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Dynamics of fungi and related mycotoxins during cereal storage in silo bags

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ABSTRACT

The aim of this work was to investigate the dynamics of fungi and related mycotoxins during cereal storage in silo bags. A 2-year trial was organised (2009–2011); in each year, two silo bags, filled with maize and durum wheat respectively, were prepared. During storage, meteorological data were collected from a close station and temperature, CO_2 and O_2 were measured inside the silos. Grain was sampled from silo filling (September) every 40 days until June (7/8 samples per silo per year). Water activity of grain, colony forming units (CFU) and mycotoxin content (fumonisins, aflatoxins and ochratoxin in maize, deoxynivalenol and ochratoxin in wheat) were determined.

Temperature inside the silo bags followed the trend of external temperature, with a more limited variation. A decrease of O_2 was observed from 16.4% to 2.0% in maize and from 15.7% to 15.1% in wheat; the decrease was balanced by CO_2 increase. Minor variations were registered in grain water activity and CFU and mycotoxin content did not change significant throughout the storage period; the results were consistent in the two years. On the basis of these results, silo bags prove to be suitable for safe cereal storage.

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1. Introduction

Long storage of cereals is a common practice worldwide and permanent structures, such as horizontal or vertical silos, sometimes equipped with temperature or air control, are used to safeguard product quality and safety (Lacey, 1989). Cereals must be protected in field against fungal attacks that could become the initial inoculum for further infection during storage. Post-harvest development of fungi, in fact, can cause severe product losses and furthermore mycotoxin contamination of grain could take place when toxigenic strains are present (Magan & Aldred, 2007).

Fusarium, *Penicillium* and *Aspergillus* spp. are fungi frequently isolated from stored maize and wheat worldwide, and many species of these genera are able to produce mycotoxins (Magan, Hope, Cairns, & Aldred, 2003). These fungi live in different, partially overlapping ecological niches (Giorni, Magan, & Battilani, 2009), and the environmental parameters determine the dominant species.

Fusarium spp. is commonly dominant in the field, where water activity (a_w) is not a limiting factor for most of the crop growing period; it can continue its development during storage only when grain is not properly dried (Magan & Lacey, 1984a, 1984b, 1984c). *Aspergillus*, on the contrary, is more xerophilic and could get the upper hand during storage, because of the low moisture content of grains, even if *Fusarium* was demonstrated to be more competitive than *Aspergillus* and *Penicillium* (Marin, Sanchis, Ramos, Vinas, & Magan, 1998).

The nature of substrate, a_w, temperature and air gas composition are the most important parameters able to affect fungal growth and mycotoxin production post-harvest (Magan et al., 2003; Magan & Lacey, 1984a, 1984b, 1984c) and their management is considered fundamental to prevent further contamination during storage.

Traditional storage systems, such as silos and warehouses, are often very expensive and not flexible in terms of volume. They are rigid systems, not always the correct size for the quantity of cereal to be stored; the presence of headspaces, where the environmental conditions are possibly appropriate, can favour fungal development and mycotoxin production (Magan & Aldred, 2007).

The silo bag is an alternative storage system, recently considered in Argentina (Bartosik, Cardoso, & Rodríguez, 2008; Bartosik,

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Rodriguez, Malinarich, & Maier, 2003) and Australia (Darby & Caddick, 2007), that is less expensive and more adaptable to storage requirements (Darby & Caddick, 2007; Muck & Holmes, 2006; Rodriguez, Bartosik, Malinarich, Exilart, & Nolasco, 2002). These bags are versatile, as they can be sealed up at different volumes, depending on the cereal mass to be stored. The silo bag is a hermetic storage technique; the respiration of the stored biomass produces a slightly anaerobic internal environment that results unfavourable for pathogens and parasites activity (Muck & Holmes, 2006). It was confirmed by Locatelli et al. (2010) who observed an inhibition of insect growth and a rise of their mortality along with CO_2 increase in durum wheat stored in silo bags.

The aim of this study was to investigate the dynamics of fungi, of related mycotoxins and of environmental parameters in maize and wheat stored in silo bags as useful parameters to evaluate cereals safety.

2. Materials and methods

2.1. Silo bag preparation and sampling

In a grain warehouse located in San Giovanni di Ostellato (Ferrara province), in northern Italy, both in 2009 and 2010, two large bags, of a triple polyethylene layer of 240 μm thickness, were used for the silage trial. The silo bags were 75 m long and 2.70 m in diameter; they were filled with about 240 tons of cereals and then hermetically sealed. The storage period started after the cereal harvest (July and September, respectively for wheat and maize), with the filling of the silo bags, and lasted until late May–early June of the following year. The differences between the life cycle of the two cereals selected for the study explain the different dates in which the two silo bags were filled; so the maize silo bags were prepared on 24th August and 8th September, and the durum wheat silo bags on 2nd July and 17th August, in 2009 and 2010, respectively. Grain sampling was planned during the storage period every 40–45 days (Table 1). Due to the delay in maize silo bag preparation, only six samples were withdrawn in 2010. A code number (1–7) was attributed to each sampling date; the same code identified very close sampling dates in the 2 years.

Three holes were made in order to sample the cereals, 120 cm above ground level, at the start, in the middle and at the end of each silo bag; they were closed with a circular foldout window.

The internal gas concentration was measured using a Check-Point (Dansensor, Glenn Rock, NJ, USA), a portable gas analyser for modified atmosphere packaging, able to determine both oxygen (O_2) and carbon dioxide (CO_2) concentration. It was equipped with a syringe that could be inserted through the bag. Gas composition measurement was done close to the holes before sampling. A gas sample was automatically drawn into the analyser, where electrochemical and infrared analysers determined the O_2 and CO_2 concentrations, respectively. The hole produced by the syringe needle was then closed with sticky tape in order to inhibit any gas exchange with the open air.

Table 1
Dates (and codes) of maize and wheat sampling during the 2009–2010 and 2010–2011 storage.

Sampling code	Sampling dates			
	2009–2010		2010–2011	
	Maize	Wheat	Maize	Wheat
1	21 Sept	21 Sept		16 Sept
2	04 Nov	04 Nov	03 Nov	03 Nov
3	17 Dec	17 Dec	13 Dec	13 Dec
4	04 Feb	04 Feb	28 Jan	28 Jan
5	24 Mar	24 Mar	15 Mar	15 Mar
6	29 Apr	29 Apr	02 May	02 May
7	31 May	31 May	08 June	08 June

A thermal probe (Thermometer HD 9010 Delta OHM S.r.l., Padova, Italy) was used for temperature measurement immediately before each sampling.

Grains were sampled using a probe, a cylindrical rod composed of two long hollow tubes (150 × 5 cm). Five sub-samples were collected inserting the probe with different slopes, from each sampling point and at each date, to obtain 6–7 kg of grains. Samples were stored at −4 °C until shipping to the laboratory, managed in less than 24 h.

Water activity (a_w) of grain, fungal colony forming units (CFU) and mycotoxin content (fumonisins, FBs; aflatoxins, AFs; and ochratoxin A, OTA in maize; deoxynivalenol, DON and OTA in wheat) were determined in all the samples collected.

Meteorological data, intended as air temperature, were recorded by a meteorological station placed close to the silo bags.

2.2. Determination of water activity

AquaLab LITE (version 1.3© Decagon devices Inc., WA, USA) equipment was used to determine a_w in kernels. This equipment uses a dielectric humidity sensor to measure sample a_w (accuracy ±0.015 a_w). Approximately 6 g of grains, 20 kernels for wheat and 10 for maize, were randomly selected from each sample and a_w was measured immediately after sample delivery.

2.3. Quantification of fungi

Samples were milled using a Universal Cutting Mill PULVERISETTE 19 (Fritsch Gmbh, Idar-Oberstein, Germany) with a 1 mm sieve. In the grinding chamber a rotor fitted with knives comminutes the sample in combination with 3 fixed knives. A FRITSCH Cyclone (Fritsch Gmbh, Idar-Oberstein, Germany) connected to the mill helps in keeping the sample temperature stable and in recovering the flour produced.

Sub-samples (10 g) of maize and wheat flour were blended with 90 mL of sterile 0.1% peptone:water (w/v) using a homogenizer (Bagmixer® 400, Interscience, Paris, France) and serial dilutions from 10^{-2} until 10^{-7} were plated in Petri dishes with PDA (Oxoid LTD., Basingstoke, Hampshire, England) added with 50 mg of chloramphenicol and incubated at 25 °C for 6 days.

The total number of fungal colonies was counted using a colony counter (Suntex Colony Counter 570, Suntex Instruments Company Ltd, Taipei, Taiwan) and the identification at genus level was carried out based on colony morphology (Leslie & Summerell, 2006). The result was expressed as CFU/g of flour.

2.4. Quantification of mycotoxins

2.4.1. Fumonisin standards

FB_1 and FB_2 standards were obtained from Sigma–Aldrich (St. Louis, MO, USA). FB_1 and FB_2 (1 mg) were separately dissolved in 10 mL acetonitrile/water (1:1 v/v); the concentration was calculated using the weight indicated by the manufacturer. These solutions were diluted to obtain HPLC calibrant solutions in acetonitrile/water (3:7 v/v) at individual concentrations of FB_1 and FB_2 between 2.5 and 50 μg/L.

2.4.2. Fumonisin analysis

Fumonisins were extracted from 20 g of sample with 200 mL of 0.4 M phosphate buffer [840 mL Na_2HPO_4 (56.8 g/L) + 160 mL $NaH_2PO_4 \times H_2O$ (55.2 g/L), pH 7.5] (Pietri & Bertuzzi, 2011). After extraction for 45 min using a rotary-shaking stirrer and filtration

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