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

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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.

34

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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, 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 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; Abbas et al., 2013; Bowen et al., 2014; Ostry 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 3000GT provides limited suppression of these pests (Buntin and Flanders, 2015).

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 (0-5cm) 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 8.1m by 12.2m in area. Corpus Christi plots were 3.9m by 12.2m 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 cm² 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. Individual colonies from the MDRB were transferred to β -cyclodextrin-amended potato dextrose agar and characterized as toxigenic or non-toxigenic based on fluorescence and production of a yellow pigment on the underside of the colony (Abbas et al., 2004).

2.3 Mycotoxin analytical methods

Two fifty-gram subsamples of ground corn were extracted with 250 mL 70% methanol and subject to analysis of aflatoxin, fumonisin and cyclopiazonic acid.

Aflatoxin: Corn extracts were cleaned with 0.45 μ L nylon syringe filters. Twenty microliters of the cleaned sample were separated on a Waters Nova-pak C18 column in a Dionex Ultimate high-pressure liquid chromatography (HPLC) system with post-column photochemical derivatization (PHRED, Aura Industries, New York, NY) and

fluorescence detection (Abbas et al., 2015). The mobile phase consisted of water : methanol : acetonitrile : 1-butanol (665 : 195 : 130 : 10) (A) and water : methanol : acetonitrile : 1-butanol (660 : 130 : 200 : 10) (B), beginning at 100% A for 6.2 minutes, changing to 100% B at 6.7 minutes, holding at 100% B until 8.7 minutes before returning to 100% A at 9 minutes. Samples were compared with commercially available aflatoxin standards (aflatoxin B₁, B₂, G₁ and G₂, Sigma Chemical Company, St. Louis, MO) with a limit of quantitation of 0.2 ng g⁻¹.

Fumonisin: Corn extracts were purified by passing 2.5 mL through with a Bond-Elute SAS columns (Varian, Harbor City, CA) that had been pre-equilibrated with 2.5mL of 100% methanol and 2.5 mL of 75% methanol. The columns were then washed with 2.5mL of 100% methanol and 2.5 mL of 75% methanol before eluting the samples in 2.5mL of 2% acetic acid in methanol. This cleaned sample was dried under nitrogen at 50°C using a Turbo Vap LV (Biotage, Charlotte, NC), and stored at -20°C until analysis. The clean samples were reconstituted in 2.5mL of 30:70 acetonitrile/water, and analyzed by HPLC with post-column derivatization. Samples were injected into an Agilent 1200 system consisting of a binary pump (model G1312A), autosampler (model G1367B), and a fluorescence detector (model G1321A) equipped with a 150mm x 4.6mm i.d. 5µm Zorbax Eclipse CDB-C18 column at a temperature of 45°C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) in a gradient flow rate of 1.2mL min⁻¹ for 18 minutes beginning at 68% A and 32% B for 8 minutes, following was a flow of 60% A and 40% B for 3 minutes, with the rate returning to 68% A and 32% B for the duration of the analysis. A secondary pump (WATERS Reagent Manager) was attached to the system in-line after the column and

before the detector to facilitate addition of the o-phthaldialdehyde (OPA) post-column derivitization solution, consisting of 40.7g L⁻¹ sodium carbonate, 13.4g L⁻¹ boric acid, 18.8g L⁻¹ potassium sulfate, 1g L⁻¹ N-acetyl-L-cysteine, and 0.8g L⁻¹ OPA at a constant flow rate of 0.45mL min⁻¹. The detector was set at 335nm (excitation) and 440nm (emission). Standards were prepared using fumonisin B₁ and B₂ (Sigma-Aldrich) in 30% acetonitrile in water, with 30% acetonitrile used as blanks for the analysis. The limit for quantitation using this process was 0.025ng µL⁻¹.

Cyclopiazonic acid: Corn extracts were mixed with 5% ammonium hydroxide (1:1), sorbed to Oasis Max solid phase extraction columns (Waters, Milford, MA) and eluted in 2 mL of 2% formic acid in methanol. Detection was via HPLC MS/MS with a Thermo LTQ XL MS, attached to a Thermo Finnigan Surveyor MS Pump and Thermo Finnigan Surveyor MS Autosampler (Thermo Electron Corp., West Palm Beach, FL). Separation was performed with a Polar RP column (Phenomenex Synergi 4µ, Hydro-RP 80A, 4.6 x 150mm, C18 Column w/ polar end-capping, Phenomex, Torrance, CA) using with a gradient from ammonium acetate (10 mM, pH 5.7)/methanol (30:70, v/v) to ammonium acetate (10 mM, pH 5.7)/methanol (5:95, v/v) over 5 min, then held constant for 7 min. Cyclopiazonic acid eluted at 6 min with a limit of detection of 0.125ng µL⁻¹.

2.4 Economic returns and statistical methods

Economic returns from each plot were determined from the measured yield and published commodity values. Corn was assigned a value of \$166.92 and \$174.00 metric ton⁻¹ for Mississippi and Texas, respectively (\$4.24 and \$4.42 bushel⁻¹, United States Department of Agriculture, National Agriculture Statistics Service, 2015)(all values in US dollars). The value of the corn was reduced by \$0.394 per metric ton (one

cent per bushel) for each 1 ppb of aflatoxin detected from the lower threshold of 20 ppb to the upper threshold of 300 ppb (personal communication, Farmer's Express Grain, Greenwood, Mississippi)(advisory and action levels for mycotoxins established by US FDA, 2011). Corn over 300 ppb is unmarketable and was assigned a zero value (Weaver et al., 2016). Seed costs were included at a rate of \$206.41, \$214.44 and \$222.47 ha⁻¹ for N78NGT, N78NGT3000 and N78N3111, respectively. The price of the biological control treatments was assessed at \$59.31 ha⁻¹ and all other direct expenses (fertilizer, fuel, equipment maintenance, pesticides, labor) totaled \$760.05 ha⁻¹ plus a variable cost for seed and trucking the grain to market (Falconer et al., 2015). Returns above direct expenses were calculated by multiplying the observed yield by the corn value, subtracting any discount for aflatoxin contamination and the total direct expenses.

Observations of insect damage, colonization of soil and grain by *A. flavus*, mycotoxin contamination, yield and economic returns were subject to analysis of variance (ANOVA) by JMP (version 11, SAS Institute) to test for interaction and main fixed effects of the biocontrol and transgenic insect protection treatments. If the interaction was not significant, then the least squared means of the main effects were separated by Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Analysis was repeated for each year for each field independently and for each year and for all fields combined. In three fields aflatoxin was too uncommon to facilitate ANOVA, and these fields were excluded from the analysis.

3 Results and Discussion

There were no significant interactions between transgenic insect protection and biocontrol on any of the experimental measurements ($P > 0.05$). Therefore the results below focus on corn ear injury response to the transgenic insect protection treatment (section 3.1), *A.flavus* response to the biocontrol treatment (section 3.2), and mycotoxin and yield response to both of these factors (sections 3.3 and 3.4),

3.1 Corn ear injury responses to hybrid and biocontrol treatments.

Others have noted that a high level of control of corn earworm was achieved only recently by comparatively small number of *Bt* corn hybrids, notably those that included Vip3Aa20 or Cry1A.105 + Cry2Ab2 (Siebert et al., 2012; Reisig et al., 2015; Yang et al., 2015). Here, we were able to directly compare the earworm management efficacy of the Cry 1Ab + Cry 3A gene stack (N78N3000GT) and the Cry 1Ab + Cry 3A + Vip3Aa20 gene stack (N78N3111) with a non-BT, glyphosate-tolerant hybrid (N78NGT) with the same genetic background. The results presented in Figure 1 include observed injury to corn ears attributed to corn earworm activity over 7 site-years. In both years, and in every field, N78N3111 had less area of ear-tip damage, fewer damaged kernels and reduced depth of penetration compared to the N78NGT and N78N3000GT hybrids ($F = 14.8, 45.7, 138.0$; d.f. = 2, 69; 2, 159; 2, 339; $P < 0.0001, < 0.0001, < 0.0001$, respectively). The N78N3000GT hybrid was associated with reduced damage in only 2 of the 7 fields, while in the other 5 fields there were no significant differences compared to N78NGT.

Fall armyworm and corn earworm populations in Corpus Christi were counted in 2015 by averaging from 80 ears per hybrid picked from 8 randomly selected plots (10 ears per plot). For N78N3111, there were an average of 0.5 corn earworm per 10 ears,

and no detected fall armyworm, for N78N3000GT there were an average of 5.9 corn earworms and 1.4 fall armyworms, and in N78NGT there were an average of 2.6 corn earworm, and 2.4 fall armyworms. While corn earworm and fall armyworms were found in N78N3111, no larva exceeded a medium classification size of 7-23mm. Although the 3111 trait package is known to be highly efficacious for corn earworm control, the survival through mid-sized larvae was consistent with the minor tip-feeding in ears. These data are consistent with Yang et al. (2015) who also noted that some larvae survived to 2nd instar on 3111 corn.

3.3 Effect of biocontrol treatments on *A. flavus* populations

Introduction of nontoxigenic *A. flavus* inoculum to these plots shifted the *A. flavus* soil population to a significantly less toxigenic state in just one of the seven field-years (Table 2). Several factors could have contributed to this failure. First, the Stoneville sites were dominated (>90%) by nontoxigenic strains of *A. flavus* even in the absence of biocontrol treatments. This could have been influenced by historical cropping patterns (Jaime-Garcia and Cotty, 2006 and 2010) or other management factors. The Corpus Christi field had > 40% toxigenic *A. flavus*. In 2015, the repeated heavy rains created periods of extensive flooding at the Corpus Christi site and it is possible that some inoculum movement occurred, obscuring the actual effect of the biocontrol treatments, a problem exacerbated by modest plot size and plot buffers. Applications of strain 21882 resulted in reduced colonization of the grain by toxigenic *A. flavus* strains in all fields when compared to the no biocontrol treatments ($F = 4.8$, d.f. = 2, 141; $P = 0.0093$).

3.3 Effect of transgenic insect protection and biocontrol treatments on mycotoxin contamination

The response of fumonisin and aflatoxin contamination of corn to the transgenic insect protection and biocontrol treatments is summarized in Table 3. There was zero cyclopiazonic acid detected in any of these samples. There was no significant effect of the biocontrol program on fumonisin contamination. The 3111 transgenic insect protection, however, significantly reduced fumonisin contamination ($F = 13.6$; d.f. = 2, 339; $P < 0.0001$) (Table 4). Additionally, through the entire experiment, every sample from hybrid N78N3111 contained less than 4 ppm fumonisin, the FDA advisory level for corn for human consumption (data not shown) (U.S. Food and Drug Administration, 2015). Hybrid N78N3111 had a 60% reduction in fumonisin relative to N78NGT, which was statistically similar to hybrid N78N3000GT in all 7 field trials (Table 3).

The use of transgenic insect protection as a means to reduce fumonisin contamination of corn is a well-established practice (Hammond et al., 2004, 2006; Munkvold et al., 1999; Munkvold, 2003; Wu, 2006), although the level of protection is not entirely predictable. The variance can be attributed to the environment, level of insect pressure and host plant genotype (de la Campa et al., 2005). In the agroecosystem of the current study the 3111 and 3000GT insect protection gene stack confer different levels of protection against corn earworm. The feeding damage incurred in N78N3000GT may have facilitated the entry of *F. verticilloides* and subsequent production of fumonisin. The observations noted in this study and others that transgenic insect protection is more effective in mitigating fumonisin contamination than aflatoxin contamination could reflect the different environments that are associated

with these mycotoxins or the relative importance of insects as vectors for *F. verticillioides* and *A. flavus*. The highly effective insect suppression by N78N3111 merits ongoing evaluation as a component of a mycotoxin prevention program.

While some aflatoxin was observed in every field, it was a major contaminant in only one of the seven fields. The Corpus Christi field trial in 2014 had detectable aflatoxin in 100% of the plots. Only 1 of the 12 plots that did not receive a biocontrol treatment had an aflatoxin concentration less than the 20 ppb threshold for conventionally marketed corn and 6 of the 12 exceeded the 300 ppb limit (data not shown). Application of biocontrol products based on strain 21882 and 30797 resulted in significant reduction relative to the no biocontrol treatments in the aflatoxin concentration in that field by 94% and 57%, respectively. No significant effect of the biocontrol products was observed in the remaining 6 fields. In total, across all fields in both years, applications of strain 21882 reduced aflatoxin by more than 90% compared to the no biocontrol treatments. The other six fields experienced unusually high rainfall, and even flooding at the Corpus Christi location in 2015. Abundant moisture coupled with moderate temperatures may have contributed to the nonconductive environment for aflatoxin contamination. None of the biocontrol or insect protection traits had a significant effect in on aflatoxin contamination in those six fields.

Corn with the 3111 insect protection trait package consistently had less aflatoxin contamination than the corn without transgenic insect protection, but this difference (>50%) was not statistically significant (Table 3). Regardless, the trend of very low ear feeding and low fumonisin in N78N3111 is consistent with a lower (although not significant) aflatoxin level and the hypothesis that transgenic insect protection at a

sufficient level can contribute to its mitigation. Selected transgenic insect protection, such as N78N3111, is worthy of additional study in aflatoxin mitigation. In 2004 Buntin et al. reported on the efficacy of Cry1Ab in suppressing earworm damage and aflatoxin contamination in corn grown in Georgia. They noted reduced grain damage in the *Bt* corn, but no effect on aflatoxin concentration (Buntin et al., 2004). Bowen et al., (2014) recently compared 8 *Bt*-corn hybrids expressing single, double and triple stacked *Bt* with near isogenic non-*Bt* hybrids, planted at 8 sites in Alabama over 3 years. After this extensive testing no consistent effect could be attributed to genotype or the presence/absence of a specific *Bt* trait. Similar results were reported by Abbas et al. (2016). It is possible that these *Bt*-hybrids cannot kill insects fast enough to prevent the minor kernel wounding that is necessary for *A. flavus* entry. Alternatively, the insect species that are suppressed by transgenic corn are only one component of the *A. flavus* infection process and other insects may play a role as *A. flavus* vectors. Separating and quantifying the various factors that contribute to aflatoxin contamination can be experimentally difficult (Farias et al., 2014; Weaver et al., 2016). Field experiments in aflatoxin are often limited by variance between fields, between plots and even within a single ear (Binder and Krska, 2012; Brera et al., 2010; Johansson et al., 2000).

3.4 Yield and economic return from investments in insect protection and mycotoxin biocontrol

The corn hybrids differing in transgenic insect protection had a significant effect on yield in 3 of the 7 fields ($F = 4.3, 8.8, 4.3$; d.f. = 2, 35; 2, 51; 2, 51 ; $P < 0.0212, 0.0005, 0.0186$ Corpus Christi in 2015, Field 2 in 2014 and Field 2 in 2015, respectively) (Table

4). In two of these fields N78N3111 was the highest-yielding hybrid. When this yield was converted to an economic return, N78N3111 had the highest net returns above direct expenses at \$125 ha⁻¹ while N78NGT and N78N3000GT hybrids returned \$34 ha⁻¹ and \$66 ha⁻¹, respectively, but the difference was not statistically significant. Corn borers (European or Southwestern) were not important pests at the sites included in this experiment, minimizing the opportunity to confer an economic advantage. Application of a biological control product never had a significant effect on yield, but had an effect on net returns in 2 fields: Biocontrol strain 21882 improved net returns in 2014 at the Corpus Christi site by \$407 ha⁻¹ compared to the no biocontrol treatments. Both biocontrol products were negatively associated with net returns at field 2 in 2015, where there was effectively zero aflatoxin and no opportunity to recover the additional input cost of the biocontrol product.

These same biological control treatments were recently reported to be cost effective in a similar study (Weaver et al., 2015). The main difference between the two studies was the value of the corn. The previous study, conducted in 2011 to 2013 had higher overall yields and value of the crop was around 50% higher than in the present study.

3 Conclusions

Consistent with other published reports of the 3111 trait package (Aguirre et al., 2016; Bowen et al., 2014; Buntin and Flanders, 2015) we observed statistically significant control of corn earworm in N78N3111 hybrid corn while the N78N3000GT hybrid corn had a lesser degree of suppression that was only significant in some trials. This transgenic insect protection was also associated with significant reduction in

fumonisin, but not aflatoxin contamination. Overall, the insect protection in this study did not confer significantly higher yields or higher economic returns. Biological control was effective in reducing aflatoxin contamination, and resulted in statistically significant positive economic returns, but only in one of seven trials.

Transgenic insect protection and biological control of aflatoxin are inherently 'defensive' strategies; neither is capable of increasing yields and can only increase economic returns indirectly by limiting losses. Both of these protective measures come at some cost to the corn producer and, unlike many crop protection products, the decision to use this technology must be made in advance of knowing the level of pest pressure that will occur in a given field or year. The range of the results encountered in this study emphasizes the value of repeating experiments of this nature in multiple sites and years and underscores the challenging decisions made by corn farmers to decide which crop management practices are worthwhile. Within the present study, with modest mycotoxin pressure, support could be found for application of biocontrol and transgenic insect protection. Aflatoxin reduction was observed with biocontrol treatments, particularly with strain 21882, while fumonsin reduction was associated with the transgenic insect protection, particularly N78N3111. These two approaches mitigated the risk of different mycotoxins and could be integrated for the greatest level of protection from mycotoxin contamination. Although the two strategies did not positively interact, their benefits appeared to be additive with selective reductions in mycotoxins associated with each strategy. Across the entire study there was no significant loss in economic returns from either of these control methods. External

economic factors such as commodity prices, input prices and individual tolerance of risk will determine the value of these technologies.

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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 Delta Branch Experiment Station (2017)

^b November-March includes two months of the preceding year.

658 Table 2. Effect of biocontrol treatments on *A. flavus* populations in soil and grain.

	Corpus Christi		Leland		Field 2		Field 3	Average		
	2014	2015	2014	2015	2014	2015	2015	2014	2015	Both Years
Biocontrol Treatment	% toxigenic <i>A. flavus</i> in soil at harvest stage ^{a, b}									
No biocontrol	43 (8)	41(5)	3 (1)	8 (2) B	2 (1)	5 (2)	11 (2)	12 (3)	14 (2)	13 (2)
Strain 21882	20 (7)	33 (8)	4 (2)	1 (1) A	1 (1)	8 (1)	10 (3)	7 (2)	11 (2)	9 (1)
Strain 30797	27 (7)	35 (6)	4 (2)	3 (1) AB	4 (1)	10 (3)	12 (2)	10 (2)	13 (2)	12 (2)
Biocontrol Treatment	% toxigenic <i>A. flavus</i> in corn grain samples ^{a, c}									
No biocontrol	45 (7) B	38 (11)	13 (4)	14 (3)	11 (3)	7 (2)	12 (3)	20 (3) B	16 (3)	18 (2)
Strain 21882	10 (3) A	27 (8)	9 (4)	20 (4)	9 (5)	4 (1)	11 (3)	9 (2) A	15 (2)	12 (2)
Strain 30797	18 (3) A	30 (6)	13 (3)	18 (5)	13 (4)	9 (3)	16 (3)	14 (2) AB	17 (2)	16 (2)

659

660 ^a Values within a section with the same letter are not significantly different at the 0.05 level by Tukey's test. No letter indicates no
 661 significant differences. Numbers in parentheses are the standard error of the mean.

662 ^b Soil samples collected at harvest and the percent toxigenic *A. flavus* determined by cultural methods

663 ^c Percent toxigenic *A. flavus* measured in ground grain samples immediately following harvest.

664

665 Table 3. Effect of transgenic insect protection and biocontrol treatments on mycotoxin contamination.

	Corpus Christi		Leland		Field 2		Field 3	Average		
	2014	2015	2014	2015	2014	2015	2015	2014	2015	Both Years
Hybrid	Fumonisin (ppm) ^a									
N78NGT	1.5 (0.5)	0.4 (0)	1.0 (0.2)	0.2 (0.1)	1.2 (0.1) B	2.0 (0.2) B	0.6 (0.2)	1.2 (0.1) B	0.9 (0.1) B	1.0 (0.1) B
N78N3000GT	1.8 (0.9)	0.8 (0.3)	0.8 (0.1)	0.2 (0.1)	1.0 (0.1) B	1.9 (0.3) B	0.6 (0.1)	1.1 (0.2) AB	0.8 (0.1) B	1.0 (0.1) B
N78N3111	0.3 (0.2)	0.2 (0.1)	1.2 (0.2)	0 (0)	0.1 (0) A	0.5 (0.1) A	0.2 (0)	0.6 (0.1) A	0.2 (0) A	0.4 (0.1) A
Biocontrol										
No biocontrol	1.3 (0.5)	0.3 (0.1)	1.0 (0.1)	0.2 (0.1)	0.6 (0.1)	1.3 (0.2)	0.6 (0.1)	0.9 (0.1)	0.6 (0.1)	0.8 (0.1)
Strain 21882	1.4 (0.8)	0.3 (0.1)	1.0 (0.2)	0.1 (0)	1.0 (0.2)	1.5 (0.3)	0.3 (0.1)	1.1 (0.2)	0.6 (0.1)	0.8 (0.1)
Strain 30797	0.9 (0.5)	0.7 (0.3)	1.1 (0.1)	0.2 (0.1)	0.7 (0.1)	1.5 (0.3)	0.5 (0.2)	0.9 (0.2)	0.7 (0.1)	0.8 (0.1)
Hybrid	Aflatoxin (ppb) ^a									
N78NGT	279 (102)	1 (0)	2 (2)	0 (0)	0 (0)	3 (2)	1 (1)	112 (47)	9 (1)	57 (25)
N78N3000GT	218 (76)	4 (3)	0 (0)	1.4 (1)	5 (5)	9 (8)	3 (3)	90 (35)	7 (5)	48 (19)
N78N3111	126 (55)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	50 (24)	0 (0)	25 (13)
Biocontrol										
No biocontrol	419 (107) B	2 (1)	2 (2)	0 (0)	5 (5)	0 (0)	0 (0)	170 (56) B	1 (1)	85 (30) B
Strain 21882	26 (8) A	1 (0)	0 (0)	1.4 (1.4)	0 (0)	3 (3)	0 (0)	11 (4) A	5 (5)	8 (3) A
Strain 30797	178 (42) A	2 (2)	0.2 (0)	0 (0)	0 (0)	8 (8)	4 (3)	72 (23) AB	3 (2)	37 (12) AB

666 ^a Values within a section with the same letter are not significantly different at the 0.05 level by Tukey's test. No letter indicates no
667 significant differences. Numbers in parentheses are the standard error of the mean. Fields with minimal aflatoxin and an abundance
668 of 'zero' observations were excluded from analysis and represented with italicized values.

669 Table 4. Effect of transgenic insect protection and biocontrol treatments on yield and economic returns over direct costs.

	Corpus Christi		Leland		Field 2		Field 3	Average		
	2014	2015	2014	2015	2014	2015	2015	2014	2015	Both Years
Hybrid	Yield (kg hectare ⁻¹) ^a									
N78NGT	3,537 (473)	4,327 (261) B	6,762 (458)	9,063 (151)	11,670 (211) B	9,680 (155) A	5,077 (438)	7,901 (506)	7,283 (318)	7,543 (282)
N78N3000GT	3,968 (553)	4,520 (253) AB	7,212 (322)	9,036 (152)	11,405 (186) B	9,054 (92) B	4,888 (326)	8,046 (444)	7,089 (288)	7,492 (253)
N78N3111	4,092 (553)	5,324 (247) A	7,267 (407)	9,125 (157)	12,544 (206) A	9,405 (190) AB	4,566 (460)	8,479 (535)	7,291 (312)	7,792 (293)
Biocontrol										
No biocontrol	3622 (373)	4,815 (183)	7157 (416)	9,177 (176)	11974 (243)	9,441 (187)	4,631 (416)	8,140 (512)	7,216 (315)	7,605 (284)
Strain 21882	4094 (550)	4,612 (250)	7012 (407)	9,033 (170)	11844 (196)	9,523 (153)	4,844 (424)	8,171 (489)	7,220 (312)	7,621 (276)
Strain 30797	3881 (469)	4,745 (382)	7071 (385)	9,103 (107)	11797 (255)	9,154 (133)	5,056 (399)	8,114 (492)	7,226 (292)	7,600 (269)
Hybrid	Net return (dollars hectare ⁻¹) ^{a, b}									
N78NGT	-639 (93)	-369 (44)	-17 (75)	347 (28)	759 (34) B	439 (24) A	-281 (70)	118 (89)	71 (50)	91 (47)
N78N3000GT	-670 (91)	-349 (46)	45 (52)	332 (23)	692 (38) B	312 (29) B	-326 (49)	109 (84)	23 (45)	59 (44)
N78N3111	-533 (117)	-221 (38)	48 (63)	355 (26)	881 (35) A	386 (34) AB	-378 (72)	216 (91)	59 (49)	125 (48)
Biocontrol										
No biocontrol	-810 (92) B	-260 (29)	74 (66)	395 (29)	821 (47)	437 (30) A	-322 (66)	133 (100)	92 (49)	109 (51)
Strain 21882	-403 (89) A	-350 (41)	-3 (64)	316 (26)	759 (31)	386 (38) AB	-346 (67)	183 (77)	29 (48)	94 (43)
Strain 30797	-628 (87) AB	-329 (62)	6 (60)	325 (16)	752 (40)	332 (20) B	-316 (62)	127 (86)	33 (45)	73 (45)

670 ^a Values within a section with the same letter are not significantly different at the 0.05 level by Tukey's test. No letter indicates no
 671 significant differences. Numbers in parentheses are the standard error of the mean.

Figure 1. Effect of transgenic insect protection on damage from ear feeding insects.

Legend:

Effect of transgenic insect protection on depth of penetration (A), area of damage (B) and number of damaged kernels (C). Values within a section with the same letter are not significantly different at the 0.05 level by Tukey's HSD test. No letter indicates no significant differences. Depth of penetration is the 10 plant average of length to the lowest point of continual kernel damage measured from the tip of the ear in centimeters. Area of damage is the 10 plant average of square centimeters of ear damage, kernel and tip damage that was attributed to earworm feeding.

A

B

C

Figure 1