ROLE OF *FUSARIUM* SPECIES IN MYCOTOXIN CONTAMINATION OF MAIZE

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ABSTRACT
Mycotoxin contamination of maize and other cereals is a globally important risk for human and animal health. The most frequently detected mycotoxins in maize are trichothecenes, zearalenone and fumonisins, which are causative agents for various diseases in domestic animals and they are a threat to humans either directly or indirectly.
The main producers of these mycotoxins, *Fusarium* species are considered the most important pathogens in the temperate climate zone of maize. Between 2011 and 2014 numerous incidence of *Fusarium* species and mycotoxins were assessed from Hungarian maize samples after harvest. Samples were collected from 8-10 maize growing areas of Hungary from hybrids with various resistant levels. The isolated *Fusarium* strains were identified using morphologic and sequence-based methods. In 2011 and 2012 14.3% and 9.2% of maize samples were found to be contaminated with potentially toxigenic isolates. The ratios of *Fusarium* isolates were 60.3% and 68.1%, respectively. In 2013 and in the highly humid 2014, 50.1% and 71.4% of maize grains were contaminated, while 46% and 84.9% of the isolated fungal strains belonged to genus *Fusarium*. *F. verticillioides* isolates were identified in the largest proportion of the samples, which are potential fumonisin producers. Along with these species *F. graminearum, F. proliferatum, F. subglutinans* and *F. sporotrichioides* isolates were also observed, which can produce trichothecens, beauvericin, moniliformin and toxin T-2. Representatives of *Fusarium culmorum* were not detected in any of the years examined.

Keywords: *Fusarium,* maize, species distribution, mycotoxin, fumonisin

INTRODUCTION

Maize (*Zea mays* L.) is the most important ingredient of feeds, and one of the most important agricultural export product of Hungary. Food and feed safety problems have increasing significance in maize production. Several fungal pathogens are able to infect maize, many of them are able to cause mycotoxin contamination. The most important mycotoxin producers are members of the *Fusarium* and *Aspergillus* species. Although *Fusarium* toxins, including trichothecenes, zearalenone and fumonisins, are considered to be the most important in cereals in regions with temperate climate (*Table 1*; LOGRIECO ET AL., 2002), mycotoxins produced by Aspergilli are also frequently identified in cereal products (HALT ET AL., 2004; GIORNI ET AL., 2007; TABUC ET AL., 2009).
In Hungary, the main toxin producer species are *F. verticillioides* and *F. graminearum*, but many toxin producing species of smaller significance can also be found (VARGA ET AL., 2004).

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Table 1. Nomenclature of *Fusarium* species and their mycotoxins

***Fusarium* species**	**Mycotoxins**
Fusarium acuminatum	T2, MON, HT2, DAS, MAS, NEO, BEA
Fusarium avenaceum	MON, BEA
Fusarium cerealis	NIV, FUS, ZEN, ZOH
Fusarium chlamydosporum	MON, BEA
Fusarium culmorum	DON, ZEN, NIV, FUS, ZOH, AcDON
Fusarium equiseti	ZEN, ZOH, MAS, DAS, NIV, DAcNIV, FUS, FUC, BEA
Fusarium graminearum	DON, ZEN, NIV, FUS, AcDON, DAcDON, DAcNIV
Fusarium oxysporum	MON, BEA
Fusarium poae	DAS, NIV, FUS, MAS, T2, HT2, NEO, BEA
Fusarium proliferatum	FB1, BEA, MON, FUP, FB2
Fusarium sambucinum	DAS, T2, NEO, ZEN, MAS, BEA
Fusarium semitectum	ZEN, BEA
Fusarium sporotrichioides	T2, HT2, NEO, MAS, DAS
Fusarium subglutinans	BEA, MON, FUP
Fusarium tricinctum	MON, BEA
Fusarium verticillioides	FB1, FB2, FB3

AcDON, monoacetyldeoxynivalenols; AcNIV, monoacetylnivalenol; BEA, beauvericin; DAcDON, diacetyldeoxynivalenol; DAcNIV, diacetylnivalenol; DAS, diacetoxyscirpenol; DON, deoxynivalenol; FB1, fumonisin B1; FB2, fumonisin B2; FB3, fumonisin B3; FUP, fusaproliferin; FUS, fusarenone-X; FUC, fusachromanone; HT2, HT-2 toxin; MAS, monoacetoxyscirpenol; MON, moniliformin; NEO, neosolaniol; NIV, nivalenol; T2, T-2 toxin; ZEN, zearalenon; ZOH, zearalenols

Fusarium verticillioides (Sacc.) Nirenberg, (teleomorph = *G. fujikuroi* (Sawada) Ito in Ito & K. Kimura) is a widely distributed pathogen of maize which is able to cause maize seedling blight, root rot, stalk rot and ear rot, and also can infect maize as an endophyte, without any symptom development. Both symptomatic and asymptomatic kernel infections by *F. verticillioides* can result decreased quality and economic losses due to contamination by fumonisins. These polyketide derived mycotoxins causing various diseases in animals including kidney and liver cancer in rodents, pulmonary edema in pigs, leukoencephalomalacia in horses and may have postulated role in human esophageal cancer. Many of the current maize hybrids are susceptible to ear rots, therefore those are exposed to fungal and toxin contamination under epidemic conditions. *Fusarium graminearum Schwabe (teleomorph = Gibberella zeae (Schwein.) Petch) is also an important pathogen of cereal crops causing primarily head blight in wheat and stalk and ear rot of maize. They produce mainly deoxynivalenol and zearalenone toxins.*

Several researchers have recently investigated the effects of climate change on mycotoxin contamination (DOBOLYI ET AL., 2011). For *Fusarium* infections the moderately warm, humid weather is optimal. The average summer temperature with higher humidity favors the spread of *F. graminearum*, but at a higher temperature *F. verticillioides* expansion can take place. Contrary, *Aspergillus flavus* requires especially dry and hot conditions to develop significant toxin contamination. Therefore, in maize some of the toxin contamination is a risk nearly every year. Maize products are frequently contaminated by fumonisins which are principally produced by *Fusarium* species, although black Aspergilli also occur frequently on maize kernels. In this study, we investigated the occurrence of these species and their mycotoxins on maize in various maize growing areas in Hungary in four consecutive years after harvest.

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MATERIAL AND METHOD

The samples were collected in various maize growing regions in 2011-2014. The samples were surface sterilized using ethanol (*v/v%: 70%*), and plated onto dichloran rose Bengal agar (DRBA) media (KING ET AL., 1979). Plates were incubated at 25 °C and after two weeks outgrowing mycelia were purified and transferred to malt extract agar (MEA) and potato dextrose agar (PDA) media. Isolates were subcultured as single conidia on MEA, Czapek-yeast extract agar (CYA) and potato dextrose agar (PDA) plates. Morphological identification of Aspergilli/Penicillia isolated from maize grains have been done according to standard textbooks and monographs (RAPER AND FENNELL, 1965, SAMSON ET AL., 2004, 2010). For sequence based identification, the cultures used for the molecular studies were grown on malt peptone (MP) broth for 7 days, and DNA was extracted from the mycelia using Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Species level identification of the selected isolates was performed by sequence analysis using genomic regions (*Fusarium* species: translation elongation factor gene; *Aspergillus* species: calmodulin gene; in section of other species: ITS region, PILDAIN ET AL., 2008). Sequences were compared using nucleotide-nucleotide BLAST (blastn) with default settings (http://blast.ncbi.nlm.nih.gov; ALTSCHUL ET AL., 1990) to the Genbank database, and to our own sequence database.

RESULTS AND DISCUSSION

In 2011 and 2012, the weather conditions were hot and dry. In 2012, extreme weather conditions were observed in Central Europe, when July and August were almost 3 °C warmer than the 100 years' average, and the amount of precipitation in August was only 13% of the average. In 2011, the average infection rate of maize grains was 14.3%. The proportion of *Fusarium* species were nearly 60.3% (*Figure 1*) and most isolates belonged to species *F. verticillioides* (70%), but *F. graminearum* (4%), *F. proliferatum* (24%), *F. subglutinans* (1%) and *F. oxysporum* (1%) have also occurred in the samples. The ratio of presence of Aspergilli was 8%. Besides the potential aflatoxin producer *A. flavus,* representatives of *A. niger* were also identified which can produce fumonisins and ochratoxins.

Due to the hot and dry climate of 2012, fungal infection of grains was reduced to 9.2%, while 68.1% of the isolated fungus belonged to genus *Fusarium* and 16% belonged to genus *Aspergillus*. Most isolates belonged to species *F. verticillioides* (74%), but *F. graminearum* (3%) and *F. proliferatum* (23%) also occurred in the samples. Within genus Aspergilli *A. flavus* isolates dominated, and we isolated some *A. clavatus* in smaller proportion which can produce patulin. In that year, the southern parts of the country had serious aflatoxin contamination in maize.

2013 was characterized by a dichotomy, in regards of volume of precipitation and temperature. The average fungal contamination was lower than in previous years (50.1%). The rate of *Fusarium* species was 46% - 69% of the isolates belonged to *F. verticillioides*, 28% to *F. proliferatum*, 3% to *F. subglutinans* and no isolate of *F. graminearum* was found. 9.6% of the isolated fungi belonged to genus *Aspergillus*. Most of these were the potential aflatoxin producer *A. flavus*, and a smaller proportion was *A. niger* and *A. ochraceus*.

An infection rate of 71.4% of maize samples was observed due to the extremely high precipitation in 2014. The rate of *Fusarium* isolates was 84.9%. 44% of the isolates belonged to *F. verticillioides*, 29% to *F. graminearum*, 23% to *F. proliferatum*, 3% to *F.*

subglutinans and 1% belonged to the species *F. sporotrichioides*. We couldn't isolate any *A. flavus* from the maize samples in 2014. *Aspergillus* contamination was not detected among the maize samples.

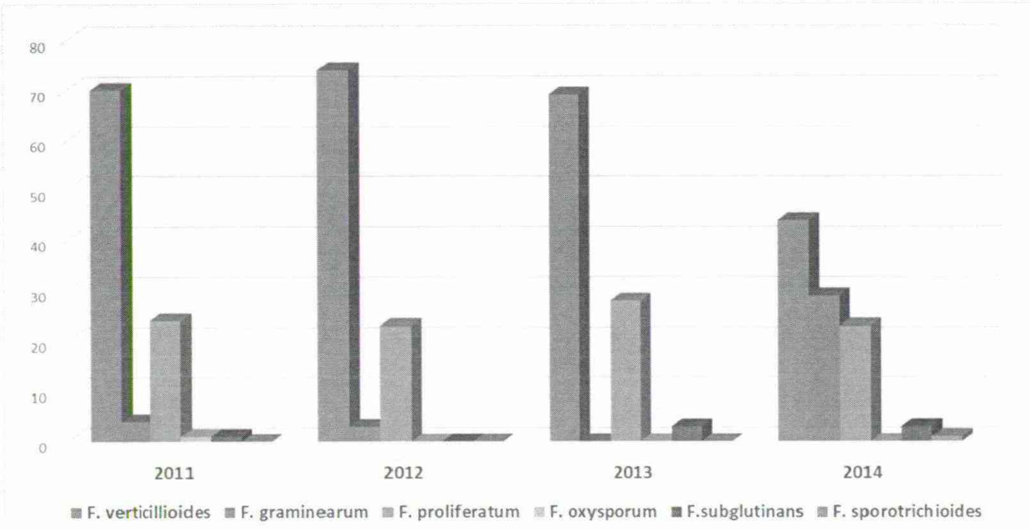

Figure 1. Incidence of mycotoxinogenic fungi in Hungarian maize samples between 2011-2014

In 2011, we identified several endophytic *Sarocladium zeae* (synonym: *Acremonium zeae*) isolates as well. This species is an antagonist of mycotoxinogenic *A. flavus* and *F. verticillioides*. This was the first data of occurrence in Hungary. In antimicrobial activity tests, *S. zeae* extracts containing pyrrocidin showed inhibition on these two pathogenic fungi.

Results of this survey verified, that according to the change of growing conditions the pathogen species distribution can change. Presence of *F. verticillioides* dominates when the temperature conditions are not favorable for *F. graminearum*. Representatives of *Fusarium culmorum* were not detected in any of the years examined. The possible reason is that warmer weather conditions strongly limit the spread of the pathogens.

Our results indicated that black Aspergilli had little role in the fumonisin contamination of maize, however the *Aspergillus flavus* infection represents a potential risk for aflatoxin contamination of Hungarian agricultural products.

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