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Mycotoxins in maize grains grown in organic and conventional agriculture

Jose Ignacio Ruiz de Galarreta [a], Ana Butrón [b], Amaia Ortiz-Barredo [a], Rosa Ana Malvar [b], Amando Ordás [b], Arsenio Landa [c], Pedro Revilla [b,*]

[a] NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, 46, 01080-Vitoria, Spain
[b] Misión Biológica de Galicia (CSIC), Spanish Council for Scientific Research, Apartado 28, 36080 Pontevedra, Spain
[c] Promotora Orxeira S.A., Ermille, 32897 Lobeira, Ourense, Spain

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ABSTRACT

Maize is traditionally used for bakery in several countries, and autochthonous varieties are increasingly demanded particularly for organic agriculture, but one of the dangers of cereal consumption is mycotoxin contamination. Mycotoxins are dangerous for health and might be present in any grain depending on genotypes and environments. In the present work we assess the natural levels of fumonisin and deoxynivalenol (DON) contaminations in nine diverse open-pollinated maize varieties grown in four different locations, under organic or conventional conditions, in two regions from the humid Spain during two years. Differences were significant among locations and among varieties for fumonisin contamination but not for DON content. Locations were the main environmental source of variation affecting fumonisins while DON was more affected by years. The Basque locations had more fumonisin than the Galician locations, but there were no differences between organic and conventional environments. Fumonisin contamination was more variable than DON among locations and among varieties. Fumonisin and DON were highly correlated on average but correlations were low for each particular environment. Mean fumonisin and DON were below the threshold allowed by the EU, but the white-kernel medium late variety Rebordanes(P)C2 had more than 4.00 mg/kg of fumonisin in one location, while the early yellow variety Sarreaus had the lowest contamination. We conclude warning producers of the danger of natural contamination with mycotoxins for some varieties in specific environments.

1. Introduction

Varieties of maize are traditionally used for bakery in northern Spain, Portugal and other countries (Landa, Revilla, Malvar, Butrón, & Ordás, 2006; Revilla et al., 2008; Vaz Patto, Moreira, Carvalho, & Pego, 2007). Besides quality and flavor, these traditional varieties are interesting due to their potential value as functional foods (Rodríguez, Soengas, Landa, Ordás, & Revilla, 2013). Moreover, there is an increasing interest for reintroducing improved varieties for food, particularly under organic agriculture (Landa et al., 2006, Revilla, Landa, Rodríguez, Ordas, Malvar, 2012; Revilla et al., 2008). Although the amount of maize used for food is lower than for feed, the economic value of maize for food is high and poses some health and safety problems, for example, reduced levels of contaminants are allowed compared with maize. In this context, organic agriculture is considered safer than conventional agriculture because inorganic fertilizers and phytosanitary synthetic products are forbidden. No final conclusion about which is the best agricultural system for reducing the risk of contamination with mycotoxins have been drawn (Ariño, Estopanan, Juan, & Herrera, 2007; Cirillo, Ritieni, Visone, & Cocchieri, 2003; Magkos, Arvaniti, & Zampelas, 2006).

Mycotoxins are produced by several species of fungi, being *Fusarium* the most common genus in the European Atlantic coast; in southern regions, the most frequent fungus found in maize grains is *Fusarium verticillioides* Saccardo (=*Fusarium moniliforme* Sheldon) Nirenberg that produces fumonisins (Logrieco, Bottalico, Mule, Moretti, & Perrone, 2003), while in colder regions, *Fusarium graminearum* Schwabe that produces deoxynivalenol (DON) could be predominant (Hooker & Schaafsma, 2005). DON is also produced by *Fusarium culmorum* and *Fusarium cereals* (Marin,

* Corresponding author. Tel.: +34 986854800.
E-mail address: previlla@mbg.csic.es (P. Revilla).

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Ramos, Cano-Sancho, & Sanchis, 2013). The Rapid Alert System for Food and Feed of the European Union has registered 14 alerts of fumonisins and 24 of DON between 2008 and 2012 in cereals and bakery products; most of these alerts were for maize samples (RASSF, 2006). Breakfast cereals and baby foods also contained DON, although at lower levels than unprocessed maize kernels (Marin et al., 2013). Fumonisins cause diverse health problems in animals and, in humans, fumonisins could be related with increased incidence of esophageal cancer and neural tube defects and are considered as probably carcinogenic (Bennett & Klich, 2003; IARC, 1993). There are no data supporting the possible mutagenic or carcinogenic effects of DON. IARC has included DON in Group 3 and fumonisins in group 2.4. Although DON is not as toxic as other mycotoxins, it is one of the most common contaminants of cereals worldwide. Acute effects of food poisoning in humans are abdominal pains, dizziness, headache, throat irritation, nausea, vomiting, diarrhea, and bloody stools.

During the bakery process, DON is considerably reduced, but fumonisins are fairly heat-stable as there is little degradation during fermentation and toxin content is significantly reduced only during processes in which the temperature exceeds 150 °C (Marin et al., 2013). Therefore, there is an increasing amount of legislation limiting the amount of fumonisins (FAO, 2004) and the European Union establishes that the threshold for fumonisin contents is 4.00 mg/kg and 1.75 mg/kg for DON in non processed maize (UE, 2006, 2007).

Mycotoxin contamination depends on the fungi isolate, but also on environmental and genetic background of the maize crop (Picot et al., 2010; Warfield & Gilchrist, 1999). Poor agricultural and harvesting practices, improper drying, handling, packaging, storage, and transport conditions promote fungal growth, increasing the risk of mycotoxin production (Marin et al., 2013). These authors stated that fumonisins are the most important mycotoxins found in maize, particularly when grown in warmer regions, as *F. verticillioides* and *Fusarium proliferatum* grow over a wide range of temperatures, but only at relatively high water activities. Cao et al. (2013) evaluated fungal infection and fumonisin accumulation at different kernel development stages and during kernel drying in three white maize hybrids and found that *Fusarium*, especially *F. verticillioides*, was the most prevalent genera compared to *Aspergillus* and *Penicillium*. Kernel damage by insects and suboptimal temperatures for fungal growth when kernel humidity is low favored an increased rate of fumonisin accumulation (Cao et al. 2013). Cao et al. (2014) also concluded that the fungal growth rate significantly increased with temperature and water activity and found variability for genetic resistance to fungal infection and fumonisins accumulation.

Several authors have searched variability for resistance to fumonisin contamination among maize inbreds or hybrids. For example, Clements, Maragos, Pataky, and White (2004) evaluated a collection of inbred lines crossed to a tester under artificial infestation with *F. verticillioides* and found significant differences for fumonisin contamination. Similarly, Afolabi, Ojiambo, Ekpo, Menkir, and Bandyopadhyay (2007) evaluated a collection of maize inbred lines per se under natural infestation and found differences among maize inbreds and genotype × environment interaction for fumonisin contamination as well as positive correlation between fungi infection and fumonisin contamination only in one of the two Nigerian localities used. Presello, Iglesias, Botta, and Eyherabide (2007) evaluated a group of maize hybrids and found positive correlations between symptom severity and concentration of fumonisins, indicating that genotypic effects for concentration of fumonisins in grain mainly depended on genotypic effects for disease resistance. Löffler, Miedaner, Kessel, and Ouzunova (2010) analyzed correlations between mycotoxin concentrations and ear rot rating of 50 inbred lines under artificial infestation and found that the early maturity group flint lines were more susceptible and there were broad ranges and significant genotypic variances as well as genotype × environment interaction variances, but also high heritabilities for ear rot and mycotoxin concentrations. Henry, Williams, Windham, and Hawkins (2009) evaluated a selected group of inbred lines inoculated with either *A. flavus* or *F. verticillioides* and found significant variability for resistance to aflatoxin and fumonisin contamination among maize inbred lines and inbreds resistant to aflatoxin were also resistant to fumonisin contamination. Santiago, Cao, Malvar, Reid, and Butron (2013) evaluated 240 maize inbred lines under kernel inoculation with *F. verticillioides* and found differences for resistance to fumonisin contamination across environments. Bolduan, Miedaner, Schipprack, Dhillon, and Melchinger (2009) evaluated in Germany a collection of maize inbreds for mycotoxin contamination, including DON, and found significant genotypic and genotype × environment interaction variances, moderate heritabilities, and high correlations between disease severity and mycotoxin concentrations. DON has been found in naturally infected ears across nine locations in Germany (Magg, Melchinger, Klein, & Bohn, 2002). Relatively high contaminations of DON were reported in maize genotypes by Hart, Gendloff, and Rossman (1984).

Even though it is clear that there are differences among locations and genotypes for resistance to fumonisin contamination, as far as we know, no previous report has been published comparing contamination levels in different open-pollinated maize varieties and in different locations. In the present work we assess the levels of mycotoxin contamination in nine diverse open-pollinated maize varieties with different grain colors grown in four different locations, under organic or conventional conditions, in two regions from northern Spain.

2. Materials and methods

We evaluated nine open-pollinated maize varieties in two farmers' fields in Galicia and in the Basque Country under organic and conventional agriculture in 2010 and 2011. Among the varieties evaluated there was one with early cycle (Sarreaus(P)C2), six with medium cycle (Carballeira, DonostiaC1, Martikoenea, Oroso, Tuy(S) C3 and Txalin) and two with medium–late cycle (Meiro(P)C2 and Rebordanes(P)C2). Concerning grain color, six varieties had yellow endosperm and three had white endosperm, one of them with transparent pericarp (Rebordanes(P)C2) and two with black pericarp (Carballeira and Meiro(P)C2). The locations included two organic fields [Lobeira, 600 masl (Galicia) and Heredia, 567 masl (Basque Country)] and two conventional fields [Pontevedra, 20 m masl (Galicia) and Arkaute, 550 masl (Basque Country)].

The varieties were evaluated following a randomized complete block design with three replications. The experimental plots of 10 m^2 had a density of 60,000 plants ha^{-1}, with rows separated 0.8 m and plants within rows 0.21 m. Agricultural practices followed the recommendations of organic agriculture, i.e. nutrients were supplied by adding manure, weeds were removed mechanically, and no chemical treatment was used. Under conventional agriculture, current practices were the usual in the area with inorganic fertilizers, use of herbicide and no irrigation.

Ears from each plot were collected when grains were dry. A representative kernel sample of approximately 200 g was ground and the resulting flour sample was maintained at 4 °C until performing chemical analyses. Kernels were ground through a 0.75 mm screen in a Pulverisette 14 rotor mill (Fritsch GmbH, Oberstein, Germany). We have performed three replicates of each sample. Mycotoxins were determined using the commercial kit Veratox (Neogen Corp., Lansing MI) a competitive direct ELISA (CD-