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# Organic *vs* conventional farming: Differences in infection by mycotoxin-producing fungi on maize and wheat in Northern and Central Italy

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ABSTRACT

This study aimed to monitor the main toxigenic fungi in neighbouring organic and conventional maize and wheat fields in Italy in 2010 and 2011. The *Fusarium* species mainly isolated were: *Fusarium poae*, sometimes predominant on *Fusarium graminearum* in wheat, and *Fusarium verticillioides* competing with *Fusarium proliferatum* and *Fusarium subglutinans* in maize. The incidence of *Fusarium* spp. was similar for both conventional (6%) and organic (4%) wheat, but it was influenced by weather conditions. 2010 was the most favourable for *Fusarium* species, with 10 times the incidence of 2011. *Fusarium* infection was significantly different between farming systems in maize (20% *vs* 35% in conventional and organic, respectively), while in 2010 the incidence was significantly higher than in 2011 (43% *vs* 25%). *Aspergillus* and *Penicillium* incidence was not linked to the farming system but to weather conditions, with moderately higher incidence in 2010.



## 1. Introduction

Organic farming, defined in Europe by the Commission Regulation (EC) No. 1991/2006 (a), amending Regulation (EEC) 2092/91, has significantly increased worldwide in the last two decades. Italy is the second largest area, after Spain, with 1.1 million ha of organic area, of European Countries (EC, 2013). Organic cereals are the second main aggregated crops cultivated in Italy, covering about 17% of the total organic area, with soft and durum wheat, and maize, accounting for 55% of the total organic cereals cultivated (SINAB, 2012).

It is estimated that 25% of the world's food production, including many basic foods, is affected by mycotoxin-producing fungi, with cereals (CAST, 2003), especially maize and wheat, contaminated at the highest levels. A review on mycotoxin occurrence between 2010 and 2013 on different cereals and related foodstuff showed that maize and wheat are, respectively, the first and the second most contaminated crops worldwide (Pereira et al., 2014).

The main mycotoxin-producing fungi affecting wheat and maize belong to the *Fusarium*, *Aspergillus* and *Penicillium* genera. In particular, *Fusarium* Head Blight (FHB) of wheat is caused by a complex of species responsible mainly for the accumulation in the kernels of trichothecenes, a family of potent mycotoxins causing inhibition of protein synthesis, and zearalenone (ZEA), an estrogenic compound (Desjardins, 2006). *Fusarium graminearum* is the main species producing deoxynivalenol (DON), the most common contaminant among the trichothecenes, and causing FHB of wheat and red-ear rot in maize (Logrieco et al., 2003). Moreover, the predominant occurring species can vary in different geographical areas and years, according to environmental conditions and agronomic practices, and each species can have its own mycotoxin profile (Logrieco et al., 2003). *Fusarium* ear rot of maize, one of the main diseases of this crop worldwide, is also caused by a complex of species, *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium subglutinans* and the recently described *Fusarium temperatum* (syn. *F. subglutinans* group1, Scauflaire et al., 2011) being associated with the so called pink-ear rot (Logrieco et al., 2003). Among these species, *F. verticillioides* and *F. proliferatum* are the main species responsible for the production of fumonisins on kernels (Desjardins, 2006).

*Aspergillus* species belonging to section *Flavi* are known to produce aflatoxins (AFs), and are frequently reported worldwide to

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occur in maize kernels, especially in the tropical areas (Fandialan and Ilag, 2003; Kana et al., 2013). Conversely, few reports have shown contamination of wheat by AFs and at levels lower than 15 μg/kg (Biomin, 2013; Riba et al., 2010). In Italy, the occurrence of these mycotoxins on maize is an emerging problem and it is associated with seasons causing high water stress to maize plants (Piva et al., 2006). Finally, in agreement with Pitt et al. (2000), the main species associated with the accumulation of ochratoxin A (OTA) in cereal grains worldwide is *P. verrucosum*. In Italy this species has been rarely reported and OTA contamination of wheat can be considered a minor problem in the Italian environment (Logrieco and Moretti, 2008).

Several studies have been focused on the influence of farming systems on mycotoxin contamination in cereals. These reports have shown contrasting data on the level of *Fusarium* mycotoxin accumulation in organic *versus* conventional farming (reviewed in Köpke et al., 2007). In a Norwegian study, *Fusarium* species were isolated from oat, barley and wheat harvested in 2002–2004. Organic cereals were less infected by *Fusarium* and with a lower content of trichothecenes than conventional ones; moreover *Fusarium avenaceum*, *F. graminearum* and *Fusarium poae* were the predominant species (Bernhoft et al., 2010). The lack of crop rotation and the use of mineral fertilisers and pesticides, which are agricultural practices characterizing conventional farming *versus* organic, seem to be the most relevant reasons that cause the differences between the two growing systems (Bernhoft et al., 2012).

Regarding Italian cereals, as far as we are aware, there are no studies which focus on the comparison of fungal incidence in different farming systems. Infantino et al. (2012) described the *Fusarium* community associated with FHB in wheat harvested in organic farming located in different geographical areas in a three-year period (2004–2006). The study showed a low *Fusarium* incidence, *F. poae* being the most occurring species in all the three years. With respect to maize, there is a complete lack of data. In Europe, only one study was carried out in Spain by Ariño et al. (2007) on the fungal occurrence in maize harvested in 2001–2003, showing that total fungal contamination was higher in organic than in conventional maize, but *Fusarium* species predominated in the latter.

Due to this scarce information, knowledge regarding the occurrence of toxigenic fungi in both maize and wheat cultivated in organic farming would be welcome.

Therefore, the aims of this study were: i) to monitor the fungal population, in particular the main mycotoxin producing fungi, associated with organic maize and wheat collected from farms located in northern and central Italy; ii) to compare the incidence of mycotoxin producing fungi on maize and wheat cultivated following conventional and organic farming.

## 2. Materials and methods

### 2.1. Wheat and maize sample collection

Wheat and maize samples were collected in 2010 and 2011 from farms located in northern and central Italy. For each crop, neighbouring fields of conventional and organic farming were chosen, in order to reduce the variables influencing fungi associated with kernels.

A total of 101 wheat samples were collected in 2010 from 91 farms: 85 samples were cultivated as organic wheat (72 of soft wheat and 13 of durum wheat) and 16 were cultivated as conventional wheat (15 of soft wheat and 1 of durum wheat). In 2011, a total of 138 wheat samples were collected from 101 farms: 121 samples were cultivated as organic wheat (110 of soft wheat and 11 of durum wheat) and 17 were cultivated as conventional wheat (13 of soft wheat and 4 of durum wheat). For maize, 30 samples were collected in 2010 from 27 farms: 24 samples were cultivated as organic maize and 6 were cultivated as conventional maize. In 2011, 39 samples were collected from 33 farms, with 35 samples from organic cultivation and 4 from conventional maize fields. For each sample, all the farmers were asked to fill in a specific form which included relevant cropping system information: geographical coordinates, wheat variety or maize hybrid, soil texture, previous crop, debris management, tillage and other agronomic operations, sowing period and investment, mineral nutrition, weed control, flowering period, biotic and abiotic crop injuries, chemical control of pest (*Ostrinia nubilalis*, the European Corn Borer-ECB) and/or disease (FHB), harvesting period and moisture of kernels at harvesting.

Sampling was performed following the protocol described by the Commission Regulation (EC) N° 401/2006 (b); incremental samples of 100 g each were collected *in continuum* during harvest combine discharge to obtain a final sample of 10 kg. The samples were sent to the laboratory for mycological analysis; subsamples of 30 g were prepared and immediately processed.

### 2.2. Fungal isolation and morphological characterization

Fifty kernels were randomly selected from each subsample and surface sterilized by washing in ethyl alcohol (70%) for 10 min and with NaCl (1%) for 2 min followed by rinsing twice with sterile double distilled water. The kernels were then dried on sterile absorbent paper. Sterilized kernels were plated in 90 mm Ø Petri dishes filled with DCPA (Dichloran Chlorampenicol Peptone Agar) (Andrews and Pitt, 1986; modified using 20× less quantity of chloramphenicol) and incubated at room temperature for 5 days under ambient light.

Based on their phenotypic characteristics, colonies identified at genus level as *Aspergillus*, *Fusarium* and *Penicillium* were selected and single-spored (5 fold-serial dilution in peptone:water 1:100). *Aspergillus* and *Penicillium* single spores were transferred onto Czapek Agar (CZ) and, after 7 days, colonies were identified at section level for *Aspergillus* cultures and genus level for *Penicillium*, based on their morphology (Raper and Fennell, 1965; Pitt, 1979). Isolates belonging to the *Fusarium* genus, were identified at species level, according to Leslie and Summerell (2006), by using 3 media: Potato Dextrose Agar (PDA) (HIMEDIA, Mumbai, India), Carnation Leaf Agar (CLA) (Fisher et al., 1982) and Spezieller Nährstoffarmer Agar (SNA) (Nirenberg, 1976). The culture observations were performed on a Nikon Eclipse E50i microscope (Nikon, Japan; 600 X).

### 2.3. Molecular identification of Fusarium spp.

#### 2.3.1. Fungal isolates and inoculum preparation

In order to confirm the morphological characterization results, a qualitative PCR analysis was run on randomly selected samples (10% of total isolates), covering all the morphologically identified species, recovered from both wheat and maize samples. Reference strains, stored at the Institute of Entomology and Plant Pathology-UCSC, Piacenza (MPVP) and the Institute of Sciences of Food Production-CNR, Bari (ITEM fungal collection, http://server.ispa.cnr.it/ITEM/Collection), were used as positive control for each species.

The strains were grown on PDA at 25 °C for 7 d in the dark. At the end of incubation, 10 mL of sterile distilled water was added to each plate and it was gently scraped to collect fungal conidia. The suspension was adjusted to $10^6$ conidia/mL and 100 μL were inoculated in 100 mL Malt Extract Agar (MEA) (Pitt, 1979) liquid medium. The static cultures were incubated for 14 days at 25 °C in the dark, then freeze-dried overnight and stored at 4 °C. Two biological replicates were performed for each sample.