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Effect of cropping systems and crop successions on fumonisin levels in corn from Northern Paraná State, Brazil

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Abstract In this study the effect of different cropping systems and crop successions was evaluated on natural *Fusarium* sp. contamination and fumonisin levels in corn. The cropping systems consisted of a conventional and no-tillage area cultivated with corn in the summer following either oats or fallow in the winter (2006 and 2007 growing seasons). In addition, the effect of applying nitrogen fertilizer (0, 22.5, 45.0, 90.0 and 90.0 kg ha^{-1} nitrogen supplemented with potassium oxide) on fumonisin contamination was evaluated in the 2006 growing season. *Fusarium* sp. was detected in 90% samples in 2006 and in 100% samples in 2007. In both growing seasons, no-till corn following oats showed the highest mean fumonisin levels and differed significantly ($P<0.05$) from all the others (2006) and from conventional till corn following either oats or fallow in the winter (2007). Fumonisin levels ranged from 0.13 to 19.52 μg g^{-1} (mean 6.97 μg g^{-1}) and from 3.70 to 7.75 μg g^{-1} (mean 6.29 μg g^{-1}) in no-till corn following oats from the 2006 and 2007 growing seasons, respectively. Plots treated with 0 kg ha^{-1} and 22.5 kg ha^{-1} nitrogen showed the highest mean fumonisin levels and differed significantly from those with 45.0 and 90 kg ha^{-1} nitrogen. Fumonisin levels correlated negatively ($P<0.05$) with the nitrogen fertilization rates. Although no-till is advantageous from a soil conservation standpoint, it may enhance the potential for fumonisin contamination in corn.

Keywords Agricultural practices · Conventional tillage · Mycotoxin · Nitrogen fertilization · No tillage

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Introduction

Corn (*Zea mays* L.) is one of the main crops in Brazil. Corn production reached 56 million tons in the 2008/2009 harvest season, ranking the country as the third largest corn producer in the world (CONAB 2010).

Southern Brazil produces 45% of Brazilian corn and the State of Paraná accounts for 23.5% of the national production (CONAB 2010).

Corn grains can be contaminated by a variety of toxigenic mould species. *Fusarium verticillioides* (Sacc.) Niremberg is a primary corn pathogen and one of the main fumonisin producers. Although 28 fumonisin analogues have been characterized since 1988, fumonisins B_1 (FB_1) and B_2 (FB_2) are the most frequent in corn and corn-based products (Rheeder et al. 2002).

Fumonisins cause leukoencephalomalacia in equines (Marasas et al. 1988), pulmonary edema in swine (Ross et al. 1990), and hepatocarcinoma in rats (Norred and Voss 1994). In humans, epidemiological studies indicate its association with esophageal and primary liver cancer (Gelderblom et al. 1992; Ueno et al. 1997) and neural tube defects (Missmer et al. 2006).

Several researchers have reported high frequency of fumonisin contamination in corn and corn-based products from Brazilian States (Van der Westhuizen et al. 2003; Caldas and Silva 2007; Moreno et al. 2009). Van der Westhuizen et al. (2003) analyzed 76 corn samples from Santa Catarina State, Brazil and detected fumonisins in 100% of the samples. Caldas and Silva (2007) analyzed 208 corn-based products from the Federal District of Brazil and detected fumonisins in 80.7% of the samples. Moreno et al. (2009) detected fumonisins in 100% freshly harvested corn samples (n=150) from Northern Paraná State, Brazil, and previous study indicated high fumonisin contamination in corn samples from this region (Ono et al. 1999).

Taking into account that fumonisins are heat stable and cannot be removed by industrial processing, the best strategy for effective control of fumonisin contamination is preventing *F. verticillioides* infection and fumonisin production in the field and during storage.

The main strategies for corn phytosanitary control involve pesticides and agricultural practices including tillage and crop rotation. The latter affect the chemical and biological characteristics of soil and, therefore, the cereal microflora (Rothrock 1992).

In Brazil, two cropping systems, conventional tillage and no-till are used in the corn producing area. No-till is preferred nowadays due to the increase in organic matter in the soil, soil moisture retention and slow and gradual decomposition (Bockus and Shroyer 1998). Conventional tillage provides adequate conditions for seed germination, root growth and development to increase productivity (Schultz 1978) and reduces fungal inoculum with the inclusion of debris into the soil. While no-till offers new possibilities to reduce production cost and erosion (Bockus and Shroyer 1998), toxigenic fungi survive on previous crop residues which is probably the most important inoculum source for kernel infection (CAST 2003).

Nitrogen, an important macronutrient, plays a significant role in plant nutrition and disease resistance. It has been shown that stalk rot incidence increases with high nitrogen fertilizer applications (Bottalico and Logrieco 1988). Crop nutrition stresses, e.g., nitrogen deficiency, have been associated with high aflatoxin levels (Lisker and Lillehoj 1991). Therefore, balanced N fertilizer application is the best approach for low mycotoxin contamination (Blandino et al. 2008).

There are few reports on the impact of agricultural practices on fumonisin contamination in Brazil so the objective of the present study was to evaluate the effect of different cropping systems and crop successions on natural *Fusarium* sp. contamination and fumonisin levels in corn.

Material and methods

Characterization of experimental site

This study was carried out on the State University of Londrina experimental farm (Oxisol), Northern Paraná State, Brazil during the 2006 and 2007 growing seasons. Londrina municipality (23°37′00″S; 51°17′00″W-GR, altitude 585 m) is characterized as a mesothermic humid subtropical climate with hot, rainy summers (December to February, average temperature >22°C), and sporadic frost during winters (May to July, average temperature <18°C) (Paraná Cidade 2008).

Experimental design

The corn hybrid AGROESTE 33 was cultivated sowing six seeds/m and the experimental units consisted of four 5 m-rows and 0.90 m row spacing. A randomized block design was used arranged in four

soil strips for the cropping systems with four replications. Two cropping systems and crop successions were evaluated, the conventional and no-till area cultivated with corn in the summer following either oats or fallow in the winter (2006 and 2007 growing seasons). In the no-till area sowing was carried out directly through the residue of the previous crop, opening only a narrow furrow in the sowing row, whereas in the conventional tillage the soil was prepared with a disc plough and heavy-disc harrow followed by a light-disc harrow. In addition, the effect of nitrogen (N) fertilizer application on fumonisin contamination was evaluated in the 2006 growing season. The N rates were distributed randomly on each strip, with four replications, at the eighth unfolded leaf growth stage. The N fertilization rates applied as urea were 0, 22.5, 45.0, 90.0 and 90.0 kg ha^{-1} supplemented with potassium oxide (K_2O) using potassium chloride as potassium source (90.0 kg ha^{-1} N+13.2 kg ha^{-1} K_2O), totalling 20 samples for each cropping system.

Sampling was carried out collecting 10 corn ears per plot at the end of maturity (grain moisture content 22%–26%) and pooled. After homogenization, the samples (1.5 kg) were sent to the laboratory and maintained at 4°C for a maximum of 7 days for *Fusarium* sp. count. For fumonisin determination, 200 g of each corn sample were ground to 50 mesh and stored at −20°C.

Protein determination

The corn kernel nitrogen content was determined by the Kjeldahl method (AOAC 1984, method 7015), using 6.25 as the factor to obtain the total protein content.

Fusarium sp. count

Two hundred grams of each corn sample were ground to 50 mesh. Sub-samples (10 g) of ground corn were blended with 90 ml sterile 0.1% peptone water (v/v), and serial dilutions were carried out with 9.0 ml of the same diluent to 10^{-6}. One millilitre of each dilution was transferred into a Petri dish and pour-plated with potato dextrose agar (PDA, pH 4.0) added to 50 μg ml^{-1} chloramphenicol and incubated at 25°C for 6 days. The *Fusarium* genus was identified and enumerated according to Nelson et al. (1983).

Fumonisin analysis

Fumonisins B_1 and B_2 were analyzed according to Shephard et al. (1990) with some modification (Ueno et al. 1993). A 10 g sample of the ground kernels was mixed with 30 ml methanol:water (3:1, v/v). After standing for 10 min at room temperature, the suspension was shaken at 150 rpm for 30 min and centrifuged at 4500 × g for 10 min. The crude extract (1.0 ml) was applied to preconditioned Sep Pak accell plus QMA cartridge (Waters Co., Ltd.). After washing the cartridge with methanol-water (3:1, 6 ml) followed by methanol (3 ml), fumonisins were eluted with 10 ml methanol containing 0.5% acetic acid. The eluate was evaporated to dryness under a stream of nitrogen at 45°C, the residue was dissolved in methanol-water (3:1, 800 μl) and a 200 μl aliquot dried under nitrogen. After derivatization with 200 μl O-phthaldialdehyde reagent (40 mg OPA, 1 ml methanol, 5 ml 0.1 M sodium borate and 50 μl 2-mercaptoethanol), HPLC injections were made within 1 min. Fumonisins were analyzed by a reversed-phase isocratic HPLC system (Shimadzu LC-10 AD pump and RF-10A XL fluorescence detector), using a Shim-pack CLC-ODS (M) column (4.6×250 mm, Shimadzu). Excitation and emission wavelengths were 335 nm and 450 nm, respectively. The eluent was CH_3OH:0.1 M NaH_2PO_4 (80:20, v/v) adjusted to pH 3.3 with ortho-phosphoric acid. The flow rate was 1 ml min^{-1}. The detection limits for FB_1 and FB_2 were 27.5 ng g^{-1} and 35.3 ng g^{-1}, respectively. The recoveries of FB_1 and FB_2 from spiked corn in the range 100–400 ng g^{-1} FB_1 and 250–450 ng g^{-1} FB_2 averaged 95.6% (mean CV 8%) and 96.9% (mean CV 10%), respectively, based on duplicate spiking and duplicate analyses. Corn samples provided by the Agronomic Institute of Paraná (IAPAR) with non-detectable fumonisin levels were used for corn spiking.

Statistical analysis

Differences in *Fusarium* sp. count, fumonisin levels and protein content among the conventional and no-till corn following either oats or fallow in the winter were evaluated using ANOVA for factorial analysis (2006) and totally randomized analysis (2007) followed by the Duncan and *t*-tests ($P<0.05$). The Duncan test was used to compare means of those parameters for the

same growing season, and the *t*-test was used to compare means between the same treatments in different growing seasons. The *Fusarium* sp. count of every sample was transformed to log (x +1) and log x for samples from the 2006 and 2007 growing seasons, respectively, to reduce the variability among the data. Transformation to log (x+1) was necessary for samples from the 2006 growing season because *Fusarium* sp. was not detected in some samples. The Pearson correlation coefficient between N fertilization rates and protein content or fumonisin levels was calculated. Statistical analysis was performed using the 'Statistica' software version 6.0.

Results

Effect of cropping systems and crop successions on *Fusarium* sp. contamination

Table 1 shows the *Fusarium* sp. count in 96 freshly harvested corn samples from Northern Paraná State in the 2006 (n=80) and 2007 growing seasons (n=16).

In the 2006 growing season *Fusarium* sp. was detected in 90% samples and in 100% samples in 2007. *Fusarium* sp. was detected in the 10^2 to 10^4 CFU g^{-1} range in no-till corn following fallow, but in the 10^2 to 10^5 CFU g^{-1} range in other cropping systems (2006). In 2007, the *Fusarium* sp. count ranged from 6.0×10^4 to 1.4×10^6 CFU g^{-1} in no-till corn following fallow and from 2.0×10^5 to 7.5×10^5 CFU g^{-1} in no-till corn following oats, but in other cropping systems this genus was detected in the 10^4 to 10^5 CFU g^{-1} range. There was no significant difference by the Duncan test ($P<0.05$) in the *Fusarium* sp. count mean values between no-till and conventional till corn following either oats or fallow in either growing season, but *Fusarium* sp. contamination in corn samples from the 2007 growing season was higher than that of the 2006 growing season ($P<0.05$).

Effect of cropping systems and crop successions on fumonisin levels in corn

Table 2 shows the total fumonisin levels (FB_1 + FB_2) in corn grown in conventional and no-till area in the summer following either oats or fallow in the winter (2006 and 2007 growing seasons).

Fumonisin levels ranged from 0.13 to 19.52 μg g^{-1} (mean 6.97 μg g^{-1}) and from 3.70 to 7.75 μg g^{-1} (mean 6.29 μg g^{-1}) in no-till corn following oats from the 2006 and 2007 growing seasons, respectively (Table 2). In both growing seasons, no-till corn following oats

Table 1 *Fusarium* sp. count in corn grown in conventional and no-till area in the summer following either oats or fallow in the winter (2006 and 2007 growing seasons) in Northern Paraná State

Growing season	Cropping system and crop succession	*Fusarium* sp.		
		Positive/total samples	Count (CFU g^{-1})	
			Mean[x]	Range
2006	NTO	20/20	2.7×10^4 [aB]	1.0×10^2–1.0×10^5
	NTF	17/20	6.2×10^3 [aB]	1.0×10^2–1.5×10^4
	CTO	19/20	4.6×10^4 [aB]	1.0×10^2–5.0×10^5
	CTF	16/20	2.8×10^4 [aB]	1.0×10^2–2.0×10^5
2007	NTO	4/4	4.5×10^5 [aA]	2.0×10^5–7.5×10^5
	NTF	4/4	5.0×10^5 [aA]	6.0×10^4–1.4×10^6
	CTO	4/4	2.3×10^5 [aA]	6.5×10^4–5.0×10^5
	CTF	4/4	1.2×10^5 [aA]	5.0×10^4–2.0×10^5

NTO No-till corn in succession to oats in the winter; *NTF* No till-corn in succession to fallow soil in the winter; *CTO* Conventional till corn in succession to oats in the winter; *CTF* Conventional till corn in succession to fallow soil in the winter

[x] Means followed by the same lowercase letter are not significantly different by the Duncan test ($P<0.05$) for each growing season

[x] Means followed by the same uppercase letter are not significantly different by the *t*-test ($P<0.05$) between the same treatment in the two growing seasons

Table 2 Fumonisin levels and protein content in corn grown in conventional and no-till area in the summer following either oats or fallow in the winter (2006 and 2007 growing seasons)

Growing season	Cropping system and crop succession	n	Total FB * (μg g^{-1})		Protein content (%)	
			Mean[x]	Range	Mean[x]	Range
2006	NTO	20	6.97 aA	0.13–19.52	7.91abA	5.29–9.19
	NTF	20	3.28 bA	0.16–9.93	7.59 bA	5.01–9.09
	CTO	20	3.29 bA	0.08–15.33	8.04 aA	5.86–9.83
	CTF	20	2.97 bA	0.03–8.60	8.09 aA	7.05–8.97
2007	NTO	4	6.29 aA	3.70–7.75	6.43 abA	6.11–6.90
	NTF	4	2.74 abA	1.57–3.85	5.58 bB	4.47–6.50
	CTO	4	2.14 bA	0.99–3.41	6.24 abB	5.38–6.93
	CTF	4	2.24 bA	1.41–3.60	7.64 aA	7.45–7.89

* Total FB=FB_1+FB_2 (Detection limit: FB_1=27.5 ng g^{-1}, FB_2=35.3 ng g^{-1})

NTO No-till corn in succession to oats in the winter, *NTF* No-till corn in succession to fallow soil in the winter, *CTO* Conventional till corn in succession to oats in the winter

n number of samples

[x] Means followed by the same lowercase letter in the same column are not significantly different by the Duncan test ($P<0.05$) for each growing season

[x] Means followed by the same uppercase letter in the same column are not significantly different by the t-test ($P<0.05$) between the same treatment in the two growing seasons

showed the highest mean fumonisin levels and differed significantly ($P<0.05$) from all the others (2006) and from conventional till corn following either oats (2.14 μg g^{-1}) or fallow (2.24 μg g^{-1}) in the winter (2007). Nevertheless, there was no significant difference in mean fumonisin levels from either cropping system or crop succession between the two growing seasons.

Protein content and fumonisin levels in corn kernels

The corn kernel protein content (2006 and 2007 growing seasons) was evaluated because of the role of nitrogen in plant nutrition and disease resistance (Table 2). Mean protein content ranged from 7.59% to 8.09% (2006) and from 5.58% to 7.64% (2007). The corn kernel protein content correlated positively with the N fertilization rates (ρ=0.583) ($P<0.05$) (Fig. 1).

Fumonisin levels correlated negatively ($P<0.05$) with corn kernel protein content (ρ=−0.359) (Fig. 2)

Effect of N fertilization rates on fumonisin levels

The correlation was evaluated between the N fertilization rates in coverage and fumonisin levels in corn grown in conventional and no-till area in the summer following either oats or fallow in the winter (2006) (Fig. 3).

Fumonisin levels correlated negatively ($P<0.05$) with the N fertilization rates (ρ=−0.313), i.e., the mean fumonisin level increased as the N-rate application

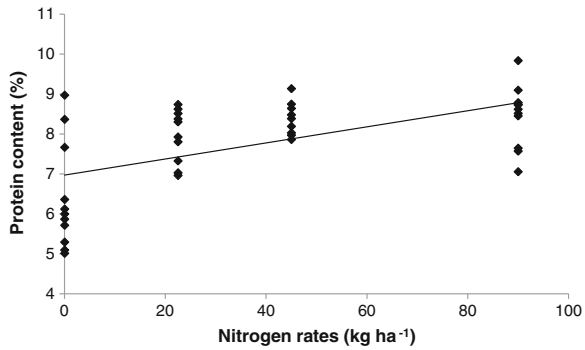

Fig. 1 Correlation between nitrogen fertilization rates in coverage and protein content in freshly-harvested corn grown in a conventional and no-till area in the summer following either oats or fallow in the winter from Northern Paraná State (2006 growing season). Linear regression equation y=6.9884+0.02034× was obtained with correlation coefficient (ρ) of 0.583 ($P<0.05$)

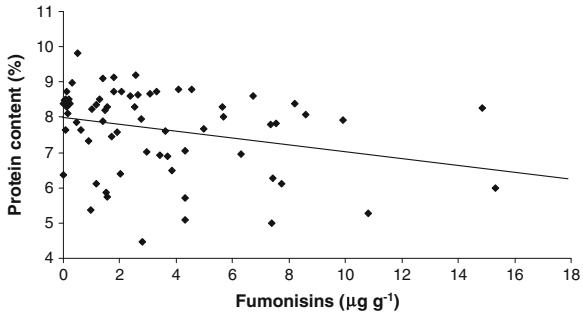

Fig. 2 Correlation between fumonisin levels (FB_1+FB_2) and protein content in freshly-harvested corn grown in a conventional and no-till area in the summer following either oats or fallow in the winter from Northern Paraná State (2006 and 2007 growing seasons). Linear regression equation $y=8.2793–0.1085\times$ was obtained with correlation coefficient (ρ) of −0.359 ($P<0.05$)

decreased (Fig. 3). The highest mean fumonisin levels were detected in the plots that received 0 kg ha^{-1} N (6.13 μg g^{-1}) and 22.5 kg ha^{-1} N (6.59 μg g^{-1}). There was a significant difference ($P<0.05$) between these treatments and the plots that received 45.0 and 90 kg ha^{-1} N (Table 3), but there was no significant difference ($P<0.05$) in the mean fumonisin level between the plot that received 90 kg ha^{-1} N and this N-rate supplemented with potassium chloride (13.2 kg ha^{-1}).

Discussion

The high *Fusarium* sp. frequency (Table 1) is in accordance with the data reported by Moreno et al. (2009), who analyzed 150 corn samples from Paraná

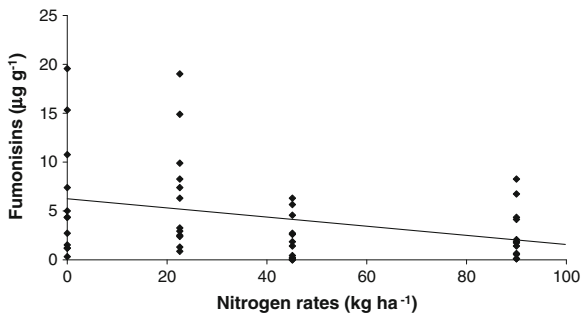

Fig. 3 Correlation between nitrogen fertilization rates in coverage and fumonisin levels (FB_1+FB_2) in corn grown in a conventional and no-till area in the summer following either oats or fallow in the winter from Northern Paraná State (2006 growing season). Linear regression equation $y=6.3401–0.481\times$ was obtained with correlation coefficient (ρ) of −0.313 ($P<0.05$)

Table 3 Mean fumonisin levels in corn grown in conventional and no-till area in the summer following either oats or fallow in the winter in different nitrogen fertilization rates in coverage (2006 growing season)

N rate (kg ha^{-1})	Total FB (μg g^{-1})[x]
0	6.13 [a]
22.5	6.59 [a]
45.0	2.17 [c]
90.0	2.65 [bc]
90.0+13.2 (K_2O)	3.11 [bc]

Total $FB=FB_1+FB_2$ (Detection limit: $FB_1=27.5$ ng g^{-1}, $FB_2=35.3$ ng g^{-1})

[x] Means followed by the same letter are not significantly different by the Duncan test ($P<0.05$)

State, Brazil, and detected *Fusarium* sp. in 100% of the samples. The absence of significant tillage effects on the incidence of *Fusarium* sp. is in accordance with data reported by Flett et al. (1998) but in disagreement with Byrnes and Carroll (1986). Flett et al. (1998) showed that alternating tillage practices had no effect on ear rots caused by *Fusarium* sp. while *Fusarium* sp. was isolated more frequently from corn in conventionally tilled fields in the sandy soils of southern Delaware (Byrnes and Carroll 1986).

In both growing seasons (2006 and 2007), no-till corn following oats showed the highest mean fumonisin levels (Table 2), probably because oats can support *Fusarium* sp. growth and could be a substratum for fumonisin production. Many *Fusarium* species survive in crop debris and become a hazard to further crops because these microorganisms act as sources of inoculum (CAST 2003). Similarly, the highest fumonisin level (6.84 μg g^{-1}) was detected in no-till corn (Schiabel 2004), regardless of crop year, N fertilization and crop succession. Dill-Macky and Jones (2000) reported that no-till after wheat or corn significantly increased deoxynivalenol contamination of the following wheat crop compared to conventional tillage, but no-till had no effect when the previous crop was soybean. On the other hand, there was no significant effect of no-till compared with conventional tillage on fumonisin occurrence in corn monoculture in Northern Italy (Marocco et al. 2008). Ariño et al. (2009) analyzed corn samples from 16 fields in Northeastern Spain and detected no clear effect of tillage system, type of irrigation, and harvest date on fumonisin levels.

Fumonisin levels increased as the corn kernel protein content decreased, probably due to the stress conditions caused by the plant-pathogen competition for nutrients, which trigger fumonisin production. These results are in agreement with Lisker and Lillehoj (1991), who reported that high aflatoxin levels have been associated with fertility-related stresses, mainly N deficiency.

Plots treated with 0 kg ha^{-1} N and 22.5 kg ha^{-1} N fertilizer rates showed the highest mean fumonisin levels. Blandino et al. (2008) reported that stress due to N deficiency and high N rates significantly increased FB_1 contamination in corn. Hassegawa et al. (2008) demonstrated that the combination of nitrogen (100 kg/ha) with zinc (0 and 1.0 kg/ha) and boron (0 and 0.5 kg/ha) reduced FB_1 and FB_2 levels. On the other hand, Jones and Duncan (1981) and Tubajika et al. (1999) reported that a higher rate of N fertilizer application reduced aflatoxin levels in corn.

Potassium supplementation showed no significant effect on fumonisin levels, probably because the KCl dose was low. No significant correlation was observed between deoxynivalenol levels in wheat and soil potassium (Teich and Hamilton 1985) but aflatoxin B_1 production was decreased by increasing KCl concentration in the culture medium (Rusul et al. 1986).

In summary, no-till corn following oats, and plots treated with low nitrogen fertilizer rates showed the highest mean fumonisin levels. Although no-till is advantageous from an environmental and economical standpoint, it may enhance the potential for fumonisin contamination in corn.

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