Occurrence of *Fusarium* toxins in stored maize in southern Brazil

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Abstract

Fusarium toxins are produced in maize in the field (before and during harvesting) and in storage. During two consecutive crop seasons of maize, 1991–92 and 1992–93, high percentages of mould damaged kernels were observed. Besides unfavourable weather conditions during plant development, stalk rot, and corn borer damage were also high. Harvesting was delayed and the crop processed under wet and cool weather. Some hybrids presented the tips of the ear heavily invaded with shrunken and reddish kernels. Concerns about mycotoxin contamination grew when *Fusarium moniliforme* and *F. graminearum* were retrieved from plated kernels. To evaluate the extent of the problem, random samples were taken every 30 days during six months, from three silos with corn from the 1992–93 crop. Samples from each silo were tested for mycotoxins, moisture content, mould damaged kernels and mould damaged germs. All the samples taken for mycotoxin analysis were positive for fumonisins, deoxynivalenol and zearalenone, with levels above their controls of 5 ppm, 1 ppm, and 1 ppm, respectively. None of the samples was positive for aflatoxins B_1 or B_2. Moisture content was at a safe level for storage, although mould damaged kernels and mould damaged germs were high. The results showed the necessity of monitoring programs to check the levels of *Fusarium* toxins and adoption of appropriate management of contaminated corn lots to be used for food or feed.

Introduction

Several species of *Fusarium* are responsible for seedling blight, root rot, stalk rot, ear rot, and kernel rot on maize (Cardwell and Tuite; CAST 1989; Cullen and Caldwell 1983). Besides the rots, some species produce mycotoxins that are harmful to domestic animals when present in the feed, and reduce the commercial value of infected and contaminated crops (Abbas et al. 1986; Luo et al. 1990; Marasas and Nelson 1987; Ross et al. 1990, 1991; Sydenham et al. 1991).

The economic losses caused by mycotoxin contaminated maize are high, widespread and difficult to assess (Abbas et al. 1986; Luo et al. 1990; Soares and Rodriguez-Amaya 1989; Sydenham at al. 1992; Tanaka et al. 1985). *Fusarium moniliforme*, and *F. graminearum (Gibberella zeae)*, produce several mycotoxins that might be found at different concentrations and combinations: zearalenone, deoxynivalenol (vomitoxin), and fumonisins (Abbas et al. 1986, 1992; CAST 1989; Cullen and Caldwell 1983; Marasas and Nelson 1987). Zearalenone is more frequently encountered in maize, maize-based products (breakfast cereals and cornmeal), and feeds. It is associated with hyperoestrogenism in swine (Abbas et al. 1986; CAST 1989; Marasas and Nelson 1987; Soares and Rodriguez-Amaya 1989).

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Kernels of maize infected by *F. moniliforme* and *F. graminearum* show a pink, reddish, and purplish discoloration allowing their visual identification in a maize sample and separation from sound kernels (Lazzari 1993; Marasas and Nelson 1987).

The objective of this work was to monitor the quality of stored maize by evaluating for the presence of mycotoxins, moisture content, mould-damaged kernels and mould-damaged germs, which are relatively new procedures in Brazil for quality control in maize marketing.

Materials and Methods

A total of 210 samples of 1 kg each was taken over a 6 month period in 1993, from the conveyor belt of three 1100 t silos in Paraná State, southern Brazil. The stored maize was from the 1992-93 crop which suffered from *Fusarium* spp. infection and adverse weather conditions during maturation and harvesting.

Every 30 days, samples were checked for mycotoxin contamination, moisture content, mould-damaged kernels and mould-damaged germs.

For mycotoxin analysis, mould-damaged kernels (shrunken, pink, reddish, or purplish), were hand picked from the 10 samples from each silo, ground and used as the working sample. Because of the great variability associated with the sampling and testing procedures it is difficult to accurately estimate mycotoxin concentrations in large bulks of stored maize; thus, it was decided to sample only mould-damaged kernels.

For detection of aflatoxins B_1+B_2, fumonisins, deoxynivalenol, and zearalenone, immunodiagnostic kits were used. The kits were purchased from Neogen Corporation, MI, USA. The sample preparation, extraction, and analysis were carried out according to the manufacturer's instructions.

For moisture content determination, 10 samples were checked every 30 days from each silo. The moisture content was determined by an electronic moisture meter and the results recorded as the average moisture content of the 10 samples analysed.

To analyse mould-damaged kernels, 10 samples of 250 g from each silo were inspected according to the Brazilian commercial standards for maize quality.

To determine mould-damaged germs, 750 kernels were randomly taken from each silo, cut lengthwise, and examined under a microscope. Discoloured, brown or deteriorated germs were considered damaged.

Results

Table 1 presents the mycotoxins detected in stored maize during the 6-month period. All the samples were positive for fumonisins, deoxynivalenol (vomitoxin) and zearalenone. None of them were positive for aflatoxins B_1 + B_2. For the toxins of *Fusarium* spp., the levels were above the controls.

Table 2 shows the average moisture content of the samples of maize during the 180 days of storage. It can be seen that the moisture content of all samples was within the safe level for storage, ranging between 12.7% and 14.1%.

Table 1. Mycotoxins detected[a] in stored maize, in Guarapuava, PR, Brazil, in 1993.

Mycotoxin	Sampling days						
	1	30	60	90	120	150	180
Aflatoxins B1+B2 (Control 20 ppb)	—	—	—	—	—	—	—
Fumonisins (Control 5 ppm)	+	+	+	+	+	+	+
Deoxynivalenol (Control 1 ppm)	+	+	+	+	+	+	+
Zearalenone (Control 1 ppm)	+	+	+	+	+	+	+

[a]- No sample was positive, i.e. above the control level, + Samples were positive, i.e. above the control level.

Table 3 presents the percentage of mould-damaged kernels. The level of mould-damaged kernels was considered high relative to the Brazilian commercial standards for maize. The averages of all samples were above the limit of acceptance (6.0%).

Table 4 gives the percentage of mould-damaged germs in the three silos during the storage period. Although, the average moisture contents were safe for storage maize without risk of spoilage by moulds, the percentage of mould-damaged germs increased as the period of storage increased.

Discussion

The results indicate that maize in southern Brazil might be heavily contaminated with *Fusarium* toxins and that action should be taken to reduce the risks, especially in years when weather conditions are favourable for fungus development.

Although there is no regulatory tolerance levels for fumonisins, deoxynivalenol and zearalenone in Brazil, it is very important to have an efficient monitoring program to assess the occurrence, predominance and levels of these toxins in maize, maize-based products, and feeds.

Due to the fact that people and domestic animals do eat 'actual doses' of mycotoxins daily and not 'average levels', a biased sampling procedure can provide a better idea of the real contamination level. For this reason the sampling procedure adopted in this study was to analyse only mould-infected kernels of maize for the immunodiagnostic and germ analysis. This procedure presents advantages because less time is expended on sampling and for a lower cost of analysis.

The moisture contents of the maize were not high enough to allow growth of *Fusarium* spp. in storage. The maize came contaminated with *Fusarium* toxins from the field, as a result of the favourable weather conditions for fungal development.

The mould-damaged germ technique is more accurate than the mould-damaged kernel inspection to check the extent of infection by fungi. By splitting kernels of maize and checking directly the germ under a microscope, a better view of the condition of the grain is possible. The commercial inspection for maize grading can lead to an underestimation of the real level of mould-damaged kernels because it is based on only the external appearance of the kernels.

In conclusion, the combination of hand-picked damaged kernels, germ inspection and immunodiagnostic test can greatly enhance the detection of mycotoxin-contaminated kernels and guide management actions, such as segregation, dilution, or blending, and marketing decisions.

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Table 2. Average moisture content (%)[a] of stored maize in Guarapuava, PR, Brazil in 1993.

Silos	Sampling days						
	1	30	60	90	120	150	180
Silo 1	13.8	13.3	13.2	12.7	13.0	13.2	13.0
Silo 2	14.1	13.9	13.7	13.8	13.8	14.1	13.5
Silo 3	13.5	13.7	13.5	13.5	13.2	13.7	13.2

[a] Each value is an average of 10 samples.

Table 3. Percentage of kernels damaged by mould in stored maize in Guarapuava, PR, Brazil, in 1993.

Silos	Sampling days						
	1	30	60	90	120	150	180
Silo 1	7.3	8.4	9.8	9.1	7.0	10.4	6.7
Silo 2	6.8	6.0	7.5	7.2	5.8	9.8	6.3
Silo 3	5.5	5.7	7.1	6.8	6.2	8.2	8.1
Average	6.5	6.7	8.1	7.7	6.3	9.4	7.0

Table 4. Percentage of mould-damaged germs in stored maize, in Guarapuava, PR, Brazil, in 1993.

Silos	Sampling days						
	1	30	60	90	120	150	180
Silo 1	10.6	9.2	14.0	24.0	15.5	9.15	12.6
Silo 2	7.3	7.0	7.7	13.7	9.0	15.9	20.9
Silo 3	6.8	7.3	5.1	12.8	11.7	11.1	12.8
Average	8.2	7.8	8.9	16.8	12.1	12.0	15.4

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