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Full Length Research Paper

Interaction between maize phenology and transgenic maize hybrids on stalk rots following European corn borer infestation

Motshwari Obopile[1]* Ronald B. Hammond[1] and Pierce A. Paul[2]

[1]Department of Entomology, Ohio Agricultural Research and Development Canter, The Ohio State University, 1680 Madison Ave, Wooster, Ohio 44691, USA.
[2]Department of Plant Pathology, Ohio Agricultural Research and Development Canter, The Ohio State University, 1680 Madison Ave, Wooster, Ohio 44691, USA.

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Field studies were carried out at Hoytville and Wooster, Ohio, USA from 2006 to 2008 to determine the influence of planting date and transgenic Bt maize on incidence and severity of stalk rots of maize. Transgenic and non-transgenic Bt maize hybrids with different maturity ratings were planted on late April/early May, mid-May and early June each year. The incidence and severity of the maize stalk rot complex were compared among planting dates and between Bt and non-Bt genotypes of different relative maturities. Significant linear correlations between European corn borer (*Ostrinia nubilalis* Hub.) injury to stalk and stalk rots were observed in 2006 and 2007, but not in 2008. We observed significant reduction in stalk discoloration and incidence of maize stalk rot on Bt hybrids compared with non-Bt genotypes. Short season hybrids had significantly lower stalk rots than full season hybrids. The benefit of using Bt hybrids to reduce stalk rots was more evident as planting was delayed than early planting.

Key words: Transgenic hybrids, relative maturity, planting date, stalk rots.

INTRODUCTION

Stalk rots are significant problems on maize (*Zea mays* L.) in many parts of the world (Nordby et al., 2006; Gatch et al., 2002), with stalk rots often resulting in annual losses estimated at 5 to 10% in Ohio, USA (OSU Extension,1998). Yield losses also occur due to reduced grain fill caused by pathogens invading maize stalks prior to physiological maturity (Koehler, 1960). Destruction of the pith tissue also predisposes the plants to lodging. The most common fungal pathogens that cause stalk rot in the United States are *Gibberella zeae* (Schwein.) Petch (Anamoprph: *Fusarium graminearum* (Schwabe), *Colletotrichum graminicola* (Ces.) G. W. Wils., *Stenocarpella maydis* (Berk.) Sutton, and other members of the genus *Fusarium*, including *F. moniliforme* (=*F. verticillioides* (Sacc.) Nirenberg, *F. proliferatum* (T. Matsushima) Nirenberg, and *F. subglutinans* (Wollenweb. and Reinking) (Kommendahl and Windels, 1981; Smith and White, 1988).

Stalk rot fungi are aided in the colonization and decay of maize stalks by low moisture and high temperature stresses that heighten the plant’s susceptibility to infection, particularly following anthesis when carbohydrate shortages can occur (Dodd, 1980). Injury due to feeding by European corn borer (ECB) (*Ostrinia nubilalis* Hub) is one stress that can promote the progression of stalk rot (White 1999), contributing to the development of stalk rot when larvae tunnel into stalks and create points for fungal invasion. European corn borer can also serve as a vector of fungal pathogens. ECB larval feeding also causes physiological stress that predisposes the maize to stalk rot development (Bergstrom and Nicholson, 1999; Jarvis et al., 1984).

Often, the planting of field maize is delayed beyond the optimum late April to early May time frame by excessive soil moisture (Nielsen et al., 2002; Jarvis et al., 1986). Occasionally, maize fields planted during the optimum

*Corresponding author. E-mail: mobopile@gmail.com.

Table 1. Planting and harvest dates at Wooster and Hoytville, Ohio from 2006 to 2008.

Location		Planting dates		
Wooster	**Year**	**Early**	**Middle**	**Late**
	2006	4 May	25 May	7 June
	2007	7 May	23 May	8 June
	2008	1 May	21 May	9 June
Hoytville	2006	28 April	24 May	7 June
	2007	7 May	23 May	8 June
	2008	*np*	22 May	9 June
			Harvest dates	
Wooster	2006	10 October	16 October	23 October
	2007	11 October	11 October	29 October
	2008	8 October	8 October	21 October
Hoytville	2006	18 October	24 October	27 October
	2007	10 October	10 October	17 October
	2008	*np*	14 October	14 October

np =Not planted due to continuous high soil moisture during late April until mid May 2008.

time frame require replanting at later dates after low moisture stress and high temperature or when pests cause excessive plant mortality (White, 1999). One of the more important factors growers need to consider if planting late is hybrid maturity. Short season hybrids suitable for a shortened growing season have been developed and can be planted during late planting conditions. Short season hybrids may facilitate earlier harvest and reduce frost risk and minimize drying costs when planted late. Late planting subjects' maize plants to pest and disease pressure (Wiatrak et al., 2005). Date of planting is an important factor in developing disease management strategies because it influences occurrence of diseases (Paul and Munkvold, 2004; Wiatrak et al., 2005). This study investigated the influence of planting date in combination with transgenic maize on stalk rots following ECB injury. The study had the following questions in mind; (a) does planting transgenic hybrids resistant to European corn borer significantly reduce incidences of stalk rots in late planted crop? (b) Does hybrid maturity influences severity and incidences of stalk rots s? (c) is there an interaction between planting date and Bt maize on incidence and severity of stalk rots?

MATERIALS AND METHODS

The experiment was carried out at the Northwest Agricultural Research Station near Hoytville (41° 12´ N, 83° 45´ W) and at the Wooster Campus (40° 46´N, 81° 55´W) of The Ohio State University in Ohio from 2006 to 2008. The land was prepared by ploughing in the spring followed by disc harrowing to break up the sod. The fields were previously planted with a crop of soybean. Individual plots were 10 m long and 12 rows wide. Maize, planted with a 4-row planter, had row spacing of 0.76 m and a seeding rate of 79 000 seeds ha^{-1}. The maize seeds planted were hybrids commonly used by farmers that had comparable agronomic characteristics. Experimental design was a 3 × 4 treatment factorial replicated 4 times in a randomized complete block arranged in split plot layout. The first factor (main plot) was planting date, randomized within each replication. There were three planting dates (early, middle and late), targeting late April/ early May, late May and early June each year (Table 1). The second factor (subplot) was four maize hybrids (Table 2) assigned randomly within each planting date. The hybrids represented two maturity groups, short and full season hybrids. Each maturity group was then represented by two Bt hybrids containing Bt endotoxin gene that confers resistance against European corn borer and two non Bt hybrids. All seeds were obtained from Dekalb, and were treated with clothianidin (Poncho 250™) (Gustafson LLC, Dallas TX) at rate of 0.25 mg a.i. per seed to control secondary soil pests.

Stalk rot sampling

Maize stalks were sampled at physiological maturity (R6) as indicated by formation of a black layer on the kernel. A total of 10 stalks were sampled from rows three and ten to avoid border effect which left the middle rows for yield collection. The stalks were split longitudinally and evaluated for the length (cm) of stalk discoloration. The incidence (presence or absence) of pith discoloration was determined by counting the number of stalks infected which was then expressed as the percentage of total stalks sampled. The isolation of fungal pathogens was done by cutting four pieces from one side of the stalk and then surface sterilized for two minutes with 10% sodium hypochlorite (NaOCl) solution. The stalk pieces were then rinsed with deionized water and excess water removed by squeezing them between double paper towels (Gatch and Munkvold, 2002). The four pieces from each stalk were then placed in water agar and incubated in the dark at 25°C until recognizable fungal colonies were formed after approximately three days. The colonies were transferred to Komada and fresh potato

Table 2. Maize hybrids planted at Wooster and Hoytville, Ohio from 2006 to 2008.

2006	Bt Event	Maturity days	Maturity GDDs
Dekalb DKC50-20	Mon810	101	2528
Dekalb DKC51-45	Non Bt	101	2530
Dekalb DKC63-81	Mon810	113	2790
Dekalb DKC63-80	Non Bt	113	2790
2007 and 2008			
Dekalb DKC52-63	Mon810	102	2540
Dekalb DKC52-62	Non Bt	102	2540
Dekalb DKC63-81	Mon810	113	2790
Dekalb DKC63-80	Non Bt	113	2790

dextrose agar (Difco, Becton Dickinson and Co., Sparks, MD). The identification of fungal pathogens to the genus level was based on morphological characteristics.

Data analysis

Data on stalk rots were analyzed using mixed model analysis (PROC MIXED) (SAS Institute, 2003). Planting dates and hybrids were considered fixed variables, while replications and interactions were assumed to be random effects. The interaction between planting date and hybrids was tested. To compare disease severity, incidence and ECB damage between long vs. short season and transgenic vs. non transgenic hybrids, planned orthogonal contrasts were performed using contrast option within PROC MIXED. Multiple comparisons were performed on least square means of the fixed effects using the PDIFF option of the LSMEANS statement in SAS. All the comparisons were based on Fisher's protected least significant difference and considered significant at P = 0.05. Correlation analysis (PROC CORR) was performed to determine the relationship between European corn borer damage and stalk rot symptoms. Percentages were arcsine transformed to stabilize variance and length of discoloration was transformed to log (×+1).

RESULTS

Stalk discoloration

Length of stalk discoloration and incidence of stalk rot (percentage of infected stalks) were affected by hybrid treatments and planting date, but these effects varied with year and location (Figures 1 and 2). Stalk discoloration and incidence of stalk rot were significantly correlated to stalk tunnelling, percentage of stalk injured, and number of European corn borer larvae per plant (not reported here); however, they were not consistent from year to year (Table 3). In 2006, a significant planting date × hybrid interaction was observed at each location (Hoytville: $F_{6, 27} = 3.22$; P = 0.016, Wooster: $F_{6, 27} = 2.61$; P = 0.033). Hybrid treatment significantly affected the length of stalk discoloration at both locations (Hoytville: $F_{3, 27} = 66.47$; P <0.0001, Wooster: $F_{3, 27} = 13.31$; P <0.0001). A significant reduction in stalk discoloration occurred on Bt hybrids compared with non-Bt hybrids at Hoytville ($F_{1, 27} = 50.86$; P <0.0001) and Wooster ($F_{1, 27} = 15.12$; P = 0.0004). Multiple comparisons showed significantly longer discoloration on the short season non-Bt hybrid compared to the full season non-Bt hybrid when maize was planted in mid May and early June at Hoytville (Figure 1A). At Wooster, significant increase in stalk discoloration was observed on the short season non-Bt hybrid than full season non-Bt hybrid only when maize was planted on 24 May (Figure 1B).

In 2007, significant planting date × hybrid interaction effects were observed at Hoytville ($F_{6, 35} = 4.71$; P = 0.001) and Wooster ($F_{6, 22} = 9.38$; P <0.0001). Significantly less stalk discoloration was observed on early planted maize compared to late plantings at Hoytville and at Wooster (Figure 1C and D). The length of stalk discoloration was affected by hybrid treatments at each location (Hoytville: $F_{3, 22}$; = 26.09; P<0.0001, Wooster: $F_{3, 35} = 11.16$; P <0.0001). A significant decrease in stalk discoloration was observed on Bt maize than non-Bt hybrids at Hoytville ($F_{1, 22} = 44.68$; P = <0.0001) and at Wooster ($F_{1, 35} = 27.68$; P <0.0001). Multiple comparisons of least square means indicated no significant differences among hybrids on early planted maize at both locations (Figure 1B). When maize was planted in middle and late plantings, the short season non-Bt hybrid showed a significant increase in stalk discoloration compared to full season non-Bt hybrid at Hoytville and Wooster (Figure 1C and D).

In 2008, a significant planting date × hybrid interaction was not observed at Hoytville ($F_{3, 12} = 1.14$ P = 0.3708), but at Wooster ($F_{6, 27} = 2.59$; P = 0.041). Delaying planting resulted in significant reduction in stalk discoloration on maize planted on 9 June at both Hoytville and at Wooster (Figure 1E and F). Stalk discoloration varied among hybrid treatments at Hoytville ($F_{3, 12} = 11.93$; P = 0.0007) and at Wooster ($F_{3, 27} = 45.25$; P <0.0001). Stalk discoloration did not differ between Bt and non-Bt hybrids at Hoytville ($F_{1, 12} = 2.95$; P = 0.112), but a significant decrease was observed on Bt hybrids at Wooster ($F_{1, 27} = 17.69$ P = 0.0003). At both locations, a significant reduction in stalk discoloration was observed on full season hybrids compared to short season ones

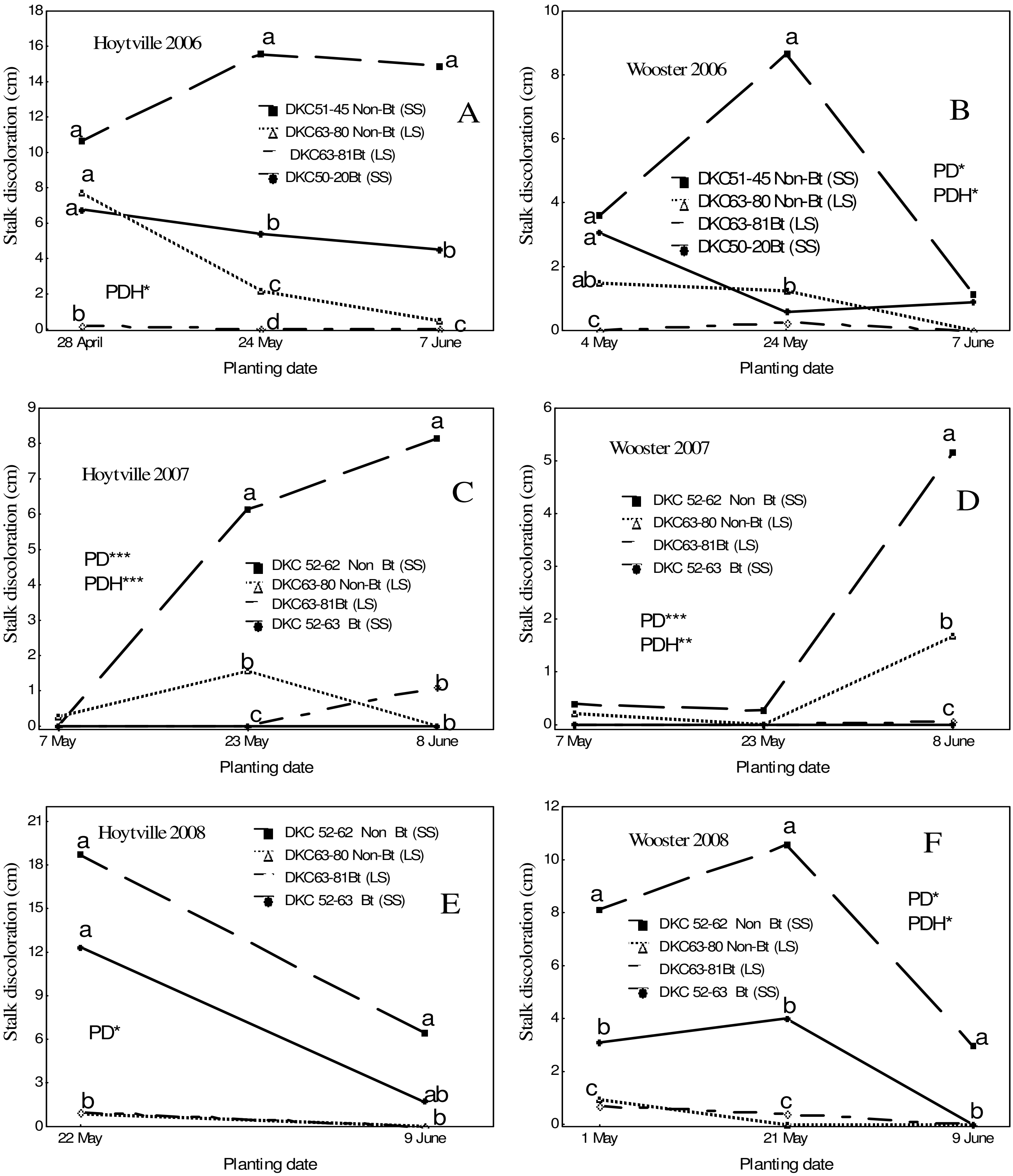

Figure 1. Mean length of discoloration per stalk among hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, α = 0.05). PD = planting date; PDH = planting date x hybrid interaction;*, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. SS = short season, LS= full season.

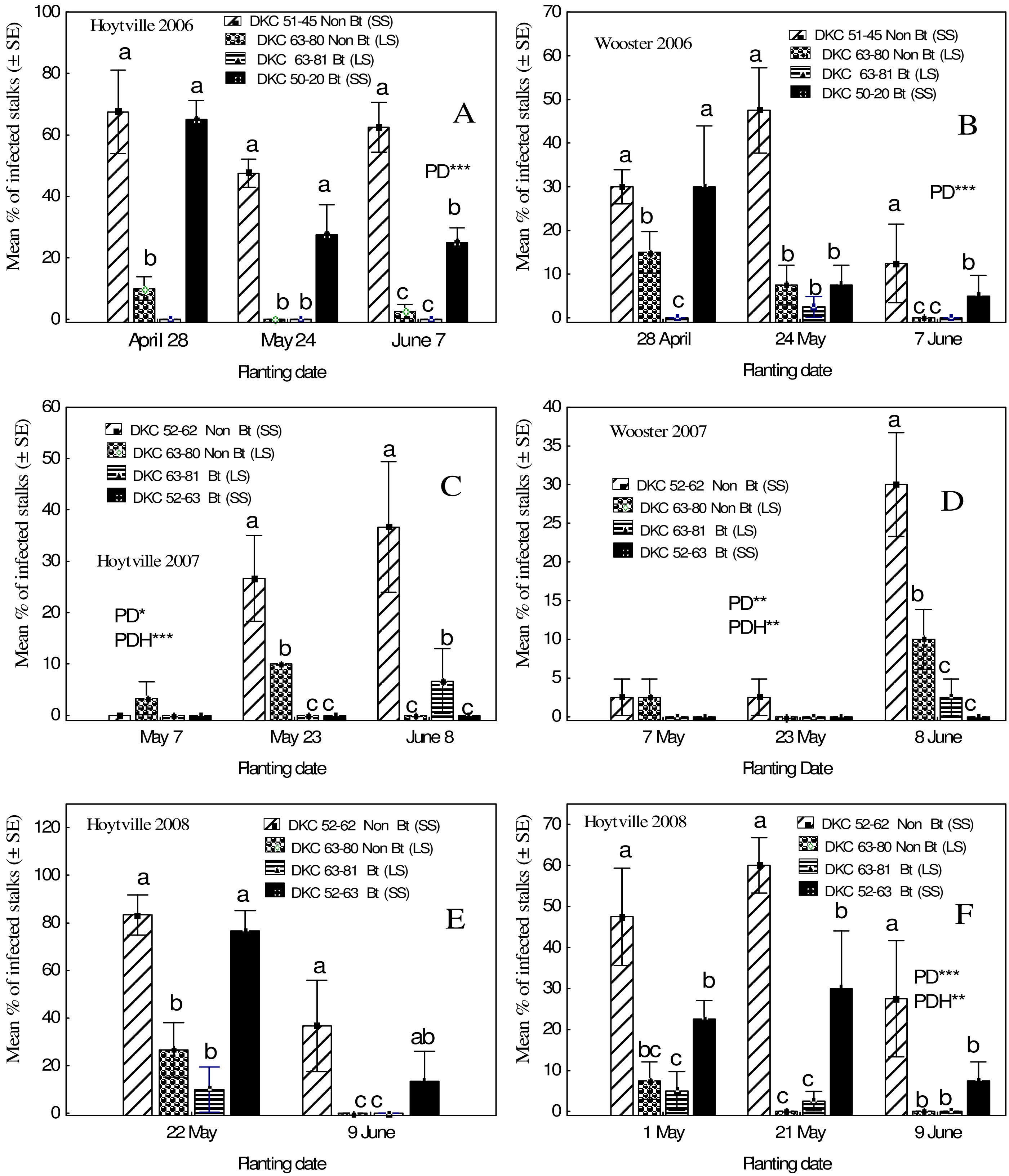

Figure 2. Mean percentage of stalks with discoloration symptoms from hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, α = 0.05). PD = planting date; PDH = planting date x hybrid interaction;*, ** and *** denote significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001 respectively. SS = short season, LS= full season.

Table 3. Linear correlation coefficients between European corn borer injury and maize stalk rot symptoms at Hoytville and Wooster in 2006, 2007 and 2008.

Location	Year	Variable [‡]	Stalk discoloration (cm)	Stalk discoloration (%)
Hoytville	2006	Tunneling	0.37**	0.21
		% tunneled stalk	0.42**	0.23
		No. larvae	0.47**	0.30*
	2007	Tunneling	0.72***	0.72**
		% tunneled stalk	0.59**	0.60**
		No. larvae	0.52**	0.50**
		No tunnels[§]	0.72***	0.70***
	2008	Tunneling	0.17	0.18
		% tunneled stalk	0.18	0.19
		No. larvae	0.05	0.11
		No tunnels	0.12	0.13
Wooster	2006	Tunneling	0.42*	0.33*
		% tunneled stalk	0.40**	0.43**
		No. larvae	0.38**	0.17
	2007	Tunneling	0.51**	0.49***
		% tunneled stalk	0.39*	0.36*
		No. larvae	0.30*	0.27
		No tunnels	0.42**	0.40*
	2008	Tunneling	0.15	0.07
		% tunneled stalk	0.2	0.16
		No. larvae	0.06	0.01
		No tunnels	0.14	0.08

*, ** and *** denote significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001 respectively. [§]Data not collected in 2006. [‡] European corn borer data not presented here.

(Hoytville: $F_{1, 12}$ = 32.34, P = 0.0001, Wooster: $F_{1, 27}$ = 99.12 P<0.0001). A significant increase in stalk discoloration on short season hybrid DKC 52 -62 was consistent among the three planting dates at Wooster (Figure 1F).

Incidence of stalk rot

In 2006, no planting date × hybrid interaction with respect to incidence of stalk rot (% of infected plants) was observed at either location (Hoytville: $F_{6, 33}$ = 2.16; P = 0.072; Wooster: $F_{6, 36}$ = 1.91; P = 0.1057). Incidence of stalk rot was significantly higher on earlier planted maize than late planted maize at Hoytville ($F_{2, 33}$ = 10.67; P = 0.0003) and also at Wooster in 2006 ($F_{2, 36}$ = 7.49; P = 0.002) (Figure 2A and B). The incidence varied among hybrid treatments at each location (Hoytville: $F_{3, 33}$ = 92.80; P<0.0001; Wooster: $F_{3, 36}$ = 11.76; P<0.0001). This difference was due to a significant reduction in stalk rot incidence found on Bt hybrid compared with non-Bt hybrids at Hoytville ($F_{1, 33}$ = 16.60 P = 0.0003) and Wooster ($F_{1, 36}$ = 12.17; P = 0.001). The incidence was also significantly reduced on full season hybrids compared with short season hybrids at Hoytville ($F_{1, 33}$ = 259.85 P<0.0001) and at Wooster ($F_{1, 36}$ = 22.54; P<0.0001).

In 2007, significant planting date × hybrid interaction effects were observed at both locations (Hoytville: $F_{6, 18}$ = 6.81; P = 0.0007; Wooster: $F_{6, 35}$ = 4.39; P = 0.0021). The interactions showed that significantly less stalk rot incidence was obtained on early planted maize than middle and late plantings at both locations (Figure 2C and D). Hybrid treatment significantly affected the incidence of stalk rot at both locations (Hoytville: $F_{3, 18}$ = 16.83; P <0.0001; Wooster: $F_{3, 35}$ = 11.09; P <0.0001). Planting Bt hybrids significantly reduced incidence of stalk rot compared with non-By hybrids at Hoytville ($F_{1, 18}$= 32.64; P <0.0001) and at Wooster ($F_{1, 35}$ = 24.94 P <0.0001). At both locations, significant planting date ×

hybrid interaction effects were observed (Hoytville: $F_{6, 18}$ = 6.81; P = 0.0007; Wooster: $F_{6, 35}$ = 4.39; P = 0.0021). Examination of planting date × hybrid interaction showed that the short season non-Bt hybrid had significantly longer discoloration compared to full season non-Bt hybrid on later planted maize than early planted maize at both locations (Figure 2C and D).

In 2008, planting date × hybrid interaction was not significant at Hoytville ($F_{3, 12}$ = 1.92; P = 0.1799), but significant effects occurred at Wooster ($F_{6, 33}$ = 3.52; P = 0.0084). Significantly lower percentages of infected stalks were obtained on late planted maize than earlier plantings at both Hoytville ($F_{1, 4}$ = 10.80; P = 0.030) and Wooster (Figure 2E and F). Stalk rot incidence varied among hybrids at Hoytville ($F_{3, 12}$ = 12.37; P = 0.0006) and at Wooster ($F_{3, 33}$ = 29.97; P <0.0001). There was no significant difference in stalk rot incidence between Bt and non-Bt hybrids at Hoytville ($F_{1, 12}$ = 3.24; = 0.0973), but a significant reduction was observed on Bt hybrids at Wooster ($F_{1, 33}$ = 13.48; P = 0.0008). Compared with short season hybrids, full season hybrids showed significant reduction in stalk rot incidence at both locations (Hoytville: $F_{1, 12}$ = 33.86; P <0.0001; Wooster: $F_{1, 33}$ = 62.96; P<0.0001). Full season hybrids consistently showed low incidence among the three planting dates (Figure 2E and F).

DISCUSSION

The influence of planting date on stalk rot varied among years and locations during this study. In 2007, stalk rot incidence and discoloration significantly increased as planting was delayed at both Hoytville and Wooster (Figure 1C and D). In three out of six comparisons, an increase in stalk rot was observed on maize planted mid May but significantly declined on late planted maize (Figure 1B, E and F). A similar trend was observed on the percentage of plants that were infected with stalk rot pathogens (Figure 2). Variability in the effects of planting date on stalk rots between years and locations in this study illustrate the difficulties of explaining or predicting the distribution of stalk rot of maize across location and time (Dodd, 1980). While late planting is known to subject maize plants to pest and disease pressure (Wiatrak et al., 2005; Jarvis et al., 1986), delayed planting has also been reported to decrease the incidence of stalk rot (Pappelis and Boone, 1966; Whitney and Mortimore, 1957). The stalk rot incidence is mostly related to the growth stage of the maize plant (Whitney and Mortimore, 1957) and the interactions of biotic and abiotic stresses that predispose the plants to infection, particularly following anthesis when carbohydrate shortages can occur (Dodd, 1980; Smith and White, 1988; Sobek and Munkvold, 1999).

One of the most important biotic factors that predispose maize plants to infection by stalk rot pathogens is the infestation by European corn borer larvae (Chiang and Wilcoxson, 1961). Because of the association between European corn borer injury and fungal stalk rot, management of this insect is recommended as one component of an integrated stalk rot management strategy (Munkvold, 1996). Transgenic field maize has been genetically engineered to express an insecticidal protein from *Bacillus thuringiensis* (Bt) to prevent damage by stalk-boring pests, in particular European corn borer, southwestern corn borer, and southern cornstalk borer (Koziel et al., 1993). Gatch et al. (2002) reported a significant reduction in stalk rot on Bt hybrids compared to their non Bt isolines, but the overall effect of Bt maize on stalk rot occurrence was variable among Bt hybrid types. In our study, we included three sequential plantings of short and full season hybrids, each maturity represented by a Bt and non-Bt hybrid. Significant correlations between European corn borer injury and stalk rot symptoms occurred, indicating that European corn borer significantly contributed to occurrence of the disease observed. When stalk rots were higher on late planted maize (in 2007) at both locations, the correlation coefficients were higher because tunnelling also increased on late planted maize in that year.

Apart from 2008 at Hoytville, we observed a significant reduction in stalk discoloration and incidence of maize stalk on Bt hybrids compared with non-Bt genotypes. We also observed a significant reduction in stalk rot on full season hybrids compared with short season hybrids. Early studies by Whitney and Mortimore (1957) reported that early maturity hybrids of maize became infested before late maturing ones. They suggested that although root and stalk rot pathogens may be present in roots, stalk rotting does not occur until the host reaches physiological maturity. The mechanism to explain the differences in stalk rot between full season and short season hybrids was beyond the scope of this research because apart from injury by European corn borer, we did not quantify other stress factors that predispose plants to stalk rot pathogens. Stalk injury by European corn borer did not show any significant reduction on full season hybrids compared with short season hybrids (not reported here), and therefore, is unlikely to have significantly contributed to the differences in stalk rot between hybrid maturity groups.

The significant reduction in stalk rot on Bt hybrids could be caused by factors other than the Bt gene. The correlations between ECB tunnelling and stalk rot symptoms were significantly positive in two out of three years, and in some cases low. The most likely factor that could influence the results of this study would be the complex interaction between stalk rot and the environment (Dodd, 1980). In the absence of insect damage, there are still many plant stresses that contribute to stalk rot development, and they vary unpredictably between years and locations (Gatch et al., 2002). In 2006, many areas in Ohio experienced an extended period of dry weather during the grain fill period

Table 4. Monthly rainfall totals (mm) from May to September at three locations from 2006 to 2008.

Environment location	Year	May	June	July	August	September	Total
	2006	148.2	103.6	164.8	32.1	72.8	521.5
Wooster	2007	67.5	50.3	147.7	121.3	55.5	442.3
	2008	67.9	147.1	131.1	32.4	72.6	451.1
	2006	146.4	109.2	147.4	56.4	94.8	554.2
Hoytville	2007	39.8	39.3	60.9	255	53.9	448.9
	2008	107.2	119.7	100.8	6.4	102.4	436.5
	2006	71.6	68.4	90.5	114.2	133.2	477.9
South Charleston	2007	326.6	74.3	62.2	81.5	137.9	682.5
	2008	87.5	189.1	102.1	63.1	36.4	478.2

Table 5. Monthly average air temperatures (Celsius) from May to September at three locations from 2006 to 2008.

Environment location	Year	May	June	July	August	September
	2006	14.62	19.00	22.92	22.16	16.11
Wooster	2007	17.08	20.37	20.83	22.55	18.25
	2008	13.51	20.76	22.15	20.08	18.05
	2006	15.52	20.01	23.34	22.01	16.26
Hoytville	2007	17.81	22.02	21.78	22.83	19.32
	2008	14.61	22.30	23.98	22.33	19.97
	2006	15.51	20.06	23.40	22.52	16.34
South Charleston	2007	18.95	21.86	21.29	24.10	19.92
	2008	15.01	21.82	22.61	21.23	19.66

(Tables 4 and 5) therefore predisposing plants to increased infection by stalk rot pathogens compared with most years of the study (Figure 1A and B). Albeit these complicating factors, our results showed that Bt maize hybrids have the potential to contribute to stalk rot management especially when European corn borer tunnelling is higher and planting is delayed.

This research has shown that transgenic maize with the Bt gene consistently reduced incidence and infection of stalks by fungal pathogens associated with stalk rots of maize compared with non-Bt hybrids. Transgenic Bt hybrids significantly reduced stalk injury by European corn borer, thus hindering an important pathway for infection by fungal pathogen complex associated with stalk rots. When the interaction between planting date and hybrid treatment was significant, the benefit of using Bt hybrids to reduce stalk rots was more evident as planting was delayed. Reduction in stalk rot on Bt maize is important because yield losses are known to occur due to reduced grain fill caused by pathogens invading maize stalks prior to physiological maturity (Koehler, 1960).

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