Influence of Cry1Ab Protein and Hybrid Genotype on Fumonisin Contamination and Fusarium Ear Rot of Corn

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ABSTRACT

Fusarium ear rot of corn (*Zea mays* L.) is associated with feeding damage from the European corn borer (ECB), *Ostrinia nubilalis* Hübner, and the corn earworm (CEW), *Helicoverpa zea* Boddie. Specific transformation events encoding for Cry1Ab protein from *Bacillus thuringiensis* Berliner (Bt) may reduce Fusarium ear rot and fumonisin concentration in grain by minimizing damage from certain insects. The objective of this study was to determine if effects from Cry1Ab protein in kernels and silks on fumonisin concentration in grain vary depending on the genotype of the hybrid or the predominant insect species. Four Bt corn hybrids and their corresponding nontransgenic, near-isogenic hybrids were compared for ear rot severity and fumonisin concentration in grain in four environments. Treatments included inoculation with *F. verticillioides* (Sacc.) Nirenb. (Syn = *F. moniliforme* J. Sheld.) and *F. proliferatum* (Matsushima) Nirenb., infestation with ECB larvae, infestation with CEW larvae, and controls. Cry1Ab protein from the Mon810 transformation event was associated with reduced ear rot severity when hybrids were not inoculated with *Fusarium* spp., regardless of whether hybrids were infested or not infested with insects. Cry1Ab protein was associated with reduced fumonisin concentration in grain when ECB was the predominant insect, but not when CEW was the predominant insect. Cry1Ab protein was not associated with reduced fumonisin concentration in grain for the most resistant hybrid pair in this study. Results suggest that Bt hybrids can reduce fumonisin concentration in grain during seasons when ECB is favored, but not during seasons when CEW is favored. Hybrid genotype was an important factor in reducing fumonisin concentration in grain.

Fusarium verticillioides, *F. proliferatum*, and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas reduce yield and seed quality of corn worldwide by causing seedling blights and stalk, root, and kernel rots. Although the ear rot caused by these fungi has been recognized since 1904 (Sheldon, 1904), Fusarium ear rot of corn has only recently become a significant concern of the corn producing and processing industries.

Fusarium verticillioides and *F. proliferatum* produce a family of mycotoxins known as fumonisins. Fumonisins were identified in association with *F. verticillioides* (Gelderblom et al., 1988) and a neurotoxological condition in horses in 1988 (Marasas et al., 1988). Since 1988, a number of fumonisin homologs have been identified, of which FB_1, FB_2, and FB_3 are the most prevalent in corn (Cawood et al., 1991; CFSAN, 2001a; Musser and Plattner, 1997; Sydenham et al., 1992; Thiel et al., 1992). Fumonisins are a causal agent of equine leukoencephalomalacia (Kellerman et al., 1990; Marasas et al., 1988; Ross et al., 1990; Ross et al., 1993; Wilson et al., 1992), porcine pulmonary edema (Colvin and Harrison, 1992; Harrison et al., 1990; Haschek et al., 1992; Kreik et al., 1981; Osweiler et al., 1992; Ross et al., 1990), cardiac failure in baboons (Kreik et al., 1981), atherogenic effects in vervet monkeys (Fincham et al., 1992), hepatic cancer in mice and rats (Gelderblom et al., 1991; Gelderblom et al., 1993; NTP, 1999), neural tube defects in mice (Gelineau-vanWaes et al., 2001), and renal cancer in rats (NTP, 1999). Fumonisins also have been associated with esophageal cancer in several human populations (Cheng et al., 1985; Chu and Li, 1994; Doko and Visconti, 1994; Rheeder et al., 1992; Sydenham et al., 1990) and with neural tube birth defects in one human population where corn was a major component of the diet and grain consumed by humans was commonly contaminated with Fusarium ear rot (Stack, 1998). Evidence of clinical toxicoses in animals and the possibility of human health disorders associated with consumption of fumonisin contaminated grain have prompted the United States Food and Drug Administration (FDA) to issue a "Guidance for Industry" for fumonisins in corn and corn products intended for food and feed (CFSAN, 2001a; CFSAN, 2001b).

Current FDA Guidance levels between 2 and 4 μg of fumonisins per gram of grain have been suggested for various cleaned and dry milled corn products (CFSAN, 2001a) pending a more permanent risk management policy. Grain from the Midwest corn crop is frequently contaminated with fumonisins at concentrations between 1 and 5 μg/g, and concentrations above 5μg/g are common in some years (Anderson and Dolezal, 1993; Munkvold and Desjardins, 1997; Murphy et al., 1993; Scott et al., 1992; Woloshuk et al., 2001). Thus, a substantial portion of the U.S. corn crop could be affected if fumonisins are regulated by "Action" at or below levels currently suggested by the Guidance.

The feeding habits of several insect species have been associated with an increase in incidence and severity

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Abbreviations: Bt, *Bacillus thuringiensis*; CEW, corn earworm; ECB, European corn borer; ELISA, enzyme linked immunosorbent assay; FDA, Food and Drug Administration; FB_1, fumonisin B_1; FB_2, fumonisin B_2; FB_3, fumonisin B_3; HPLC, high-performance liquid chromatography; PBS, phosphate buffered saline; PDA, Potato dextrose agar; R2, blister developmental stage of corn; R4, dough developmental stage of corn; USDA-ARS-NCAUR, United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research; V8, developmental stage of corn when eight leaves with collars are visible; VT, tasseling developmental stage of corn.

of Fusarium ear rot. Nitidulid beetles, *Glischrochilus quadrisignatus* Say, may harbor *F. verticillioides* externally and internally during all life stages; therefore, beetles feeding on corn ears may serve as vectors for the fungus (Windels et al., 1976). Reductions in intraear populations of thrips (*Frankliniella occidentalis* Perg.) with insecticides have been associated with reductions in incidence of Fusarium ear rot (Davis et al., 1989; Farrar and Davis, 1991). Thrips may act as vectors of the fungus by dispersing *F. moniliforme* beneath husk leaves and creating wounds that favor fungal infection (Farrar and Davis, 1991). Damage to ears and shanks caused by endemic populations of ECB have been associated with high incidence of kernels infected with *F. verticillioides* (Christensen and Schneider, 1950; Koehler, 1942). *Fusarium* spp. have been isolated from internal and external structures of the ECB, as well as from shank and ear tissues damaged from ECB feeding (Christensen and Schneider, 1950). Christensen and Schneider (1950) hypothesized that wounds created by ECB tunneling into the ear shank provided an entrance through which fungi might colonize the ear, and that frass deposited after feeding might serve as a source of nutrients for the continued growth and sporulation of *Fusarium* spp. Sobek and Munkvold (1999) observed that incidence of kernel infection by *F. verticillioides* was higher when hybrids were infested with ECB larvae than when hybrids were treated by wounding or not treated. They demonstrated that ECB can acquire spores of *F. verticillioides* from plant surfaces and transmit them to kernels. Symptomatic or asymptomatic development of *F. verticillioides* also was influenced by ECB infestation (Sobek and Munkvold, 1999). Applying DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] to the silks of 20 varieties of corn that had been inoculated with *F. verticillioides* reduced infestation by CEW and decreased the percentage of ears damaged by Fusarium ear rot (Smeltzer, 1959).

Associations between insect feeding, Fusarium ear rot, and fumonisins have stimulated interest in reducing ear rot and mycotoxin contamination by reducing kernel damage from insects. Transgenic hybrids with *cry* gene insertions from the bacterium *Bacillus thuringiensis* have been proposed as one alternative to the application of insecticides. Several Bt hybrids have been developed with the *cry1Ab* gene. These products have been designed to specifically target the ECB, the predominant pest in the seed corn industry (Mason et al., 1996) and the most damaging pest of corn in the USA (Ostlie et al., 1997). Plant tissues of corn hybrids expressing Cry1Ab and other Cry proteins exhibit a high degree of resistance to the ECB and other susceptible Lepidopteran species (Armstrong et al., 1995).

Specific Bt transformation events encoding for Cry1Ab protein in kernels and silks (MON810 and BT11 events) were associated with reduced incidence and severity of Fusarium ear rot and a reduced fumonisin concentration in grain when kernel damage from ECB (Bakan et al., 2002; Dowd, 2000; Munkvold et al., 1997; Munkvold et al., 1999) and other Lepidopteran insects (Hammond et al., 2001) was reduced. Low fumonisin concentration in grain also has been associated with Bt hybrids expressing Cry1Ab protein in an experiment in which kernel damage from insects was not measured (Masoero et al., 1999). Conversely, Magg et al. (2002) reported that Bt hybrids expressing Cry1Ab protein in kernels and silks and nontransgenic hybrids did not differ significantly for fumonisin concentration in grain when plots were infested with ECB larvae. Only one location of five in the study by Magg et al. (2002) had a mean concentration of fumonisin in grain greater than 1 μg/g. Transformation events encoding for Cry protein production in plant tissues other than the kernels and silks (e.g., event 176) are not commonly associated with a reduction in ear rot severity and fumonisin concentration in grain (Dowd, 2000; Munkvold et al., 1997; Munkvold et al., 1999). Munkvold et al. (1997) observed that incidence of Fusarium ear rot was higher when plants were manually infested with ECB than when plants were naturally infested with insects, and that disease incidence was positively correlated with insect feeding damage. Bakan et al. (2002) observed low incidence of kernels infected by *Fusarium* spp., low number of ECB larvae per plant, and low fumonisin concentration in grain for two Bt hybrids compared to their nontransgenic, near-isogenic lines in naturally infected and naturally infested plots in Spain and France. Dowd (2000) reported less feeding, decreased growth and higher mortality of ECB on Bt hybrids than non-Bt hybrids. Non-Bt hybrids tended to have greater severity of Fusarium ear rot, higher fumonisin concentration in grain, and more damage from ECB than non-Bt hybrids (Dowd, 2000). Incidence of visibly molded kernels and number of insect damaged kernels were positively correlated in the study by Dowd (2000), as were number of insect damaged kernels and fumonisin concentration in grain for several hybrids.

Cry1Ab protein in kernels and other plant tissues has had variable effects on reducing kernel damage from CEW (Dowd, 2001; Gould, 1998; Lynch et al., 1999a; Lynch et al., 1999b; Pilcher et al., 1997; Rice and Pilcher, 1998). In a study of hybrids naturally infested with a number of insect species, Dowd (2001) observed that number of kernels damaged by CEW larvae at R3 (milk) and harvest was generally lower for Bt hybrids that express *cry* genes in kernels and silks (e.g., MON810 and BT11) than for non-Bt hybrids and Bt hybrids that do not express *cry* genes in kernels and silks. Incidence of CEW-infested ears did not differ between Bt and non-Bt hybrids at R3. With few exceptions, MON810 and BT11 hybrids slowed feeding by CEW, but they did not significantly reduce incidence of CEW infestation or fumonisin concentration in grain. Dowd (2001) hypothesized that large infestations of CEW larvae may limit effectiveness of Bt hybrids as a means of indirectly reducing fumonisin concentration in grain, but that Bt hybrids should provide some control of fumonisin in seasons when CEW populations are low.

Although Cry1Ab protein does not control CEW as effectively as it controls ECB (Dowd, 2001; Marçon et al., 1999), Cry1Ab protein in kernels and silks may reduce kernel feeding by CEW and indirectly reduce fu-

monisin concentration in grain under some conditions. The presence of Cry1Ab protein does not guarantee, however, that fumonisin will be reduced to a concentration below FDA Guidance levels when ECB or CEW is the predominant pest.

A comparative study of Bt and non-Bt hybrids that have been manually infested with CEW and inoculated with *Fusarium* spp. is not available in literature. Inoculation and manual infestation should increase disease severity and reduce variability encountered in experiments that rely on natural infection and infestation. Information on the interaction between hybrid genotype and *cry* gene expression in relation to Fusarium ear rot and fumonisin concentration has been published only in studies monitoring damage from ECB (Munkvold et al., 1997; Munkvold et al., 1999). These studies evaluated hybrids with different Bt transformation events (i.e., different *cry* gene-promoter combinations) or different levels of Cry protein production in plant tissues. Hybrids with different genetic backgrounds were evaluated in these studies, but hybrids with different genetic backgrounds and an identical transformation event were not compared. Information of this type from a study monitoring damage from CEW and ECB will be useful to breeders considering the costs and benefits of developing transgenic hybrids for disease and insect resistance.

The objective of this study was to compare severity of Fusarium ear rot and concentration of fumonisin in grain of hybrids with different genetic backgrounds and an identical Bt transformation event when *Fusarium* spp. are prevalent and CEW or ECB is the predominant pests.

MATERIALS AND METHODS

Seed of four Bt corn hybrids and their corresponding nontransgenic, near-isogenic hybrids were planted at Urbana and Monmouth, IL, on 28 April and 3 May, 2000, respectively, and 2 April and 23 May, 2001, respectively. Seed was provided by Monsanto Company (Monmouth, IL). Pairs of hybrids examined were DKC58-52BtY/DK585, DK621BtY/DK621, RX697YG/RX697, and RX730YG/RX730. Transgenic hybrids were iso-line conversions with greater than 98% genetic similarity to their nontransgenic counterparts. Detailed information on these hybrids is proprietary information of Monsanto Company and is not available to public sources. All plant tissues of transgenic hybrids expressed Cry1Ab protein from the MON810 Bt event.

The experimental design was a split block with four replicates. The treatment design was a 2×3×2×4 factorial with two Fusarium treatments, three insect treatments, two Bt treatments, and four hybrid genotypes. Fusarium treatments (inoculated or not inoculated with *Fusarium* spp.) were applied to main plots. The 24 combinations of insect treatments, Bt treatments and hybrids were randomized within Fusarium treatments as subplots. Experimental units at Urbana consisted of three 5.3-m rows (±0.5-m fallow ally) spaced 0.76 m apart. Experimental units at Monmouth consisted of four 5.3-m rows (±0.5-m fallow ally) spaced 0.76 m apart. Each row included 20 to 24 plants.

Inoculum was prepared from two isolates of *F. verticillioides* (numbers 42 and 150) and three isolates of *F. proliferatum* (numbers 19, 37-2, and 310) that produced severe Fusarium ear rot and a high concentration of fumonisin in grain when injected into corn ears in a concurrent study (Clements, 2002; Clements et al., 2002). Inoculum also was prepared from one isolate of *F. verticillioides* (number 152) that does not produce fumonisin in grain (Clements, 2002). Isolates were obtained from corn grain collected throughout Iowa and Illinois by Gary Munkvold, Department of Plant Pathology, Iowa State University. All six isolates are maintained at the University of Illinois. Isolates were grown on potato dextrose agar (PDA) (Becton Dickinson and Company, Sparks, MD) at approximately 25°C under 12 h of diurnal fluorescent light for approximately 7 d. Inoculum was prepared by blending an equal number of cultures of the six isolates in deionized water. The resulting propagule suspension was strained through two layers of cheesecloth, further diluted with water to a concentration of 10^6 conidia/mL as determined with a hemacytometer, and amended with 0.2 mL/liter Tween 20 surfactant (polyoxyethylene 20-sorbitan monolaurate; Fisher Biotech, Fairlawn, NJ). Ten milliliters of the spore suspension were injected down the silk channel of the primary ear of plants at the R2 (blister) growth stage. Inoculum was delivered from backpack sprayers (model 425; Solo Inc., Newport News, VA) equipped with an injection device consisting of a Tee-Jet brand meter jet gun assembly (model 23623-31; Spraying Systems Co, Wheaton, IL) fitted with a 0.64-cm Tee-Jet outlet adaptor (model 4676) and a grease needle (model 5803; Lincoln Automotive, St. Louis, MO) with the orifice enlarged to 1.6 mm.

Three insect treatments included infestation with ECB, CEW, or a nontreated control. Insect populations were provided by Monsanto Company. Plants were infested with first-instar ECB in the center row of each 3-row subplot at Urbana and in the center two rows of each 4-row subplot at Monmouth. Larvae mixed with corn cob grit were distributed on plants with volumetric applicators (Custom Bio-Products, Maxwell, IA). Forty-five to fifty larvae per plant per day were placed in the whorl at the V8 (8 leaves with collars) growth stage on two consecutive days. European corn borer larvae also were placed at a rate of approximately 25 larvae per axil in the leaf axils immediately above and below the primary ear, and at a rate of 45 to 50 larvae in the leaf axil of the primary ear of the same plants at the R2 growth stage. Plants infested with ECB larvae were rated for leaf feeding damage at VT (tasseling) by the Guthrie scale (Guthrie et al., 1960), where 0 = no visible leaf feeding, 1 = a small amount of pin or fine shot-hole injury on a few leaves, 2 = a small amount of shot-hole injury on a few leaves, 3 = shot-hole injury common on several leaves, 4 = several leaves with shot-hole and elongated lesions, and 5 = several leaves with elongated lesions.

Primary ears were infested with first-instar CEW in all rows of an experimental unit at Urbana and Monmouth. A waxed-paper shoot bag (model 217; Lawson Bags, Northfield, IL) with the sealed end removed was fitted to primary ears so that silks were exposed within a paper corral. Larvae mixed with corn cob grit were distributed onto silks within the corral with volumetric applicators at a rate of 5-10 larvae per ear when plants reached approximately R2. Shoot bags were removed from primary ears one week after infestation. Feeding damage from CEW and number of live larvae per ear were determined from primary ears of the outermost row of CEW infested plots and control plots when plants reached approximately R4 (dough). Feeding damage from CEW at harvest was determined on primary ears from the center row at Urbana, and the center two rows at Monmouth. Ear damage was estimated using the Widstrom scale (Widstrom, 1967), where 0 = no damage, 1 = damage to silks only, and kernel damage to n cm beyond the ear tip is represented by $n + 1$.

All primary ears from the center row of each plot at Urbana

and the center two rows of each plot at Monmouth were hand-harvested at approximately 180 g kg^{-1} grain moisture. Primary ears were rated visually for severity of Fusarium ear rot as percentage of the ear with symptoms. Ears from Monmouth were rated in 12.5% increments in 2000. Ears from Urbana in 2000 and 2001 and from Monmouth in 2001 were rated on a continuous scale from 0 to 100%. Ears were dried to approximately 140 g kg^{-1} grain moisture and shelled. Shelled grain was bulked by experimental unit and ground with a Romer grinding–subsampling mill (model 2A; Romer Labs, Inc., Union, MO) to pass through a 1-mm mesh. A 25-g sample of ground corn from each experimental unit was analyzed for fumonisin concentration with a competitive-direct enzyme-linked immunosorbent assay (ELISA) described previously (Clements et al., 2002). Concentration of fumonisin in grain was determined by comparison of sample absorbance to a standard curve established with eight standards (equivalent to 0, 0.1, 0.45, 1.0, 4.5, 10, 45, and 100 μg/g in sample extract) on each plate.

Year and location effects were treated as environments in the statistical analyses. The effects of environments, Fusarium treatments, insect treatments, Bt treatments, hybrids, and the interactions between these factors on fumonisin concentration and ear rot severity were analyzed with analysis of variance via the Mixed procedure of Statistical Analysis System software (SAS institute, NC). Significant interaction terms were analyzed with single degree of freedom contrasts. Least squares means were sorted with the pdmix800 macro devel-

Table 1. Analysis of variance for four environments, two Fusarium treatments, three insect treatments, two Bt treatments, and four hybrids evaluated for fumonisin concentration in grain and severity of Fusarium ear rot at Urbana and Monmouth, IL, in 2000 and 2001.

	Fumonisin†			Fusarium ear rot‡		
Source of variance	df	MS		df	MS	
Env§	3	36.7		3	27.9	
Rep(Env)¶	12	1.4		12	2.2	
Fusarium#	1	88.1	*	1	268.1	*
Fusarium×Env	3	5.2		3	9.4	
Fusarium×Rep(Env)	12	0.8		12	2.0	
Insect††	2	3.7	*	2	4.8	
Insect×Env	6	0.5		6	1.0	
Insect×Rep(Env)	24	0.4		24	0.3	
Insect×Fusarium	2	0.8		2	0.1	
Insect×Fusarium×Env	6	0.5		6	0.3	
Insect×Fusarium×Rep(Env)	24	0.4		24	0.2	
Bt‡‡	1	10.1	***	1	24.1	*
Bt×Env	3	0.0		3	1.3	
Bt×Rep(Env)	12	0.7		12	0.3	
Bt×Fusarium	1	1.0		1	4.5	
Bt×Fusarium×Env	3	0.1		3	1.1	
Bt×Fusarium×Rep(Env)	12	0.5		12	0.3	
Bt×Insect	2	1.8		2	1.0	
Bt×Insect×Env	6	0.4		6	0.3	
Bt×Insect×Rep(Env)	24	0.4		24	0.2	
Bt×Insect×Fusarium	2	1.2	**	2	0.4	*
Bt×Insect×Fusarium×Env	6	0.1		6	0.1	
Bt×Insect×Fusarium×Rep(Env)	24	0.4		24	0.4	
Hybrid§§	3	10.4	*	3	24.7	**
Hybrid×Env	9	1.7		9	2.3	
Hybrid×Rep(Env)	36	0.3		36	0.5	
Hybrid×Fusarium	3	3.8	**	3	9.2	**
Hybrid×Fusarium×Env	9	0.0		9	1.0	
Hybrid×Fusarium×Rep(Env)	36	0.3		36	0.4	
Hybrid×Insect	6	0.2		6	0.6	
Hybrid×Insect×Env	18	0.5		18	0.5	
Hybrid×Insect×Rep(Env)	72	0.3		72	0.3	
Hybrid×Insect×Fusarium	6	0.6		6	0.2	
Hybrid×Insect×Fusarium×Env	18	0.4		18	0.4	
Hybrid×Insect×Fusarium×Rep(Env)	72	0.4		72	0.2	
Hybrid×Bt	3	1.0	*	3	2.6	
Hybrid×Bt×Env	9	0.2		9	0.7	
Hybrid×Bt×Rep(Env)	36	0.3		36	0.5	
Hybrid×Bt×Fusarium	3	0.6		3	0.5	
Hybrid×Bt×Fusarium×Env	9	0.4		9	0.4	
Hybrid×Bt×Fusarium×Rep(Env)	36	0.3		36	0.6	
Hybrid×Bt×Insect	6	0.2		6	0.2	
Hybrid×Bt×Insect×Env	18	0.3		18	0.5	
Hybrid×Bt×Insect×Rep(Env)	72	0.3		72	0.3	
Hybrid×Bt×Insect×Fusarium	6	0.1		6	0.2	
Hybrid×Bt×Insect×Fusarium×Env	18	0.3		18	0.4	
Residual	64	0.3		71	0.4	

*, denotes significance at the 0.05 probability level.
**, denotes significance at the 0.01 probability level.
***, denotes significance at the 0.001 probability level.
† Fumonisin concentration in grain was analyzed as the natural log of the quantity one plus μg fumonisin/gram corn.
‡ Severity of Fusarium ear rot was analyzed as the natural log of the quantity one plus the percent of the ear with symptoms.
§ Environments evaluated were Urbana, IL, in 2000 and 2001 and Monmouth, IL, in 2000 and 2001.
¶ Replicates nested within environment. Four replicates per environment.
# Fusarium treatments included inoculation with *Fusarium* spp. and no inoculation.
†† Insect treatments included infestation with European corn borer larvae, infestation with corn earworm larvae, and no insect infestation.
‡‡ Bt treatments included cry1Ab gene expression in all plant tissues and no *cry* gene expression.
§§ Four near-isogenic pairs of hybrids were evaluated.

oped by Saxton (Saxton, 1988). Fumonisin concentration and severity of Fusarium ear rot were transformed to the natural log (1 + μg/g fumonisin) and the natural log (1 + percent of the ear symptomatically affected), respectively, to normalize residuals. Environments and replicates were considered random terms, and all other terms were considered fixed. Means ± standard deviations are reported untransformed in the text. Pearson's correlation coefficients were calculated on transformed treatment means (Gomez and Gomez, 1984, p. 388–390). Weather information for central Illinois was obtained from the Midwest Regional Climate Center, Champaign, IL.

RESULTS

Fumonisin concentration in grain ranged from <1 to 210 μg/g with a mean of 9 ± 17 μg/g. Fumonisin concentration in grain was not significantly different among the four environments evaluated in this study (Table 1). Interactions among environments and main effects (Fusarium treatments, insect treatments, Bt treatments and hybrids) were not significant. Fumonisin concentration in grain differed significantly for all four main effects and for the Bt × insect × Fusarium interaction; the hybrid × Fusarium interaction; and the hybrid × Bt interaction (Table 1).

Fumonisin concentration in grain was significantly different between ears that were inoculated with *Fusarium* spp. and ears that were not inoculated (Table 1). The average fumonisin concentration was 13 ± 21 μg/g and 5 ± 9 μg/g in grain from inoculated and noninoculated ears, respectively. Fumonisin concentration was significantly greater in grain from ears that were infested with ECB than in grain from noninfested ears and ears that were infested with CEW. Average fumonisin concentration in grain from ears infested with ECB, ears infested with CEW and ears not infested with insects was 10 ± 14, 9 ± 19 and 8 ± 17 μg/g, respectively.

Fumonisin concentration in grain of Bt and non-Bt hybrids averaged 8 ± 13 μg/g and 10 ± 20 μg/g, respectively. Significantly lower fumonisin concentration in grain was associated with Cry1Ab protein when Fusarium inoculated (Fig. 1A) or noninoculated (Fig. 1B) ears were infested with ECB. Conversely, fumonisin concentration in grain was not affected by Cry1Ab protein when Fusarium inoculated or noninoculated ears were infested with CEW (Fig. 1A and 1B, respectively). Fumonisin concentration in grain was not affected significantly by Cry1Ab protein when ears that were not infested with insects were inoculated with *Fusarium* spp., but fumonisin concentration was affected by Cry1Ab protein when ears were not inoculated with *Fusarium* spp. and not infested with insects.

Fumonisin concentration in grain was affected significantly by hybrids (Table 1). Grain from one nontransgenic hybrid, RX697, had a significantly lower fumonisin concentration than the other hybrids evaluated in this study. Fumonisin concentration in grain also was affected by the hybrid × Fusarium interaction (Table 1 and Fig. 2A) and the hybrid × Bt interaction (Table 1 and Fig. 3A). The most resistant pair of hybrids, RX697/RX697YG, was the only pair for which fumonisin concentration in grain was not lower for the hybrid expressing Cry1Ab protein.

Severity of Fusarium ear rot ranged from 0 to 57% with a mean of 8 ± 10%. Ear rot severity did not differ

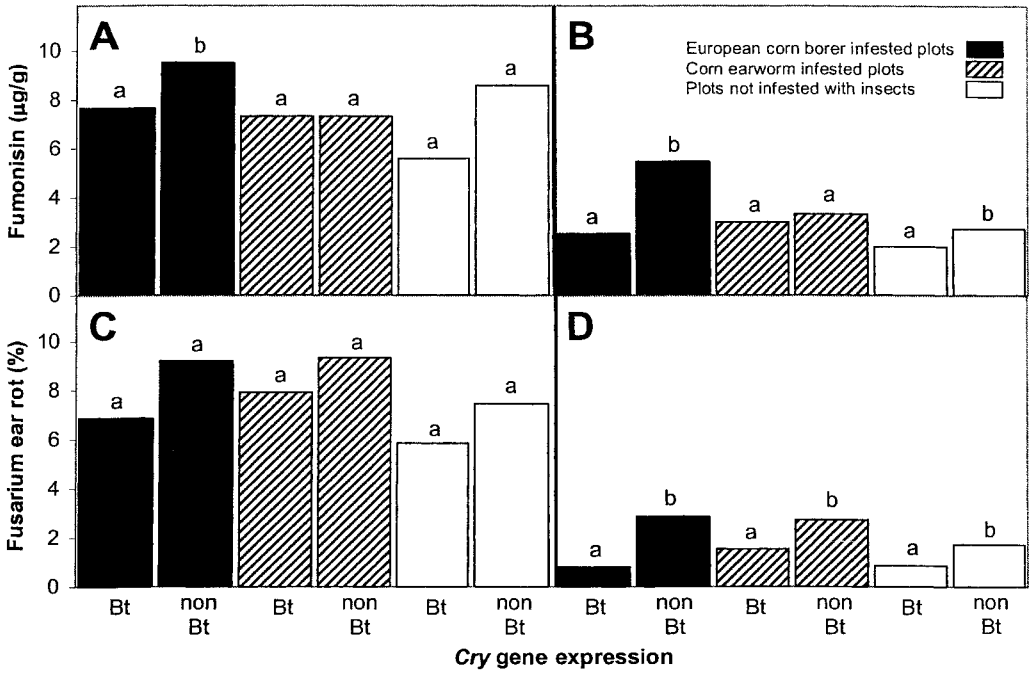

Fig. 1. Fumonisin concentration in grain (A and B) and the severity of Fusarium ear rot (C and D) for Bt and non-Bt corn hybrids infested with European corn borer larvae at V8 (8 leaves with collars) and R2 (blister), or infested with corn earworm larvae at R2, or not infested with insects. Primary ears were injected with a spore suspension of *F. verticillioides* and *F. proliferatum* down the silk channel at R2 (A and C), or were not inoculated with *Fusarium* spp. (B and D). Lowercase letters above columns designate differences at α = 0.05 between Bt and non-Bt hybrids within treatments.

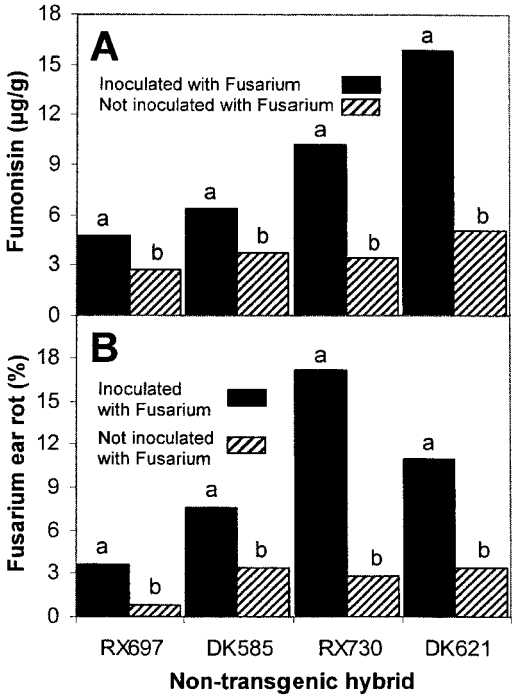

Fig. 2. Effect of Fusarium treatments on four nontransgenic corn hybrids evaluated for fumonisin concentration in grain (A) and severity of Fusarium ear rot (B) at Urbana, IL and Monmouth, IL in 2000 and 2001. Primary ears in inoculated plots were injected with a spore suspension of *F. verticillioides* and *F. proliferatum* down the silk channel at R2 (blister). Lowercase letters above columns designate differences at $\alpha = 0.05$ between inoculated and noninoculated plots of the same hybrid.

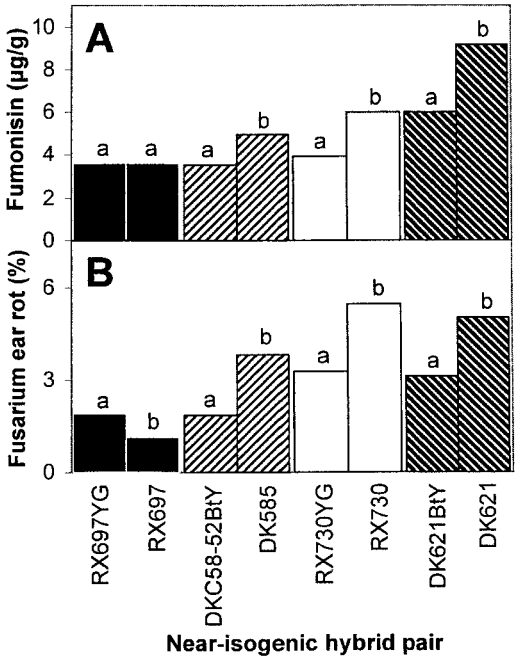

Fig. 3. Fumonisin concentration in grain (A) and severity of Fusarium ear rot (B) for Bt and non-Bt corn hybrids evaluated at Urbana and Monmouth, IL, in 2000 and 2001. Lowercase letters above columns designate differences at $\alpha = 0.05$ between Bt and non-Bt hybrids within hybrid pairs.

significantly among the four environments evaluated in this study (Table 1). Ear rot differed significantly among the main effects of Fusarium treatments, Bt treatments, and hybrids, but did not differ significantly among insect treatments (Table 1). Ear rot also was affected significantly by the hybrid × Fusarium interaction; and the Bt × insect × Fusarium interaction.

Average severity of Fusarium ear rot in Fusarium inoculated and noninoculated plots was 13 ± 12% and 3 ± 3%, respectively. Severity of Fusarium ear rot for Bt and non-Bt hybrids averaged 7 ± 10% and 9 ± 11%, respectively. Severity of Fusarium ear rot did not differ among hybrids with and without expression of Cry1Ab protein when ears were inoculated with *Fusarium* spp. (Fig. 1C); however, less severe Fusarium ear rot was associated with Cry1Ab protein when ears were not inoculated with *Fusarium* spp., regardless of the insect treatment (Fig. 1D).

Ear rot severity was affected significantly by hybrids (Table 1 and Fig. 2B). Three nontransgenic hybrids, RX697, DK585, and DK621 had significantly less severe Fusarium ear rot than RX730. Ear rot severity was affected significantly by the interaction between hybrids and Fusarium treatments (Table 1 and Fig. 2B), and hybrids and Bt treatments (Table 1 and Fig. 3B). The most resistant pair of hybrids, RX697/RX697YG, was the only pair for which Fusarium ear rot severity was not significantly lower for the hybrid expressing Cry1Ab protein (Fig. 3B).

When all treatment means were evaluated, fumonisin concentration in grain and ear rot severity were not significantly correlated in plots that had been inoculated with *Fusarium* spp., but were significantly correlated in plots that had not been inoculated with *Fusarium* spp. ($r = 0.38$, $P = 0.0002$) (Table 2). Low ear rot severity (≤5%) and a high fumonisin concentration in grain (greater than 5 µg/g) occurred for 85% of treatment means from inoculated plots and 26% of treatment means from naturally infected plots (Table 3).

Fumonisin concentration in grain and ear rot severity were significantly correlated in ECB-infested plots, CEW-infested plots, and controls, ($r = 0.59$, $P < 0.0001$; $r = 0.39$, $P = 0.0016$; and $r = 0.54$, $P < 0.0001$, respectively) (Table 2). However, correlations of fumonisin concentration in grain and ear rot severity from insect treatments within specific Fusarium and Bt treatment combinations were not significant. Fumonisin concentration in grain and ear rot severity were significantly correlated in plots expressing Cry1Ab protein and plots not expressing Cry1Ab protein ($r = 0.55$, $P < 0.0001$; and $r = 0.44$, $P < 0.0001$, respectively). Fumonisin concentration in grain and ear rot severity were significantly correlated in plots of hybrids DK621BtY, DK621, RX697YG, RX697, and RX730YG ($r = 0.56$, $P = 0.0046$; $r = 0.50$, $P = 0.0121$; $r = 0.67$, $P = 0.0004$; $r = 0.45$, $P = 0.0273$; and $r = 0.69$, $P = 0.0002$, respectively) but were not significantly correlated in plots of hybrids DKC58-52BtY, DK585, and RX730. Fumonisin concentration in grain and ear rot severity were significantly correlated in plots that were not inoculated with Fusarium for hybrid pair RX730YG/RX730 ($r = 0.53$, $P = 0.008$), but were not significantly correlated for other Fusarium treatment, hybrid pair combinations.

Amount of CEW feeding damage at R4 and number

Table 2. Pearson's correlation coefficients for the concentration of fumonisin in grain and severity of Fusarium ear rot in various combinations of two Fusarium treatments, three insect treatments, two Bt treatments, and four hybrid genotypes evaluated at Urbana and Monmouth, IL, in 2000 and 2001.

Fusarium†	Insect‡	Bt§	Hybrid genotype¶	r#		n††
Inoculated	CEW	Bt	All Bt	−0.19		16
Inoculated	Control	Bt	All Bt	0.19		16
Inoculated	ECB	Bt	All Bt	0.06		16
Natural infection	CEW	Bt	All Bt	0.12		16
Natural infection	Control	Bt	All Bt	0.26		16
Natural infection	ECB	Bt	All Bt	0.34		16
Inoculated	CEW	Non-Bt	All Non-Bt	0.11		16
Inoculated	Control	Non-Bt	All Non-Bt	0.09		16
Inoculated	ECB	Non-Bt	All Non-Bt	0.43		16
Natural infection	CEW	Non-Bt	All Non-Bt	0.13		16
Natural infection	Control	Non-Bt	All Non-Bt	0.44		16
Natural infection	ECB	Non-Bt	All Non-Bt	0.41		16
Inoculated	All	All	DKC58-52BtY/DK585	0.37		24
Inoculated	All	All	DK621BtY/DK621	0.02		24
Inoculated	All	All	RX697YG/RX697	0.12		24
Inoculated	All	All	RX730YG/RX730	0.02		24
Natural infection	All	All	DKC58-52BtY/DK585	0.22		24
Natural infection	All	All	DK621BtY/DK621	0.05		24
Natural infection	All	All	RX697YG/RX697	0.34		24
Natural infection	All	All	RX730YG/RX730	0.53	**	24
All	All	Bt	DKC58-52BtY	0.01		24
All	All	Bt	DK621BtY	0.56	**	24
All	All	Bt	RX697YG	0.67	***	24
All	All	Bt	RX730YG	0.69	***	24
All	All	Non-Bt	DK585	0.09		24
All	All	Non-Bt	DK621	0.50	*	24
All	All	Non-Bt	RX697	0.45	*	24
All	All	Non-Bt	RX730	0.38		24
Inoculated	All	All	All	0.14		96
Natural infection	All	All	All	0.38	***	96
All	CEW	All	All	0.39	**	64
All	Control	All	All	0.54	***	64
All	ECB	All	All	0.59	***	64
All	All	Bt	All	0.55	***	96
All	All	Non-Bt	All	0.44	***	96

*, denotes significance at the 0.05 probability level.
**, denotes significance at the 0.01 probability level.
***, denotes significance at the 0.001 probability level.
† Fusarium treatments included inoculation with *Fusarium* spp. and no inoculation.
‡ Insect treatments included infestation with European corn borer larvae, infestation with corn earworm larvae, and no insect infestation.
§ Bt treatments included cry1Ab gene expression in all plant tissues and no *cry* gene expression.
¶ Four near-isogenic pairs of hybrids were evaluated.
# Pearson's correlation coefficients were calculated on transformed data. Severity of Fusarium ear rot was transformed to the natural log of the quantity one plus the percent of the ear with symptoms. Fumonisin concentration in grain was transformed to the natural log of the quantity one plus μg fumonisin/gram corn.
†† Number of observations.

of live CEW larvae collected from primary ears at R4 differed significantly between ears that had been infested with CEW and ears that had not been infested with insects. Corn earworm feeding damage at harvest did not differ significantly between CEW and noninfested treatments. Feeding damage on ears infested with CEW and ears not infested with insects averaged 2 ± 2 and 0 ± 1 at R4, and 2 ± 1 and 1 ± 1 at harvest, respectively. Number of live CEW larvae collected from primary ears at R4 averaged 2 ± 4 larvae per ear for ears infested with the CEW and 0 larvae per ear for ears not infested with insects.

Leaf feeding damage from ECB at VT, kernel damage from CEW at R4, number of live CEW larvae collected from primary ears at R4, and kernel damage from CEW at harvest were significantly, although weakly correlated

Table 3. Relative frequency† of fumonisin concentration in grain for hybrids with different severities‡ of Fusarium ear rot at Urbana and Monmouth, IL, in 2000 and 2001.

Concentration of fumonisin μg/g§	Hybrids inoculated with *Fusarium* spp.				Hybrids not inoculated with *Fusarium* spp.			
	≤5	>5-7	>7-10	>10	≤5	>5-7	>7-10	>10
≤5	0.15	0.08	0.44	0.21	0.74	0.33	1.00	0.33
>5–20	0.75	0.83	0.50	0.56	0.26	0.77	0	0.77
>20	0.10	0.08	0.06	0.23	0	0	0	0
n¶	20	12	16	48	87	3	3	3

† Treatment means were calculated from untransformed data before computing frequencies.
‡ Categories represent U.S. No. 1 and 2, U.S. No. 3, U.S. No. 4, and inferior grades of corn based on standards established by part 810.401, subpart D, United States Standards for Corn (GIPSA, 1996).
§ Categories approximate safe levels established by the U.S. Food and Drug Administration for humans and equids, swine, and other livestock in the 'Guidance for Industry' for corn and corn products (CFSAN, 2001a; CFSAN, 2001b).
¶ Number of treatment means out of 96 (four environments × three insect treatments × two Bt treatments × four hybrids) per Fusarium treatment (inoculated or not inoculated).

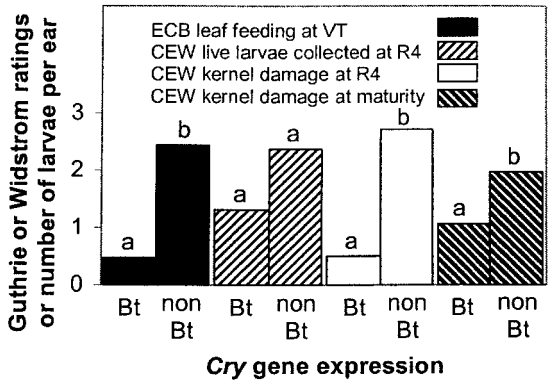

Fig. 4. Comparison of Bt and non-Bt corn hybrids for the presence of corn earworm (CEW) larvae, or damage from insects at Urbana and Monmouth, IL, in 2000 and 2001. Widstrom ratings for corn earworm damage were recorded as 0 = no damage, 1 = damage to silks only, 2 = kernel damage to 1 cm beyond the ear tip, and 3 = kernel damage to 2 cm beyond the ear tip. Guthrie ratings for European corn borer (ECB) damage were recorded as 0 = no visible leaf feeding, 1 = a small amount of pin or fine shot-hole injury on a few leaves, 2 = a small amount of shot-hole injury on a few leaves, and 3 = shot-hole injury common on several leaves. Lowercase letters above columns designate differences at $\alpha = 0.05$ between Bt and non-Bt hybrids within insect observations.

with the severity of Fusarium ear rot ($r = 0.17$, $P = 0.0065$; $r = 0.15$, $P = 0.0138$; $r = 0.15$, $P = 0.0162$; and $r = 0.18$, $P = 0.0049$, respectively), but were not significantly correlated with fumonisin concentration in grain. Leaf feeding damage from ECB at VT, kernel damage from CEW at R4, and kernel damage from CEW at harvest were significantly lower for Bt hybrids than for non-Bt hybrids; however, number of live CEW larvae collected from primary ears at R4 did not differ significantly between Bt and non-Bt hybrids (Fig. 4).

Leaf feeding damage from ECB at VT, CEW feeding damage at R4 and number of live CEW larvae collected from primary ears at R4 were not affected significantly by hybrids. Feeding damage from ECB at VT averaged 1.4 ± 1.2. Feeding damage from CEW at R4 and number of live CEW larvae collected from primary ears at R4 averaged 1.1 ± 1.6 and 1.2 ± 2.8, respectively. Feeding damage from CEW at harvest was affected significantly by hybrids. Two hybrids, RX697 and DK585 had significantly less feeding damage from CEW at harvest than the other hybrids evaluated in this study. Hybrids RX697 and DK585 averaged 1.1 ± 0.9, while hybrids DK621 and RX730 averaged 1.6 ± 1.0 for feeding damage from CEW at harvest.

Temperature in central Illinois was above average during the spring and below average during the summer of 2000. Precipitation was below average during the summer of 2000. Temperature in central Illinois was above average during the spring and near average during the summer of 2001. Precipitation was below average from March through July of 2001.

DISCUSSION

Bt hybrids that express Cry1Ab protein in kernels and silks may have lower fumonisin concentration in grain and lower severity of Fusarium ear rot than their nontransgenic, near isogenic counterparts in some situations. Lower fumonisin concentration in grain was associated with Cry1Ab protein when ECB was the predominant insect, but not when CEW was the predominant insect. Differences in ear rot severity were associated with Cry1Ab protein when plants were not inoculated with *Fusarium* spp.

Inability of Cry1Ab protein to rapidly reduce CEW populations, coupled with a tendency for CEW to feed sparingly on a large number of kernels, may explain why fumonisin concentration in grain did not differ significantly between Bt and non-Bt hybrids when CEW was the predominant insect. Dowd (2001) and Pilcher et al. (1997) reported no significant difference in incidence of CEW larvae on ears of Bt hybrids and non-Bt hybrids. Pilcher et al. (1997) observed lower incidence of damage from CEW to ears of Bt hybrids than non-Bt hybrids. Dowd (2001) noted that CEW larvae on ears of Bt hybrids expressing high levels of Cry protein often fed sparingly on a large number of kernels. This phenomenon was not observed on ears of non-Bt hybrids or Bt hybrids expressing low levels of Cry protein. Small amounts of feeding damage on a large number of kernels may have been present in our study; however, our method of evaluating ears for kernel damage did not detect this type of damage. Like Pilcher et al. (1997), we observed less feeding damage from CEW on Bt hybrids than non-Bt hybrids. Like Pilcher et al. (1997) and Dowd (2001), we observed no significant reduction in number of live CEW larvae on ears of Bt hybrids compared to non-Bt hybrids.

It is unlikely that our technique of placing a shoot bag around the ear for CEW infestations had an effect on humidity levels and fungal growth within the ear. The sealed end of the waxed-paper shoot bags were opened to facilitate infestations, and the bags were removed from ears 1 wk after infestation. High ear rot severity and greater concentration of fumonisin in grain were not evident on ears inoculated with *Fusarium* spp. and covered with an intact waxed-paper shoot bag from R2 to harvest in another study (Clements, 2002).

Previous studies have associated reduced insect feeding damage with reduced severity of Fusarium ear rot (Christensen and Schneider, 1950; Davis et al., 1989; Dowd, 2000; Dowd, 2001; Farrar and Davis, 1991; Munkvold et al., 1997; Munkvold et al., 1999; Smeltzer, 1959; Sobek and Munkvold, 1999). In our study, Cry1Ab protein significantly reduced insect feeding damage (leaf damage from ECB at VT, kernel damage from CEW at R4, and kernel damage from CEW at harvest) in plots that were inoculated and not inoculated with *Fusarium* spp. Cry1Ab protein also was associated with reduced severity of Fusarium ear rot in plots that were not inoculated with *Fusarium* spp. Reductions in ear rot severity associated with Cry1Ab protein in the absence of inoculation may have been obscured by high levels of rot on inoculated ears. We do not attribute the lack of a significant effect on ear rot by Cry1Ab protein in plots that were inoculated with *Fusarium* spp. to be due to insufficient insect populations, since differences between insect treatments and controls in these plots

were detected for fumonisin concentration in grain and insect feeding damage.

Hybrids inoculated with *Fusarium* spp. had greater severity of Fusarium ear rot and a greater fumonisin concentration in grain than hybrids that were not inoculated. Inoculation increased disease development for some hybrids more than others, although rank order of Bt hybrids and rank order of corresponding non-Bt hybrids were identical for fumonisin concentration. A similar trend was observed for severity of Fusarium ear rot. Cry1Ab protein was not associated with significant reductions in fumonisin concentration or severity of Fusarium ear rot for the most resistant hybrid pair in this study, RX697/RX697YG. Our observation that the nontransgenic hybrid RX697 had significantly lower severity of Fusarium ear rot than RX697YG is not understood. Since resistance to disease from hybrid pair RX697/RX697YG was not associated with resistance to insect damage imparted by Cry1Ab protein, RX697/RX697YG may have genes for resistance to insect damage or genes that affect disease development in the presence and absence of insect damage. Since rank order of all Bt and non-Bt hybrids were similar for fumonisin concentration in grain and severity of Fusarium ear rot, background or genotypic resistance to disease and insects serves as an important mechanism in the overall susceptibility of Bt and non-Bt hybrids to fumonisin concentration in grain and ear rot severity. This supports work by Magg et al. (2002), who hypothesized that mycotoxin concentration in grain may be largely independent of ECB feeding, and that ECB feeding may be less important than plant morphology and environment in influencing fungal colonization of plant tissues.

Although Cry1Ab protein was associated with a reduction in severity of Fusarium ear rot and less fumonisin for three of the four pairs of hybrids evaluated in this study, Cry1Ab protein was associated with fumonisin concentration in grain below FDA Guidance levels for only two of the hybrid pairs. Munkvold et al. (1999) also observed Bt hybrids with fumonisin concentration in grain above FDA Guidance levels in plots that had been naturally or manually infested with ECB.

Commercially available Bt transformation event MON-810 can be effective in indirectly reducing fumonisin concentration in corn grain by reducing damage from the predominant insect pest of corn in the USA, ECB (Mason et al., 1996, Ostlie et al., 1997). MON810 does not have the same effect when secondary insect pests, e.g., CEW, that are less sensitive to Cry1Ab protein are prominent. These results suggest that Bt transformation events like MON810 are a useful supplement to hybrid resistance to fumonisin contamination and Fusarium ear rot. However, insertion of the *cry1Ab* gene into moderately resistant or resistant hybrids may not reduce fumonisin concentration in grain or Fusarium ear rot below levels already achieved by hybrid genotype. Insertion of the *cry1Ab* gene into susceptible hybrids may be beneficial for reducing fumonisin concentration in grain.

Very little information is available on the effect of feeding damage from non-Lepidopteran insects on fumonisin concentration in grain of Bt hybrids. Additionally, very little information is available on nontransgenic sources of resistance to the production and/or accumulation of fumonisin in corn grain. Ear rot severity of ≤5% was associated with fumonisin at levels of concern (greater than 5 μg/g) for 26% of treatment means in plots that were naturally infected with endemic *Fusarium* spp. Correlation coefficients for ear rot severity and fumonisin concentration in grain were not significant for plants inoculated with *Fusarium* spp., but were significant for plants that were not inoculated with *Fusarium* spp. Five of the six isolates used in this study were chosen on the basis of their ability to produce fumonisins in grain under field conditions; therefore, the range of fumonisin concentration in grain was greater in inoculated plots than naturally infected plots. Severity of Fusarium ear rot also tended to be greater in inoculated plots (approximately 21% of treatment means with ≤5% ear rot) than in noninoculated plots (approximately 91% of treatment means with ≤5% ear rot). Plants that were not inoculated, however, had greater fumonisin concentration in grain than most reports of naturally infected grain sampled throughout the Midwest. A small number of treatment means with severe ear rot and high concentration of fumonisin in grain from plots that were not inoculated with *Fusarium* spp. may have contributed to the significant correlation value associated with this treatment. The lack of a significant correlation between ear rot severity and fumonisin concentration in grain from plots that were inoculated with *Fusarium* spp. is different from previous reports (Clements et al., 2002; Munkvold et al., 1999) and likely due to variation associated with main effects other than Fusarium treatment. Our results emphasize the need to select for low ear rot severity and a low fumonisin concentration in grain when breeding corn for disease resistance. Further research is needed to identify inbred sources of resistance to Fusarium ear rot and the production–accumulation of fumonisin in grain.

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