Toxigenic fungi in corn (maize) stored in hermetic plastic bags

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DOI: 10.5073/jka.2010.425.323

Abstract

In Argentina, 35 million tonnes are stored in hermetic plastic bags. Inside the bags, the modified atmosphere has an effect on stored grains, insects and fungi. Fungi taxa and species typically isolated from stored grains consist of species of the genera *Aspergillus*, *Penicillium*, *Fusarium* and xerophilic fungi, some of which are potential producers of mycotoxins. The province of Entre Rios, Argentina, is an important area of study because much of the production of maize stored in bags is supplying the poultry industry demand. The objective of this study was to identify mycotoxigenic fungi species in maize stored in hermetic plastic bags, located in three Departments of Entre Rios province. A total of 176 samples of maize were analyzed, stored in 23 bags located in the Departments of Paraná (west region), La Paz (northern region) and Tala (central region). Two potential producers of aflatoxins (*A. flavus* and *A. parasiticus*) and a potential producer of fumonisins (*F. verticillioides*) were identified in all the plastics bags evaluated. In La Paz Department, *Aspergillus* spp. and *F. verticillioides* were detected in 66.7% and 54% of samples, respectively. In Tala Department, *Aspergillus* spp. were detected in 63.3% and *F. verticillioides* in 43.6%, while in Parana, *Aspergillus* spp. was detected in 88.2% of the evaluated samples and *F. verticillioides* in 41.4% of the samples. The results revealed that mycotoxigenic fungi can develop in maize stored in hermetic plastic bags. This implies a potential risk of contamination with aflatoxins and /or fumonisins in the grain lots stored inside these bags.

Keywords: Mycotoxigenic fungi, Grain, Plastic silo-bag, Grain quality, Fungi spoilage

1. Introduction

The total storage capacity of Argentina is estimated 73 million tonnes, which represents about 75% of the total production. As a result, the efficiency of the postharvest system is largely compromised. To overcome these unfavorable circumstances, a new storage technique has gained popularity among farmers to store dry grains (wheat, maize, soybean, etc) in hermetic plastic bags. These bags can hold approximately 200 tonnes of grains, and since plastic enclosure material are 230 µm thick, they are airtight to water and gasses (O_2, CO_2 and water vapor). The respiration of grain, fungi, insects and other live organisms consumes the oxygen and generates carbon dioxide. This modified atmosphere has effects on the seeds, insects and fungi (Cardoso et al., 2008; Rodriguez et al., 2008).

The fungi genera typically found in stored grains are *Aspergillus*, *Penicillium*, *Fusarium* and some xerophilic species, several of them with capabilities of producing toxins (Christensen, 1987; Lacey, 1989). The development of these fungi can be affected by moisture content of the product (Giorni et al., 2009; Hell et al., 2000), temperature, storage time, degree of fungal contamination rate prior to storage and insect and mite activity that might facilitate fungi dissemination. Those facts lead to the importance of identifying the fungi species in stored grain, with a special consideration for micotoxigenic ones, since they can be a potential threat for people and animal health. The goal of this study was to characterize the fungi species found in maize grain stored in hermetic plastic bags. This maize is to be used by the poultry industry in Entre Rios province, Argentina. This province concentrates 47% of the poultry and eggs industry in the country. The results of this study aim at helping farmers and the industry to better produce and store properly their quality grains, especially in terms of good hygiene practices and sanitation.

2. Materials and methods

2.1. Geographical situation of maize production area in Argentina

This study was carried out from maize samples collected in hermetic plastic bags located in the Departments of Parana (West), La Paz (North) and Tala (Center) in Entre Ríos province, Argentina. Entre Rios province limits to the North with Corrientes province, to the West, separated by the Paraná River, with the Santa-Fe province, to the South with Buenos Aires province, and to the East, separated by the Uruguay River, it limits with the Uruguay Republic.

2.2. Grain Sampling

The maize harvest was in April, and the grain sampling was done at the end of July, after 3 to 4 months of storage.

One sample was collected every 10 m along the bag (in general the plastic bags are 60 m long) with a compartmented sampling spear probe of 1.8 m long which allows to take samples from the entire vertical profile of the grain mass. The collected grains were separated according their location in the profile of the grain mass: i/ upper layer (0-0.1 m) and ii/ middle and lower layers (0.1-1.8 m).

Grain temperature and moisture content were also recorded for each grain layer and sampling location. Temperature was determined with a portable temperature sensor that can be inserted in the grain mass and measure grain temperature at 0.1; 0.7 and 1.6 m from the top of the bag. Grain moisture content was determined with a moisture meter (Dickey-John, GAC 2100,).

The collected maize samples were placed in plastic bags with hermetic sealing and shipped to the Microbiology Laboratory of the Balcarce Integrated Unit (INTA-Agronomy College of Mar del Plata University), and stored at 4°C until processed.

2.3. Fungi identification

2.3.1. Fungi isolation

The presence of filamentous fungi was determined implementing the direct plate technique. In a laminar flow cabinet, 110 kernels of each sample were selected from each grain sample and placed in a Petri dish (10 kernels per dish) on agar culture medium supplemented with 18% of glycerol (DG18 medium). The DG18 medium has a water activity of 0.95 that allows the growth of non xerophylic species (*Penicillum, Aspergillus*), as well as xerophylic fungi (*Eurotium* spp.) or yeast (Pitt and Hocking, 1997). The remaining 10 kernels were placed in a Petri dish on dichloran chloramphenicol peptone agar (DCPA), in order to detect *Fusarium* spp.

The Petri dishes were incubated at 25°C during 7 days. Then, the percentage of fungi contaminated kernels was determined for each sample, and all the colonies with a visual difference in morphology were removed in sterile conditions to be deposited in Petri dishes with DG18 and DCPA culture media. The isolated fungi strains were incubated at 25°C during 7 days to obtain a pure monospecific isolated culture.

2.3.2. Fungus species identification

The isolated fungi of the genus *Penicillium*, *Aspergillus* and *Eurotium* were identified using the taxonomic key of Pitt and Hocking (1997), based on morphological and biochemical characteristics. For the species belonging to *Fusarium* genus, in addition to the Pitt and Hocking key, the key of Samson et al. (1995) and the *Fusarium* genus atlas (Gerlach and Nirenberg, 1982) were also used. For conducting these identification procedures in rigorous conditions of comparison, the pure cultures were all grown on both DCPA and potato dextrose agar (PDA) medium (Pitt and Hocking, 1997).

2.4. Determination of the Isolation Frequency

The isolation frequency of the fungi genus and of the potentially aflatoxigenic species in each plastic bag was determined as the ratio between the number of samples with positive isolation and the total number of samples analyzed from each bag. The frequency of isolation for each one of the identified species was also determined as the ratio between the number of samples with positive isolation for each species and the total number of samples analyzed from each Department.

3. Results

A total of 176 samples of maize collected from 23 different hermetic plastic bags were analyzed. The genera *Penicillium*, *Aspergillus*, *Fusarium* and *Eurotium* were isolated and identified from each bag in this study. Even though *Penicillium* and *Aspergillus* had the higher proportion of positive isolation, only two species of *Aspergillus* genus (*A. flavus* and *A. parasiticus*) and one of *Fusarium* genus (*F. verticillioides*) were identified in the literature as potentially mycotoxigenic. Therefore, those are considered of importance for the human and animal health. It was also determined than 90% of *Aspergillus* spp. isolation cases corresponded to *A. flavus*, a potentially mycotoxigenic species.

Table 1 shows the isolation frequency of the potentially mycotoxigenic species the most important for stored maize, aggregated by Department.

Table 1 Isolation frequency (in percentage of analyzed samples), of potentially mycotoxigenic species from three Departments of Entre Rios province, Argentina.

Department	**Frequency (%)**		
	Fusarium verticillioides	***Aspergillus* spp.(#)**	***F. verticilloides* and *Aspergillus* spp.(#)**
La Paz (North)	54.0	66.7	52.4
Paraná (West)	41.4	88.2	36.4
Tala (Central)	43.6	63.3	ND

(#) Aspergillus flavus; Aspergillus parasiticus; ND: not determined

The location of the grain in the profile of the grain mass (in upper or inner layer of the storage enclosure) did not have influence in the type of fungi species identified, since in the two layers evaluated (upper and middle-bottom) the same species were identified, even though the isolation frequency was different.

Mycotoxigenic species of the *Aspergillus* and *Fusarium* genus were isolated from all the bags. Grain samples coming from the Paraná Department had the higher percentage of isolation of afatoxigenic fungal species, while samples from La Paz Department had the higher proportion of *F. verticillioides* (fumonisin producer). In this Department 52.4% of the samples were contaminated with the three potentially mycotoxigenic species (*A. flavus*, *A. parasiticus* and *F. verticillioides*). It was also observed that in samples in which *A. flavus* and *F. verticilloides* were present, the proportion of *F. verticillioides* was always higher (data not presented).

4. Discussion

Storing grain in hermetic plastic bags requires frequent monitoring in order to early detect grain spoilage and/or mycotoxigenic fungi development. In this study some potentially mycotoxigenic species of the *Aspergillus* and *Fusarium* genus were identified in grain samples, in addition to other species from the *Eurotium* and *Penicillium* genus. *A. flavus* and *F. verticilloides* were the most important species due to their capacity to produce mycotoxins (aflatoxins and fumonisins, respectively) and to contaminate the grain (Lino et al., 2007; Logrieco et al., 2007).

Previous studies identified *Aspergillus* and *Fusarium* mycotoxigenic species in stored grains, as well as their mycotoxins, aflatoxins and fumonisins, in different concentrations (Pacin et al., 2009; Moreno Cunha et al., 2009). There is a general trend to increase the consumption of cereal grains and cereal products. On the other hand, the consumption of contaminated grain with mycotoxins causes different problems, including death (Lerda et al., 2005; Voss et al., 2007). There is a general agreement that the consumption of contaminated grains with mycotoxins is a risk for the animal and human health, and may lead to an important economic problem in the near future.

The study of the conditions that lead to the development of fungi during storage and the production of mycotoxins indicated that the grain moisture content is one of the most important factors (Hell et al., 2000; Giorni et al., 2009). In maize, for instance, it was determined that a storage moisture content of 13% is sufficiently low to prevent fungus development and mycotoxin production (water activity below 0.65). In this study, all the grain samples presented moisture content above 13%, reaching values as high as 25.1% in samples from La Paz Department (Northern region). This unsafe storage conditions would indicate that the grain stored in plastic bags can be contaminated with different levels of mycotoxins. In fact, *Fusarium*, which is a genus that typically affects the grain in the field, was found with a frequency of 40% in the samples collected from the bags, most likely due to fungal activity during storage of extremely high moisture content grain.

In Argentina, storing grain in hermetic plastic bags is a common practice. It became an important tool for agriculture, both from the logistic and the economical point of view, since it is a simple and inexpensive storage system. However, proper storage conditions should be considered and evaluated, especially those affecting the grain sanitary and hygienic quality, such as moisture content, temperature and storage time. These seem to be the most important factors conditioning the development of mycotoxigenic fungi during storage, which are of importance for the management of hazards to human and animal health they represent.

Acknowledgements

The authors want to thank to Enrique Behr and Diego Croce for their help collecting the grain samples from the silo-bags. The authors also thanks to the National Institute of Agricultural Technologies, through the project INTA-PRECOP: Eficiencia de poscosecha and the Mar del Plata University, through the project A-15-297, for the financial support of this study.

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