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Original Research

Effects of planting date and transgenic maize hybrids on Fusarium ear rot severity at Hoytville and Wooster, Ohio, USA:

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ABSTRACT

A three year study was conducted at Hoytville and Wooster, Ohio from 2006 to 2008 to find out how sequential planting of transgenic and non-transgenic maize influence Fusarium ear rot of maize. Maize hybrids with different maturities (short vs. long season), with and without *Bacillus thuringiensis* Berliner (Bt) gene were planted on different dates in late April/early May, late May and early June each year and the severity of ear rot caused by Fusarium fungal species were compared among planting dates and between maize genotypes. The study also investigated the association between European corn borer and Fusarium ear rot. A significant effect of planting date ($P < 0.05$) and the interaction with hybrid ($P < 0.05$) on Fusarium ear rot severity was only observed at Wooster in 2008. Transgenic Bt maize had significantly ($P \leq 0.05$) lower severity of Fusarium ear rot than non-Bt hybrids in most cases during this study. A significant effect of hybrid maturity was only observed at Hoytville ($P \leq 0.05$) in 2007 where higher severity was observed on late planted maize. The damage to maize kernels caused by European corn borer was significantly correlated ($r^2 = 0.29$, $P \leq 0.01$, to 0.43, $P \leq 0.001$) with severity of Fusarium ear rot at both sites and in all years except in 2008 at Hoytville. The study showed that using Bt hybrids which vastly reduce kernel injury leads to reduced Fusarium ear rot severity.

Keywords *Bacillus thuringiensis*, relative maturity, *Ostrinia nubilalis,* Bt genotype

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INTRODUCTION

Maize ear rots are considered a major constraint to maize (*Zea mays* L.) production in many parts of the world (Nordby *et al.,* 2007). One of the most common ear or kernel rots is caused by *Fusarium* species (J. Sheldon) (Kommedahl and Windels, 1981). Kernel infection by *Fusarium* species can reduce grain yield and quality, and results in the accumulation of mycotoxin in grain (Munkvold, 2003). The pathogen can enter the maize kernels through wounds created by insect feeding (Schulthess *et al.,* 2002) or by the growth of mycelium from spores germinating on the silks. Injuries to plants caused by insects such as the European corn borer (*Ostrinia nubilalis* Hub.) are often the initial infection sites for *Fusarium* species. Yield loss attributed to European corn borer (ECB) is often due in part to subsequent fungal decay of tissue injured by larvae (Blandino *et al*., 2008). Control of European corn borer using transgenic maize containing an insecticidal protein from Bacillus thuringiensis (Berliner) has been shown to reduce the amount of injury to ears, and subsequently lower the severity and incidence of ear rots (Munkvold, 2003).

Date of planting is an important factor in developing disease management strategies because it influences occurrence of diseases (Wiatrak *et al*., 2005). In this study the influence of planting date in combination with transgenic maize on severity of Fusarium ear rot following European corn borer injury was investigated.

MATERIALS AND METHODS

The field experiments were conducted at the Northwest Agricultural Research Station near Hoytville (41º 12´ N, 83º 45´ W) and at the Wooster Campus (40º 46´ N, 81º 55´ W) of The Ohio State University in Ohio, USA from 2006 - 2008. The fields used were previously planted with a crop of soybean for rotation purposes. 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. The experimental design was a 3 x 4 treatment factorial replicated four times in a randomized complete block arranged in a split plot layout. The first factor (main plot) was planting date, randomized within each replication. Maize crops were planted on three planting dates targeting late April/early May, late May and early June of each year (Table 1). The second factor (subplot) was four maize hybrids (Table 2) assigned randomly within planting dates. The hybrids represented two maturity groups; a short and a long season hybrid. Each maturity group was then represented by two Bt hybrids containing Bt endotoxin gene that confers resistance against European corn borer as well as two non-Bt hybrids. All seeds were obtained from Dekalb, and were treated with clothianidin (Poncho 250™) (Gustafson LLC, Dallas TX) at a rate of 0.25 mg a.i. per seed to control secondary soil pests.

Fusarium ear rot sampling

When maize had reached physiological maturity, individual ears were collected from 10 randomly selected plants (five each from row 3 and 10), husked, and visually evaluated for fungal ear rot pathogens. The number of ears damaged by European corn borer was expressed as a percentage of the ten ears sampled per plot. Samples of kernels were cultured in appropriate media and pathogens isolated to confirm the symptoms. This was done by randomly collecting twenty five kernels with ear rot symptoms which were surface-sterilized for three minutes in a 10% solution of sodium hypochlorite. After removing excess water, kernels were then cultured on Komada and fresh potato dextrose agar, incubated for five to eight days, and then observed for the presence of fungal pathogens. Fungal colonies were identified by conidial morphology to genus level. Ear rot severity was calculated as the mean number of kernels with symptoms of Fusarium ear rot (Munkvold, 2003).

Statistical analysis

Data on Fusarium ear rot 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 between long vs. short season and transgenic vs. non transgenic hybrids, planned orthogonal contrasts were performed using the 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 ear damage and ear rot severity. Percentages were arcsine transformed to stabilise variance.

RESULTS

Maize ear damage by European corn borer

In 2006, the percentage of ears damaged by European corn borer larvae was significantly lower (P = 0.005) on late planted maize than earlier plantings at Hoytville (Table 3 and Figure 1A) but no such significance occurred at Wooster (Table 3). At each location, the percentage of damaged ears in Bt maize hybrids was significantly lower (Table 3) than in non-Bt maize hybrids. An interaction between planting date and hybrid was detected at Hoytville (P= 0.036) but not at Wooster (Table 3). A significant (P = 0.005) increase in the percentage of damaged ears was obtained on short season non-Bt hybrids at Wooster (Table 3 and Figure 1B) but not at Hoytville.

In 2007, the percentage of damaged ears was significantly higher on late planted maize at both sites (Table 3). The percentage of damaged ears was also impacted significantly by the interaction between planting date and hybrid, transgenic maize and hybrid maturity (Table 3). The non-Bt hybrids had a significantly higher percentage of damaged ears than Bt hybrids at both locations (Table 3). At Hoytville, a significant increase (P = 0.0014) in ear damage occurred on the short season non-Bt hybrid compared to long season non-Bt hybrid with early and middle plantings (Fig. 1C).

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

Location	Year	Early	Middle	Late
Planting dates				
Wooster	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.

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

[¥]*Growing degree days*

Table 3. ANOVA for the effect of planting date, hybrid maturity and Bt hybrids on damage to maize kernels by European corn borer

Year	Location	Effects	*Df*	*F*	*P*
2006	Hoytville	Planting date	2, 33	6.44	0.005
		Hybrid	3, 33	61.88	<0.0001
		Planting date x hybrid	6, 33	2.59	0.036
		Bt vs non-Bt hybrids	1, 33	161.39	<0.0001
		Long vs Short hybrids	1, 33	0.89	0.353
	Wooster	Planting date	2, 36	1.09	0.347
		Hybrid	3, 36	59.53	<0.0001
		Planting date x hybrid	6, 36	0.48	0.821
		Bt vs non-Bt hybrids	1, 36	159.00	<0.0001
		Long vs Short season hybrids	1, 36	9.04	0.005
2007	Hoytville	Planting date	2, 24	12.82	0.0002
		Hybrid	3, 24	126.30	<0.0001
		Planting date x hybrid	6, 24	8.69	<0.0001
		Bt vs non-Bt hybrids	1, 24	374.02	<0.0001
		Long vs Short hybrids	1, 24	2.38	0.0014
	Wooster	Planting date	2, 27	16.07	<0.0001
		Hybrid	3, 27	200.47	<0.0001
		Planting date x hybrid	6, 27	6.52	0.0001
		Bt vs non-Bt hybrids	1, 27	586.20	<0.0001
		Long vs Short hybrids	1, 27	6.90	0.014
2008	Hoytville	Planting date	1, 2	0.31	0.635
		Hybrid	3, 12	99.77	<0.0001
		Planting date x hybrid	3, 12	0.11	0.950
		Bt vs non-Bt hybrids	1, 12	288.34	<0.0001
		Long vs Short hybrids	1, 12	5.49	0.037
	Wooster	Planting date	2, 33	0.55	0.582
		Hybrid	3, 33	59.46	<0.0001
		Planting date x hybrid	6, 33	0.93	0.486
		Bt vs non-Bt hybrids	1, 33	158.74	<0.0001
		Long vs Short hybrids	1, 33	9.81	0.004

At Wooster there was a significant reduction (P = 0.0014) in the percentage of damaged ears on long season non-Bt maize compared to the short season non-Bt hybrid with early planting (Fig. 1D).

In 2008 neither planting date nor planting date × hybrid interaction were significant at either location (Table 3). However a highly significant effect (P < 0.0001) due to hybrid was detected at both locations. This difference was due to a highly significantly higher percentage of damaged ears on non-Bt than Bt hybrids at each location (P < 0.0001). There was also a significant reduction in the percentage of damaged ears on the long season non-Bt hybrid compared with short season non-Bt at both Hoytville and Wooster (Table 3).

Fusarium ear rot severity

In 2006 the severity of Fusarium ear rot was not significantly affected by either planting date or planting date × hybrid interaction at either location (Table 4). However, a significant hybrid treatment effect (P = 0.027) was observed at Hoytville but not at Wooster. Also, ear rot severity was significantly lower (P = 0.012) on Bt than non-Bt hybrids at Hoytville but no such significant differences occurred at Wooster. At both locations, the short season non-Bt hybrid DKC 51-45 showed a significant increase (P < 0.05) in ear rot severity with late planting (Figures 2A and 2B).

In 2007, Fusarium ear rot severity was not significantly impacted by planting date or planting date × hybrid interaction at either location (Table 4). Ear rot severity was significantly affected by hybrid at Hoytville (P < 0.05) but not at Wooster

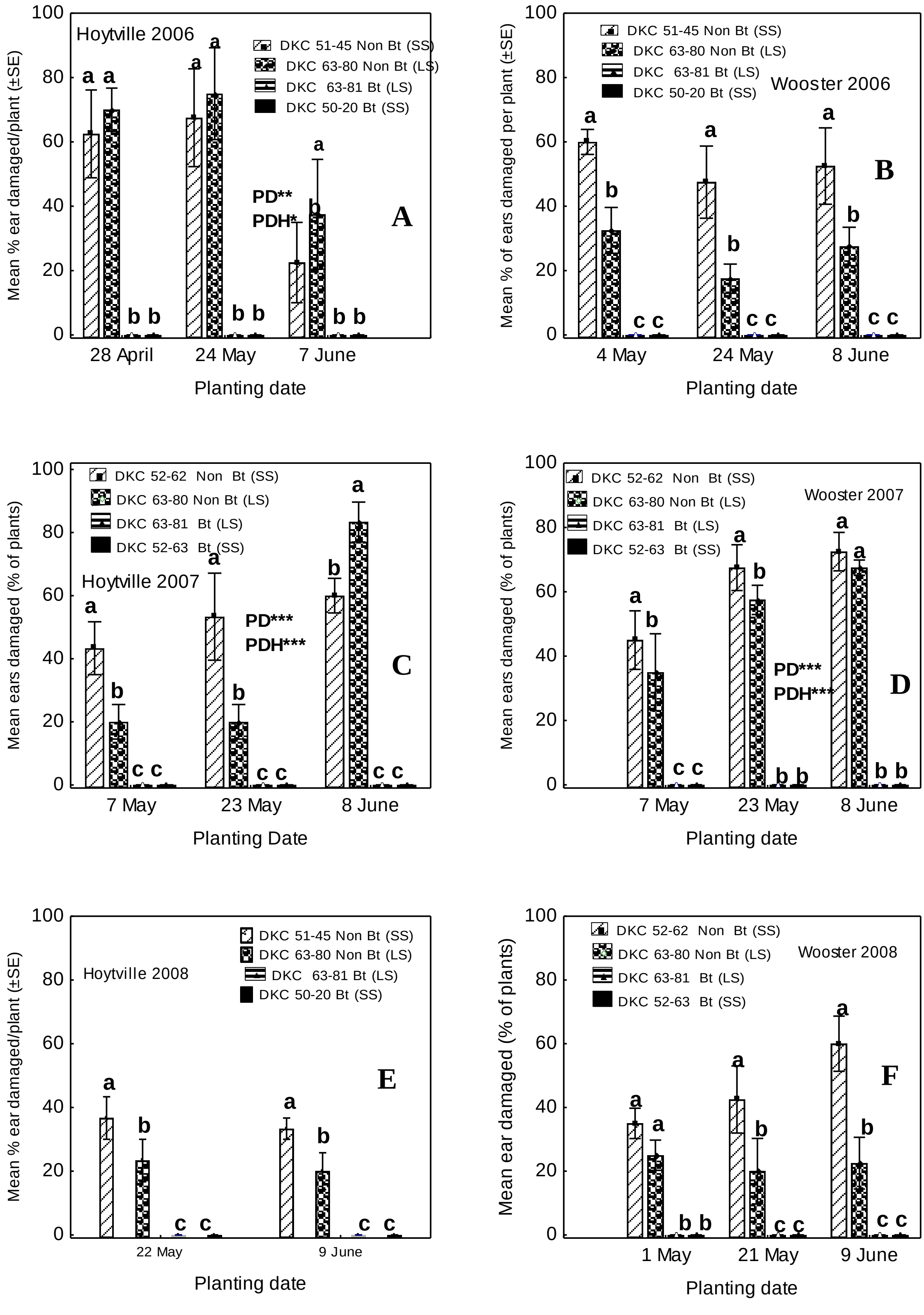

Figure 1. Mean percentage of ears per plant damaged by European corn borer on hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Means followed by the same letter within a planting date are not significantly different (Fisher protected LSD, α = 0.05). PD = planting date; 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 = long season.

Contrast analysis indicated a significant reduction (Table 4) in ear rot severity on Bt hybrids compared to non-Bt hybrids at both Wooster and only with late planting (Table 4, Fig. 2F). A significant reduction (P = 0.038) in Fusarium ear rot severity occurred on the long season non-Bt hybrid compared to short season non-Bt hybrid at Hoytville with middle and late plantings (Figure 2C). As in 2006, the increase in Fusarium ear rot severity increased on the short season non-Bt hybrid DKC 51-45 on later planted maize at both locations (Figures 2C and D). In 2008, no significant differences among planting dates occurred at Hoytville (Table 4) but there was a significant reduction (P = 0.038) in ear rot severity on early and middle planting dates compared with late planting at Wooster (Table 4 and Figure 2F). There was no significant planting date × hybrid interaction at Hoytville but a significant interaction occurred at Wooster (P = 0.012). Fusarium ear rot severity did not vary significantly among hybrid treatment at Hoytville but it did so at Wooster (P = 0.002). There was no significant difference between Bt and non-Bt hybrids at Hoytville but a significant reduction (P < 0.001) in Fusarium ear rot severity occurred on Bt hybrids at Wooster.

Table 4. ANOVA for the effects of planting date, maize hybrid maturity and Bt hybrids on severity of Fusarium ear rot

Year	Location	Effects	*df*	*F*	*P*
2006	Hoytville	Planting date	2, 9	0.78	0.486
		Hybrid	3, 27	3.56	0.027
		Planting date x hybrid	6, 27	0.47	0.821
		Bt vs non-Bt hybrids	1, 27	7.26	0.012
		Long vs Short hybrids	1, 27	3.12	0.089
	Wooster	Planting date	2, 6	0.07	0.932
		Hybrid	3, 27	1.62	0.209
		Planting date x hybrid	6, 27	1.39	0.256
		Bt vs non-Bt hybrids	1, 27	2.09	0.160
		Long vs Short season hybrids	1, 27	1.22	0.280
2007	Hoytville	Planting date	2, 24	0.60	0.559
		Hybrid	3, 24	3.89	0.021
		Planting date x hybrid	6, 24	0.34	0.910
		Bt vs non-Bt hybrids	1, 24	5.04	0.034
		Long vs Short hybrids	1, 24	4.84	0.038
	Wooster	Planting date	2, 33	0.50	0.609
		Hybrid	3, 33	2.67	0.063
		Planting date x hybrid	6, 33	1.38	0.252
		Bt vs non-Bt hybrids	1, 33	5.77	0.022
		Long vs Short hybrids	1, 33	0.14	0.709
2008	Hoytville	Planting date	1, 4	0.24	0.652
		Hybrid	3, 12	1.21	0.346
		Planting date x hybrid	3, 12	1.68	0.225
		Bt vs non-Bt hybrids	1, 12	4.08	0.066
		Long vs Short hybrids	1, 12	0.48	0.500
	Wooster	Planting date	2, 9	4.79	0.038
		Hybrid	3, 27	6.19	0.002
		Planting date x hybrid	6, 27	3.42	0.012
		Bt vs non-Bt hybrids	1, 27	16.12	0.0004
		Long vs Short hybrids	1, 27	0.34	0.563

Hybrid relative maturity did not affect the severity of Fusarium ear rot at either location (Table 4). Planting date comparisons indicated that a significant reduction (P < 0.05) in ear rot severity associated with Bt maize occurred on late planted maize (Figures 2E and 2F). Significant correlations were observed between European corn borer damage to maize kernels and Fusarium ear rot severity in all years and locations except in 2008 at Hoytville (Table 5, with r^2 varying between 0.29 with P ≤ 0.01 to 0.43, with P ≤ 0.001).

DISCUSSION

The significance of planting date effects on Fusarium ear rot levels was variable during the three years of the study. In 2008, the planting date × hybrid interaction showed a significant increase in severity of Fusarium ear rot on non-Bt hybrids as planting was delayed at Wooster, possibly because of higher incidence of European corn borer damage on the late planted crop which exposed plants to fungal infections (Wiatrack *et al*., 2005). Severity was much higher here than in previous years while at Hoytville it was much lower than previously observed. This was also indicated by a higher correlation between Fusarium severity and damage caused by the European corn borer observed at Wooster in 2008. Early planting of maize is recommend so as to avoid or reduce factors like drought stress and insect injury that create environments conducive for maize kernel infection by pathogens (Wiatrack *et al*., 2005; Blandino *et al*., 2008).

Early planting has been found to reduce the incidence and severity of stalk rot (Obopile *et al.,* 2011) because of significant reduction in stalk tunnelling by European

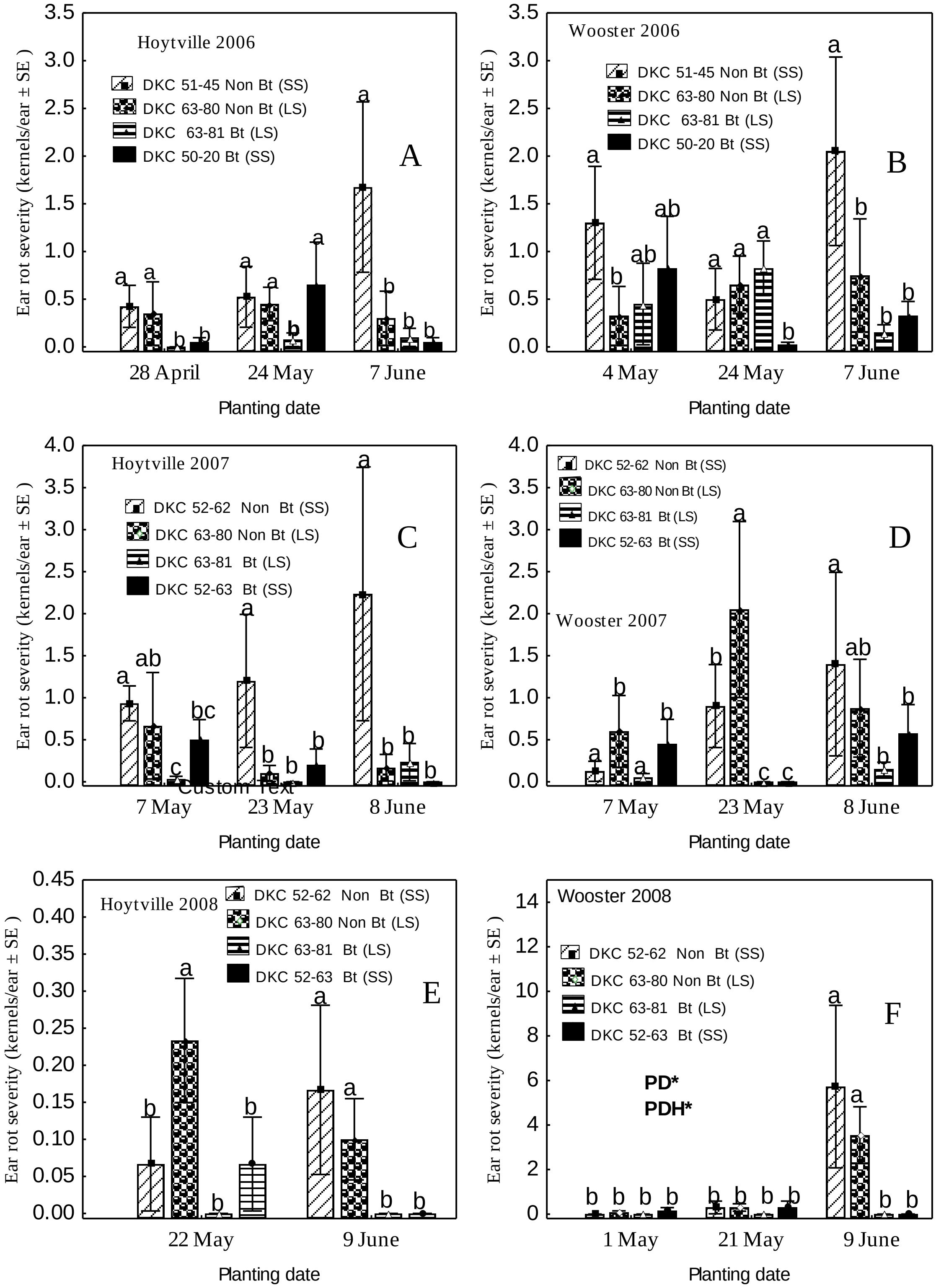

Figure 2. Fusarium ear rot severity (mean number of infected kernels/ear) recorded on hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Means followed by the same letter within a planting date are not significantly different (Fisher protected LSD, α = 0.05). PD = planting date; PDH = planting date x hybrid interaction; * and ** denote significance at P ≤ 0.05 and P ≤ 0.01 respectively. SS = short season, LS = long season.

corn borer (Obopile *et al.,* 2012). The significant correlation observed in this study between European corn borer kernel damage and Fusarium maize ear rot has also been reported by Munkvold (2003). Management of insect pests has been reported to reduce Fusarium ear rot and fumonisin contamination (Avantaggiato *et al.,* 2003). Previous studies (Logrieco *et al*., 2002; Munkvold, 2003) have not compared hybrids from different maturity groups; this study found significant reductions in both ear damage by European corn borer and severity of Fusarium ear rot on long season hybrids compared with short season only once. Given that significant correlations occurred among European corn borer kernel injury and maize ear rot it is probable that the reduction in ear rot on long season hybrids is associated with the reduced ear injury from corn borers observed on these hybrids.

Table 5. Linear correlation coefficients between severity of Fusarium ear rots and European corn borer (ECB) damage to maize ears caused by ECB at Hoytville and Wooster, OH, 2006-2008

Year	Location	ECB Ear damage
2006	Hoytville	0.35*
2007	Hoytville	0.37*
2008	Hoytville	0.25^{ns}
2006	Wooster	0.29*
2007	Wooster	0.37*
2008	Wooster	0.43**

** = P ≤ 0.01 and ** = P ≤ 0.001 and ns = not significant*

Transgenic Bt hybrids significantly reduced kernel injury by European corn borer, thus preventing an important pathway for infection by fungal pathogens that cause ear rots on maize (Logrieco *et al.,* 2002).

CONCLUSION

Maize hybrids expressing the Cry1Ab Bt protein had very low incidences of European corn borer kernel damage and consequently reduced Fusarium ear rot severity when compared to non-Bt hybrids. The study showed that the benefit of using Bt hybrids to reduce Fusarium ear rot severity would be realised more on late planted than early planted crops.

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Conflict of interest None

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