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Delaying harvest for naturally drying maize grain increases the risk of kernel rot and fumonisin contamination

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Abstract

Artificial drying, although costly, ensures a safe storage and preserves post-harvest grain quality. Alternatively, a more cost-effective measure is to delay harvest to promote natural drying, but this management may increase the risk of mycotoxigenic ear rot fungi contamination. The objective was to evaluate the effect of increasing maize harvest delay on fungal disease and fumonisin levels. Three maize hybrids (BRS1035, Attack and DKB390 YG) were sown in the field to study the effect of five harvest delays (15, 30, 45, 60 and 75 days) after the optimum date (18% grain moisture). There was a significant trend of increasing the incidence of kernel rot and total fumonisins in the grains when delaying harvest, but fungal incidence and mycotoxin contamination varied with the hybrid. The main fungi detected in the grain samples were *Fusarium verticillioides* and *Stenocarpella maydis*. The hybrid DKB390 YG showed significantly lower incidence of *F. verticillioides* contamination and lower fumonisin accumulation in the grains than the other hybrids. The hybrid Attack was the least susceptible to kernel rot incidence. Our data shows that delaying harvest for minimizing drying costs may increase the risk of mycotoxin contamination in maize in the tropics of Brazil.

Keywords *Fusarium verticillioides* · *Stenocarpella maydis* · *Zea mays* · Mycotoxins · Ear rot

Introduction

Maize crops are susceptible to a range of mycotoxigenic fungi that infect and colonize ears, thus causing yield losses, and lowering grain quality and safety during post-harvest (Hermanns et al. 2006; Yılmaz and Tuncel 2010; Kamala et al. 2016). Among the mycotoxins produced by these fungi, fumonisins, produced primarily by two maize pathogens, *Fusarium verticillioides* and *Fusarium proliferatum*, are the most important worldwide given the frequency and levels of contamination (Blandino et al. 2009; Folcher et al. 2010; Yılmaz and Tuncel 2010; Ferreira et al. 2016; Lanza et al. 2016, 2017).

The fumonisin B1 (FB1) is the most frequent and highly toxic, but FB2 and FB3 are also found eventually (Marasas 2001; Samapundo et al. 2006; Yılmaz and Tuncel 2010; Bowers et al. 2013; Guo et al. 2016). FB1 is classified as a human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC) and is associated with several animal diseases (Jackson and Jablonski 2004; Lanza et al. 2017; Lerda 2017).

In field conditions, several factors influence maize grain contamination by toxigenic fungi such as seasonal weather (De la Campa et al. 2005), host genotype (Dowd 2000; Bakan et al. 2002) and insect attack (Sobek and Munkvold 1999). In addition, planting and harvesting dates may also affect grain quality, since they are related to the occurrence of stress conditions during plant flowering and grain drying (Santiago et al. 2015).

Maize plants reach physiological maturation (black layer) at around 100 to 120 days, when the grain moisture content is at 28 to 30% (Johnson 2000; Yılmaz and Tuncel 2010). However, the high moisture content at this stage makes the

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mechanized harvest unfeasible, due to threshing difficulties and excess of moist and green parts of the plants, which can lead to severe injuries by grains kneading (Alves et al. 2001; Marques et al. 2009).

Usually, harvest is performed when the grain moisture ranging between 18 to 25%, requiring the use of artificial drying to reduce it to 13 and 14% (Yılmaz and Tuncel 2010). Artificial drying, although essential to ensure safe storage and crop quality after harvest, increases production costs (Bruns and Abbas 2004; Yılmaz and Tuncel 2010). Alternatively, farmers have adopted natural drying of grains by delaying harvest until grains reach the desirable levels of moisture (Bruns and Abbas 2004). In Brazil, harvest delay varies from 2 to 10 weeks depending on the hybrid and weather conditions (Cruz et al. 2009). Despite the advantage of reducing costs, the additional period in the field may increase the risk of mycotoxigenic ear rot fungi contamination (Bush et al. 2004; Torelli et al. 2012; Cao et al. 2013; Santiago et al. 2015). In fact, increases in kernel rot incidence and aflatoxins (Kaaya et al. 2005; Marques et al. 2009); nivalenol and deoxynivalenol (Lauren et al. 2007); *Aspergillus* spp., *Penicillium* spp., *Cephalosporium* spp., and *Fusarium* spp. (Santin et al. 2004) have been previously associated with harvest delay with yield losses ranging from 7 and 15% but eventually 50% (Kaaya et al. 2005). Little is known about the effects of harvest delay of maize crops on the fumonisins levels and incidence of kernel rot fungi. Therefore, we conducted a two-year field experiment to evaluate the effect of maize harvest delay on fungi disease and fumonisin levels in three maize hybrids grown in the tropics of Brazil.

Material and methods

Study area and experimental procedures

Field experiments were conducted in the experimental area of Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, during the 2009/2010 and 2010/2011 seasons. Fields were sown in December 2009 and in November 2010. Three hybrids were used: Attack, BRS1035 and DKB390YG hybrids. The harvest delay treatments consisted of increasing the length of harvest time after the optimum date (time 0): 15, 30, 45, 60 and 75 days. The optimum date is 18% moisture content in grains.

A randomized complete block design was used with treatments set in a 3×6 factorial design (cultivars x harvest delay) with three replicates. Each plot consisted of four rows of five meters, spaced 0.8 m between rows and around five plants per meter. Fertilization consisted of the application of 28 kg.ha^{-1} of N, 98 kg ha^{-1} of P_2O_5 and 56 kg ha^{-1} of K_2O. At 35 days after emergence (DAE), the nitrogen fertilization was conducted by applying 68 kg.ha^{-1} of urea. The experiment was conducted in a no-tillage system under maize mulching. Sowing was performed using a four-line vacuum seeder equipped with an index seed distribution system.

In both years, temperature (°C) maximum and minimum, relative humidity (%) and precipitation (mm) were gathered from a meteorological station located at the experimental site. Monthly averages were calculated for the three weather variables measured at a daily scale. Patterns of daily precipitation were inspected from 30 days before and after the average flowering date of the three maize hybrids.

At each harvesting date, ears of the two central lines of each plot were harvested, tagged and threshed separately. The moisture content of the grain mass was determined using a portable grain moisture meter model Mini Gac Plus, with a moisture resolution of 0.1%. The grain mass of each plot was homogenized and two samples of 500 g each were obtained for the analysis of fungal incidence and total fumonisin contents.

Kernel rot and fungal incidence

Kernel rot incidence was assessed visually by separating healthy from symptomatic grains and weighting symptomatic grain and expressing as percentage of the total weight of sample. Kernel infection was assessed by surface-disinfesting in 2% sodium hypochlorite for 5 min, washing twice in sterilized distilled water, and plating on the top of filter paper moistened with 5% water-agar inside a plastic box (*gerbox*). The boxes were maintained at room temperature during 24 h to promote seed germination. Thereafter, boxes were transferred to a freezer at a temperature of −5 °C, where they remained for additional 24 h. Finally, the boxes were taken to the incubation chamber with a temperature of 24 °C and a 12–12 light/dark cycle. After 15 days, fungi were identified with the aid of stereo and compound microscopes and percent incidence of each fungal species was calculated.

For the morphological identification of *Fusarium* species, isolates were grown in BDA medium and incubated at 25 °C, under 12 h of photoperiod for 14 days. Morphology of microconidia, macroconidia, conidiogenic cells (phallus) and chlamydospores were observed for each isolate and the species were identified (Leslie et al. 2006). For *Stenocarpella* spp., identification was made based on the morphological characteristics of conidia and conidiogenic cells (Sutton 1980).

Fumonisin analysis

For quantifying total fumonisins water content of grain samples was initially reduced in an oven at 65 °C under forced ventilation for 72 h. After cooling, the grains were ground in a Willey mill with 20 mesh sieve. Total fumonisins were extracted in methanol:water (80:20) solution and purified on

Fumoni Test immunoaffinity columns (VICAM), according to the manufacturer's guidelines, with laboratory validated modifications for 10 g sample and the other reagents following the appropriate proportion for this weight. The extracts were analyzed shortly after extraction and purification. The fumonisins were quantified in a VICAN fluorimeter, series 4. All analyzes were performed in duplicates and, for each analysis, a reference sample with a known content of total fumonisins (3.63 µg/g ± 1.290 µg/g, Romer Labs, code BRM 003017, lot M10203B) was added for quality control.

Statistical analysis

Total fumonisins content data (µg/g), grains moisture content (%), incidence of kernel rot (%) and fungal incidence in the grains (%) were subjected to analysis of variance. Hybrid, year and harvest delay and all possible interactions were treated as fixed effects in the model. The means of single and interaction of categorical factors (hybrid and year) were compared based on Scott-Knott test at 5% probability. Linear regression models were fitted to the data of the variables as a function of harvest times. The analyses were conducted in Sisvar 5.3 (Ferreira 2011).

Results

All single factors (hybrid, year and harvest delay) and the hybrid x year interaction significantly affected total fumonisin ($P < 0.05$) (Table 1). Total fumonisin content increased 0,02 µg/g per delaying day, ranging from 2 µg/g to 3.5 µg/g at 75 days of harvest delay (Fig. 1). As expected, grain moisture reduced from 17.5% on average to 11.5% on average during the same period, with a rate of −0.07% per delaying day (Fig. 1). Average of total fumonisin in the reference

sample ranged from 3.1 and 3.3 µg/g in the analysis conducted for the 2009/2010 and 2010/2011 samples, respectively, which was within the error (3.63 µg/g ± 1.290 µg/g, Romer Labs). Total fumonisin content varied significantly between seasons and between hybrids (Table 2).

Total fumonisins was lowest in DKB390 than the other hybrids in the 2 y of study, and did not differ between seasons. In 2009/2010 season, total fumonisins was higher in Attack compared to the other hybrids, which was also highest in 2010/2011 season but did not differ from BRS1035. For these two hybrids, total fumonisin contents were lower in 2010/2011 than in 2009/2010 season.

Two interaction factors, year x hybrid and hybrid x harvest date, significantly affected kernel rot incidence (Table 1), which was generally low across the samples (<5%). A significance increase in kernel rot incidence was found with the increase of the delay in harvest for all three hybrids, with a rate of 0.01% per day for hybrid Attack; 0.015% per day for hybrid BRS1035 and 0.04% per delaying day for hybrid DKB390 (Fig. 1). The hybrid Attack showed lower incidence of kernel rot than the others in both seasons; the highest mean was found for hybrid DKB390YG in the 2009/2010 season (Table 2), which differed from the other hybrids. In 2010/2011 season the DKB390YG and BRS1035 showed the highest kernel rot incidence and did not differ from each other. Kernel rot incidence in BRS1035 did not differ between seasons.

Fusarium verticillioides and *S. maydis* were found infecting the kernels, and their levels differed according to year and hybrid. In the 2 y, Attack and BRS1035 showed high incidence of *F. verticillioides* in the grain samples (Table 3), both differing from DKB390YG. In 2009/2010 season, incidence of *S. maydis* was lower in Attack and BRS1035 compared to DKB390YG. In the second season, only the Attack differed from the others with regards incidence of *S. maydis*, which was the lowest.

Table 1 Analysis of variance for total fumonisins (FUMON), kernel rot incidence (KR), grain moisture content (GM), *Fusarium verticillioides* incidence (FV) and *Stenocarpella maydis* incidence (SM) in experiments of harvest delay in corn conducted in Sete Lagoas in 2009/2010 and 2010/2011 seasons

Sources of Variation	DF	Mean Squares				
		FUMON	KR	GM	FV	SM
Year	1	112.159 *	19.253 *	39.120 *	1105.728 ns	177.100 ns
Hybrid	2	159.165 *	49.645 *	0.016 ns	8226.728 *	13,914.014 *
Harvest delay	5	5.802 *	7.039 *	68.533 *	832.042 ns	592.545 ns
Year x Hybrid	2	24.669 *	8.463 *	0.544 ns	2337,696 *	2704.709 *
Year x Harvest delay	5	0.251 ns	0.285 ns	3.852 *	251.578 ns	598.951 ns
Hybrid x Harvest delay	10	1.293 ns	1.765 *	0.147 ns	262.969 ns	421.697 ns
Year x Hybrid x Harvest delay	10	0.487 ns	0.407 ns	0.106 ns	738.294 ns	805.887 ns
Residual	68	0.887	0.740	0.215	276.715	630.712
CV (%)		28.3	39.6	10.9	27.2	28.7

ns Not significant

* Significant at the 5% probability level

Fig. 1 Total Fumonisins (μg/g) content (a), kernel rot incidence (b) and moisture content (c) in the grains due to corn harvest delay in 2009/2010 season, in Sete Lagoas, MG, Brazil
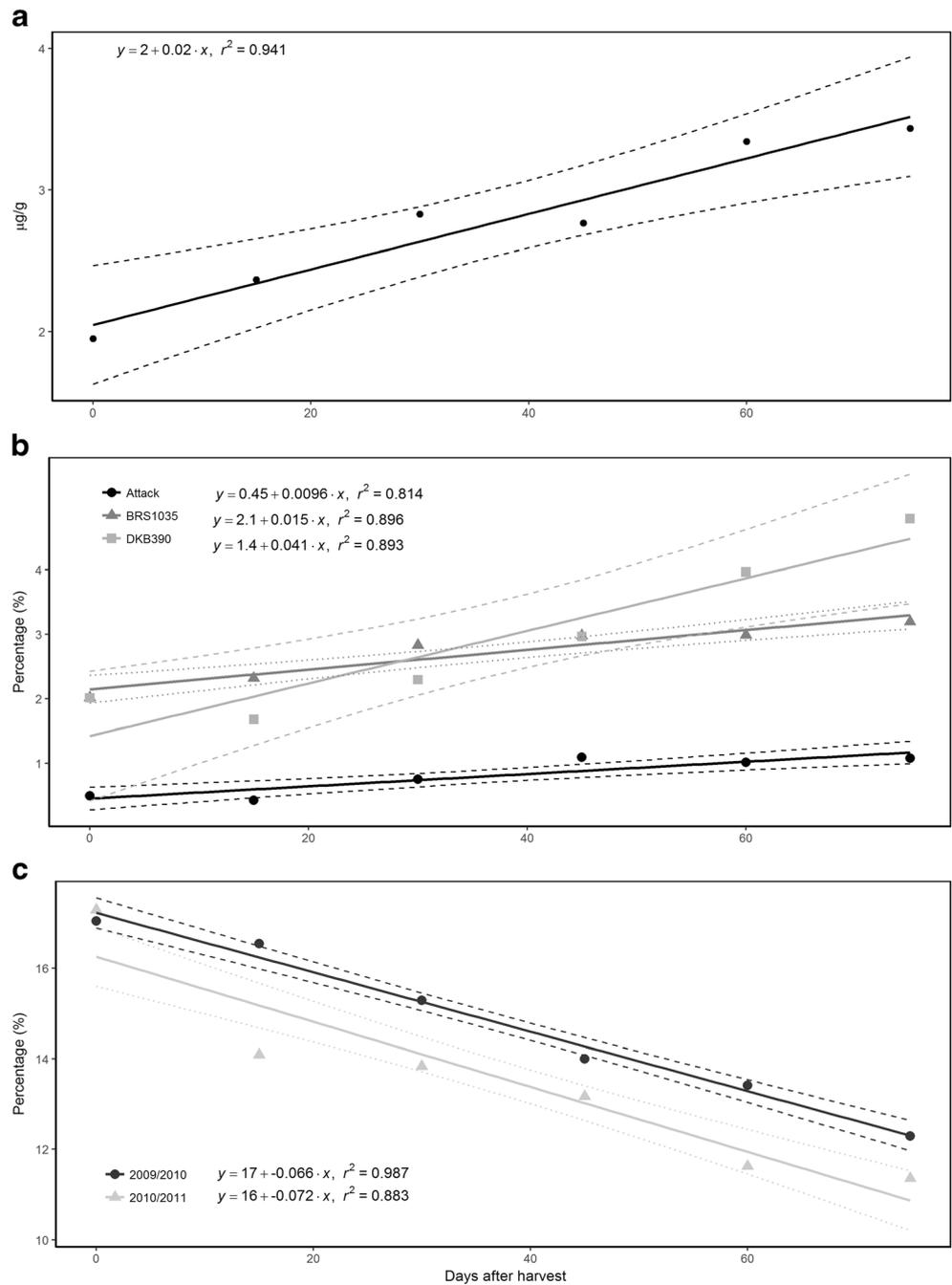
Weather conditions, especially rainfall amounts from December to May, varied dramatically between the two seasons (Table 4 and Fig. 2); accumulated rainfall in that period was 934.7 and 256.1 mm in the first and second seasons, respectively. For the other weather variables differences were not apparent.

Grain moisture content was consistently higher in 2009/2010 season than in the 2010/2011 season, which may be due to increased rainfall, mainly from March/April, when the first harvests were completed. The difference in total volume of rainfall for the period, between the both years, was 679 mm.

Table 2 Fumonisin (μg/g) and kernel rot (%) contents in three corn hybrids in 2009/2010 and 2010/2011 seasons, in Sete Lagoas, MG, Brazil

Hybrids	Fumonisins (μg/g)		Kernel rot (%)	
	2009/ 2010	2010/ 2011	2009/ 2010	2010/ 2011
DKB390YG	0.48Aa	0.32Aa	4.00Ca	2.00Bb
BRS1035	4.90Ba	2.21Bb	2.90Ba	2.55Ba
Attack	6.03Ca	2.74Bb	0.93Aa	0.70Ab

Means followed by the same capital letters in column and lowercase in row do not differ at the 5% probability level by the Scott-Knott test

Table 3 *F. verticillioides* and *Stenocarpella maydis* incidence in three corn hybrids subjected to harvest delay in 2009/2010 and 2010/2011 seasons in Sete Lagoas, MG, Brazil

Hybrids	2009/2010		2010/2011	
	F. verticillioides	*S. maydis*	*F. verticillioides*	*S. maydis*
Attack	92.8b	4.6a	89.8b	3.7a
BRS1035	77.7b	9.7a	75.1b	28.1b
DKB390	50.6a	50.1b	68.1a	42.3b
Average	73.7	21.5	77.7	24.7

Means followed by the same letter in a column do not differ at the 5% probability level by the Scott-Knott test

Discussion

We showed that even in the first, non-delayed, harvest (17 to 18% of humidity) both fumonisins and kernel rot were detected, which confirms previous findings of early infection of maize by toxigenic fungi and fumonisins (Chulze et al. 1996; Warfield and Gilchrist 1999). According to Hermanns et al. (2006), grain contamination by toxigenic *Fusarium* spp. and fumonisins can occur at the early stages of grain development (farinaceous grains) and may increase until physiological maturation. King (1981) harvested ears at weekly intervals during 10 weeks, starting at flowering, and found *F. verticillioides* in the grains as early as 2 wk after flowering, with frequency increasing afterwards.

In our study, the total fumonisin contents and kernel rot incidence increased from the optimal harvest time to 75 days of delay. Similarly Kaaya et al. (2005) reported that a four-week harvesting delay, beyond physiological maturation, significantly increased aflatoxin content in the grains. In New Zealand, similar pattern was reported by Lauren et al. (2007), for nivalenol, deoxynivalenol and zearalenone. In North Carolina, Bush et al. (2004) found an increase in the fumonisin content from the grains physiological maturation until the average date of corn harvest in the region.

Early harvesting has been recommended to reduce fumonisins in grains in other countries (Bush et al. 2004; Kaaya et al. 2005; Hermanns et al. 2006; Lauren et al. 2007), and our data is the first for tropical conditions that confirms earlier findings and recommendations for managing fumonisin risk. Previously, Santin et al. (2004), in southern Brazil, reported an increase in kernel rot incidence when grain moisture was above 18%, with a reduction at lower (drier) levels. The authors concluded that delay in corn harvest did not influence the increase in the kernel rot incidence. In the present study, the incidence of kernel rot gradually increased in 0.02% per delaying day in hybrids average, even when the grains had moisture content below that limit. These differences may be related to a) species of fungi present, b) occurrence of rainfall after the grains physiological maturation phase and c) ear damage caused by insect attack.

Variability in fumonisin levels and kernel rot incidence was observed among the hybrids. There was not an apparent

Table 4 Maximum and minimum temperatures, relative humidity and precipitation. Monthly summary of weather variables recorded at Sete Lagoas experimental station, in 2009/2010 and 2010/2011 seasons

Month/year	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Mean air relative humidity (%)	Total precipitation (mm)
2009/2010				
Dec/09	28.40	19.00	77.65	364.00
Jan/10	31.63	19.23	65.78	153.80
Feb/10	32.40	19.22	62.80	118.20
Mar/10	30.40	19.29	73.66	190.90
Apr/10	29.05	16.77	67.27	55.30
May/10	28.14	14.37	67.45	46.20
Jun/10	26.15	10.91	65.03	6.30
Average/Sum	29.45	16.97	68.52	934.70
2010/2011				
Dec/10	30.25	19.73	72.15	34.00
Jan/11	30.25	19.33	69.47	44.30
Feb/11	32.53	19.31	59.45	54.90
Mar/11	29.28	19.28	75.40	122.00
Apr/11	29.33	17.79	67.06	0.00
May/11	28.13	15.60	61.77	0.90
Jun/11	27.31	12.09	62.90	0.00
Average/Sum	29.37	16.86	69.02	256.10

Fig. 2 Daily precipitation 30 days before and after the flowering date (arrow) of corn hybrids, in the experiments of corn harvest delay conducted in the Sete Lagoas experimental station, in 2009/2010 and 2010/2011 harvests
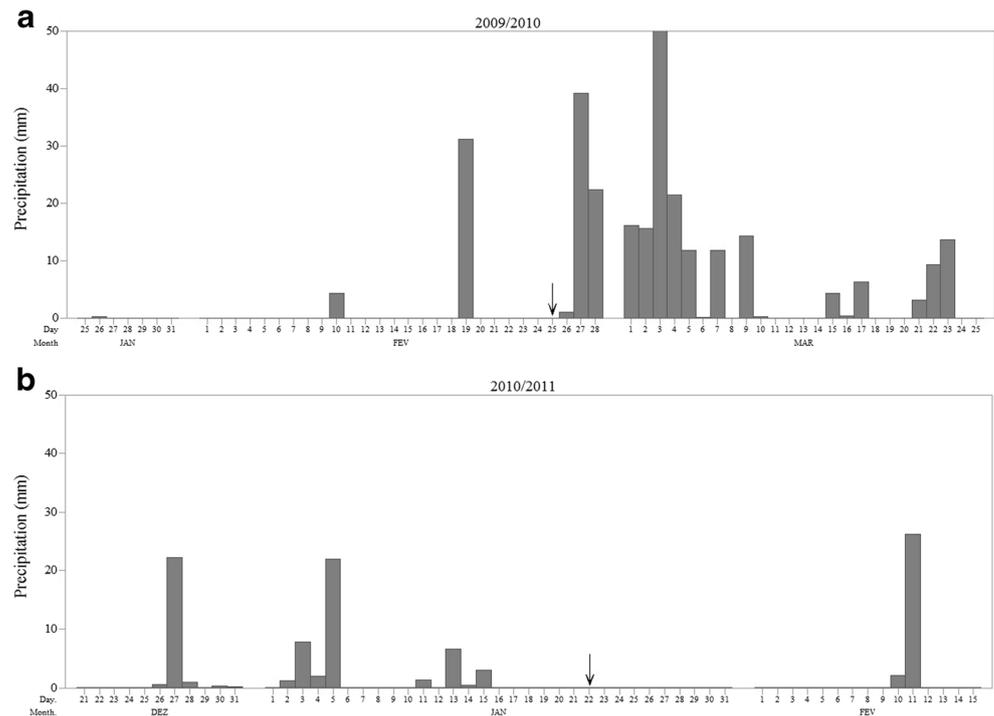
association between these two variables; hybrid that showed the lowest accumulation of fumonisins was not necessarily least affected by kernel rot. This may be partially explained by the fungi species found in the samples at different harvest times. The hybrid DKB390 YG showed lower incidence of *F. verticillioides* than the other hybrids in both years, thus explaining the lower incidence of fumonisins in the grains. *F. verticillioides* is considered one of the main species of fumonisin-producing fungi in maize grains (Nelson et al. 1991; Guo et al. 2016). According to Lanza et al. (2017), both *F. verticillioides* and fumonisins occur frequently in asymptomatic maize grains. On the other hand, the incidence of *S. maydis* in this hybrid was high and, although this fungus is not related to the occurrence of fumonisins, it is one of the main fungi causing ear rot and kernel rot. Inverse results were observed for the hybrid Attack, which presented low incidence of kernel rot and higher levels of fumonisin in the grains.

Although *F. verticillioides* and fumonisins may be found in asymptomatic corn grains (Lanza et al. 2017), high levels of fumonisins are commonly associated with ear rot incidence (Bowers et al. 2013). According to Parsons and Munkvold (2010), grains showing physical injuries have a higher incidence of ear rot and contamination with fumonisins than undamaged grains. In particular, insect-damaged grains are more likely to be contaminated with fungi and mycotoxins (Bowers et al. 2013). According to these authors, the use of transgenic hybrids expressing the Cry1AB x Vip3Aa genes reduced the fumonisin content in the grains when compared to hybrids expressing only the Cry1AB gene or non-transgenic hybrids.

In our study, the transgenic Bt hybrid, DKB390 YG, although less contaminated with fumonisins, kernel rot incidence was higher than in the other hybrids. This suggests that the damage caused by insects in the ears was not a key factor for the grains contamination with fungi and mycotoxins and, therefore, it was not possible to associate Bt hybrid with fumonision contamination.

According to Munkvold et al. (1999), transgenic insect protection events tend to be efficient in preventing grains contamination with toxigenic fungi in situations of high ears damage caused by insects' attack, which favor fungi entry. However, even in the absence of insect damage in the ears, it is possible to contaminate the grains since the fungi have other forms of grain contamination, such as infection by the style-stigma (Headrick et al. 1990), by seeds (Kedera et al. 1992) or even plant systemic infections (Munkvold et al. 1997). These results indicate that hybrid DKB390 YG shows greater genetic resistance to the infection by *F. verticillioides* and fumonisins accumulation when compared to the other evaluated hybrids.

High incidence of *F. verticillioides* and high levels of fumonisins in corn grains occurs in hot and dry climate regions (Parsons and Munkvold 2010). In the present study, however, fumonisin contents and kernel rot incidence were higher in 2009/2010 season, when total rainfall accumulation was much higher than in 2010/2011 season (934.7 and 256.1 mm, respectively). However, some studies suggest that the critical phase of corn, as regards the effect of climate conditions on the development of *F. verticillioides*, is pollination (Miller 1994; Pascale et al. 1997). Shelby et al. (1994)

reported a strong inverse correlation between precipitation close to the pollination stage and fumonisins accumulation in corn grains.

In 2009/2010 season, the number of days without rain before the flowering phase was higher than in 2010/2011 season. Inverse condition occurred in 2009/2010 season with higher rainfall accumulation after flowering. This may be explained by the known effect of water stress before flowering, followed by rainfall after this phase, favoring *F. verticillioides* infections and fumonisin accumulation in the grains (Miller 1994; Shelby et al. 1994; Pascale et al. 1997; De la Campa et al. 2005; Cao et al. 2013). However, this correlation has not been confirmed in other studies. In Spain, grain contamination with fumonisins is low in hot and dry areas of the northeast (Arino et al. 2007; Herrera et al. 2010). Meanwhile, fumonisin levels are higher in colder and wetter areas of the northwest, closer to the limit of 4 μg/g established by the European Union for human consumption (Butron et al. 2006; Cao et al. 2013). According to Fandohan et al. (2005), in wet regions, fumonisins contamination is favored by the existence of more than one crop per year, high infestation of pests and fungal infections in the field. These reports suggest the need for further studies relating infections to toxigenic species of *Fusarium* spp. and the fumonisins accumulation to weather conditions in corn producing regions in the Brazilian Cerrado. In summary, our data shows that delaying harvest for minimizing drying costs may increase the risk of mycotoxin contamination in maize in the tropics of Brazil.

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