Fungal Species Composition in Maize Stalks in Relation to European Corn Borer Injury and Transgenic Insect Protection

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ABSTRACT

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The maize stalk rot complex is comprised of several fungal pathogens, including *Gibberella zeae*, *Colletotrichum graminicola*, *Stenocarpella maydis*, and several members of the genus *Fusarium*. The European corn borer (ECB) (*Ostrinia nubilalis*) can contribute to stalk rot development by creating entry wounds and by serving as a vector of some stalk rot pathogens, particularly *Fusarium verticillioides*. Transgenic insect protection of maize hybrids with insecticidal proteins derived from *Bacillus thuringiensis* greatly reduces ECB injury and may therefore alter the species composition and diversity of the stalk rot complex. Field experiments were conducted in 1998, 1999, and 2000 to compare the species composition and diversity of fungi infecting stalks of Bt and non-Bt maize hybrids. Hybrids representing five Bt types (or "events") and their near-isogenic non-Bt counterparts were subjected to manual and natural infestations with ECB larvae. Stalk tissue samples were cultured to determine fungal species composition. At least one species was isolated from nearly every stalk and from both diseased and symptomless tissues. *G. zeae* was the most common species in 1998 and 1999, but *C. graminicola* was most common in 2000. The mean proportions of stalks infected with *F. verticillioides*, *F. proliferatum*, and *F. subglutinans* were significantly lower in Bt hybrids than in non-Bt hybrids in 2 of the 3 years. Conversely, the mean proportion of stalks infected with *G. zeae* was higher in some Bt hybrids than their non-Bt counterparts in two of the three years. *F. verticillioides* was more likely to be isolated from ECB-injured tissue, whereas *G. zeae* and *C. graminicola* were more likely to be isolated from tissue not associated with ECB injury. The overall species diversity of the stalk rot complex was lower in some Bt hybrids compared with their non-Bt counterparts in 1998 and 1999. ECB activity appeared to alter fungal species composition in stalks, reflecting the association between ECB injury and specific stalk rot pathogens, particularly *F. verticillioides*. The species composition of fungi infecting stalks of Bt hybrids differed from that of non-Bt hybrids, but the implications of this result are not yet clear.

Additional keywords: corn, GMO, *Zea mays*

Numerous fungi have been implicated in stalk rot of maize, a disease of worldwide importance whose effects include reduced yields, premature death of plants, and lodged stalks (15). The predominant members of the stalk rot complex in the United States are *Gibberella zeae* (Schwein.) Petch, *Colletotrichum graminicola* (Ces.) G. W. Wils., *Stenocarpella maydis* (Berk.) Sutton, and members of the genus *Fusarium*, including *F. verticillioides* (Sacc.) Nirenberg, *F. proliferatum* (T. Matsushima)

Nirenberg, and *F. subglutinans* (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun, and Marasas (17,30). *Bipolaris zeicola* (G. L. Stout) Shoemaker frequently is isolated from stalks but is considered a minor stalk rot pathogen (30).

Although many of these pathogens occur simultaneously in maize plants, a core

complex of several dominant pathogens often is associated with stalk rot in a given region (11,16,35). The relative prevalence of species in this complex, as well as the overall species diversity, can change both within the growing season and gradually over time as new cultural practices and hybrids are introduced, more virulent biotypes of the pathogens appear or disappear, and weather patterns change (4,16,18,34). The predominance of *S. maydis*, for example, has fluctuated greatly over the past several decades, while *C. graminicola* has steadily increased in importance (14,16).

The complex of fungal pathogens found in maize stalks resembles a sophisticated ecological community in many ways. There is evidence that competition occurs among stalk rot pathogens, particularly between *G. zeae* and *Fusarium* spp. (18,19,24). Interactions among competing fungi, insect pests, and maize stalks result in distinct mycofloral communities whose composition changes with the plant growth stage and seasonal weather fluctuations, approximating a kind of ecological succession (39).

These shifts in species composition can have implications for the type and severity of the resulting stalk rot, because stalk rot pathogens are known to vary in pathogenicity. *C. graminicola* and *G. zeae* generally are believed to cause a more severe decay than other species (11,18). In addition to this interspecific variation, strains within species vary in their ability to cause stalk rot (11,38). There are conflicting reports of the pathogenicity of *F. verticillioides* in maize stalk tissue because this fungus is associated with both stalk rot

Table 1. Maize hybrid representatives of five Bt transformation types (events) and their near-isogenic, non-Bt counterparts used in field experiments conducted near Ames, IA in 1998, 1999, and 2000

Bt event	1998	1999	2000
176 (KnockOut) ^a	NK (Ciba) MAX21	NK (Ciba) MAX21	NK (Ciba) MAX454
Non-Bt	NK (Ciba) 4349	NK (Ciba) 4394	NK (Ciba) 4494
Bt11 (Yieldgard) ^b	NK 4640Bt	NK 7070Bt	NK 7070Bt
Non-Bt	NK 4640	NK 7070	NK 7070
MON810 (Yieldgard) ^c	Pioneer 34R06	Pioneer 34R06	Pioneer 34R06
Non-Bt	Pioneer 3489	Pioneer 3489	Pioneer 3489
DBT418 (BtXtra) ^d	DK 566Bt	DK 595Bt	DK 595Bt
Non-Bt	DK 566	DK 595	DK 595
CBH351 (StarLink) ^e	Garst 8600Bt	Garst 8539 BLT	Garst 8539 BLT
Non-Bt	Garst 8600	Garst 8539 IT	Garst 8539 IT

^a Ciba Seeds, Greensboro, NC.

^b Northrup King Co., Golden Valley, MN.

^c Monsanto Co., St. Louis, MO.

^d DeKalb Plant Genetics, DeKalb, IL.

^e Garst Seeds, Inc., Slater, IA.

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and symptomless infection of stalk tissue (11,25,38).

Stalk rot fungi are aided in the colonization and decay of maize stalks by environmental stresses that heighten the plant's susceptibility to infection, particularly following anthesis when carbohydrate shortages can occur (9). Injury due to the feeding of the European corn borer (ECB), *Ostrinia nubilalis* Hübner, can significantly stress maize plants and encourage stalk rot development in several ways (6,7,15). By tunneling into stalk tissue, larvae create entry points for fungal pathogens. Extensive tunneling compromises maize plants physiologically and can hasten the development of stalk rot symptoms. Finally, ECB larvae can serve as vectors of some stalk rot fungi, particularly *F. verticillioides* (31).

Because of the association between ECB injury and certain stalk rot pathogens, measures taken to control ECB infestations could affect the species composition and diversity of the stalk rot complex. ECB tunneling is only one of several infection pathways for stalk rot pathogens. Given our knowledge of fungus–insect dynamics, it is possible that the fungal community in ECB-injured stalk tissue differs from other rotted tissue in both composition and overall diversity. The concept of species diversity has numerous definitions and applications in ecological investigations. Several mathematical indices have been developed to gauge species diversity (8,12,29), and some have been used in plant pathology research. The Simpson diversity index (29) is suitable for assessing both species richness (total number) and species evenness (relative abundance).

In the past, ECB management tools were limited to foliar applications of insecticides, which are frequently ineffective due to difficulty in scouting and timing treatments to control the larvae before they bore into plants. In response to the need for improved ECB control, transgenic Bt hybrids were developed. Bt hybrids are created by the insertion into the maize genome of a gene sequence derived from the bacterium *Bacillus thuringiensis* that codes for production of a lepidopteran-specific insecticidal protein (5,13). Six different transformation types, defined by the specific Bt protein and the promoter sequence used, have been registered by the U.S. Environmental Protection Agency for commercial use.

Bt hybrids have been widely adopted by U.S. farmers since their introduction in 1996 for the protection they provide against ECB injury. Area planted with Bt hybrids increased from 1.4% of all maize in 1996 to 25.9% in 1999; farmers planted 22% Bt hybrids in 2002 (33). As controversy over the use of these and other transgenic crops has escalated, so has the need for further investigation of how Bt hybrids will influence the com-

Table 2. *P* values for the effects of brand, Bt transformation (Bt), manual or natural European corn borer infestation (Trt), and interactions among these variables on the incidence of selected fungal pathogens in stalks of maize from field experiments conducted near Ames, IA in 1998, 1999, and 2000^a

Year	<i>Gibberella zeae</i>	<i>Fusarium verticillioides</i>	<i>Fusarium proliferatum</i>	<i>Fusarium subglutinans</i>	<i>Colletotrichum graminicola</i>
1998					
Brand	<0.01	0.23	0.18	0.44	<0.01
Bt	0.48	<0.01	<0.01	0.55	0.58
Trt	<0.01	<0.01	0.01	0.88	0.86
Brand x Bt	<0.01	0.98	0.17	0.51	0.96
Brand x Trt	0.07	0.67	0.21	0.47	0.22
Trt x Bt	0.15	0.03	0.90	0.24	0.73
1999					
Brand	<0.01	0.46	0.97	0.18	0.02
Bt	<0.01	0.38	0.41	0.04	0.78
Trt	0.01	<0.01	0.06	0.05	0.75
Brand x Bt	0.20	0.65	0.60	0.11	0.10
Brand x Trt	0.09	0.85	0.99	0.33	0.49
Trt x Bt	0.75	0.06	0.32	0.13	0.94
2000					
Brand	<0.01	0.21	0.20	0.02	<0.01
Bt	<0.01	<0.01	0.20	<0.01	0.18
Trt	0.83	<0.01	0.66	0.41	0.89
Brand X Bt	<0.01	0.34	0.68	0.90	0.25
Brand X Trt	0.96	0.30	0.62	0.21	0.33
Trt X Bt	0.40	0.89	0.03	0.62	<0.01

^a Brand is the company of origin of each hybrid. Each brand represents a different Bt type. Manual infestation consisted of applying 50 neonatal larvae to the whorl (at growth stage V8 to V9) or ear-leaf axil (at stage R1) of each plant.

Fig. 1. Mean proportions of stalks infected with selected stalk rot pathogens in Bt and non-Bt maize hybrids in field experiments in central Iowa in 1998, 1999, and 2000. *G.* = *Gibberella*, *F.* = *Fusarium*, and *C.* = *Colletotrichum*. Values are based on eight replicates of four stalks each. Asterisks indicate significant differences ($P \leq 0.05$) between Bt and non-Bt counterparts, according to planned orthogonal contrasts.

plex interactions among insect pests, diseases, and the environment. Previous studies examining the potential role of Bt hybrids in maize stalk rot management have not had consistent results, although there is some evidence that certain Bt transformation types may be more effective than others in reducing stalk rot (3,10,20,26). These experiments were based on visual assessments of stalk rot symptoms; to date, there have been no attempts to examine the effects of Bt hybrids on the fungal communities responsible for stalk decay. The objectives of this study were to assess the influence of ECB injury on fungal species composition in maize stalks, and to determine whether the species composition and diversity of the stalk rot complex are altered by the use of Bt hybrids.

MATERIALS AND METHODS

Field experiments were conducted in 1998 through 2000 in the vicinity of Ames, IA. Each year, five Bt hybrids and their near-isogenic non-Bt counterparts (Table

1) were evaluated in field plots arranged in a randomized complete block design with eight replications. Fields were located at the Iowa State University Johnson farm (Story County) in 1998, Woodruff farm (Boone County) in 1999, and the Agronomy and Engineering Research Farm (Boone County) in 2000. All fields were in a maize-soybean rotation and were field cultivated prior to planting between mid- and late May. Standard fertilization and weed management practices were applied in all fields. Plots consisted of four rows 5.33 m in length planted at a density of 123,000 seeds/ha and thinned to approximately 69,000 plants/ha, with plants spaced 15 to 20 cm apart to standardize competition with neighboring plants. Each plot was subjected to one of two treatments: (i) manual infestation, consisting of two ECB infestations, one at growth stage V8 to V9 (mid-whorl) and one at growth stage R1 (silk stage) (27); and (ii) natural infestation (no manual infestations). Larvae for the manual infestations were reared at the United States

Department of Agriculture-Agricultural Research Service Corn Insects Laboratory in Ames and applied to plants using an applicator that delivered approximately 50 neonatal larvae mixed with ground maize cobs to the whorl at V8 to V9 and to the axil of the ear shank at R1 (21). Each of the infestations was applied twice within a 1-week period. No inoculation of stalk rot pathogens was made, because these fungi are present naturally in crop residue and soil.

Stalks were sampled at physiological maturity, as indicated by formation of a black layer on the kernel. Sampling consisted of systematic selection of four stalks (every fifth or sixth stalk) from an inner row of each four-row plot. Stalks were cut off at the base with a pruning shears, trimmed back four nodes above the ear node, and split longitudinally.

Tissue cross sections approximately 5 cm in length were excised aseptically from each stalk. If disintegrated pith was observed, the decayed portion of the stalk was sampled. If no pith disintegration was present, a section of discolored tissue was sampled. In the absence of either pith disintegration or stalk discoloration, a section of healthy tissue was sampled. If ECB injury had occurred, tissue associated with the injury also was sampled and classified as disintegrated, discolored, or symptomless. Tissue samples were subsequently divided into four pieces, which were surface disinfested for 1 min in a 0.5% NaOCl solution, placed onto potato dextrose agar (Difco, Becton Dickinson and Co., Sparks, MD) and incubated in the dark at 25°C until recognizable fungal colonies were formed (approximately 4 days). The colonies were transferred to carnation leaf agar for identification. *Fusarium* spp. were identified based on morphology, as described by Nelson et al (22) or Nirenberg (23). Non-*Fusarium* stalk rot pathogens such as *C. graminicola*, *Bipolaris zeicola*, and *S. maydis* also were identified by morphology (1,32). The fungi detected can all grow rapidly on potato dextrose agar; however, slower-growing fungi may have escaped detection.

The SAS statistical package (Statistical Analysis System; SAS Institute, Inc., Cary, NC) was used for all analyses. Analyses of variance were conducted on the mean proportions of stalk tissue samples per plot from which a given species was isolated. Four-way analysis of variance was carried out to determine the main effects of hybrid brand (company of origin of each hybrid), presence of Bt proteins (Bt), manual or natural infestation (treatment), and replicate block, as well as interactions among these variables. Each hybrid brand contained a different Bt type; therefore, the brand by Bt interaction was interpreted to represent differential effects among Bt types. Analyses of variance also were conducted to assess whether mean proportions

Fig. 2. Mean proportions of stalks infected with *Gibberella zeae* in tissue samples collected from five Bt maize hybrids and their non-Bt counterparts in field experiments in central Iowa 1998, 1999, and 2000. Each Bt transformation type is represented by a different hybrid brand. Values are based on eight replicates of four stalks each. Asterisks indicate significant differences ($P \leq 0.05$) between Bt and non-Bt counterparts, according to planned orthogonal contrasts.

of stalks infected by each fungal species differed significantly among tissues that were disintegrated, discolored, or symptomless, and between tissues that were or were not associated with ECB injury. The Simpson Diversity Index, which is a measure of both species evenness (relative abundance of each species) and, to a lesser extent, species richness (total number of species), was calculated for Bt transformation types and their non-Bt counterparts (8,12,29). Mean separation was performed using orthogonal contrast analysis or Duncan's multiple range test.

RESULTS

Stalk rot pathogens were isolated from nearly every stalk sampled. The pathogens isolated in highest proportions were *G. zeae*, *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, and *C. graminicola*. *G. zeae* was the most common species in 1998 and 1999, isolated from 87 and 70% of sampled stalks, respectively. *C. graminicola* was the most common in 2000, isolated from 64% of sampled stalks. Less prevalent fungi recovered included *F. sporotrichioides* (3-year mean, 16%), *F. semitectum* (8%), *F. acuminatum* (10%), *F. equiseti* (8%), *S. maydis* (5%), and *B. zeicola* (14%).

The composition of fungal stalk rot species differed among hybrid brands, between Bt and non-Bt hybrids, between manual and natural ECB infestation, and among years (Table 2). The mean proportion of stalks infected with *F. verticillioides* was significantly lower in Bt hybrids than in non-Bt hybrids in 1998 (manual infestation) and 2000 (Fig. 1). Similarly, the proportions of stalks infected with *F. proliferatum* and *F. subglutinans* were significantly lower in Bt hybrids compared with non-Bt hybrids in 2 of the 3 years, depending on the infestation treatment (Fig. 1). In 1999 and 2000, the proportions of stalks infected with *G. zeae* were significantly higher in Bt hybrids compared with non-Bt hybrids (Fig. 1), but these differences were dependent upon hybrid brand (and therefore Bt type) in 2000 (Fig. 2). The proportion of stalks infected with *C. graminicola* was significantly higher in Bt hybrids than in non-Bt hybrids in 2000 (manual infestation; Fig. 1).

Comparisons of species composition among three types of stalk tissue (disintegrated, discolored, and symptomless) were carried out in 1999 and 2000 (Fig. 3). Disintegrated tissue had significantly higher proportions of most stalk rot pathogens than other tissue types in both years. There were significantly higher proportions of *G. zeae* in disintegrated stalk tissue compared with discolored tissue in both 1999 and 2000. Proportions of *C. graminicola* in disintegrated and discolored tissue were significantly higher compared with symptomless tissue in 2000. Also in 2000, the

proportions of *F. subglutinans* and *F. acuminatum* in disintegrated tissue were significantly higher compared with symptomless tissue. In 1999, the proportions of *F. verticillioides* and *F. subgluti-*

nans were significantly higher in disintegrated compared with discolored tissue, and were higher for *F. verticillioides* in discolored tissue compared with symptomless tissue.

Fig. 3. Mean proportions of maize stalks infected with selected stalk rot pathogens in tissue samples showing either discoloration, pith disintegration, or lack of symptoms (healthy) in field experiments in central Iowa in 1999 and 2000. *G.* = *Gibberella*, *F.* = *Fusarium*, *B.* = *Bipolaris*, *S.* = *Stenocarpella*, and *C.* = *Colletotrichum*. Values are based on eight replicates of four stalks each. Within each species, bars with the same letter indicate values that are significantly different ($P \leq 0.05$), according to Duncan's multiple range test.

Species composition of the stalk rot complex also was influenced by whether or not the stalk tissue sample was associated with ECB injury, a distinction recorded in 1999 and 2000 (Fig. 4). The proportion of

stalks infected with *F. verticillioides* was higher in ECB-associated stalk rot compared with stalk rot not associated with ECB injury in both 1999 and 2000, whereas the proportion of *G. zeae* was

lower in ECB-associated stalk rot in 2000. Proportions of *F. proliferatum* and *F. subglutinans* were higher in ECB-associated stalk rot compared with non-ECB-associated stalk rot in 2000, whereas the proportion of *F. subglutinans* was lower in ECB-associated stalk rot in 1999. Proportions of *B. zeicola*, *S. maydis*, and *C. graminicola* were lower in ECB-associated rot in 2000.

Certain Bt transformation types (176 and CBH 351) had significantly reduced species diversity indices compared with their non-Bt counterparts, depending on the year (Table 3).

DISCUSSION

The management of maize stalk rots is complicated by the little-understood dynamics of the fungal populations responsible for stalk decay. Previous work on the role of environmental stresses such as leaf disease, overpopulation, and insect injury in stalk rot development have tended to focus on visual symptoms of decay, with little investigation of how these factors affect the complex fungal communities found in both diseased and healthy stalk tissue. We would be remiss to continue overlooking these fluctuations in stalk rot species composition, because stalk rot pathogens differ in dispersal mechanisms, environmental preferences, aggressiveness, mycotoxin production, resistance to plant defenses, and other traits of epidemiological consequence (4,16,35,37).

Bt hybrids provide a unique tool for examining the species composition of the stalk rot complex in relation to ECB injury, one of the stresses known to contribute to stalk rot development. Studies demonstrating a vector relationship between ECB larvae and several stalk rot pathogens provided a rationale for our hypothesis that Bt hybrids might have reduced proportions of these pathogens in their stalk tissue (31). The results of the present study substantiate this hypothesis with respect to some species, because the relative proportions of *F. verticillioides*, one of the species transmitted by ECB larvae, were significantly reduced in Bt hybrids compared to non-Bt hybrids in 2 out of 3 years. A similar but less-pronounced pattern was observed in the reduced proportion of Bt stalks infected with *F. proliferatum* and *F. subglutinans*, species whose vector relationship with ECB larvae is suspected but not confirmed (31). These reductions in the occurrence of *Fusarium* spp. in Bt hybrids may account for the decreased species diversity observed in some Bt hybrids compared with their non-Bt counterparts.

A comparison of the fungal complex isolated from rotted stalk tissue both with and without ECB injury proved useful in the interpretation of species composition variation between Bt and non-Bt hybrids. In each of the 2 years this comparison was made, the proportion of stalks infected

Fig. 4. Mean proportions of maize stalks infected with selected stalk rot pathogens in stalk rot not associated with ECB injury and stalk rot associated with ECB injury in field experiments in central Iowa in 1998, 1999, and 2000. *G.* = *Gibberella*, *F.* = *Fusarium*, *B.* = *Bipolaris*, *S.* = *Stenocarpella*, and *C.* = *Colletotrichum*. Values are based on eight replicates of four stalks each. Asterisks indicate significant differences ($P \leq 0.05$) between Bt and non-Bt counterparts, according to planned orthogonal contrasts.

with *F. verticillioides* was significantly higher in the ECB injury-associated stalk rot than stalk rot not associated with ECB injury. This is consistent with observations of Koehler (16), who reported that *F. verticillioides* (reported as *F. moniliforme*) was the most common fungus recovered from stalk injured by ECB, and that infections of *S. maydis* (reported as *Diplodia zeae*) and *G. zeae* were not increased by stalk injury. This result reflects the established vector relationship between *F. verticillioides* and ECB larvae (31) and may explain why Bt hybrids, which have greatly reduced levels of ECB injury, also had significantly lower proportions of *F. verticillioides* than non-Bt hybrids in 2 out of 3 years. It also is possible that ECB injury predisposed the plants specifically to *F. verticillioides* by providing entry wounds (6,7) and physiological stress associated with stalk injury (9). Alternatively, it is possible that stalks infected by *F. verticillioides* were more attractive to ECB adults or that ECB larvae were more successful in stalks already infected by *F. verticillioides*. In West Africa, maize stalks inoculated with *F. verticillioides* had a higher incidence of infestation by *Eldana saccharina*, an insect pest in the same family (Pyralidae) as ECB (28). The authors proposed several explanations, including the production of volatiles by the fungus, which have been shown to attract some insects (2).

The significantly higher proportion of stalks infected with *G. zeae* in Bt hybrids versus non-Bt hybrids in 1999 and 2000 also may be explained by the ECB-associated versus non-ECB-associated stalk rot comparison. Stalk rot that was not associated with ECB injury (the type usually found in Bt hybrids) had significantly higher proportions of *G. zeae* in 2000 than ECB-associated stalk rot. Non-ECB-associated stalk rot also had significantly higher proportions of *S. maydis*, *B. zeicola*, and *C. graminicola* in 2000 compared with ECB-associated stalk rot. It should be noted that, in 2000, the effects of Bt transformation on *G. zeae* were dependent upon hybrid brand and that, in 1998, two Bt transformation types showed the opposite

effect of lower proportions of *G. zeae* compared with their non-Bt counterparts. During the 3 years of our study, there was a pattern of above-average spring rainfall, below-average late-summer rainfall, and above-average late-summer temperatures. The magnitude of these departures from normal differed among seasons, with 2000 having the most extreme conditions. The variable weather among seasons seems to have influenced the results; *G. zeae* infection apparently was not favored by the very dry late-season conditions in 2000. Overall, the effect of Bt transformation on *G. zeae* occurrence in maize stalks was not as clear as the effect on *F. verticillioides*.

Proportions of stalks infected by *G. zeae* and *C. graminicola* differed among hybrid brands. This probably reflects differences in genotype susceptibility among the hybrids, independent of the presence or absence of Bt transformation. Each brand also represents a different Bt type; therefore, the significant interaction for *G. zeae* infection in 2000 suggests that the effect of Bt transformation on infection by this fungus depends on the Bt type.

Further research is necessary to explain the increased proportions of *G. zeae* and non-*Fusarium* stalk rot pathogens in tissue unaffected by ECB injury. It has been speculated, however, that interspecific competition may occur in stalk tissue (18,36). Reduced infection of Bt maize stalks by *F. verticillioides* may provide an unoccupied niche that can be filled by other pathogens. The results of this project suggest that *Fusarium* spp. compete with other fungal pathogens in maize stalks, and that the shifts in species composition we observed in Bt hybrids are a reflection of the effects of ECB on fungal interactions in stalk tissue. The consequences of such a shift in species composition are not yet known.

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Table 3. Values for Simpson Diversity Index for maize stalk tissues sampled from field experiments conducted near Ames, IA, in 1998, 1999, and 2000^a

Bt type		1998	1999	2000
176	Bt	0.67	0.81	0.77
	Non-Bt	0.76*	0.85	0.81
BT11	Bt	0.82	0.85	0.80
	Non-Bt	0.83	0.85	0.83
MON810	Bt	0.71	0.86	0.86
	Non-Bt	0.72	0.85	0.86
DBT418	Bt	0.72	0.81	0.86
	Non-Bt	0.75	0.86*	0.83
CBH351	Bt	0.67	0.84	0.86
	Non-Bt	0.77*	0.83	0.84

^a Simpson index $D = 1 - [\sum n_i(n_i - 1)]/[N(N - 1)]$; S = number of species, n_i = number of individuals belonging to species, N = total number of individuals. Values followed by an asterisk are significantly different from non-Bt counterparts at $P \leq 0.05$.

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