization method before evaluations. Five randomly selected kernels were plated on both Modified Dicholoran Rose Bengal (MDRB) (Selective for *Aspergillus* spp. (Abbas et al., 2007)) and Malachite Green Agar (MGA) (Selective for *Fusarium* spp. (Leslie and Summerell, 2007)) replicated 5 times and were allowed to incubate at 32ºC for 3-5 days before evaluation for presence of pathogens. Colony morphology and spore/structure characteristics were used to identify pathogens under a microscope.

Evaluation of Mycotoxins

Quantification and detection of mycotoxins (FUM and AF) were performed using EnviroLogix's TotalTox Kits and the QuickScanII (EnviroLogix, Inc., Portland, ME), a rapid testing lateral flow device (LFD) that delivers quick, easy, and accurate quantification results for mycotoxins. Frozen grain samples were ground in a grinder for 1 min, followed by bleach sterilization between samples. Ground samples were filtered through a 20-mesh sieve, and a 25g subsample of ground corn was used to extract AFs and FUMs using EnviroLogix mycotoxin test strips/kits according to the manufacturer's protocol for extraction. Mycotoxin levels were measured by inserting the LFD strips into the QuickScan II to obtain a quantified reading of the sample in: AF, parts per billion (ppb), with a base detection limit of 2.7 ppb to 30 ppb and FUM, parts per million (ppm), with a base detection limit of 0.1ppm to 10 ppm.

Results and Discussion

While both pathogens were detected in harvested ears from Tennessee, *Fusarium* species were the predominant pathogen for both years. Damage type or Bt Hybrid was not significant for the presence of either pathogen. Though, it is to be noted that presence of these pathogens does not always equal mycotoxin production, some species/strains of these pathogens can be atoxigenic (doesn't produce toxins) and/or environmental conditions are not conducive for production. Mycotoxin results were limited to FUM , as AF was not detected in the 2020 samples. 2021 samples are in the process of being screened for mycotoxins. The amount of damage did have an impact on FUM levels in 2020 and 2021, where the ears with CEW and Southwestern corn borer damage having significantly greater FUM than ears with no damage. The amount of FUM in Bt corn hybrids with different Bt traits was not significant, with all hybrids exceeding the regulatory threshold for humans, except for the TRECEPTA treatment in the Bt Late Milan Trial, although statistically there was no difference in FUM across hybrids within any trial. The location of these trials appears to influence FUM levels, but this could be due to the irrigation at the Jackson location, creating an environment more conducive to FUM production.

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