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SHORT COMMUNICATION

Symptomatological aspects associated with fungal incidence and fumonisin levels in corn kernels

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Abstract Toxigenic fungi that infect corn grains are among the major problems in Brazil as they are associated with toxicological implications for both humans and animals. Mechanical-physical methods have been used to separate contaminated grains in lots of corn kernels based on the symptoms caused by fungi. The objective of this study was to assess whether the presence or the type of symptoms were associated with fungal incidence and total fumonisin levels in corn kernel samples from the field. Kernels of two hybrids were separated visually into three groups: 1) asymptomatic or symptomatic exhibiting 2) kernel rot or 3) kernel streak symptoms. A high frequency of *Fusarium* spp. was observed in asymptomatic (69.5%) and kernel rot (69.9%). *Stenocarpella* sp. and *Penicillium* sp. were found in kernel rot (16.0%) and kernel streak (44.1%), respectively. High fumonisin levels (7.24 μg.g-^{1}), above the maximum tolerated limit, were detected only in kernel rot. No association between fumonisin levels and the frequency of *Fusarium* spp. in kernels was found.

Keywords *Zea mays* · Corn grain symptomatology · Fungal incidence · Mycotoxins

Brazil is the third largest producer of corn with 84 million tons of corn grain produced in the 2014/15 growing season (CONAB 2016). However, the sanitary quality of grains creates barriers to export and poses serious risks to human health (Koffi-Nevry et al. 2013). In recent years, there is a growing public and private demand for high quality and safe grains for food and feed. As a result, regulatory guidelines became more severe for the presence of contaminants in grains for both the domestic consumption and export, particularly for mycotoxins not only in Brazil but also worldwide (ANVISA 2017; EURL 2006; FDA 2001).

In Brazil, corn is grown all over the country and under a wide range of climatic conditions, and is a host for a large number of pathogens that can cause reductions in grain quality, some of which can also produce toxins dangerous to humans and animals (Oliveira et al. 2004). Fumonisins are the most important group of mycotoxins found in Brazilian corn and are known to be harmful to animals and to cause certain types of cancer in humans (Munkvold and Desjardins 1997; Gelderblom et al. 1988; Jackson and Jablonski 2004). Although fumonisins are produced by several *Fusarium* species, *Fusarium verticillioides* is the most commonly fungi infecting corn kernels and causing ear and kernel rot (Munkvold and Desjardins 1997; Lanza et al. 2014).

Considering the importance of fumonisins to human health, government and food processing industry of many countries have implemented programs to monitor mycotoxin levels in corn grain and products. In Brazil, the food industry adopted a 6% tolerance for the presence of kernel rot in commercial lots of corn grain (Pinto et al. 2007). In addition, the National Agency for Sanitary Surveillance (ANVISA), have established a maximum tolerance limit (MTL) of 5 μg.g-^{1} of total fumonisins (B_1 + B_2) for corn grain (ANVISA 2017).

Considering the new guidelines imposed by agribusinesses and government agencies in Brazil to improve the quality of corn grain, the development of new management strategies is

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necessary to reduce fumonisin contamination in grains (Munkvold and Desjardins 1997; Shetty and Bhat 1999; Pearson and Wicklow 2006). The use of more resistant hybrids is the principal strategy used by Brazilian farmers for controlling corn diseases (Costa et al. 2012). Furthermore, post-harvest strategies, such as mechanical separation of symptomatic grains or separation of fungal-infected corn kernels by density difference are important to improve the sanitary quality of grain (Shetty and Bhat 1999; Pearson and Wicklow 2006). However, little is known about the relationship between fumonisin levels and disease symptoms in corn grain. Kernel rot symptoms such as damaged kernels or streaked grains are typical of fungal infections that reduce grain quality (Pearson and Wicklow 2006; Pinto et al. 2007). Thus, the goal of this study was to investigate the association between symptoms induced by fungal pathogens in corn kernels with the fungal incidence and total fumonisin levels.

The experiment was performed at the Embrapa Maize and Sorghum Research Center, Sete Lagoas, Minas Gerais, Brazil. Two commercial hybrids, BRS1010 (Embrapa) and 2B710 (Dow AgroSciences), both rated as moderately resistant to fungal diseases, were grown during the 2011/12 crop season. The planting date was 11/11/2011 and the experimental design was a randomized complete block design in a factorial arrangement (2 genotypes × 3 symptoms), with three replications. Each plot consisted of four rows measuring five meters in length, with 0.8 m between rows and an average of five plants per meter. A one-meter distance between the sides and the ends of each plot was kept. Crop fertilization consisted of the application of 350 $kg.ha^{-1}$ of NPK fertilizer (8-28-16) + Zn at planting. Two applications of urea (150 $kg.ha^{-1}$) were made at 30 and 45 days after planting (DAP), respectively.

Grains without any apparent symptoms were scored as asymptomatic (Fig. 1a), while those showing at least 25% of the surface with discoloration, whose hue varied from light brown to purple or bright red to red-intense, were scored as kernel rot (Fig. 1b) (Pinto 2005; Trento et al. 2002; Pinto et al. 2007). The kernel streak (starburst symptoms) symptom type was assigned to grains showing unidirectional white spots, also known as streaks, on a portion of the grain surface (Fig. 1c).

By the time of crop maturity (150DAP), all ears from the two center rows of each plot were harvested and threshed, and the total grain from each plot was mixed thoroughly to obtain a random distribution of the kernels. A grain sample of 500 g from each plot was taken to determine the frequency of symptom type and the amount grain fumonisins levels. Grain from each sample was separated into three classes based on the presence and type of symptos: asymptomatic grains, kernel rot, and kernel streak (Fig. 1).

The incidence of fungi associated with asymptomatic grain, kernel rot and kernel streak was determined using a blotter test with freezing (Pinto et al. 2007). Two hundred kernels previously desinfeted with 2% sodium hypochlorite for five minutes were placed into germination plastic boxes (eight replicates, with 25 kernels in each box) containing three filter paper layers moistened with 5% water-agar. The boxes were incubated at 25 °C and 12 h photoperiod for 10 days. Kernels were then examined individually using a stereomicroscope to determine fungal incidence.

For the quantification of total fumonisins in corn grain, the water content of grain samples was reduced in an oven at 65 °C under forced ventilation for 72 h. After cooling, grain samples were ground and sieved to mesh size 20. Total fumonisins were extracted in methanol:water (80:20) and purified by FumoniTest® (VICAM Inc.) immunoaffinity column, and total fumonisin levels were determined by fluorometer according to the manufacturer's instructions (VICAM, 2011). A reference sample of fumonisins (TR-F100, Trilogy Analytical Laboratory, Inc.) quantified by High-Performance Liquid Chromatography (HPLC) (4.1 $\mu g.g^{-1} \pm 0.5$) was used to assure the accuracy test of the immunoaffinity column method.

The variables fungal incidence and concentration of fumonisins were subjected to analysis of variance by SISVAR statistical program (Ferreira 2011). Data were arcsine-transformed prior to analyses and treatment means were compared by the Tukey test at 5% probability.

Fig. 1 Symptoms of fungal infection in corn kernels. **a** asymptomatic grain; **b** kernel rot; **c** kernel streak

Table 1 Incidence of *Fusarium sp*., *Penicillium sp*. and *Stenocarpella sp.* in corn grain types classified according to the presence and type of symptom

Grain type	Incidence (%)*		
	Fusarium sp.	*Penicillium* sp.	*Stenocarpella* sp.
Asymptomatic	69.5 bC	29.0 aB	1.0 aA
Kernel streak	60.8 aC	44.1 bB	1.6 aA
Kernel rot	69.9 bC	22.5 aB	16.0 bA

*Means followed by the same letter, lowercase (vertically) and uppercase (horizontally), do not differ by Tukey test at 5% probability

Four fungal genera were associated with corn kernels were identified: *Fusarium*, *Penicillium*, *Stenocarpella*, and *Aspergillus*. For fungal incidence, significant effects were found for genotype and grain type, for *Fusarium* ($P = 0.015$ and 0.001 respectively), *Penicillium* ($P = 0.001$ and 0.001 respectively), and *Stenocarpella* ($P = 0.022$ and 0.001 respectively), but not for *Aspergillus* ($P = 0.43$ and 0.65 respectively). The interaction effect was not significant for any of the fungi ($P > 0.55$).

Genotype 2B710 showed higher incidence of *Fusarium* sp. (75%) compared to the BRS1010 genotype (61%) ($P = 0.001$). Conversely, the incidence of *Penicillium* sp. and *Stenocarpella* sp. were higher in BRS1010 (42.3% and 8.7%, respectively) than in 2B710 (21.4% and 3.8%, respectively) ($P < 0.001$). With regards to grain type, the incidence of *Fusarium* sp. in asymptomatic grains and kernel rot was higher than in kernel streak grains (Table 1). For *Penicillium* sp. a higher incidence was observed in kernel streak, while for *Stenocarpella* sp. higher incidences were observed in kernel rot (Table 1). For *Aspergillus* sp., the mean incidence across the genotype and grain type was 0.71%.

Analysis of variance of total fumonisins showed a significant effect only for grain type ($P = 0.001$). The highest total fumonisin level was detected in kernel rot with the average value of 7.24 $\mu g.g^{-1}$ (Fig. 2). There was no significant difference between the means of total fumonisins for asymptomatic grains and kernel streak, which averaged 1.39 $\mu g.g^{-1}$ and 1.08 $\mu g.g^{-1}$, respectively.

In our study, the highest incidences of *Stenocarpella* sp. and *Fusarium* sp. were found associated with kernel rot, corroborating previous reports (Pinto 2005, Pinto et al. 2007). The fact that kernel streak was associated with the incidence of *Penicillium* sp. contradicts Duncan and Howard (2010) who reported that kernel streak was mainly associated *Fusarium* sp., most often *F. verticillioides*. Those authors also reported that kernel streak originates from the insertion point of the style attachment to the kernel in the hilar region. We found that the development of the streaks initiated at the kernel insertion point into the corn cob (hilum) and progressed to the upper part of the kernel (Fig. 1c). This may be a key for differentiating symptoms by *Penicillium* sp. and *Fusarium* sp.

Aspergillus spp. and *Penicillium* spp. were identified both in symptomatic grain and kernel rot, but the incidence of *Penicillium* was higher in kernel streak. The incidence of those pathogens in corn kernels is worrisome because of their toxigenic potential, which are associated with some types of cancer in humans and animals (Machinski et al. 2001). Although in Brazil high levels of aflatoxins and incidence of *Aspergillus* spp. have been reported (Machinski et al. 2001; Ramos et al. 2010), we found a lower incidence of this fungal genus compared to previous reports. The mycotoxins aflatoxins and ochratoxins, which are mainly produced by *Aspergillus* and *Penicillium*, respectively, were not quantified in this study.

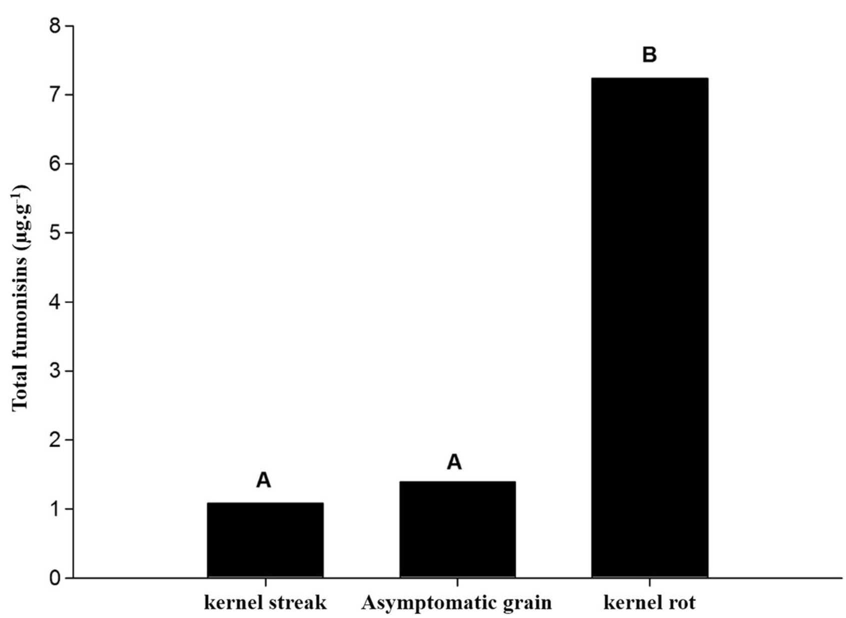

Fig. 2 Total fumonisin levels in corn grain samples with different types of symptoms: kernel streak, asymptomatic grain and kernel rot. Means followed by the same letter do not differ by Tukey test at 5% probability

Literature reports on the association between symptoms on grains and fungal incidence are limited. The incidence of *Fusarium* sp. did not differ between asymptomatic kernels and kernel rot, and it was lower in kernel streak, corroborating previous reports (Munkvold and Desjardins 1997). Nonetheless, we found low levels of fumonisins in samples of asymptomatic grain, while Munkvold and Desjardins (1997) suggested that asymptomatic grain infected with *Fusarium* sp. may contain high levels of fumonisin. The contrasting results may be due to differences in corn genotypes, pathogen variability or environmental conditions (Picot et al. 2010).

For the two commercial hybrids tested in our study, the high incidence (> 69%) of *Fusarium* sp. in both asymptomatic and kernel rot kernels suggests that this fungus is the main rotting agent of corn kernels. Regarding *Stenocarpella* sp., we found low incidence in asymptomatic kernels (1%) and kernel streak (1.6%), and higher incidence in kernel rot (16%) (Table 1), which suggest its secondary role in kernel rot and ear rot for the conditions of our experiments. Further studies with a larger number of genotypes and different environmental conditions are needed to confirm this hypothesis.

Duncan and Howard (2010) reported that streaks in corn kernels were mainly associated with *Fusarium* infection. We found that streak symptoms were associated only with higher incidence of *Penicillium* sp. Futhermore, our results showed that higher fumonisin levels were not always associated high incidence of *Fusarium* sp., once the level of this toxin was low in asymptomatic grain and high in kernel rot, while the *Fusarium* sp. incidence did not differ between these two grain types. Also important was that high fumonisin level was detected only in kernel rot, while the fumonisin levels in asymptomatic grains and kernel streak was below to the Brazilian regulation of maximum tolerated limit of 5 $\mu g.g^{-1}$ (ANVISA 2017). Thus, under conditions conducive for fumonisins production, the *Fusarium*-infected asymptomatic or kernel streak symptom may contain fumonisins at levels of risk to human health (Munkvold and Desjardins 1997; Picot et al. 2010).

We confirmed that the highest frequencies of *Fusarium* sp., *Penicillium* sp., and *Stenocarpella* sp. were associated with kernel rot and asymptomatic grains, kernel streak, and kernel rot, respectively. Higher levels of fumonisins were found in rotted kernels and there was no association between fumonisins and the incidence of *Fusarium* sp. in the grain. Nevertheless, the assessment of symptoms on healthy grain may be useful for improving methods of mechanical-physical separation as proposed previously (Shetty and Bhat 1999; Pearson and Wicklow 2006). To best or our knowledge, this is the first study on the association between symptoms on kernels and fungal incidence and fumonisins in Brazilian corn.

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