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Lower Fumonisin Mycotoxin Levels in the Grain of Bt Corn Grown in the United States in 2000−2002

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Fumonisins were monitored in corn grain collected from Bt hybrids grown in 107 locations across the United States in 2000−2002. Bt corn hybrids contain the Cry1Ab protein from *Bacillus thuringiensis* that controls European corn borers and other stalk-boring pests. Fumonisin levels were frequently lower in grain from Bt hybrids grown in field trials under conditions of natural (FACT trials) or manual insect infestation (university trials). Over three years of FACT trials, there were 126/210 comparisons when fumonisin levels in grain from control hybrids were >2 ppm, exceeding U.S. FDA guidance levels of 2 ppm for human food. Grain from Bt hybrids was at or below 2 ppm of fumonisins for 58 of the 126 comparisons. The use of Bt hybrids can increase the percentage of corn grain that would be suitable for use in food and feed.

KEYWORDS: Corn (*Zea mays*); fumonisins; mycotoxins; Cry1Ab protein; Bt corn; biotechnology

INTRODUCTION

Corn (*Zea mays* L.) is grown on every continent except Antarctica with a worldwide production of 591 million tons a year, second only in cereal production to rice (*1*). Wherever corn is grown, it can be infected with mycotoxigenic fungi that produce toxic secondary metabolites called mycotoxins. *Fusarium verticillioides* (Sacc.) Nirenberg (synonym = *F. moniliforme* J. Sheld.) and *Fusarium proliferatum* (T. Matsushima) Nirenberg, which produce fumonisin mycotoxins, are the most common fungi that infect corn; other *Fusarium* species such as *Fusarium graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein.) Petch] produce the mycotoxins zearalenone and deoxynivalenol (*2*). Dietary exposure to mycotoxins can cause a variety of adverse health effects in farm animals and humans (*3*, *4*). Various environmental factors such as insect damage, heat and drought stress, and genetic susceptibility predispose corn plants to infection with mycotoxigenic fungi (*2*, *5*).

Control of insect pests that damage corn can reduce fungal infection. Damage to the ear or stalk resulting from insect feeding provides ports of entry for fungi, and some insect pests also serve as vectors of mycotoxigenic fungi (*6*, *7*). Within the past few years, an effective insect pest control strategy has been developed with the introduction of coding sequences for the Cry1Ab protein derived from *Bacillus thuringiensis* into corn plants (event MON 810, Yieldgard Cornborer, trademark of Monsanto Technology LLC) (*8*). The Cry1Ab protein controls lepidopteran insect pests such as the European corn borer (ECB), *Ostrinia nubilalis* Hübner, the most important stalk-boring and ear-damaging insect pest of corn in the United States (*6*, *9*−*11*). It also controls southwestern corn borer (*Diatraea grandiosella* Dyar) and other related corn-boring insects (*12*). The CaMV 35S gene promoter enables constitutive expression of the Cry1Ab protein in hybrids during the growing season, thus providing season-long protection against corn borers. The *B. thuringiensis*-based microbial pesticides that contain Cry proteins such as Cry1Ab have been used commercially in agriculture for over 40 years to control larval insect pests (*8*, *13*). They have a record of safe use because their insecticidal mode of action is highly specific against target lepidopteran insect pests. Cry1Ab protein has no activity against nontarget organisms such as mammals and birds (*8*, *13*−*16*).

Munkvold and co-workers were the first to report that, in Iowa, corn hybrids protected with the Cry1Ab protein had

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significantly lower fumonisin mycotoxin levels in the grain (*17*, *18*). This was most evident in corn plants that expressed Cry1Ab protein throughout all tissues during the growing season. Reduction in fumonisin contamination of corn grain could have important health consequences for farm animals and humans. Fumonisins cause a variety of toxic effects in laboratory animals and farm animal species. Epidemiology studies have also suggested a link between high levels of fumonisin exposure and elevated rates of liver and/or esophageal cancer in farmers from certain regions of Africa, China, Italy, and Brazil (*4*, *19*).

MATERIALS AND METHODS

Field Trials. A large study was undertaken to determine if fumonisin levels in Bt hybrids (derived from event MON 810) would be lower in other geographical regions across the U.S. corn belt. Grain was collected for mycotoxin analysis from Bt hybrids and their near-isogenic controls grown in FACT trials (Field Analysis Comparison Trials) and university trials at various locations in the United States. FACT trials were carried out under conditions of natural insect infestation, whereas test plots in university trials were manually infested with European corn borer and southwestern corn borer and, in some locations, corn earworm and fall armyworm. Monitoring of mycotoxin levels was originally intended to be carried out for two consecutive years (2000 and 2001) because environmental conditions can vary from year to year and across geographical regions. In 2002, there was persistent drought and high European corn borer pressure in parts of Iowa. Corn grain samples were collected from FACT trials in southern Iowa to assess the impact of Bt corn on fumonisin levels under more extreme environmental conditions.

FACT Trials. Asgrow and Dekalb precommercial and commercial corn hybrids are evaluated annually in FACT strip trials at multiple locations across the United States. FACT trials are conducted to determine yield and to collect agronomic data on the hybrids. In all years, experimental units were 91.4 m long strips that were four−eight rows wide. Rows were on 0.76−0.91 m centers, and one hybrid strip per entry was evaluated at each location. Management practices (i.e., row spacing, plant population, fertility, weed and insect control) were determined by the farmer cooperator according to local practice and needs. Bt hybrids and the respective near-isogenic non-Bt hybrids (controls) typically were grown at the same locations for comparison purposes. During the year 2000 FACT trial, grain samples were assayed for mycotoxins at the USDA Animal and Plant Inspection Service, Veterinary Services Laboratory. Fumonisin data from 53 comparisons (21 Bt hybrids) were at or above the level of quantitation (0.5 ppm) for the USDA Ames testing laboratory.

Approximately 0.9−2.3 kg of grain samples from Bt hybrids and the respective non-Bt controls were obtained from each strip by collecting one sample from the corn grain stream being transferred from the combine to the weigh-wagon. Grain samples were collected in mesh bags, labeled, and shipped overnight to the Monsanto Agronomy Center in Monmouth, IL. Grain was dried to ∼14% moisture and ground with a Romer grinding/subsampling mill (series II; Romer Labs, Inc., Union, MO) to pass through a 1 mm mesh screen. A subsample of the ground grain was coded, labeled, and shipped to the USDA mycotoxin testing laboratory in Ames, IA.

In the 2001 FACT trial, grain samples were collected, ground with a Romer mill, and subsampled in the same manner for mycotoxin analysis. Ground grain samples were shipped to Romer Labs for analysis. There were 130 comparisons (23 Bt hybrids) in which fumonisin levels were at, or greater than, the limit of quantification (0.2 ppm) for the Romer testing laboratory.

In 2002, grain samples that were collected from southwestern Iowa were ground and analyzed at Romer Labs in the same manner as the 2001 FACT trials. All 27 comparisons (5 Bt hybrids) had fumonisin levels at, or greater than, the limit of quantification (0.2 ppm) for the Romer testing laboratory.

In 2001 and 2002, the identity of all grain samples was confirmed by testing the grain for the presence/absence of the Cry1Ab protein using lateral flow strips (*20*).

University Field Trials. Field trials with Bt hybrids were conducted in 2000 and 2001 in cooperation with universities across several states. Mycotoxin levels were monitored in the grain as part of the trials. Experimental units were four rows, 3 m wide by 6.1 m long, and were arranged in a randomized complete block (RCB) design with four replications. Final plant populations ranged from 49400 to 64200 plants per hectare depending upon the location. Treatments included one Bt hybrid and a nontransgenic (lacking the Cry1Ab protein coding sequence) near-isogenic control hybrid. The center two rows of each plot were artificially infested with European corn borers (ECB) at a rate of 50 larvae/infestation to the whorl at growth stage V8 using a bazooka applicator (*21*). Second-generation ECB larvae subsequently were applied at the same rate to the first and second leaf axil above the ear at V_T. Depending on geographic location, some plots were also infested with first- and second-generation southwestern corn borer (same timing as ECB), corn earworm (5 larvae/ear at R1 plant stage), or fall armyworm (30 larvae/plant at V6 in the whorl and at R1 when applied to the green silks). At harvest, ears were collected from the middle two rows (∼50 ears/plot) and shelled. A 227 g subsample of grain from the middle two rows was collected for each replicate for mycotoxin analysis. Samples were sent to Monmouth, IL, and maintained at 8 °C until they were shipped to the USDA laboratory (2000 trial) or to Romer Labs (2001 trial), for mycotoxin analyses. At each location, there were usually four samples of Bt hybrid and control grain collected from different parts of the test plot. Each replicate sample was separately analyzed for mycotoxins.

Mycotoxin Analyses. The methods used to analyze mycotoxins at the USDA Animal and Plant Health Inspection Service, Veterinary Services Laboratory, are as follows. Fumonisins B_1, B_2, and B_3 were analyzed according to published procedures (*22*). Briefly, 10 g samples were extracted with 50 mL of 50% acetonitrile for a minimum of 30 min. After filtration, the extract was purified using a 500-mg C18 solid-phase extraction (SPE) column (Waters/Millipore, Milford, MA). Fluorescent derivatives of the fumonisins were formed using *o*-phthalaldehyde (OPA) and detected using a fluorescence detector connected to a model 250 Perkin-Elmer HPLC containing a C18 column (Perkin-Elmer) to separate the derivatized fumonisin species. Quantification was made using external standards and peak height measurements; standards were run at the beginning and end of each assay. Fumonisin B_1 and B_2 standards were purchased from Sigma Chemical Co. (St. Louis, MO), and fumonisin B_3 standard was obtained from Ron Plattner (USDA-ARS, NCAUR, Peoria, IL). Detection limits were 0.1 ppm for fumonisin B_1 and 0.5 ppm for fumonisins B_2 and B_3. Because the detection limits for fumonisins B_2 and B_3 were higher than that for fumonisin B_1, only data for fumonisin levels $\geq$0.5 ppm are presented.

Aflatoxin and zearalenone were measured on TLC plates. Corn grain samples were ground and extracted with solvent, filtered, evaporated to dryness, and redissolved in spotting solvent. Analytical standards were added to the plates, and the plates were examined under long-wave UV to observe aflatoxin B_1 and under short-wave UV for zearalenone. Aflatoxin B_1 and zearalenone positives were quantified according to AOAC methods (*23*, *24*). Samples were considered to be positive if they had greater than 20 ppb of aflatoxin B_1 or 1 ppm of zearalenone, respectively. Aflatoxin positives were further quantified using the Veratox-AST ELISA-based test kit (Neogen Corp.).

Deoxynivalenol (DON) was measured by HPLC using extract remaining from the HPLC analysis. The sample was dried and redissolved in acetic acid buffer and injected into an HPLC. Analytical standards were run before and after each assay. Aflatoxin B_1, zearalenone, and DON standards were purchased from Sigma Chemical Co.

The methods used to analyze mycotoxins at Romer Labs, are as follows. Samples were analyzed for fumonisins B_1, B_2, and B_3 according to method fum-lc-02-00.1, "Fumonisin B_1, B_2, & B_3 HPLC Method" (Romer Labs, Inc.), which was adapted from published methods (*25*). From the ground samples received in the laboratory, 25 g of corn from each was weighed into a 250 mL Erlenmeyer flask and was then extracted with 100 mL of 50:50 (v/v) acetonitrile/water. Each extract was filtered and purified using a MultiSep 211 cleanup column (Romer Labs, Inc.). The eluant was evaporated to dryness, and fumonisins, if present, were derivatized using naphthalene 2,3-dicarboxaldehyde,

Table 1. Total Fumonisin Levels in Bt Corn Hybrids and Their Controls in FACT Trials in 2000

		fumonisin (ppm)				fumonisin (ppm)	
trial site location	Bt hybrid no.	Bt hybrid	control	trial site location	Bt hybrid no.	Bt hybrid	control
Imperial, NE	DK618BtY	1.4	6.4	Jacob, IL	DKC061-A	5.1	nd
Lexington, NE	DK618BtY	2.3	6.9	Florida, IL	DK618BtY	nd	15.8
Kearny, NE	DKC53-52	0.9	5.3	Staunton, IL	Rx730YG	2	15.4
Hampton, NE	DK595BtY	2	6	Staunton, IL	DK618BtY	9	4.7
Grand Island, NE	DK595BtY	1.6	7.4	Stonington,[b] IL	DK679YG	0.8	4.5
Ogden, IA	DKC58-52	nd[a]	0.5	Stonington,[c] IL	DK679YG	1.7	9.2
Ogden, IA	DK595BtY	nd	0.9	Stonington,[d] IL	DK679YG	4.3	11.9
Jasper, IA	DK618BtY	1.6	2.5	Stonington,[e] IL	DK679YG	2.1	8.1
Storm Lake, IA	DK595BtY	0.7	nd	Princeton, IN	DK618BtY	0.8	1.9
Ahrens, IA	DK618BtY	0.6	0.7	Princeton, IN	DKC61-24	0.7	5
Bonnichsen, IA	DK618BtY	nd	0.7	northern IN	DKC58-52	nd	0.5
Volga, IA	DKC58-52	nd	0.7	Ashville, OH	DK618BtY	nd	0.9
Wall, IA	DKC58-52	nd	0.8	Ashville, OH	DKC58-52	nd	3.2
Centralia, KS	DK621BtY	0.7	0.6	Ashville, OH	RX730YG	nd	0.5
Onaga, KS	DK647BtY	nd	0.6	Lockbourne, OH	DKC58-52	0.6	nd
Onaga, KS	DK621BtY	0.8	1.8	Lockbourne, OH	RX730YG	0.5	0.5
Brookings, SD	DKC47-42	0.6	0.5	York, PA	DK618BtY	1	1.4
Clinton, IL	H8692Bt	0.5	nd	Milton, PA	DKC58-52	1.2	1.4
Clinton, IL	H9285Bt	1.7	2.6	Milton, PA	DK647BtY	0.8	5.4
Clinton, IL	H9221Bt	3.8	2.6	Salisbury, MD	DK618BtY	nd	2.2
Clinton, IL	R500511Bt	0.6	1.2	Galena, MD	DKC58–52	nd	0.6
Clinton, IL	Ex09247Bt	nd	2.1	Hampstead, MD	DK618BtY	0.9	0.5
Clinton, IL	R447583Bt	1.4	2.1	Lexington, SC	DK626BtY	14.3	10.4
Clinton, IL	Ex09488Bt	1	1.1	Lexington, SC	DK626BtY	nd	1
Mount Carmel, IL	DK618BtY	nd	0.7	Lexington, SC	DKC53-32	7.3	4.3
Mount Carmel, IL	Exp061A	nd	1.5	Elk Point, SD	DK618BtY	1.2	4.9
Jacob, IL	DK618BtY	2.1	4				

[a] Not detectable, <0.5 ppm for total fumonisins. The mean difference in fumonisin levels between Bt and control hybrids was highly significant, $p = 0.0007$. [b–e] Same hybrid planted at four different times.

according to a published procedure (*25*). Derivatized samples (80 μL) were injected onto a Shimadzu HPLC system with a fluorescence detector and fumonisin derivatives separated on a 100 × 4.6 mm, 5 μm, Spheri-5, RP-18 (PJ Cobert Associates, Inc., St. Louis, MO). The mobile phase was 47:52:1 (v/v/v) acetonitrile/water/acetic acid, and the flow rate was 2 mL/min. Excitation was at 420 nm, and emission was at 500 nm. Fumonisin standards were run at the beginning and end of each assay.

Samples were analyzed for aflatoxins B_1, B_2, G_1, and G_2 according to method afl-lc-01-00.1, "Kobra Cell Method for Aflatoxin by HPLC" (Romer Labs, Inc.). From the ground samples received in the laboratory, 25 g of corn from each was weighed into a 250 mL Erlenmeyer flask and was then extracted with 100 mL of 84:16 (v/v) acetonitrile/water. Each extract was filtered and purified using a MultiSep 224 cleanup column [as adapted by Romer Labs, Inc., from published methods (*25*)]. The eluant was transferred to a silanized amber autosampler vial, deionized water was added, and an aliquot was injected onto a Shimadzu HPLC system with a fluorescence detector. Aflatoxins were separated on a 100 × 4.6 mm Brownlee cartridge C-18 column (PJ Cobert Associates, Inc.). The mobile phase was 5:1:1 (v/v/v) water/acetonitrile/methanol, and the flow rate was 2 mL/min. One hundred milliliters nitric acid and 0.3 g of potassium bromide were added to each liter of mobile phase for postcolumn derivitization with the Kobra cell. Excitation was at 360 nm, and emission was at 440 nm. The Kobra cell was 200 μA. Aflatoxin standards were run at the beginning and end of each assay run.

Statistical Analysis. The differences in total fumonisin levels between Bt hybrids and the near-isogenic control hybrids in 2000–2002 FACT and 2000 and 2001 university trials were computed for each site, replication, and/or planting period. A paired *t* test was used to determine if the mean differences in the 2000–2002 FACT trials were significantly different from zero ($p < 0.05$). All other means (university trials) were compared using a mixed-model analysis of variance ($p < 0.05$). SAS version 8.2 software was used to perform all analyses (*26*).

RESULTS

The levels of total fumonisins ($FB_1 + FB_2 + FB_3$) in corn grain from Bt hybrids and their near-isogenic controls grown in FACT trials are found in **Tables 1–3**. When all three fumonisin derivatives were detectable in grain, FB_2 and FB_3 levels were approximately 20 and 3%, respectively, of FB_1 levels. This is consistent with published data showing that the FB_1 homologue is normally the most abundant form found in corn grain (*27*, *28*). In the 2000 FACT trial, 53 of ~194 comparisons had detectable levels of fumonisins. In the 2001 FACT trial, 130 of ~161 comparisons had detectable fumonisin levels. In the 2002 FACT trial in Iowa, all 27 comparisons had detectable fumonisin levels. When the levels of total fumonisins were averaged across all sites for the 2000 and 2001 FACT trials, total fumonisin levels were ~50% lower for Bt hybrids.

When the mean differences in total fumonisin levels between the Bt and control hybrids were subjected to statistical analysis for each year of study, the mean differences for the 2000 and 2001 FACT and university trials were statistically significantly different. For the university trials, mean differences for each replicate (Bt/control comparison) were included in the statistical analysis. Results from the FACT trial in southwestern Iowa in 2002 also showed a trend for lower fumonisins in Bt hybrids when compared to controls, although the mean differences between Bt hybrids and their controls were not statistically different.

The reduction in fumonisin levels in Bt hybrids compared to controls was compared and calculated as a percentage of control fumonisin levels. The control hybrids in the FACT trials (2000–2002) were divided into two groups; fumonisin levels were between 5 and 10 ppm for group 1 or >10 ppm for group 2. The mean differences in fumonisin levels between Bt hybrids and controls were calculated for the two groups and then divided

Table 2. Total Fumonisin Levels in Bt Corn Hybrids and Their Controls in FACT Trials in 2001

		fumonisin (ppm)				fumonisin (ppm)	
trial site location	Bt hybrid no.	Bt hybrid	control	trial site location	Bt hybrid no.	Bt hybrid	control
Brady, NE	DKC53-32	0.7	2.8	Appleton, MN	RX452YG	0.4	1.3
Brady, NE	DKC57-72	2.6	6.2	Brewster, MN	RX452YG	0.7	0.1
Monroe, NE	DKC57-72	0.7	2.6	Clarkfield, MN	RX452YG	3.2	4.1
Plainview, NE	RX730IMIYG	6.9	0.5	Fairmount, MN	RX452YG	nd	1.5
Spalding, NE	DK551BtY	2.3	6.5	Madison, MN	RX452YG	0.4	0.7
Spalding, NE	RX730RR/BTY	nd[a]	5.5	Clear Lake, SD	DKC44-42	0.3	0.7
Arlington, IA	DKC58-78	4.7	1.1	Bob Starke, SD	DKC44-42	2.6	1.4
Arlington, IA	DKC61-25	0.3	0.9	Carrollton, MO	DK61-11	0.3	1.7
Deon, IA	DK551BtY	1	4.3	Carrollto,n MO	RX730YG	0.5	1.4
Deon, IA	RX452YG	7.9	7.8	Carrollton, MO	DKC65-26	0.3	3.3
Donnellson, IA	DKC61-25	2.3	nd	Aledo, IL	DKC53-32	nd	9.6
Lohrville, IA	DKC53-32	7	15.3	Aledo, IL	DKC58-78	0.1	7.9
Lohrville, IA	DKC57-78	3.1	12.4	Alexis, IL	DKC53-32	0.2	2.1
Manning, IA	DKC58-78	3.5	4.3	Alexis, IL	DKC58-78	0.6	3
Marble Rock, IA	DKC44-42	nd	1.2	Alhambra, IL	DKC61-25	0.4	nd
Marble Rock, IA	DKC53-32	1.2	4.4	Assumption, IL	DKC61-25	2.6	0.9
Marshalltown, IA	DKC53-32	7.3	11	Dundas, IL	DKC58-53	0.3	nd
Mount Vernon, IA	DKC53-32	0.3	7.7	Dundas, IL	DKC61-25	0.7	0.8
Norway, IA	DKC61-25	0.9	1.9	Flannigan, IL	DKC53-32	0.6	1.3
Norway, IA	DKC53-32	0.4	4.1	Flannigan, IL	DKC58-52	0.5	1.2
Ogden, IA	DKC53-32	7.5	25.3	Flannigan, IL	DKC58-52	0.1	4.2
Oskaloosa, IA	DKC61-25	0.2	2.2	Flannigan, IL	DKC58-78	0.9	4.2
Orange City, IA	DK551BtY	8.5	9.9	Flannigan, IL	DK551BTY	0.4	1.2
Orange City, IA	DKC58-78	15.7	20.3	Flannigan, IL	DKC58-78	nd	3.5
Primghar, IA	DK551BtY	5.6	3.9	Flannigan, IL	DKC61-25	9.5	2.2
Primghar, IA	RX452YG	10.7	10.3	Flannigan, IL	DKC61-25	5.1	2.8
Shellrock, IA	DKC53-32	2.7	1.8	Flannigan, IL	DKC53-32	1.7	13.6
Sloan, IA	DKC58-78	4.8	8	Girard, IL	DKC61-25	0.6	2
Somers, IA	DKC61-25	1.4	4.8	Girard, IL	DKC58-78	2.9	4.3
Somers, IA	DKC53-32	6.1	4.8	Gridley, IL	DKC53-32	0.3	4
Somers, IA	DK551BtY	6.1	12.5	Gridley, IL	DKC58-78	0.6	0.9
Spencer, IA	RX452YG	7.4	8.8	Monmouth, IL	RX730YG	2.2	0.6
Spencer, IA	DK551BtY	0.7	7	Monmouth, IL	RX697YG	nd	1.9
Storm Lake, IA	RX452YG	31.2	26.3	Monmouth, IL	DKC61-25	4.2	1.7
Storm Lake, IA	DK551BtY	18.2	27.1	Monmouth, IL	DKC58-52	0.3	2.7
Storm Lake, IA	DKC61-25	4.2	17.6	Monmouth, IL	DKC58-78	10.7	15.5
Thorman, IA	DKC61-25	4.1	2	Monmouth, IL	DKC56-71	2.8	2
Tripoli, IA	DKC53-32	4	6.9	Monmouth, IL	DKC60-08	12.2	0.9
Westside, IA	DKC58-78	3.1	25.5	Morris, IL	DKC58-78	0.8	0.4
Washington, IA	DKC61-25	2.8	2.8	Morris, IL	DKC61-25	nd	2.5
Woodborg, IA	DKC58-78	8.1	8.9	Morris, IL	DKC58-52	1.5	0.7
Woodborg, IA	DKC53-32	5	9.2	New Berlin, IL	DKC61-25	nd	0.6
Roshman, TX	DK68-70YG	3	5.9	Princeton, IL	DKC60-08	nd	4.2
Roshman, TX	DKC69-70	3.4	9.6	Princeton, IL	DKC53-32	0.4	5.3
Shelby, MS	DKC68-70	0.6	7.1	Princeton, IL	DKC58-78	5	1.9
Shelby, MS	DKC68-70	1.6	5.2	Raymond, IL	DKC61-25	nd	3.9
Shelby, MS	DKC68-70	3.6	10.2	Seaton, IL	P33G30	1	1.3
Shelby, MS	DKC68-70	6.8	4.3	Seaton, IL	DKC61-25	0.2	3.6
Shely, MS	DKC69-70	0.5	5.4	Wyoming, IL	DKC61-25	nd	1.6
Shelby, MS	DKC69-70	4.1	7.1	Yates City, IL	DKC56-71	nd	0.9
Shelby, MS	DKC69-70	2.8	1.1	Yates City, IL	DKC58-58	nd	1.9
Shelby, MS	DKC69-70	0.9	1.2	Berne, IN	DKC60-08	nd	1.6
Shelby, MS	P31B13	1.5	10.7	Berne, IN	DKC58-78	0.3	1.2
Shelby, MS	P31B13	0.4	5.5	Evansville, IN	DKC61-25	4.3	8.5
Shelby, MS	P31B13	0.4	1.7	Monticello, IN	DKC58-52	0.5	1.3
Shelby, MS	P31B13	4.3	6.3	Monticello, IN	DKC56-71	0.3	1.2
Cheneyville, LA	DKC69-70	0.9	1.9	Rochester, IN	DKC58-78	0.6	1.3
Cheneyville, LA	DKC68-70	0.3	3.5	Rochester, IN	DKC61-25	nd	2.1
Cheneyville, LA	P31B13	2.4	1.7	West Lafayette, IN	DKC61-25	nd	2.9
Union, TN	DKC21-25	0.2	3.6	West Lafayette, IN	DKC53-32	nd	2.9
Union, TN	DKC68-70	5.3	2.4	Moline, MI	DKC56-71	3.8	5.6
Union, TN	DKC68-70	0.6	2.3	Moline, MI	DK44-42	0.2	1.1
Leitchfield, KY	DKC61-25	0.3	0.5	Moline, MI	DKC53-32	5.9	4.1
Vine Grove, KY	DKC61-25	1.7	3.1	Sand Lake, MI	RX452YG	2.4	7.7
Adrain, MN	RX452YG	3	1				

[a] Not detectable, <0.2 ppm for total fumonisins. The mean difference in fumonisin levels between Bt and control hybrids was highly significant, $p < 0.0001$.

by the mean fumonisin levels for the respective control group to determine a percentage reduction in fumonisin levels. The mean reduction in fumonisin levels between Bt and control groups for group 2 was more than twice that of group 1, 10.3 versus 4.5 ppm, respectively. The percent reductions in groups 1 and 2 were similar, 63 and 61%, respectively.

Over three years of FACT trials, there were 126/210 comparisons in which fumonisin levels in grain from control

Table 3. Total Fumonisin Levels in Bt Corn Hybrids and Their Controls in FACT Trials in Southwestern Iowa, 2002

		fumonisins (ppm)	
trial site location	Bt hybrid no.	Bt hybrid	control
Beaconfield	RX708YG	4.9	1.9
Beaconfield	DKC61-25	2.8	5
Danbury	DKC58-78	6.4	5.4
Danbury	RX634YG	5.8	2.9
Danbury	DKC53-32	6.3	7.1
Ogden	DKC58-78	1.7	7.7
Ogden	RX634YG	4.2	4.5
Ogden	DKC53-32	1.3	8.9
Persia	DKC58-78	6	9.5
Persia	DKC61-25	4.8	4.4
Persia	DKC53-32	16	12.2
Sloan	RX634YG	0.7	3.8
Sloan	DKC53-32	17.5	15.7
Elliott	DKC58-78	nd[a]	0.3
Elliott	DKC61-25	0.5	1.6
Elliott	RX708YG	0.4	1.3
Perry	DKC53-32	3.9	2.3
Perry	DKC58-78	6.9	3.4
Pacific Junction	RX708YG	10	16.8
Pacific Junction	DKC58-78	4.3	25.7
Pacific Junction	DKC61-25	17.8	8.6
Westside	DKC58-78	7.3	13.5
Westside	DKC61-25	14.7	10.1
Westside	DKC53-32	11.6	22.5
Lohrville	DKC53-32	4.4	12.4
Lohrville	DKC58-78	3.8	8
Lohrville	RX634YG	4.3	4.6

[a] Not detectable, <0.2 ppm for total fumonisins. The mean difference in fumonisin levels between Bt and control hybrids was not significant, $p = 0.1065$.

hybrids were >2 ppm, exceeding FDA guidance levels of 2 ppm for human food (degermed dry milled corn products) (*29*). Fumonisin levels in grain used to feed horses should not exceed 5 ppm (*30*). In 58 of the 126 comparisons, grain from Bt hybrids were at or below 2 ppm fumonisins as shown in **Figure 1**.

With regard to the measurement of other mycotoxins in the year 2000 FACT trial, little aflatoxin was detected and deoxynivalenol was found in 34 of 53 (64%) of comparisons. There were no apparent differences in deoxynivalenol as the average levels across comparisons were approximately 0.46 and 0.44 ppm for Bt and control hybrids, respectively. No zearalenone was detected. In the university trials, small amounts of aflatoxin and deoxynivalenol were detected, but there were no apparent differences between the Bt and control hybrids.

DISCUSSION

The significantly lower fumonisin levels in Bt hybrids observed in the FACT and university trials are consistent with those reported with Bt hybrids grown in the United States (*18*, *31*), France and Spain (*32*), Italy (*33*), and Turkey and Argentina (*34*, *35*). This indicates that in many environments, insect damage is an important contributor to mycotoxin contamination of corn grain. Reducing insect damage decreases the ports of entry for fungal infection, leading to decreased fungal biomass on corn grain (*32*, *33*). In general, there is a positive correlation between total fungal biomass and the accumulation of mycotoxins in corn grain (*2*), although some studies found a poor correlation between ear rot symptoms and fumonisin levels (*6*).

In the FACT trials, one sample from the grain stream was collected for each hybrid that was tested. Because mycotoxin contamination is not uniformly distributed throughout the grain, it would have been preferable to collect multiple subsamples from the grain stream for each hybrid and pool the subsamples for analysis to reduce sampling variability (*4*). This would have provided greater assurance that the sample tested was representative of the fumonisin contamination in the weigh-wagon. However, in consideration of the large number of samples (764) that were collected over the three years of FACT trials, collection of multiple subsamples was not practical. The authors' intent was to sample many Bt hybrids grown in a large variety of geographical locations over consecutive years to determine the overall impact on fumonisin levels. Fumonisin levels tended to be lower in Bt hybrids for the majority of samples summarized in **Tables 1**−**4**. As shown in **Figure 1**, the differences in fumonisin levels were of sufficient magnitude at a number of locations to confirm that protection of corn plants against corn-boring insects could substantially reduce fumonisin contamination of the grain. In the university trials, four replicate

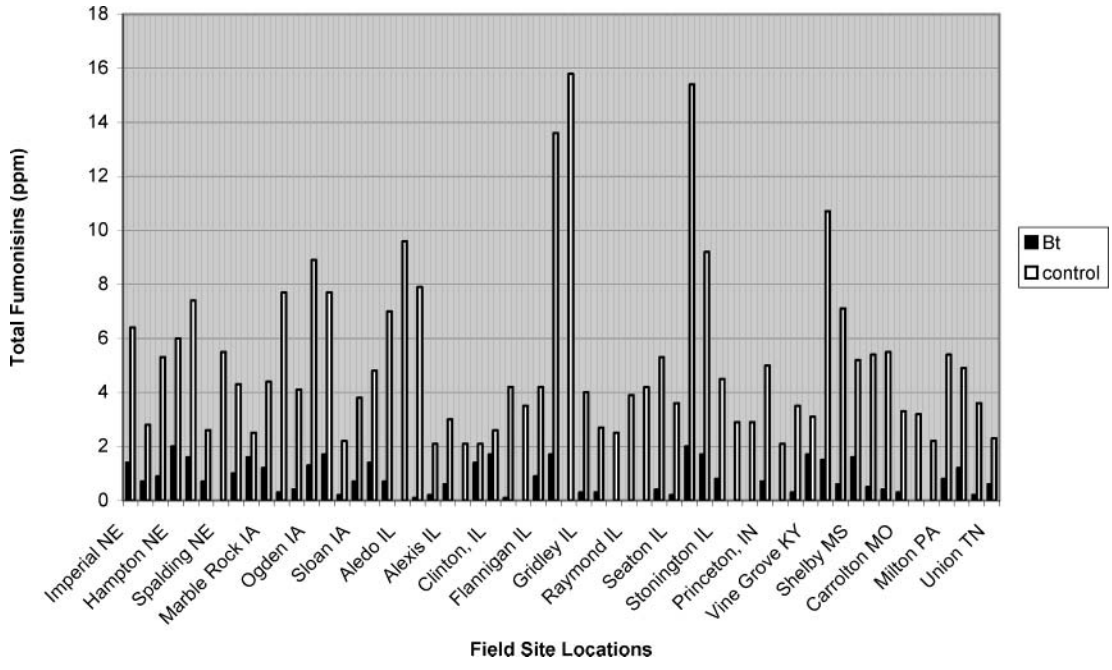

Figure 1. FACT trial comparisons of total fumonisins >2 ppm (control hybrids) and ≤2 ppm (Bt hybrids).

Table 4. Total Fumonisins, Aflatoxins, and Deoxynivalenol in Bt Corn Hybrids and Their Controls in University Trials Conducted in 2000/2001[a]

2000 university trial location	fumonisins (ppm)		aflatoxin (ppb)		deoxynivalenol (ppm)	
	Bt hybrid	control	Bt hybrid	control	Bt hybrid	control
Oklahoma	0.8	0.6	nd[b]	nd	1.3	0.6
Alabama 1	22.4	42.1	1975	2200	nd	nd
Alabama 2	0.7	4.9	>3000	>3000 (2 reps)		
				1500 (2 reps)	0.1	0.4
Texas 1	nd[c]	0.48	nd	nd	nd	nd
Texas 2	0.8	0.1	190	265	nd	0.4
Georgia	31.8	77	nd	369	0.4	1
Tennessee	3.6	40	nd	nd	nd	nd
Nebraska	0.4	3.9	nd	nd	nd	nd
Kansas 1	0.5	2.2	nd	nd	nd	0.2
Kansas 2	1.1	1.5	nd	nd	nd	nd
Missouri 1	1.3	2.8	nd	nd	nd	nd
Missouri 2	0.6	4.6	nd	nd	0.1	0.1
Missouri 3	0.2	1.8	nd	nd	nd	nd
Iowa	0.4	2.3	nd	nd	nd	0.3
Illinois 1	2.3	36.9	42	nd	nd	0.1
Illinois 2	1.1	2.4	nd	nd	nd	nd

2001 university trial location	fumonisins (ppm)		aflatoxin B_1 (ppb)	
	Bt hybrid	control	Bt hybrid	control
Raymond, MS	4.1	5.3	30	nd
Washington Co., NC, early plant	2.9	6.8	nd	nd
Washington Co., NC, late plant	5.8	4.4	nd	nd
Spalding Co., GA	1.6	2.5	1025	435
Colombia, MO	2.7	1.4	nd	41
Jerseyville, IL	1.7	7.9	nd	29
Massac Co., IL	3.8	40	nd	nd
Pope Co., IL	2.6	16.9	nd	nd
Garden City, KS	9	11.3	nd	nd

[a] Data represent the mean of four replicates/location. The mean difference in fumonisin levels between Bt and control hybrids was highly significant, $p < 0.0001$ (year 2000) and $p = 0.0005$ (year 2001). [b] Not detected; detection limit = 20 ppb. [c] Not detected; detection limit < 0.5 ppm total fumonisins.

grain samples were collected from different locations of the field at each site for each hybrid that should have reduced sampling variability. Results from the university trials demonstrated a similar trend for lowered fumonisin levels in Bt hybrids.

There were some comparisons in which fumonisin levels were not lower in Bt hybrids compared to controls or were increased. This did not appear to be related to any particular germplasm, as it occurred in several different Bt hybrids. As stated earlier, the Cry1Ab protein provides excellent protection against the corn borers and suppression (partial control) against other corn pests such as corn earworm (*Helicoverpa zea* Boddie), common stalk borer (*Papiapema nebris* Guenee), and armyworm (*Pseudaletia unipunctata* Hawthorn) (*10*, *36*−*39*). The Cry1Ab protein is not active against certain lepidoptera (western bean cutworm) or nonlepidopteran pests (e.g., sap beetles, Coleoptera: *Nitidulidae*). When the predominant insect pest is corn earworm and not corn borers, fumonisin levels are not reduced as significantly in Bt hybrids (*5*, *39*). Lepidopteran pests not controlled by Cry1Ab protein, such as the western bean cutworm (*Loxagrotis albicosta*) can also damage corn grain. In the 2001 FACT trial in Orange City and Primghar, IA, the field cooperator at those sites reported significant western bean cutworm damage in both Bt and control plots. Fumonisin levels were similar or greater in Bt hybrids than in controls at Primghar. The lack of reduction in fumonisin levels in Bt hybrids observed at certain sites may be due to the predominance of insect pests not controlled by Cry1Ab protein.

Parts of southwestern Iowa where the 2002 FACT trials were conducted experienced severe drought during the summer (*40*) and substantially higher first-generation corn borer pressure than was typically observed in these locations in Iowa (personal observation, Steve Spangler, Monsanto Co. Technology Development Manager in Iowa). The more extreme environmental conditions may have influenced the amount of fumonisin contamination that was observed in both control and Bt hybrids.

Impact of Bt Corn on Aflatoxin Contamination. Aflatoxin contamination annually occurs in southern regions of the United States and periodically in the Midwest where a variety of insect pests in addition to corn borers feed on corn. There have been reports that Bt hybrids had lower aflatoxin levels in southern Texas (*41*) and in Mississippi when corn borers were the principal insect pest (*42*). However, in other studies where insect pests were present that are not as well controlled by the Cry1Ab protein, there was no evidence of decreased aflatoxin levels in the grain of Bt hybrids (*43*, *44*). Second-generation Bt hybrids under development that control a broader spectrum of insect pests may have a greater impact in lowering aflatoxin contamination of corn grain.

Impact of Bt Corn on Deoxynivalenol and Zearalenone. Virtually no zearalenone was detected in the 2000 FACT and university trials. Deoxynivalenol was frequently detected in the 2000 FACT trials, and there was no difference in grain levels between Bt hybrids and their controls. However, studies in Ontario in 1996−1999 found that the levels of deoxynivalenol were decreased ~59% in the grain of Bt hybrids when compared to controls when there was high corn borer pressure (*45*). In Germany, deoxynivalenol levels were also significantly lower in the grain of Bt hybrids in 1999 field trials, whereas, in 2000, there was less apparent difference as the background levels of deoxynivalenol contamination were much higher in 2000 (*46*).

Significance of Lower Fumonisin Levels in Bt Corn. Fumonisins exert a variety of toxic effects on the heart, brain, kidney, and liver of different animal species (*4*, *29*, *30*, *47*, *48*). They are carcinogenic in rats and mice and have been linked to

high rates of esophageal and liver cancer in humans that consume large amounts of corn grain highly contaminated with fumonisins (*4*, *47*). In consideration of the toxic potential of fumonisins and their widespread occurrence in corn grain, regulatory agencies in the United States and Switzerland have proposed limits for contamination of corn grain with fumonisins (*4*, *29*, *30*). JECFA established a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg of body weight/day (120 μg/day for a 60 kg adult) for fumonisin exposure in humans (*49*). Although the PMTDI is seldom exceeded in regions of the world where dietary intake of corn is low and fumonisin contamination is minimal, fumonisin exposures can considerably exceed the PMTDI in other localities where high corn consumption and fumonisin contamination prevail (*50*). Bt corn may prove to be a useful tool to lower dietary intake of fumonisins, particularly in regions of the world where chronically high exposures persist. It can also increase the percentage of corn that would be suitable for consumption.

Supporting Information Available: Tables of corn grain consumption and fumonisin levels. This material is available free of charge via the Internet at http://pubs.acs.org.

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