Crop Protection 98 (2017) 108–115

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Integration of biological control and transgenic insect protection for mitigation of mycotoxins in corn

Mark A. Weaver [a, *], Hamed K. Abbas [a], Michael J. Brewer [b], Luke S. Pruter [b], Nathan S. Little [c]

[a] USDA ARS, Biological Control of Pests Research Unit, National Biological Control Laboratory, Stoneville, MS, USA
[b] Texas A&M AgriLife Research and Department of Entomology, Texas A&M AgriLife Research and Extension Center at Corpus Christi, Corpus Christi, TX, USA
[c] USDA ARS, Southern Insect Management Research Unit, Stoneville, MS, USA

ARTICLE INFO

Article history:
Received 11 July 2016
Received in revised form
18 March 2017
Accepted 20 March 2017

Keywords:
Aflatoxin
Aspergillus flavus
Biopesticide
Bt-corn
Fumonisin
Helicoverpa zea

ABSTRACT

Biological control is known to be effective in reducing aflatoxin contamination of corn, and some transgenic corn hybrids incur reduced damage from corn earworm (*Helicoverpa zea)* than comparable non-*Bt* hybrids. We conducted seven field trials over two years to test the hypothesis that transgenic insect protection and biological control could be integrated to prevent mycotoxin contamination of corn. Corn hybrid N78N3111, expressing the highest degree of transgenic insect protection, was nearly 100 percent free from corn earworm damage and generally had less than half as much contamination from fumonisin compared to N78NGT, a near isogenic corn hybrid without insect protection. This insect protection, however, did not significantly prevent aflatoxin contamination. Soil application of non-aflatoxigenic biocontrol strains of *Aspergillus flavus* significantly reduced aflatoxin concentrations in corn. Biocontrol strain 21882 of *A. flavus* was especially effective and reduced aflatoxin contamination by about 90 percent over the seven field trials. There was no significant interaction between the insect protection and biocontrol treatments. Although no synergies were detected, the reduction of mycotoxins by both strategies supports application of both strategies in tandem. Economic factors external to the cost of the technologies will be a major determinant if the mycotoxin mitigation attained by use of these technologies will have a positive economic benefit.

Published by Elsevier Ltd.

1. Introduction

Aflatoxins are among the most potent carcinogens found in nature (Wogan et al., 2004). These mycotoxins are produced primarily by the opportunistic plant pathogens *Aspergillus flavus* (Link) and *A. parasiticus* (Speare) (Payne, 1992). These aflatoxin-producing pathogens can be found on many crops (Shephard, 2008), but corn, peanuts, and cotton are of the commodities most commonly associated with aflatoxin contamination (Cotty, 1994; Dorner, 2004; Abbas et al., 2006). Aflatoxin in corn is closely monitored and layers of regulation and monitoring have largely transformed this health threat into an economic risk in the developed world (Otsuki et al., 2001; Wu, 2004). To protect the health of humans and livestock, aflatoxin contamination in foodstuffs is limited to 20 and 300 parts per billion, respectively (United States Food and Drug Administration, 2011; United States Department of Agriculture, 2015). In other parts of the world acute aflatoxicosis is occasionally lethal: more frequently aflatoxicosis is sublethal and involves chronic exposure causing impaired immune function and certain cancers (e.g., Lewis et al., 2005; Marechera and Ndwiga, 2015; Probst et al., 2007; Wagacha and Muthomi, 2008). Other mycotoxins, including cyclopiazonic acid (King et al., 2011; Miller et al., 2011) and fumonisin (Abbas et al., 2007; Abbas et al., 2016; National Toxicology Program, 2001) periodically contaminate corn, although they are less rigorously monitored.

Improvements in corn production management might effectively reduce aflatoxin contamination. Drought and heat stress during flowering are thought to increase the risk of aflatoxin occurrence, and aflatoxin contamination is much more common in the Southern U.S (Diener and Davis, 1977; Jones et al., 1981; Payne, 1992). Hybrid selection, increased irrigation and fertility, and earlier planting dates may be advised, but are generally insufficient to prevent all infection by *A. flavus* and concomitant aflatoxin

* Corresponding author.
E-mail address: Mark.Weaver@ars.usda.gov (M.A. Weaver).

http://dx.doi.org/10.1016/j.cropro.2017.03.020
0261-2194/Published by Elsevier Ltd.

contamination (Bruns, 2003; Wiatrak et al., 2005). In growing regions where risk persists or is variable, the most effective strategy for the prevention of aflatoxin contamination is the application of biological control products using strains of *A. flavus* that do not produce aflatoxin. While the mechanism of biocontrol is under investigation, it is thought to include competitive displacement (Cotty, 1994; Dorner, 2004; Abbas et al., 2006). The efficacy of this biopesticide approach has been well tested in numerous conditions (e.g. Atehnkeng et al., 2008; Dorner, 2010) and has recently been reported to be cost effective for corn growers in the Southern U.S. (Weaver et al., 2015).

Visible infection of corn by *A. flavus* had been seen in the kernels with damage from insect activity, therefore there was some expectation that transgenic insect protection would confer resistance to *A. flavus* infection (Windham et al., 1999; Dowd, 2001; Abbas et al., 2013). In fact, genetically modified corn expressing Bt δ endotoxins (*Bt* corn) has been effective in preventing corn contamination with fumonisin, another mycotoxin produced by *Fusarium verticillioides* [(Sacc.) Nirenberg] (Ostry et al., 2010; Bowers et al., 2014). It has been proposed that *Bt* corn might be similarly effective in preventing aflatoxin contamination (Wu et al., 2008). To date, the research on *Bt* corn for controlling aflatoxin has been equivocal (Abbas et al., 2008, 2013; Bowen et al., 2014; Ostrý et al., 2015). The relationship between *Bt* corn and mycotoxin contamination is complicated by the fact that the corn industry continues to develop different forms of transgenic insect protection, which offer varying levels of specificity to different insect pests (Buntin and Flanders, 2015). First generation *Bt* corn hybrids were highly effective against European corn borer, *Ostrinia nubilalis* (Hübner) and subsequent hybrids have expanded the range of lepidopteran pests controlled (Buntin and Flanders, 2015). The available *Bt* transgenes also vary in toxicity to corn earworm [*Helicoverpa zea* (Boddie)] and fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] (Buntin et al., 2004; Brewer et al., 2014).

The objective of this study was to measure the relationships between corn yield, insect damage and mycotoxin contamination of corn in the context of biological control and transgenic insect protection.

2. Materials and methods

2.1. Experimental background and treatments

Field experiments were replicated in a field near Corpus Christi, TX (2014 and 2015) and two (2014) or three (2015) fields near Stoneville, MS. These locations have a history of aflatoxin contamination and occurrence of ear-feeding by corn earworm [*Helicoverpa zea* (Boddie)] and fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] (Bruns and Abbas, 2005; Adamczyk and Hubbard, 2006; Brewer et al., 2014). Specifics of the growing conditions were obtained from the National Oceanic and Atmospheric Administration (2015) and summarized in Table 1. The experimental treatments were three commercially-available, near-isogenic hybrids varying in *Bt*-based insect protectant transgenes and two *A. flavus* biocontrol products as well control plots with no biocontrol treatment. The corn was grown in a 3 (hybrids) by 3 (biocontrol) factorial treatment structure, in a randomized complete block. The corn hybrids tested were N78NGT, a glyphosate tolerant hybrid and two near-isogenic hybrids with additional transgenic insect protection (Syngenta Crop Protection, Greensboro, NC). The hybrid N78N3000GT is glyphosate and glufosinate tolerant and expresses the *Bt* proteins Cry 1Ab and Cry 3A, while the hybrid N78N3111 is glyphosate and glufosinate tolerant and expresses Cry 1Ab, Cry3A and Vip3Aa20. Corn with the 3111 trait package is considered to have excellent resistance to corn earworm and fall armyworm, while 3000 GT provides limited suppression of these pests (Buntin and Flanders, 2015).

Table 1
Observed weather conditions during test of transgenic insect protection and biocontrol for mycotoxin control (2014–2015).

	Corpus Christi, TX		Stoneville, MS		Historical Average[a]
	2014	2015	2014	2015	
Rainfall (mm)					
Nov-March[b]	159	342	523	645	650
April–May	115	525	407	335	266
June–July	55	72	280	130	146
Average Max/Min Air Temperature (°C)					
April–May	29/18	28/21	25/14	27/16	26/14
June–July	35/24	34/24	32/21	33/23	32/21

[a] Historical average for Stoneville, Mississippi (Rainfall, 99 years; Air temperature, 84 years). Historical data obtained from Mississippi State University Extension Service (2017).
[b] November–March includes two months of the preceding year.

The biocontrol treatments were a 22 kg ha^{-1} of *A. flavus* strain NRRL 30797 prepared on autoclaved wheat (Abbas et al., 2006) or NRRL 21882 (AflaGuard GR, Syngenta Crop Protection, Greensboro, NC) or sterile autoclaved wheat (no biocontrol treatment). In 2014, applications were made to Stoneville fields at the V5 growth stage and in Corpus Christi at the V10 stage. In 2015, all applications were made at the V8 stage. Surface soil (0–5 cm) samples were collected from each plot before application of inoculum and again before harvest.

Standard agronomic practices were followed at each site. Pre-plant atrazine and *s*-metolachlor and post emergent glyphosate were used for weed control. No irrigation was used at the Stoneville fields. A single drip-irrigation event was needed at the Corpus Christi site in 2014 and no irrigation occurred in 2015. Planting dates were April 1, 2014 and April 3, 2015 at Corpus Christi and May 6, 2014 (both fields) and April 16, 2016 to April 28, 2015 (three fields) at Stoneville. These comparatively late planting dates encounter higher pressure from ear-feeding larvae, which is typical for this region (Rodriguez-del-Bosque et al., 2012). Stoneville plots were 8.1 m by 12.2 m in area. Corpus Christi plots were 3.9 m by 12.2 m in area. The Corpus Christi field and Stoneville Fields 2 and 3 were used in both years, but the experiment was moved to a different portion of the field.

2.2. Experimental measurements

Near harvest, ear injury from feeding by corn earworm and fall armyworm were measured on 20 ears. Total cm^2 of ear injury and length of larval feeding (Corpus Christi) and number of damaged kernels (Stoneville sites) were recorded per ear. After maturity and in-field drying to <15% moisture, plots were harvested by hand (Corpus Christi) or combine (Stoneville) to determine yield and collect a representative sample of grain for mycotoxin analysis. Approximately 3 kg of shelled corn was ground and homogenized with a Romer (Union, MO) mill. Soil and grain samples were analyzed for the size and composition of the *A. flavus* population. Soil and ground corn samples were diluted and plated on modified dichloronitroaniline rose bengal media (MDRB) (Horn and Dorner, 1998). The *A. flavus* colonies were counted on MDRB agar.