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OCCURRENCE OF *FUSARIUM* SPECIES IN MAIZE GRAINS FOR SILAGE

ABSTRACT

Grain samples of two maize hybrids, medium early (ZP434) and late maturity (ZP704), collected during harvest in 2008 were investigated for contamination by fungi. Grains were plated on agar media and grown fungi were identified by morphological macroscopic and microscopic characteristics on potato-dextrose agar (PDA) and synthetic nutrient agar (SNA). Species of the genus *Fusarium* were the most common in both hybrids, and their presence amounted to 33.89% (ZP434) and 42.00% (ZP704). Other fungi of genera, *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Nigrospora, Penicillium*, *Rhizopus* and *Trichotecium*, were isolated from 0 to 41.00%. Four species belonging to the genus *Fusarium* were identified, of which the species *F. verticillioides* was the most common with 28.63% in ZP434 and 30.50% in ZP704 hybrids. The presence of *F. graminearum, F. proliferatum* and *F. subglutinans* ranged from 3.00% (ZP704) to 5.00% (ZP434), 0.13% (ZP434) to 7.00% (ZP704) and 0.13% (ZP434) to 7.00% (ZP704), respectively. Generally, the incidence of every particular fungus was higher in the late maize hybrid with a higher moisture content than in the medium-early hybrid with a lower moisture content.

Key words: Fusarium spp., maize, pathogenic fungi, silage

INTRODUCTION

Maize is one of the most important components of animal feed in Serbia. In animal feeding maize is used as whole grains, ground grain (bruised grain), in mixtures, as well as, silage from whole plants. Healthy grain of maize is a prerequisite for good reproduction and growth of animals.

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Maize is cultivated on approximately 1.2 M ha in Serbia (Statistical Yearbook of Serbia, 2001). Grain of maize, as well as, of other cereals, is susceptible to infestation of numerous microorganisms. Fungi of the genera *Fusarium*, *Alternaria* and *Cladosporium* are predominantly active in fields, while fungi of *Penicillium* and *Aspergillus* act in storages (Scudamore, 1993). Potentially the most important fungal toxigenic species in Serbia belong to genera *Fusarium*, *Penicillium* and *Aspergillus*, and they are isolated mainly from maize and wheat, either as field crops or components of feed and food (Lević *et al.*, 2004). *F. graminearum* Schwabe, *F. verticillioides* (Saccardo) Nirenberg (syn. *F. moniliforme* Sheldon) and *F. subglutinans* (Wollen. & Renik.) Nelson, Toussoun & Marasas, three very toxigenic fungal species of the genus *Fusarium*, are the most common pathogens in maize grain all over the world (White, 1999).

Food and feed safety and hygiene are a significant problem, and great attention is directed towards diseases that are closely related to different mycotoxicoses. Mycotoxins cause a whole range of disorders in the body of animals, ranging from biochemical changes, through the functional and morphological damages of different tissues and organs, to the appearance of clinical signs of mycotoxicoses with even possible lethal outcome (Jakić-Dimić *et al.*, 2009).

Since the maize is in Serbia one of the most important components in the food chain, the aim of this study was to investigate the microbiological quality of grain used for silage. Special attention was devoted to determining toxigenic species such as the fungi of the genus *Fusarium*.

MATERIAL AND METHODS

During September of 2008 the samples of maize grain that is used for silage were collected in the production plots of the Institute for Animal Husbandry in Belgrade-Zemun. The moisture content of samples was 16.3% in the medium early hybrid ZP434 (FAO 400) and 22.0% in the late hybrid ZP704 (FAO 700). A total of 1200 grains, 800 of ZP434 and 400 of ZP704, were surface-sterilised with 1% solution of sodium hypochlorite, rinsed three times with distilled water, dried and then five seeds were distributed on the PDA in 100-mm Petri dish. The identification of fungi was carried out after seven days of incubation under laboratory conditions. on the basis of morphological macroscopic and microscopic characteristics on potato-dextrose agar (PDA) and synthetic nutrient agar (SNA).Determination of *Fusarium* species was done after Nelson *et al.* (1983) and Burgess *et al.* (1994), while remaining fungal genera were determined after Ellis (1971) and Watanabe (1994).

RESULTS

Table 1

Incidence (%) of pathogenic fungi genera on grains in two maize hybrids

Fungal genera	Incidence (%) in maize hybrids	
	ZP434	ZP704
Acremonium	0.13	7.75
Alternaria	22.63	41.00
Aspergillus	5.20	0.50
Chaetomium	1.50	0.00
Cladosporium	0.63	0.50
Fusarium	33.89	42.00
Nigrospora	0.00	2.00
Penicillium	3.88	1.75
Rhizopus	31.63	4.50
Trichotecium	0.13	0.00

Table 2

Incidence* (%) of *Fusarium* spp. on grains in two maize hybrids

Fusarium spp.	Incidence (%) in maize hybrids	
	ZP434	ZP704
Fusarium graminearum	5.00	3.00
Fusarium proliferatum	0.13	7.00
Fusarium subglutinans	0.13	1.50
Fusarium verticillioides	28.63	30.50

*Calculated in relation to the total number of isolated fungi.

Ten genera of pathogenic fungi were isolated from grains of examined hybrids, including *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Nigrospora, Penicillium*, *Rhizopus* and *Trichotecium*. Their presence varied from 0.13% to 42.00%. Small number of grains of investigated hybrids were not contaminated with fungi (up to 0.38%). The presence of the most common genus *Fusarium* in both examined hybrids amounted from 33.89% (ZP434) to 42.00% (ZP704), followed by the presence of *Alternaria* genus (22.30% in ZP434 and 41.00% ZP704). Besides *Fusarium* and *Alternaria*, genera *Acremonium* and *Nigrospora* also were classlessness in the late

maturity hybrid ZP704 (7.75% and 2.00%, respectively) compared to the medium early hybrid ZP434 (0.13% and 0.00%, respectively) (Table 1). *F. verticillioides* was the most common species among the identified *Fusarium* species in both examined hybrids and their presence ranged from 28.60% (ZP434) to 30.50% (ZP704). *F. proliferatum* and *F. subglutinans* were classlessness in the hybrid ZP704 (7.00% and 1.50%) compared to the hybrid ZP434 (0.13%), while the species *F. graminearum* was classlessness in the hybrid ZP434 (5.00%) compared to the hybrid ZP704 (3.00%) (Table 2).

DISCUSSION

The quality of livestock feed is required for the maintenance of physiological function and the defence of animals against the disease. Based on the analysis of certain components of feeds, an overall quality can be assessed. In this paper microbiological quality testing of medium early and late maturity maize hybrids established the presence of pathogenic fungal species from 10 genera. *Fusarium* species known as economic very important toxigenic species were the most present.

Out of four isolated *Fusarium* species, *F. verticilliodes* was the most common in both hybrids, followed by *F. graminearum*, while the species of *F. proliferatum* (Matsushima) Nirenberg and *F. subglutinans* were less present. This is in agreement with data presented by Logrieco *et al.* (1995). According to these data the *F. verticilliodes* and *F. graminearum* species in the Mediterranean countries were most frequently isolated from maize. *Fusarium* species commonly isolated from Mediterranean cereals produce high amounts of fumonisin FB_1 and zearalenone (ZEN) *in vitro* on different media. A study of FB_1 production by 28 strains of *F. verticillioides* isolated from cereals in Italy, Spain and France revealed fumonisinotoxigenic potential for all strains with a maximum produced concentration of 4100 $\mu g\ g^{-1}$ (Visconti and Doko, 1994).

The results obtained in the period 1994–1996 by Lević *et al.* (1997) indicate that *F. verticillioides* (63.0%) is predominant maize ear rot pathogen in Serbia, followed by the *F. subglutinans* (50.6%), *F. graminearum* (12.2%), *F proliferatum* (9.6%) and *F. oxysporum* Schlech. (5.8%). According to these authors, *F. solani* (Mart.) Appel & Wollen. occurred each year during the stated period, but its frequency was not so high (2.4%), and other *Fusarium* species (*F. equiseti*, *F. sporotrichiodes*, *F. chlamydosporum*, *F. crookwellense* and *F. semitectum*) occurred sporadically on a few number of samples.

In Canada, according to Xue *et al.* (1995), *F. subglutinans* was a dominant species recovered from 28.8% of the silage maize grains and its incidence increased with delayed harvesting time. The other *Fusarium* species were also isolated: *F. oxysporum* (2.6%), *F. graminearum* (2.5%), *F. proliferatum* (0.3%) and *F. sporotrichioides* (0.2%). These authors observed four silage maize hybrids and established a significantly lower occurrence of *Fusarium* species in

the hybrid NK BRAND Enefrast1 than in other tested hybrids (MAIZEX leafy 4, Pioneer 37M81 and MYCOGEN TMF94)]. In addition, they found A significant presence of *Fusarium* species in all investigated hybrids after late harvest. In our tests we found a significant presence of *Fusarium* species in the hybrid belonging to later maturity group with a higher percentage of grain moisture (22.00%) than in the hybrid belonging to medium maturity group and with a lower percentage of grain moisture (16.31%).

CONCLUSIONS

Microbiological analyses of maize grains, intended for silage, show that a small number of grains of investigated hybrids were not contaminated with fungi (up to 0.38%). The presence of 10 fungal species was determined on the collected samples. *Fusarium*, *Alternaria* and *Rhizopus* were predominant species. *F. verticilliodes* was the most dominant *Fusarium* species and was followed by *F. graminearum*, *F. proliferatum* and *F. subglutinans*.

Based on these results it can be concluded that the incidence of individual fungi was higher in the late grain hybrid with a higher moisture content than in the medium-early hybrid with a lower moisture content. Furthermore, these results indicate that investigated samples, due to the presence of toxigenic fungi in high percentage (up to 40.00%), are not safe for animal feeding.

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