

CORN

Influence of Planting Date on Aflatoxin Accumulation in Bt, non-Bt, and Tropical non-Bt Hybrids

P. J. Wiatrak,* D. L. Wright, J. J. Marois, and D. Wilson

ABSTRACT

Aflatoxin, produced by the fungus *Aspergillus flavus* Link, reduces the value of corn (*Zea mays* L.) and is usually associated with high temperatures, water stress, and insect damage. The objective of this study was to determine if Bt (*Bacillus thuringiensis*) corn hybrids or "tropical" germplasm could reduce aflatoxin accumulation with later planting dates. Aflatoxin accumulation (B_1 , B_2 , G_1 , and G_2) in corn grain was evaluated on Bt, non-Bt, and tropical non-Bt hybrids and four planting dates (March, April, May, and June) from 1998 to 2000. Aflatoxin concentration in corn varied across years but generally decreased with later planting date. In 1998, aflatoxin accumulation was lower in Bt (314 ng g^{-1}) than non-Bt hybrids (634 ng g^{-1}) but not different than tropical non-Bt hybrid (470 ng g^{-1}). However, aflatoxin contamination was lower from Bt hybrids (70 ng g^{-1}) than from the tropical non-Bt hybrid (259 ng g^{-1}) but not different in non-Bt hybrids (86 ng g^{-1}) in 1999. In 2000, aflatoxin levels were low, and hybrid had no effect on aflatoxin concentration. Temperature and irrigation effects on aflatoxin accumulation were not consistent. Increased temperature and delayed harvest may lead to aflatoxin accumulation before harvest. However, precipitation may influence aflatoxin levels in some years. The results of this study indicate that aflatoxin accumulation in corn may be decreased with later planting date, and less accumulation in Bt than non-Bt or tropical non-Bt hybrids may be indirectly explained by insect reductions.

AFLATOXIN IS A NATURALLY occurring toxin (Williams et al., 2002) and one of the strongest carcinogens found in nature (Castegnaro and McGregor, 1998). Yu et al. (1998) noted that aflatoxins B_1 (the most potent hepatocarcinogenic compound known), B_2 , G_1 , and G_2 are toxic and carcinogenic secondary metabolites produced by the filamentous fungi *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus*, found often on insects inhabiting corn ears (Lillehoj et al., 1980), produces only aflatoxins B_1 and B_2 and, *A. parasiticus* produces aflatoxins G_1 and G_2 in addition to aflatoxins B_1 and B_2 (Yu et al., 1998). Payne (1992) stated that insects may facilitate infection of preharvest ears by transporting inoculum already in the silk into ears, disseminating inoculum within the ears, and creating a

favorable *A. flavus* habitat through injury associated with feeding.

The fall armyworm (*Spodoptera frugiperda* J.E. Smith) and the corn earworm (*Helicoverpa zea* Boddie) are the most destructive insect pests of field corn in the southeastern USA (Wiseman et al., 1974). Lepidoptera infestations of ears have been linked to increased contamination of aflatoxin (Smith and Riley, 1992) due to insect damage (McMillian, 1983). If insects are controlled, mature late plantings are still susceptible to plant pathogens, and Bt varieties will still experience low yields if pathogens like mycotoxin-producing organisms (*A. flavus* and *A. parasiticus*) are not adequately controlled. However, insects that could be *A. flavus* vectors do not necessarily have to be pests that cause damage (McMillian, 1983).

A linear accumulation of aflatoxin occurs at least 40 d beyond inoculation (Widstrom, 1988). Smart et al. (1990) and Payne et al. (1988a) found that aflatoxin contamination of undamaged kernels started late in the season when the plants were at or near physiological maturity. After examination of temperature on *Aspergillus* infection of silk-inoculated corn, Payne et al. (1988b) found that with day/night regime of 34/30°C and 34/22°C, 28 and 7% of kernels were infected, respectively. However, the inoculum must be present to have silk or insect-vectored infection. The U.S. Food and Drug Administration limits corn grain sale with an aflatoxin contamination of 20 ng g^{-1} (Park and Liang, 1993). Contamination is generally considered to be more severe in the Southeast than in other regions of the USA but varies from year to year in severity (Widstrom et al., 1990).

Corn hybrids, from Midwest germplasm, have been grown for many years in the Southeast. In the last few years, however, interest has developed over the potential of tropical hybrids from South America. These tropical hybrids are adapted for planting at times that take advantage of optimal rainfall patterns in the subtropical areas of the South (Wright et al., 1991). Summer rainfall in the southeastern USA is often more optimal for corn planted from mid-May until mid-June. However, planting generally is terminated from mid-April to early May due to large insect injury (Anderson and Linker, 1991) and associated prohibitive levels of mycotoxins (Gray et al., 1982). McMillian et al. (1985) reported that crop management, including hybrid selection, planting date, and irrigation, may have a significant influence on the quality of produced grain. The development of suitable varieties could result in consistent desirable yields and reduce weather-related problems concerning quality,

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Published in Agron. J. 97:440–445 (2005).
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Abbreviations: Bt, *Bacillus thuringiensis*.

such as aflatoxin (Keisling et al., 1999). The objective of this study was to determine if Bt or tropical hybrids could reduce aflatoxin contamination over successive later planting.

MATERIALS AND METHODS

Field trials were conducted from 1998 to 2000 on a Dothan sandy loam (fine, loamy siliceous, thermic Plinthic Kandicudults) at the University of Florida's North Florida Research and Education Center in Quincy, FL. Pioneer (Pioneer Hi-Bred Int. Inc., Des Moines, IA) and Novartis (Novartis Seed Co., Minneapolis, MN) corn hybrids were selected based on the current use of hybrids by farmers and for evaluation purposes.¹ These corn hybrids were developed for production in the southeastern USA. The tropical hybrids used in this study were developed from germplasm used in the tropical region of South America.

The seedbed was prepared with a Brown Ro-till implement (Brown Manufacturing Co., Ozark, AL). Corn was planted at approximately 30-d intervals beginning in late March (Table 1). Seven corn hybrids were seeded at 64 250 kernels ha⁻¹ in strip till using a cone row planter (Table 2). Plant stands were thinned to 59 300 plants ha⁻¹ about 2 wk after planting. Plot dimensions were 6 by 11 m and consisted of 12 rows. Plot rows 1, 2, 11, and 12 were maintained as undisturbed border/buffer rows. Just after planting, fertilizer was banded on both sides of the row and 3 to 7 cm from the center of the row at 28, 24, and 70 kg ha⁻¹ of N, P, and K, respectively. Corn was sidedressed with ammonium nitrate (34-0-0 of N-P-K) at 171 kg N ha⁻¹ when plants were about 0.4 m tall. Other cultural practices, including weed control, irrigation, and harvest, were implemented according to standard production practices. Insecticides were not applied either at planting or during the season.

Ear samples were collected from Rows 3 and 8 beginning approximately 3 wk after pollination (brown silk). Data for corn earworm damage (Wiatrak et al., 2004) in the corn ears were collected based on a procedure described by Reid (1975). Corn for grain yield determination (Wiatrak et al., 2004) was harvested from Rows 6 and 7 when grain moisture was below 250 g kg⁻¹ using a modified grain combine, which occurred from late July to late September (Table 1). Tropical hybrids, due to later maturity, were harvested about 3 wk later in 1998 and 1999 and 2 wk later in 2000 than nontropical hybrids. Grain yield was adjusted to moisture content of 155 g kg⁻¹. Corn grain subsamples of 2.3 kg ha⁻¹ were collected from each plot to evaluate aflatoxin accumulation. These grain subsamples were collected from one to three planting dates in 1998 and one to four planting dates in 1999 and 2000. The grain subsample was not collected from the fourth planting date in 1998 due to technical problems. Total aflatoxin (B₁, B₂, G₁, and G₂) contamination was determined by using the Vicam

¹ Mention of a proprietary product name is for identification purposes only and does not imply endorsement or warranty to the exclusion of other products.

Table 1. Corn planting and harvest dates at Quincy, FL, from 1998 to 2000.

Planting date	1998	1999	2000
	Planting date		
1	27 March	26 March	31 March
2	24 April	23 April	27 April
3	22 May	21 May	25 May
4	19 June	21 June	22 June
	Harvest date†		
	1998	1999	2000
1	5 August	28 July	21 July
2	17 August	17 August	7 August
3	9 September	1 September	8 September
4	2 October	16 September	26 September

† Harvest dates for nontropical hybrids; tropical hybrids were harvested 3 wk later in 1998 and 1999 and 2 wk later in 2000.

immunoaffinity column method (Truckness et al., 1991). The length of vegetation period was calculated from planting to grain harvest and used to compute the correlation with aflatoxin accumulation in corn.

Weather data were collected from a weather station located at the North Florida Research and Education Center, Quincy, FL (84°33' W, 30°36' N). Generally, air temperatures were similar, and rainfall was lower during the 3-yr vegetation periods (from planting to harvest) compared with the 20-yr average (data not shown), except for higher precipitation in September of 1998 (230 mm) and July of 1998 and 1999 (75 and 28 mm, respectively). The inadequate precipitation was compensated with irrigation on an as-needed basis using a sprinkler irrigation system. Corn was irrigated when tensiometer (Irrometer Co., Riverside, CA) readings at a 30-cm soil depth indicated 40 kPa. Irrigation was included in the total precipitation from 1 to 5 wk before harvesting corn to calculate correlations with aflatoxin accumulation in corn. Air temperature and total precipitation including irrigation, at 1 to 5 wk before corn harvest, varied for planting date (Tables 3 and 4). Air temperatures across years ranged from 25.9 to 29.7°C, 26.2 to 29.6°C, 23.6 to 29.8°C, and 18.6 to 28.9°C, and precipitation ranged from 0 to 91, 93, 183, and 197 mm for Planting Dates 1 to 4, respectively.

The experimental design was a split plot in a randomized complete block with four replications. Planting date was the main plot, and hybrid was the subplot. Aflatoxin accumulation data were analyzed using PROC MIXED (SAS Inst., 1999). As years were sequential with potentially cumulative effects on soil and plant parameters, years were considered fixed effects. Planting dates and hybrids were considered fixed. Blocks and interactions including blocks (block × year and block × year × planting date) were assumed to be random effects. The PROC MIXED procedure of SAS with the LS-MEANS PDIF option was used to compare planting dates, and the CONTRAST option within PROC MIXED was used to compare hybrids. The difference between means for planting dates and hybrids was considered significant at $P \leq 0.05$. Pearson correlation coefficients (r) were calculated between aflatoxin accumulation and air temperatures, total precipita-

Table 2. Corn hybrids with relative maturity (RM) grown at Quincy, FL, from 1998 to 2000.

1998	RM	1999	RM	2000	RM
Novartis N79-L3 Bt	116	Novartis N79-L3 Bt	116	Novartis N79-L3 Bt	116
Novartis N7639 Bt	113	Novartis NX8318 Bt	118	Novartis NX8318 Bt	118
Pioneer 31B13 Bt	118	Pioneer 31B13 Bt	118	Pioneer 32K62 Bt	114
Pioneer 33Y09 Bt	113	Novartis N79-P4	116	Novartis N79-P4	116
Novartis N79-P4	116	Pioneer 3223	116	Novartis N83-N5	117
Pioneer 32K61	114	Novartis N83-N5	117	Pioneer 32K61	114
Pioneer 3098 (T)†	140	Pioneer 3098 (T)	140	Pioneer 30F33 (T)	132

† (T) = tropical corn hybrid.

Table 3. Average air temperature by planting date (PD) from 1 to 5 wk before harvest of temperate and tropical corn for grain at Quincy, FL, in 1998–2000.

Weeks before harvest	Air temperature					
	1998		1999		2000	
	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical
	°C					
1 PD						
1	26.1	26.9	28.3	28.7	29.5	26.6
2	26.9	26.6	26.4	28.3	29.6	29.3
3	28.3	27.1	26.3	29.7	26.9	29.5
4	29.0	26.1	26.9	28.3	26.4	29.6
5	29.4	26.9	25.9	26.4	27.0	26.9
2 PD						
1	26.9	26.7	28.8	26.6	27.1	27.9
2	26.5	28.1	28.8	27.5	29.0	27.6
3	26.9	27.1	29.2	28.2	29.5	27.1
4	28.3	26.9	27.7	28.8	29.6	29.0
5	29.0	26.5	26.2	28.8	26.9	29.5
3 PD						
1	26.8	26.5	27.3	24.1	26.9	23.6
2	28.4	25.3	28.2	24.8	29.8	25.4
3	26.7	24.2	28.8	25.5	28.6	26.9
4	27.1	26.8	28.6	27.3	27.3	29.8
5	26.1	28.4	29.7	28.2	27.4	28.6
4 PD						
1	26.8	23.4	18.6	24.7	22.8	21.9
2	26.4	22.2	26.1	23.1	25.3	23.0
3	25.3	24.4	26.7	23.1	26.6	22.8
4	26.4	26.8	28.1	18.6	27.6	25.3
5	28.4	26.4	28.9	26.1	27.2	26.6

tion, vegetation period, earworm damage, and grain yield of corn in 1998, 1999, and 2000.

RESULTS AND DISCUSSION

Aflatoxin data were analyzed by year because a significant planting date \times hybrid \times year interaction was observed. In 1998, planting date did not influence aflatoxin accumulation (Table 5). The same year, Bt hybrids had less aflatoxin compared with non-Bt hybrids; however, no differences were detected between Bt hybrids and

the tropical non-Bt hybrid and between non-Bt hybrids and the tropical non-Bt hybrid. In 1999, a planting date \times hybrid interaction was detected for aflatoxin due to the greater level of infestation among hybrids at the April planting date compared with other planting dates. Aflatoxin accumulation was relatively less for March, May, and June planting dates and greater for the April planting date (Fig. 1). Hybrid mostly had no effect on aflatoxin when the values were low, but Bt and non-Bt hybrids had less aflatoxin compared with the tropical

Table 4. Sum of precipitation (including irrigation) by corn planting date (PD) from 1 to 5 wk before harvest of temperate and tropical corn for grain at Quincy, FL, in 1998–2000.

Weeks before harvest	Precipitation					
	1998		1999		2000	
	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical
	mm					
1 PD						
1	34	34	13	36	54	31
2	91	16	30	86	0	76
3	31	23	89	0	0	54
4	61	34	75	13	69	0
5	52	91	40	30	49	0
2 PD						
1	19	3	81	36	24	25
2	36	29	42	32	83	0
3	52	20	0	13	25	24
4	93	19	13	81	28	83
5	69	26	61	42	0	25
3 PD						
1	29	183	47	90	0	0
2	106	85	17	0	57	51
3	19	3	36	29	13	0
4	29	29	86	47	13	57
5	36	106	0	17	5	13
4 PD						
1	197	0	0	13	11	28
2	51	0	29	3	0	0
3	50	6	44	90	51	11
4	0	197	19	0	13	0
5	3	51	36	29	45	51

Table 5. Planting date and hybrid influence on aflatoxin content in corn from 1998 to 2000.

Characteristic	Aflatoxin		
	1998	1999	2000
	ng g ⁻¹		
Planting date			
March	528 a†	13 b	55 ab
April	526 a	365 a	15 b
May	230 a	33 b	109 a
June	—‡	4 b	6 b
Hybrid			
Bt	314	70	55
Non-Bt	634	86	36
Tropical non-Bt	470	259	48
Contrast for hybrid			
Bt vs. non-Bt	**	NS	NS
Bt vs. tropical non-Bt	NS§	***	NS
Non-Bt vs. tropical non-Bt	NS	**	NS

** Significant at the 0.01 probability level.
 *** Significant at the 0.001 probability level.
 † LSMEANS within a column followed by the same letter are not different at the 0.05 probability level.
 ‡ Missing data.
 § NS, nonsignificant at the 0.05 probability level.

hybrid when the aflatoxin values were high. Averaged across hybrids, aflatoxin accumulation was less for March, May, and June planting dates than the April planting date in 1999 (Table 5). The same year, less aflatoxin infestation was observed for Bt hybrids than the tropical non-Bt hybrid and less for non-Bt hybrids than the tropical non-Bt hybrid. However, no difference in aflatoxin level was observed between Bt and non-Bt hybrids. In 2000, aflatoxin accumulation was less for April and June planting dates than the May planting date while no difference was detected between March and May planting dates. The same year, hybrid did not influence aflatoxin contamination in corn. Lillehoj et al. (1980) also reported that the levels of aflatoxin accumulation in hybrids differed among planting dates. Our results in 1998 and 1999 agree with Buntin et al. (2001), who noted that aflatoxin progressively declined to low levels with later plantings. Regressions of aflatoxin concentrations on planting date revealed a large linear decrease in concentration (200–300 ng g⁻¹ per 15-d delay in planting) from early to late (Widstrom et al., 1990). Different results in 2000, compared with other years, were mainly due to relatively less aflatoxin infestation. Comparing

Table 6. Pearson correlation coefficient (r) of aflatoxin with the average air temperature, total precipitation, vegetation period, and earworm damage at Quincy, FL, from 1998 to 2000.

Characteristic	Aflatoxin		
	1998	1999	2000
Temperature (wk before harvest)			
1	NS†	0.32**	0.20*
2	-0.27*	0.43***	0.30**
3	NS	0.40***	NS
4	0.26*	NS	-0.19*
5	0.28*	-0.27**	0.32***
Precipitation (wk before harvest)			
1	NS	0.48***	NS
2	NS	0.42***	NS
3	0.21*	-0.43***	-0.29**
4	0.24*	-0.32***	NS
5	0.24*	0.36***	NS
Vegetation period	NS	0.20*	0.27**
Earworm damage	NS	-0.21*	NS

* Significant at the 0.05 probability level.
 ** Significant at the 0.01 probability level.
 *** Significant at the 0.001 probability level.
 † NS, nonsignificant at the 0.05 probability level.

corn hybrids for aflatoxin concentration, Thompson et al. (1984) also showed that hybrids can sustain different levels of aflatoxin accumulation. Aflatoxin level may vary among genotypes (Darrah et al., 1987) due to effectively reducing Lepidoptera ear infestations, therefore reducing aflatoxin contamination of grain (Williams et al., 1998). Also, McMillian et al. (1988) noted lower concentrations of aflatoxin in European corn borer (*Ostrinia nubilalis* Hübner) resistant hybrids compared with conventional corn. Moreover, our aflatoxin data from 2000 is supported by Buntin et al. (2001), who reported that grain aflatoxin concentrations were not different between susceptible and Bt hybrids. Generally, less aflatoxin infestation in corn grain may be observed with later planting dates and less in Bt than non-Bt or tropical non-Bt corn due to less insect damage.

The Pearson correlation coefficient of aflatoxin with the average temperatures and precipitation, vegetation period, corn earworm damage, and grain yields was evaluated in 1998, 1999, and 2000 (Table 6). An increase of aflatoxin accumulation was observed with increasing air temperature at 4 and 5 wk before grain harvest in 1998, 1 to 3 wk before harvest in 1999, and 1, 2, and 5

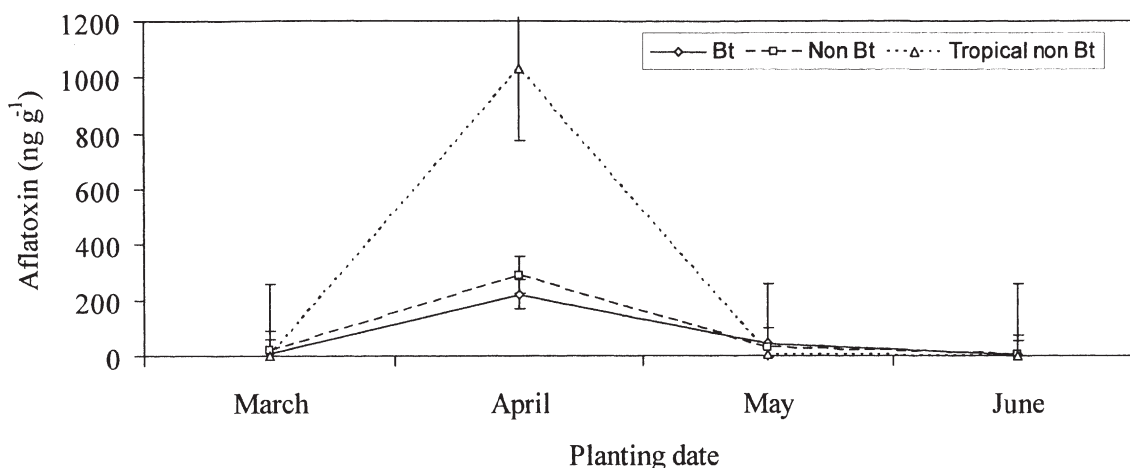


Fig. 1. Influence of planting date on aflatoxin accumulation in corn in 1999. Vertical bars indicate the standard error of four replicates.

wk before harvest in 2000. McMillian et al. (1985) also reported a positive correlation of aflatoxin with higher temperature. Higher temperatures increased aflatoxin concentrations in inoculated ears (Thompson et al., 1980) and aflatoxin accumulation in grain (Payne, 1992). Aflatoxin concentrations were correlated with maximum and minimum daily temperature during 20- to 40-d and 40- to 60-d periods following full silk (Widstrom et al., 1990). Early plantings of corn are at higher aflatoxin risk due to high seasonal maximum and minimum temperatures (Widstrom et al., 1990). However, our results also showed that air temperature at 2 wk in 1998, 5 wk in 1999, and 4 wk in 2000 before corn harvest was negatively correlated with aflatoxin accumulation. Generally, data were not consistent. However, these results suggest that increased temperature may lead to aflatoxin accumulation before harvest.

Our research showed that aflatoxin content was positively correlated with total precipitation (including irrigation) at 3 to 5 wk before grain harvest in 1998 and 1, 2, and 5 wk before harvest in 1999 (Table 6). However, increased aflatoxin content in grain was also observed with decreased precipitation at 3 and 4 wk before harvest in 1999 and 3 wk before harvest in 2000. Others found that aflatoxin contamination has been linked to availability of water in the soil (Lillehoj, 1983) and drought (Payne, 1992), which leads to an increased number of infected kernels in silk-inoculated ears (Payne et al., 1986). However, irrigation may either decrease (Jones et al., 1981) or increase aflatoxin accumulation in corn (Jones et al., 1980). Overall, aflatoxin accumulation in corn varies across years and may be influenced by precipitation.

The length of the vegetation period was positively correlated with aflatoxin accumulation in 1999 and 2000, indicating more aflatoxin in late-harvested corn (Table 6). Jones and Duncan (1981) and Jones et al. (1981) also reported a greater aflatoxin accumulation in late-harvested corn, which was reduced with early harvest. However, our research showed that aflatoxin content was not affected by the length of vegetation period in 1998. Overall, delayed corn harvest may lead to increased aflatoxin infestation.

A negative correlation of aflatoxin accumulation in corn grain was observed with corn earworm damage in 1999, suggesting that it is not necessary to have insect damage to get aflatoxin (Table 6). Another explanation would be that aflatoxin accumulation occurs early and insects may avoid aflatoxin-infected corn. No correlation of aflatoxin content with earworm damage was observed in 1998 and 2000. Our results from 1999 are different from McMillian et al. (1985), who showed that insect damage was positively correlated with the concentration of aflatoxin. The relationship between insect damage and aflatoxin contamination is related to enhanced aflatoxin production in damaged areas of the ear (Lee et al., 1980). Corn earworm has been linked to high levels of aflatoxin contamination (McMillian et al., 1985) due to feeding on and damaging developing kernels and by transporting *A. flavus* conidia into the ear. Also, insect damage and aflatoxin accumulation

increases with decreasing husk cover (Keisling et al., 1999). However, Widstrom et al. (1976) noted that insect control reduces, but does not eliminate, aflatoxin contamination. The results from 1998 and 2000 agree with Widstrom et al. (1990), who noted that aflatoxin concentrations were not related to insect damage. Generally, insect damage may increase aflatoxin infestation; however, infestation can occur without insect damage in corn.

SUMMARY

Aflatoxin accumulation in corn varied across years and generally decreased with later planting date. Hybrid had no effect on aflatoxin when aflatoxin levels were low. When the aflatoxin values were high, however, Bt hybrids had less aflatoxin when compared with non-Bt or tropical non-Bt hybrids due to effectively reducing insect infestations, therefore reducing aflatoxin contamination of grain. The influence of precipitation and air temperatures on aflatoxin contamination was not consistent. Aflatoxin accumulation was greater with increasing air temperature at 4 and 5 wk before grain harvest in 1998, 1 to 3 wk before harvest in 1999, and 1, 2, and 5 wk before harvest in 2000. However, an increase in air temperature at 2, 5, and 4 wk before corn harvest decreased aflatoxin content in 1998, 1999, and 2000, respectively. Aflatoxin content increased with precipitation at 3 to 5 wk before grain harvest in 1998 and 1, 2, and 5 wk before harvest in 1999. However, lower precipitation increased aflatoxin content in grain at 3 and 4 wk before harvest in 1999 and 3 wk in 2000. Higher aflatoxin content was observed with extended vegetation period from planting to harvest in 1999 and 2000 and increased grain yields of corn in 1999. The results of this study indicate that aflatoxin accumulation in corn may be decreased with later planting date. Also, aflatoxin contamination may be lower in Bt than non-Bt or tropical non-Bt hybrids due to insect reductions. Increased temperature and delayed harvest may lead to aflatoxin accumulation before harvest, and the precipitation may influence aflatoxin levels in some years.

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