Effect of Bt-Corn Hybrids on Deoxynivalenol Content in Grain at Harvest

A. W. Schaafsma, D. C. Hooker, T. S. Baute, and L. Illincic-Tamburic, Ridgetown College, University of Guelph, Ontario, Canada

ABSTRACT


Concentrations of the mycotoxins deoxynivalenol (DON) and fumonisin B1 in grain were compared among Bt-transformed corn hybrids and their non-Bt isolines on 102 commercial corn fields across Ontario from 1996 to 1999. Intensities of naturally occurring populations of Ostrinia nubilalis were assessed from tunneling measurements in the stalks of non-Bt isolines in 1996 and 1997. Mean concentrations of fumonisin B1 across hybrids were <0.25 µg g⁻¹ in every year of the study. Relationships between the concentration of fumonisin B1 and intensity of O. nubilalis or with the use of Bt corn hybrids could not be determined because the concentrations of fumonisin B1 were below the lower limit of detection in most fields (<0.1 µg g⁻¹). However, DON was more prevalent with mean concentrations across fields from 0.42 µg g⁻¹ in 1997 to 1.12 µg g⁻¹ in 1999. The effect of Bt hybrids on reducing concentrations of DON was mainly dependent on the intensity of O. nubilalis in each field. Where a high intensity (stalk injury) of O. nubilalis was observed (>4 cm of tunnel per stalk in the non-Bt), the use of Bt hybrids reduced concentrations of DON by an average of 59% from concentrations in the non-Bt isolate. Where the intensity of O. nubilalis was low (<4 cm of tunneling per stalk), concentrations of DON were not different among Bt and non-Bt hybrids. Concentrations of DON were low and not different between events Bt11 and 176 among Bt hybrids. A quadratic relationship was developed showing that the concentration of DON increased with intensity of O. nubilalis feeding. This study cautiously supports the use of Bt corn to reduce the risk of high concentrations of DON at harvest in Ontario.

Several fungi are toxigenic in corn; mycotoxins produced from these fungi are a threat to livestock and human health (13). In the more southern regions of corn production in North America, aflatoxins produced by Aspergillus spp. are the dominant concern (21), while in the central regions of the continent, fumonisins produced by some Fusarium spp. of the Liseola taxonomic group are of greater concern (13). However, in the northern regions of North American corn production, Aspergillus does not occur on corn in the field (13) and fumonisins are rare and generally at very low concentrations (14). Fusarium graminearum occurs frequently in northern corn production regions that include eastern Canada, producing several trichothecene toxins and zearealenone (13). The most common and important mycotoxin occurring in corn in the north is deoxynivalenol (DON).

Fusarium fungi may gain entrance to corn ears mainly through the following: infections down the silk channel (22), systemic channels as the corn plants grow from seedlings (17), or wounds caused by insects (22). European corn borer (ECB), Ostrinia nubilalis Hübner, is the dominant insect that can wound corn ears in the central and northern production areas in North America. Relationships between insect injury in corn ears and fungal ear rot have been documented for several lepidopterous and coleopterous pests of corn (1,6,7,18,25). McMillian et al. (12) were among the first to show lower concentrations of aflatoxin with ECB resistance in corn in an ECB infestation. Dowd (7) and Dowd et al. (9) demonstrated that controlling chewing insects with insecticides has not reduced mycotoxigenic ear molds. The advent of transgenic ECB resistance in corn, with the expression of Bacillus thuringiensis (Bt) endotoxins, has led to observations that there was reduced Fusarium ear rot and symptomless kernel infection in Bt-transformed ECB-resistant corn ears compared with nontransformed corn ears (16,18), and lower concentrations of fumonisin produced in transformed ECB-resistant hybrids (8,15). In a review of the safety and advantages of Bt-transformed corn, Betz et al. (3) suggested reduced concentrations of fungal toxins as one benefit of growing Bt-transformed corn. However, Dowd (8) cautioned that concentrations of fumonisin may not be reduced in ECB-resistant corn under certain environmental conditions or if ear injury has been caused by other insects.

Relationships between intensities of ECB and DON and fumonisin B1 have not been reported for northern corn production regions. The objective of this paper was to investigate the effects of Bt corn hybrids on DON and fumonisin B1 at harvest, and to complement existing Bt-corn/mycotoxin information with data from the northern corn production area of North America. This paper is an extension of the work by Baute et al. (2), who showed that Bt corn in eastern Canada was economical only when ECB injury was high, and that the benefits were directly related to ECB control.

MATERIALS AND METHODS

Plot layout. Concentrations of DON and fumonisin were measured in samples of machine-harvested grain from adjacent strips that were planted to a Bt corn hybrid, the non-Bt isoline, and a non-Bt check hybrid. Seventeen commercial corn fields were sampled in 1996, 27 in 1997, 31 in 1998, and 27 in 1999, for a total of 102 pairwise comparisons between Bt corn hybrids and isolines. The purpose of the non-Bt check hybrids was to compare the advantages, if any, in the use of other non-Bt corn hybrids for reducing mycotoxins. The non-Bt check hybrids were selected from the Ontario Hybrid Corn Performance Trials (Ontario Corn Committee 1997 and 1998) on the basis of their agronomic adaptation to the region, high yield potential, and ability to resist stalk lodging. The fields selected in the survey were among several crop heat unit (CHU) [5] regions in each year, ranging from 2,600 to 3,200 CHU. The width of each strip varied according to the width of planting equipment used by each grower, which ranged from 4 to 12 rows wide. The Bt hybrids were either event 176 or Bt11 from Syngenta Seeds Inc. All corn hybrids were popular hybrids adapted to each region across Ontario, and they were harvested when moisture content of the grain was between 20 and 30%. These sites were also used for an ECB damage and yield performance study.

Insect assessment. The main purpose for the sites was to investigate the performance of Bt corn with respect to ECB injury and yield (2). Only two ECB injury assessments were made in each field, which consisted of stalk tunneling measurements. Injury assessments were not done on corn ears because of resource limitations; therefore, stalk tunneling was used as an index of ECB population intensity. The intensity of ECB infestation was assessed in only 35 of the fields during 1996 and 1997. Ten consecutive plants in
each of four segments were selected in the center rows of non-Bt strips. The first and last segments were at least 12 m from the front and rear of the field margins to avoid any edge effects; the center two segments were spaced evenly between the first and last segment (minimum space between segments in all fields was 15 m). The stalks of selected plants were split lengthwise, and the lengths of tunnels caused by ECB were measured. Intensity of ECB in a field was assumed “low” when an average of <4 cm of tunneling was measured per plant, and “high” when >4 cm of tunneling was measured per plant. No insecticides were applied to these fields. Ear damage caused by molds or insects other than ECB was not noted.

**Sampling and mycotoxin analysis.** The strips were harvested in field with commercial harvesting equipment between mid-October and the end of November. Approximately 2 kg of grain was obtained from each hybrid by removing small samples from the grain unloading auger of the combine. The grain samples were stored in burlap sacks and hung to dry at room temperature for about 3 weeks. The entire sample was ground coarsely through a No. 60 Grist Mill (C.S. Bell Co., Tiffin, OH), with the resulting meal mixed thoroughly by hand. A 250-g subsample of the meal was finely ground through a ROMER grinding/subsampling mill (Model 2A, Romer Labs, Inc., Union, MO). For DON analysis, a 20-g subsample was extracted from each hybrid by removing small samples from the grain unloading auger of the combine. The grain samples were stored in a 100-ml mixture of methanol and water (1:9). DON was quantified using competitive direct enzyme-linked immunosorbent assay (CD-ELISA) according to Schaafsma et al. (20) using the commercial preparation EZ-Quant DON Plate Kit (Beacon Analytical Systems, Inc., Scarborough, ME). This analytical procedure for DON has a lower limit of detection of 0.2 µg g⁻¹. For fumonisin B₁ analysis, a 50-g sample of finely ground flour was extracted in 100 ml of acetonitrile, 4% KCl (9:1), and quantified by thin layer chromatography according to Schaafsma et al. (20); this procedure for fumonisin has a lower limit of detection of 0.1 µg g⁻¹.

**Statistical analysis.** PROC UNIVARIATE (SAS Institute, Cary, NC) was used to test the plausibility of assumptions for ANOVA. DON and fumonisin values were transformed using ln(x + 0.1) to satisfy assumptions of normality. The transformed data were analyzed using PROC MIXED (SAS), with individual strips as the experimental unit. All non-Bt check hybrids were removed from subsequent analyses if they were deemed not different (P = 0.05) from the non-Bt isolines. Data were combined across years when interactions with year were not significant (P > 0.05). The year, corn hybrid type (i.e., Bt versus non-Bt), and categorical intensities of ECB (i.e., low, or <4 cm of tunneling per plant versus high or >4 cm of tunneling per plant) were considered fixed effects. “Field within year” was the blocking term in the RANDOM statement in PROC MIXED. Relationships among DON and ECB tunnel lengths were investigated using PROC REG. Dummy variables were used in PROC REG (4) to test the effect of Bt versus non-Bt hybrids on this relationship.

**Weather data estimates.** A partial account of year-to-year variability in concentrations of DON and fumonisin was attempted using estimates of daily rainfall and air temperatures across field locations. Weather variables were obtained for each field using data from the nearest Environment Canada weather station within 50 km of each field location.

**RESULTS**

No relationships were found between the concentration of fumonisin B₁ and ECB injury, or with the use of Bt corn hybrids. In fact, only a small proportion of grain samples tested positive for fumonisin B₁ (i.e., >0.1 µg g⁻¹); 6% of the samples were fumonisin B₁-positive in 1996, 56% in 1997, 26% in 1998, and 44% in 1999.

Mean concentrations of fumonisin B₁ in grain samples across all hybrids were <0.1 µg g⁻¹ in 1996, 0.23 µg g⁻¹ in 1997, 0.11 µg g⁻¹ in 1998, and 0.25 µg g⁻¹ in 1999. The maximum concentration of fumonisin B₁ recorded in each year was 1.0 µg g⁻¹ in 1996, 2.1 µg g⁻¹ in 1997, 3.0 µg g⁻¹ in 1998, and 4.0 µg g⁻¹ in 1999.

DON was more prevalent than fumonisin in grain corn samples; the proportion of samples that tested positive for DON (>0.2 µg g⁻¹) varied from 47 to 95% depending on the hybrid type (transformed versus nontransformed) and year (Table 1). The non-Bt checks were removed from subsequent analyses comparing Bt hybrids and their non-Bt isolines because concentrations of DON did not differ between the non-Bt checks and non-Bt isolines when averaged across years (P = 0.34; data not shown).

Concentrations of DON were different in Bt versus non-Bt hybrids; however, the magnitude of the response interacted with year, which resulted in a significant corn hybrid type by year interaction (P = 0.0138; Table 2). Depending on the year between 1996 and 1998, the use of Bt hybrids reduced concentrations of DON from 30 to 64% (0.0001 < P < 0.02) compared with their non-Bt counterparts. Mean DON concentrations in 1999 were the same among Bt and non-Bt hybrids. Across all years, concentrations of DON were 40% lower (P < 0.0001) in Bt hybrids than in non-Bt isolines. Furthermore, we did not detect a difference in concentrations of DON among Bt hybrids containing event 176 compared with those containing Br11 in any of the 4 years (P > 0.41; data not shown).

Although Bt hybrids reduced concentrations of DON compared with non-Bt isolines, the magnitude of the reduction was dependent on the intensity of ECB activity as estimated by mean tunnel lengths. Data were combined across 1996 and 1997 because ECB injury among non-Bt hybrids was similar in both years (mean tunnel

### Table 1. Mean deoxynivalenol (DON) concentrations across corn Bt and isoflavone hybrids from farm strip trials across Ontario from 1996 to 1999

<table>
<thead>
<tr>
<th>Source</th>
<th>NDF</th>
<th>DDF</th>
<th>F</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>3</td>
<td>96</td>
<td>6.16</td>
<td>0.0007</td>
</tr>
<tr>
<td>Corn type</td>
<td>1</td>
<td>99</td>
<td>32.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year × corn type</td>
<td>3</td>
<td>99</td>
<td>3.73</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

a DON concentrations are presented as detransformed means from ln(DON + 0.1).

b Lower detection limit for DON is 0.2 µg g⁻¹.

c NDF = degrees of freedom in the numerator; DDF = degrees of freedom in the denominator.

d Bt or non-Bt isoflavone.

### Table 2. Analysis of variance for year and corn Bt versus non-Bt hybrid effects on transformed deoxynivalenol (DON) concentrations from 1996 to 1999

<table>
<thead>
<tr>
<th>Source</th>
<th>NDF</th>
<th>DDF</th>
<th>F</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>3</td>
<td>96</td>
<td>6.16</td>
<td>0.0007</td>
</tr>
<tr>
<td>Corn type</td>
<td>1</td>
<td>99</td>
<td>32.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year × corn type</td>
<td>3</td>
<td>99</td>
<td>3.73</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

a Concentrations of DON were transformed using ln(DON + 0.1) before analysis.

b Effects of source variables were fixed; field within year was the random effect in SAS PROC MIXED.

c NDF = degrees of freedom in the numerator; DDF = degrees of freedom in the denominator.

d Bt or non-Bt isoflavone.
lengths of 4.9 cm per stalk in 1996 compared with 4.3 cm in 1997, plus the response in DON to ECB injury did not interact with year (P = 0.22; data not shown). In fields where ECB injury was “low” (as assessed by <4 cm of tunneling per plant) in non-Bt hybrids, concentrations of DON in Bt versus non-Bt hybrids were similar (P = 0.1088; Table 3); mean concentrations of DON in Bt hybrids averaged 0.36 µg g⁻¹ compared with 0.54 µg g⁻¹ in non-Bt isolines. However, in fields where ECB injury was “high” (injury of >4 cm of tunneling per plant), concentrations of DON were reduced by 59% in Bt hybrids (P = 0.0011; Table 3); Bt hybrids in those fields averaged 0.39 µg g⁻¹ of DON compared with 0.95 µg g⁻¹ in non-Bt isolines (Table 3).

A quadratic relationship was identified between a measurement of ECB intensity (i.e., assessed by ECB tunnel length in stalks) and the DON content at harvest:

\[
\text{DON} = \exp(-1.298 + 0.321 \times \text{ECBTUNNEL} - 0.016 \times (\text{ECBTUNNEL}^2) - 0.1)
\]

where DON is the concentration of deoxynivalenol (µg g⁻¹) and ECBTUNNEL is the average tunnel length (cm) per stalk from ECB feeding. Even though only 18% of the variability in DON was explained by the model, the parameter estimates for the prediction were all highly significant with P < 0.0005 (Table 4). The relationship of DON and ECB intensity was not different among non-Bt and Bt hybrids (P > 0.50; data not shown). In other words, neither the proportion of variability explained by the relationship nor the parameter estimates changed when data from Bt hybrids were removed from the equation. Thus, data from all hybrids were combined for the development of one equation. In general, DON content increased as the injury from ECB feeding intensified.

It was difficult to associate mean air temperatures, rainfall amounts, and frequencies of rainfall to mean DON contents across the study area in every year. Concentrations of DON were the lowest in 1997, which was also the year with the lowest rainfall and coolest temperatures during the period from 9 July to 19 August (data not shown).

**DISCUSSION**

The fact that no relationship was found between intensities of ECB or the use of Bt hybrids and concentrations of fumonisin at harvest in our study does not contradict the findings of Munkvold et al. (15). Concentrations of fumonisin in the majority of samples were near or below the lower detection limit between 1996 and 1999 because there were no excessively hot and dry conditions during the grain fill period (data not shown). In fact, hot and dry conditions, which favor fumonisin B₁ production in corn (14), are rare in the northern corn production areas of North America. Other researchers have found ECB damage in corn to increase *Fusarium moniliforme* and fumonisin concentrations in grain at harvest (10). However, our findings suggest that the advantage of Bt corn for fumonisin management may only extend to environments where the risk of ECB injury is high and the environment is conducive to fumonisin-producing *Fusaria*.

The use of Bt corn reduced mean concentrations of DON by more than 59% compared with those in non-Bt hybrids when the intensity of ECB was high (≥4 cm on tunnels in the stalks of non-Bt plants). Other studies have also found Bt corn to reduce DON (18). We assume that all non-Bt hybrids had a similar response because there was no evidence to suggest that concentrations of DON were better (i.e., lower) in popular non-Bt hybrids compared with the non-Bt hybrids (i.e., lower) in non-Bt hybrids because there was no evidence to suggest that concentrations of DON were better in hybrids transformed with *Bt* (19). Therefore, we recommend with caution the use of Bt corn to reduce the potential concentrations of DON contamination because protection against ECB injury to corn ears will have no impact on the development of silk channel infections for *F. graminearum* or other *Fusarium* species.

No differences in concentrations of DON could be detected between event Bt11 and 176 among Bt hybrids. We expected that the concentration of DON may be lower in hybrids transformed with Bt11 because the Cry IA (b) protein is expressed in all tissue including tissue in the ear; event 176 only expresses the protein in green leaf tissue and not in the ear or kernel (23). On the other hand, other researchers have observed reduced mycotoxins with 176 compared with non-Bt hybrids because larvae feeding on the leaves or husks may not survive long enough to injure ears sufficiently for infection by *Fusaria* (G. P. Munkvold, Iowa State University, personal communication). Indeed, it is possible that differences between the events may become more evident under severe epidemics.

To avoid ill-founded expectations, however, recommendations for using Bt corn to

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**Table 3.** Mean deoxynivalenol (DON) concentrations in grain samples of corn as affected by intensities of European corn borer (ECB) in Bt and non-Bt corn hybrids from farm field strip trials from 1996 to 1999

<table>
<thead>
<tr>
<th>Corn type/contrasts</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DONb µg g⁻¹</td>
<td>No. paired observations</td>
</tr>
<tr>
<td>Corn type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Bt isolate</td>
<td>0.54</td>
<td>16</td>
</tr>
<tr>
<td>Bt</td>
<td>0.36</td>
<td>39</td>
</tr>
<tr>
<td>Mean within intensity of ECB</td>
<td>0.44</td>
<td>60</td>
</tr>
<tr>
<td>Contrasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolate vs. Bt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECB Injury: low vs. high</td>
<td>0.2263</td>
<td></td>
</tr>
<tr>
<td>Low intensities of ECB injury: isolate vs. Bt</td>
<td>0.1088</td>
<td></td>
</tr>
<tr>
<td>High intensities of ECB injury: isolate vs. Bt</td>
<td>0.0011</td>
<td></td>
</tr>
</tbody>
</table>

* Fields were classed with low ECB intensities when tunnels of ≤4 cm per stalk were observed in each non-Bt corn isolate.

* DON concentrations are presented as detransformed means from ln(DON + 0.1).

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**Table 4.** Regression statistics for predicting deoxynivalenol (DON) in grain corn from observations of European corn borer (ECB) tunnel lengths from farm strip trials in 1996 and 1997

<table>
<thead>
<tr>
<th>Parameterb</th>
<th>Parameter estimatec</th>
<th>SE</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.298</td>
<td>0.1688</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ECBTUNNEL</td>
<td>0.321</td>
<td>0.0706</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ECBTUNNEL²</td>
<td>-0.016</td>
<td>0.0044</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

* Adjusted model R² = 0.18 (P < 0.0001); mean square error (MSE) = 1.349 with 112 df.

* ECBTUNNEL = average length (cm) of ECB burrow in corn stalks from all *Bt* and non-*Bt* hybrids.

* DON concentrations were transformed using ln(DON + 0.1) before analysis.
reduce concentrations of DON at harvest must be accompanied with caution that this approach may have serious, inherent limitations. For example, rainfall and temperature at silking and during the period of ear fill for corn have been considered more important than insect injury in ear rot epidemics caused by *Fusarium* spp. in Ontario (14,19,24); different conditions cause shifts in the *Fusarium* spp. involved. Furthermore, DON was correlated to ECB injury, suggesting that the intensity of ECB can also be important for ear rot epidemics in the northern part of the corn production belt of North America. In Iowa, ECB injury is a dominant factor in *Fusarium* ear rot and the development of fumonisin (15). Therefore, this study supports the use of *Bt* corn to reduce the risk of potentially high concentrations of DON at harvest. This is particularly important if concentrations of mycotoxins cause concern for swine production (13).

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**