Pestic. Phytomed. (Belgrade), 28(3), 2013, 167–179
DOI: 10.2298/PIF1303167L

UDC: 632.4+632.7:579.64:615.9:632.11:633
Scientific paper

An Outbreak of *Aspergillus* Species in Response to Environmental Conditions in Serbia

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Received: June 18, 2013
Accepted: August 13, 2013

SUMMARY

The frequency and incidence of *A. flavus* and *A. niger* on barley, maize, soybean, sunflower and wheat grain, the abundance of European corn borer (*Ostrinia nubilalis*) moths and their interaction depending on weather conditions in the 2008-2012 period were studied. Under the agroecological conditions of Serbia, the species *A. niger* is more frequent than *A. flavus*, and concerning the crop species, its frequency is highest in kernels of sunflower, than soybean, maize, barley and wheat. *A. flavus* was extremely dominant on all plant species in 2012 regarding its frequency: 100% on soybean, 95.3% on maize, 65.2% on barley, 57.1% on sunflower and 45.8% on wheat. Furthermore, the incidence of *A. flavus* was higher in 2012 than in previous years. The uncommonly high frequency and incidence of *A. flavus* infestation of maize grain in 2012 were caused by extremely stressful agrometeorological conditions, high temperatures and drought over the period from flowering to waxy maturity of maize. The precipitation factor (Pf = precipitation sum / average monthly temperature) showed that 2012 was extremely arid in June (Pf = 0.57), July (Pf = 1.45), August (Pf = 0.15) and September (Pf = 1.42). European corn borer (ECB) was a second factor causing intensive occurrence of *A. flavus* on maize grain in 2012. The maximum flight of ECB moths was recorded as early as in July (5,149) and, as a result of this, high damage and numerous injuries were detected at harvest. Those injuries were covered by visible olive-green powdery colonies typical of *A. flavus*. In the chronology of *A. flavus* occurrence, these are the first data on its very high frequency and incidence under the agroecological conditions of Serbia. As intensive infections with *A. flavus* were rare in the past 50 years, the level of aflatoxins in maize grain was low.

Keywords: Cereals; Sunflower; Soybean; *Aspergillus flavus*; *A. niger*; *Ostrinia nubilalis*; Environment

INTRODUCTION

Species belonging to the genus *Aspergillus* are widely distributed throughout the world both in soil (Hill et al., 1983; Jaime-Garcia and Cotty, 2010) and in various agricultural crops, especially maize (Marín et al., 2012; Muthomi et al., 2012), cottonseed (Jaime-Garcia and Cotty, 2006), peanuts (Horn, 2003) and tree nuts (Bayman et al., 2002), and in plant products. *A. flavus* Link, the most widespread toxigenic species of the genus *Aspergillus*, is very important, particularly in some African countries, due to its high potential for synthesizing aflatoxins, which are very toxic to humans and animals (Probst et al., 2007; Donner et al., 2009; Muthomi et al., 2012). Consecutive outbreaks of acute aflatoxicosis in Kenya in 2004 and 2005 caused more than 150 human deaths (Strosnider et al., 2006).

Infections of standing cereal crops by *Aspergillus* species and contamination by aflatoxins are mild or very rare under the climatic conditions of many European countries: Austria (Öhlinger et al., 2004), Belgium (Chandelier et al., 2004), Germany (Curtui et al., 2004), Hungary (Varga et al., 2004), Poland (Perkowski et al., 2004), Romania (Ittu et al., 2004), the UK (Scudamore, 2004), etc. In 2003, extremely high temperatures during the whole growing season of maize and extraordinary drought from early May to early September, resulting in high levels of water stress for maize cultivated in Northern Italy, led to an intensive occurrence of *A. flavus* and aflatoxins contamination of maize crops (Moretti et al., 2004). In Europe, *A. flavus* and aflatoxins are considered an 'import problem' (Curtui et al., 2004; Ioannou-Kakouri et al., 2004; Levitin, 2004; Ostry et al., 2004; Perkowski et al., 2004; Varga et al., 2004) and a strict control system is applied to imported critical foods (pistachio, figs, hazelnuts and spices). In Serbia, 30 different species of the genus *Aspergillus* have been identified, isolated mainly from cereal grains, and later from sunflower grain, wheat flour, oilseed products, vegetable and fruit products, sugar confectionary, raw milk, meat products and from other feed and food products (Lević et al., 2004). *A. flavus* and *A. versicolor* (Vuill.) Tirab. of the genus *Aspergillus* have been mostly isolated from either feed or food products. Natural occurrences of aflatoxins, toxic metabolites of *Aspergillus* species, have been rare under Serbia's climatic conditions but there has been a possibility of an intensified presence of these mycotoxins through imports of some products. Generally, concentrations of aflatoxin B_1 ranging from 1 to 50 ppb have been most frequently detected.

The aim of this study was to analyse data obtained during years of studying the frequency and incidence of populations of *A. flavus* and *A. niger* (Fr.) P. Karst in grains of most cultivated crops in Serbia – cereals (maize, barley and wheat) and industrial crops (sunflower and soybean). The analysis was additionally motivated by an outbreak of *A. flavus* in maize in 2012 and rather high concentrations of aflatoxin M_1 in milk at the end of 2012 and the beginning of 2013 (unpublished data). Data on meteorological conditions and flight dynamics of European corn borer (ECB) (*Ostrinia nubilalis* Hbn.) moths were also analysed because those were important factors for the presence of *Aspergillus* species in fields.

MATERIAL AND METHODS

Post-harvest grain samples of barley, maize, soybean, sunflower and wheat from different environments in Serbia, were collected in the period 2008-2012. A total of 1514 grain samples were collected and analysed.

A standard method for fungal isolation and identification was applied (Lević et al., 2012). Briefly, approximately 50 kernels from each sample were surface-sterilised in 1% sodium hypochlorite for three minutes, rinsed three times with distilled water and then dried between two layers of soft paper. Depending on kernel size, five (maize, sunflower and soybean) or eight (barley and wheat) kernels were placed in Petri dishes (Ø 100 mm) on PDA and incubated for seven days under laboratory conditions at 25°C and a day/night regime. Kernels were then examined for fungal growth under a stereomicroscope (16x magnification). In order to reliably identify individual species of fungi, fragments of the colonies developed on kernels were transferred to carnation leaf agar (CLA) and synthetic nutrition agar (SNA) and incubated under the 12 h day/night regime using a combined fluorescent and near ultraviolet light during the daytime period. The CLA and SNA media were prepared according to Burgess et al. (1994) and Nirenberg (1976), respectively. Also, fungal fragments were transferred to potato dextrose agar (PDA) and incubated at 25°C in the dark for microscopic studies. This medium was prepared according to Burgess et al. (1994). Identification of *Aspergillus* species was done according to Singh et al. (1991).

Occurrence of *Aspegillus* species were analysed in 180, 1,138, 28, 89 and 79 samples of barley, maize, soybean, sunflower and wheat kernels, respectively.

The frequency (F) and incidence (I) of certain fungi were computed according to Lević et al. (2012): *F (%) = [Number of kernel samples in which a species occurred / Total number of kernel samples] x 100; I (%) = [Number of kernels in one sample in which a species occurred / Total number of kernels in the same sample] x 100].* Based on the data, frequency and incidence of fungal species in grain samples of the plants studied were classified as low (0 to ≤ 20%), moderate (>21 to ≤ 50%) or high (over 50%) (Lević et al., 2012).

An analysis of meteorological conditions during the study period was performed according to data provided by the Republic Hydrometeorological Service of Serbia (RHMS), Belgrade. Data were analysed for the Belgrade area in which the majority of grain samples were collected. Based on meteorological data, the monthly precipitation factor (*precipitation sum / average of monthly temperature*) was calculated according to Gračanin, 1950 (cited Lević, 1987).

The flight of ECB moths was monitored using light traps at the Zemun Polje Maize Research Institute, Belgrade, during the period 2008-2012. In 2012, the intensity of this pest infestation and incidence intensity of olive-yellow powdery mould (Figure 1) were evaluated on the same maize ears at 21 locations.

The correlation coefficient between the frequency and incidence of *A. flavus* and *A. niger* in all grain samples, as well as the similarities between weather conditions (precipitation factor) in 2012 and in the previous four years were determined using the Pearson product-moment correlation coefficient in Microsoft Office Excel 2007.

RESULTS

Meteorological conditions

Precipitation factors as indicators of climate conditions in the study period show that conditions were arid and semi-arid during 4-5 and 1-2 out of 6 months, respectively, in the growing seasons of 2008-2012 (Table 1). Exceptionally, climate conditions in June 2009 and May 2012 were semi-humid.

Figure 1. Olive-green powdery mould (*Aspergillus flavus*) on maize ears injured by ECB (*Ostrinia nubilalis*)

Table 1. Precipitation factors (Pf)* per month in the Belgrade region during May-October of 2008-2012

Month	Year				
	2008	2009	2010	2011	2012
May	3.12^{A}	1.77^{A}	4.75^{SA}	3.61^{SA}	7.12^{SH}
June	1.89^{A}	7.23^{SH}	0.85^{A}	1.81^{A}	0.57^{A}
July	2.26^{A}	3.31^{A}	1.75^{A}	4.45^{SA}	1.45^{A}
August	1.90^{A}	1.86^{A}	2.21^{A}	0.36^{A}	0.15^{A}
September	4.03^{SA}	0.22^{A}	2.78^{A}	2.09^{A}	1.42^{A}
October	1.24^{A}	0.78^{A}	4.17^{SA}	2.69^{A}	3.41^{SA}

*Pf = precipitation sum / average monthly temperature: A – arid climate (Pf <3.3), SA – semi-arid climate (Pf 3.4-5.0), SH – semi-humid climate (Pf >5) (Gračanin, 1950, cit. Lević, 1987)

The longest period with high temperatures and no rainfall was between June and October 2012, which resulted in arid climate conditions at that time. Climatic conditions in 2010 were most similar to the conditions in 2012, as confirmed by the coefficient of correlation between the precipitation factors for these two years (r = 0.86). A positive correlation coefficient was also found between the precipitation factor for the same months in 2011 and 2012 (r = 0.54). The greatest differences were between 2012 and 2008 (r = 0.22), as well as between 2012 and 2009 (r = -0.21).

Frequency and incidence of *Aspergillus* species

A. flavus and *A. niger* were frequently isolated and identified as species of the genus *Aspergillus* in the period 2008-2012, while other *Aspergillus* spp., including *A. calvatus* Desm., were very rarely detected. The frequency and incidence of *A. flavus* and *A. niger* varied depending on the year of study and plant species. In recording the presence of *Aspergillus* species on grain, all cases were taken into account from visualization under the microscope (16x magnification) to visible full coverage by fungus.

Depending on environmental conditions, the highest average frequency of *A. flavus* on the analysed grain was in 2012, then in 2010, and much lower, but with similar approximate values in 2008, 2009 and 2011 (Figure 2). A similar tendency was found for the frequency of *A. niger*, but with a lower frequency in 2012, compared to 2010.

The lowest maximum incidence of *A. flavus* was found in 2009, while the highest was recorded in 2012 (Figure 3). In contrast to *A. flavus*, the maximum incidence of *A. niger* had an upward trend from 2008 to 2011, and it declined in 2012.

Generally, there is a statistically significant positive correlation in frequency (r = 0.84) and incidence (r = 0.61) between *A. flavus* and *A. niger*. However, the correlation between the frequency and incidence of *A. flavus* was higher (r = 0.90) than it was for *A. niger* (r = 0.70).

Considering plant species, the frequency of *A. flavus* can be classified as low (0 ≤ 20%) in wheat grain (16.2%) and moderate (> 21 ≤ 50%) in maize (39.3%), soybean (48.1%), sunflower (37.5%) and barley (21.7%) (Table 2). At the same time, the incidence of this fungus can be classified as low in all plant species (2.7-11.5%) other than maize grain which was classified as moderate (41.5%). On average, the frequency of *A. niger* can be characterized as moderate in wheat, barley and maize grains (20.4%, 32.1% and 38.6%, respectively), and high in sunflower and soybean (60.3% and 57.7%, respectively). The incidence of *A. niger* in maize grain was only moderate (42.5%), while it was low in wheat, barley, sunflower and soybean (4.7%, 7.7%, 9.5% and 17.0%, respectively).

The analysis of *A. flavus* incidence per plant species indicates that the fungus was found in a majority of grain samples of barley (65.2%), maize (95.3%), soybean (100%), sunflower (57.1%) and wheat (45.8%) in 2012, and in soybean and sunflower in 2010 (66.7% and 50.0%, respectively) (Table 2). Similar results were obtained for the frequency of *A. niger*. A very high incidence of both *A. flavus* and *A. niger* was recorded only in maize grain in 2012 (100% and 66.7%, respectively) and in 2010 (43.3% and 53.3%, respectively).

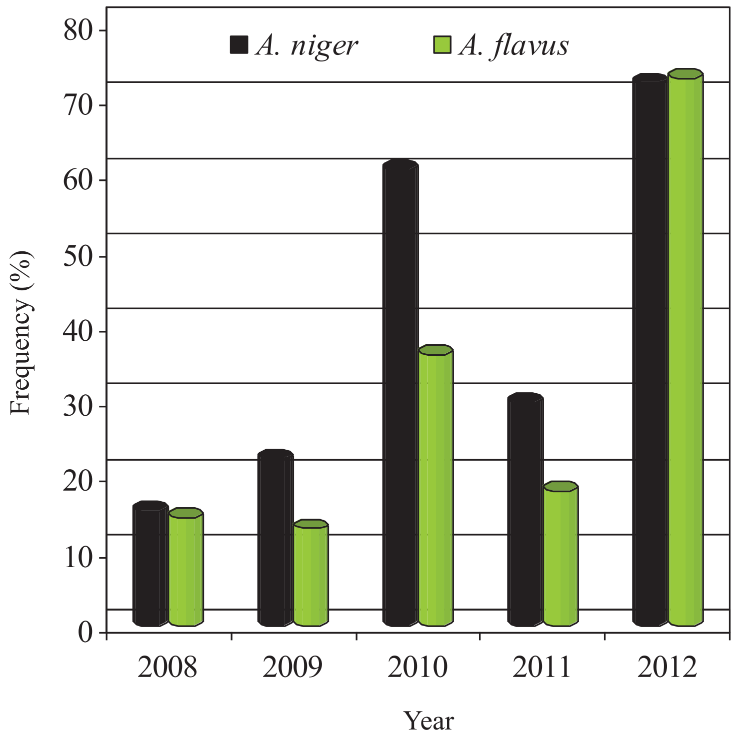

Figure 2. The average values of frequency of *Aspergillus flavus* and *A. niger* for the analyzed grains in the period 2008-2012

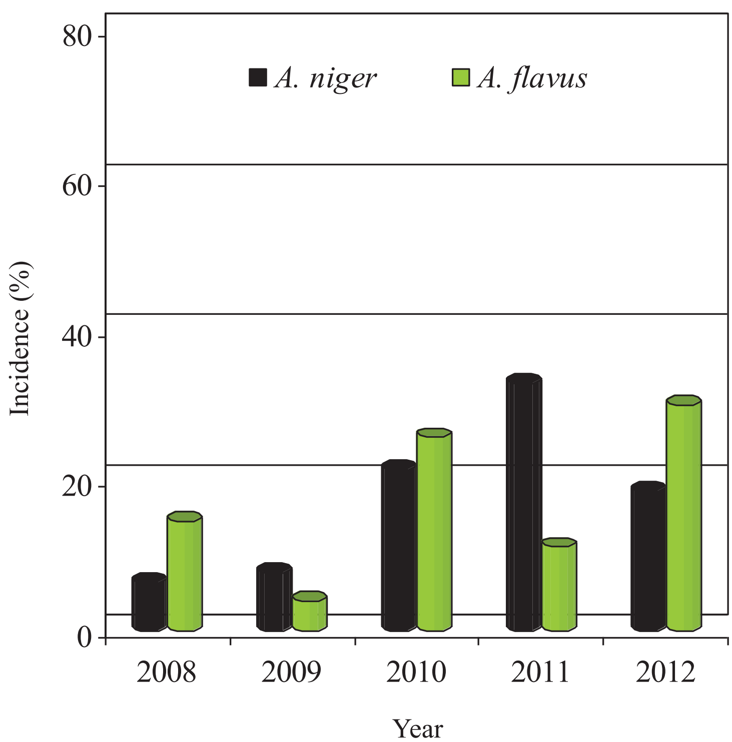

Figure 3. The average values of maximum incidence of *Aspergillus flavus* and *A. niger* for the analyzed grains in the period 2008-2012

Table 2. Frequency and maximum incidence of *Aspergillus flavus* and *A. niger* in grains of different plant species in the period 2008-2012

Plant species	Year	Number of samples	Frequency (%)		Maximum incidence (%)	
			A. flavus	*A. niger*	*A. flavus*	*A. niger*
Barley	2008	45	13.3	8.9	3.0	1.0
Barley	2009	43	2.3	9.3	1.8	3.6
Barley	2011	51	5.9	31.4	1.3	13.8
Barley	2012	41	65.2	80.0	10.7	12.5
Total/Average		180	21.7	32.4	4.2	7.7
Maize	2008	15	13.3	20.0	20.0	12.0
Maize	2009	247	23.5	3.4	12.3	16.7
Maize	2010	423	24.2	39.5	43.3	53.3
Maize	2011	126	40.0	54.8	32.0	64.0
Maize	2012	327	95.3	75.4	100.0	66.7
Total/Average		1,138	39.3	38.6	41.5	42.5
Soybean	2009	7	25.6	42.9	2.0	3.0
Soybean	2010	3	66.7	100.0	8.0	23.0
Soybean	2011	13	0	7.7	0	40.0
Soybean	2012	5	100.0	80.0	21.0	2.0
Total/Average		28	48.1	57.7	7.8	17.0
Sunflower	2008	6	16.7	16.7	20.8	6.2
Sunflower	2010	2	50.0	100.0	1.0	8.0
Sunflower	2011	74	25.7	24.3	11.3	13.8
Sunflower	2012	7	57.1	100.0	11.0	10.0
Total/Average		89	37.5	60.3	11.5	9.5
Wheat	2009	21	0	33.3	0	8.0
Wheat	2010	34	2.9	2.9	1.0	1.3
Wheat	2012	24	45.8	25.0	7.1	1.8
Total/Average		79	16.2	20.4	2.7	4.7

Occurrence of ECB populations

Monitoring of the flight dynamics of ECB showed an extremely large number of moths (5,149) in July of 2012, compared to the number of moths in the same month of previous year (Table 3). The number of moths (5,149), especially of the second generation, in July was approximately equal to the total sum of moths in the period 2008-2010 (5,121). Furthermore, the number of moths in the period May-September was higher in 2012 (7,979) than it was in all of the preceding four years (6,144). The data also show that the ECB reached maximum frequency in 2012 earlier than it did in the previous years, when maximum was commonly reached in August. The total number of moths trapped in light lamps was 72.0%, 81.3%, 82.5% and 87.2% lower in 2008, 2009, 2010 and 2011, respectively, than it was in 2012.

Table 3. Flight of European corn borer (*Ostrinia nubilalis* Hbn.) moths during the maize growing season (May-September) in the period 2008-2012, Belgrade region

Month	Number of moths of European corn borer per year					Average
	2008	2009	2010	2011	2012	
May	73	193	23	53	60	80.4
June	106	175	267	224	504	255.2
July	900	408	171	202	5,149	1,366.0
August	952	670	821	218	1,939	920.0
September	201	45	116	326	327	203
Total	2,232	1,491	1,398	1,023	7,979	

A negative correlation was detected between the occurrence of ECB moths and the precipitation factor during the growing season of maize in 2008 (r = -0.498), 2009 (r = -0.030), 2010 (r = -0.369), 2011 (r = -0.423) and 2012 (r = -0.340). A highly significant positive statistical correlation was detected between the number of ECB moths and frequency (r = 0.93) or incidence (r = 0.75) of *A. flavus.* Also, positive correlations between the number of ECB moths and frequency (r = 0.59) and incidence (r = 0.52) of *A. niger* were noticed. Based on the analysis of 21 samples of maize ears harvested in 2012 (data not presented), a statistically highly significant correlation (r = 0.714) between the ear damage caused by ECB and the incidence of olive-green powdery mould (*A. flavus*) was found.

DISCUSSION

Although *A. flavus* is mostly considered a storage fungus, it may cause infection of grain at a very high percentage under specific agroecological conditions in the field. The fungal growth generally expands from the ear tip towards the base, colonising silks, then glumes (by the late milk stage) and kernel surface but rarely penetrating the cob pith (Matsh and Payne, 1984). Silk senescence and subsequent spread of *A. flavus* down the silk is rapid (in 4 days) at 30/34°C. Internal infection of kernel did not occur until the early dent stage.

This research shows that both *A. flavus* and *A. niger* were able to infect grain of different cereals (barley, maize and wheat) and industrial crops (soybean and sunflower) at high frequency and high incidence under specific agroecological conditions in Serbia, as it was in 2012. As far as we know, this is the highest incidence of these fungi under the environmental conditions of Serbia. Bearing in mind the history of infections by *Aspergillus* species and little practical experience in controlling their occurrence in Serbia, their outbreak in 2012 and the ensuing high levels of aflatoxin M_1 (metabolite of aflatoxin B_1) detected in milk in late 2012 and early 2013 were unexpected for many agricultural, veterinary and medical researchers, as well as for food and feed manufacturers.

Data for the occurrence of *Aspergillus* species in Serbia show that they had mostly appeared at low frequency and incidence in grain in previous years. In some cases, their frequency was high but the incidence was low. *A. flavus* and a number of other fungi were for the first time found in Serbia in the winters of 1957/1958 and 1959/1960 when a strong outbreak of mould was recorded in stored maize ears (Panić and Marić, 1964). Over the period 1967-2008, the frequency of this fungus varied from 1.0 to 23.1%, and the incidence from 2.9 to 16.0% in maize grain (Lončarević et al., 1970; Lević et al., 1991, 2003, 2012). However, sometimes a higher percentage (up to 30%) of *A. flavus* was found in samples of maize-based feed (Kordic et al., 1986; Škrinjar et al., 1989; Krnjaja et al., 2007). There are even less literature data on the presence of *Aspergillus* species in wheat, barley, sunflower and soybean grain than in maize. Low frequency and low incidence of *Aspergillus* spp. were found in wheat, barley and sunflower grain, while the species were not identified in soybean (Bagi et al., 2011; Lević et al., 2012). Only in some years, *A. flavus* occurred more frequently in unshelled (up to 55%) than in shelled (up to 33.3%) sunflower grain (Dimić et al., 2006; Škrinjar et al., 2007). As a result of low incidence of *Aspergillus* species, natural occurrence of aflatoxins in Serbia was generally low (Lević et al., 2004). Although high frequency or incidence of *Aspergillus* species was found in some cases in feed, aflatoxins were not detected in feed or milk, nor their levels exceeded maximum permissible concentrations (Kordić et al., 1986; Šutić et al., 1989; Dimić et al., 2005; Jakić-Dimić et al., 2009; Polovinski-Horvatović et al., 2009; Adamović et al., 2011; Bočarov-Stančić et al., 2011; Škrinjar et al., 2011). Exceptionally, a high incidence of *Aspergillus* species was detected in 2012. Moreover, high concentrations of AFB_1 were found in maize-based feed in 2012 (Krnjaja et al., 2013). Peito and Venâncio (2004) had found a positive correlation between the occurrence of aflatoxin B_1 in different types of feeds (cattle dairy, poultry and swine feed) and the presence of *A. flavus*.

Our data show that *A. flavus* species occurred at high percentage in sunflower, soybean, barley and wheat grain under the weather conditions such as existed in 2012, which favoured the development of this fungus. Similar results for the occurrence of *Aspergillus* species in cereal grain have been reported from other countries worldwide (Gursoy and Bicici, 2004; Mateo et al., 2004; Pepeljnjak and Šegvić, 2004; Hajihasani et al., 2012). Nevertheless, in order to obtain reliable data on the importance of these species in soybean, sunflower and wheat in Serbia, a greater number of samples should be analysed. Furthermore, samples from these plant species should be collected from crops cultivated under different agroecological conditions over a longer period. Generally, more attention should be focused on grain sampling because it has a huge impact on reliability of results. Laboratory conditions for identification of these fungi should be customized according to their environmental needs, especially in terms of higher temperatures (≥30°C) during the incubation period.

The data in this research show that *A. niger* species of the genus *Aspergillus* also frequently occurred, and it was more common in some years and its incidence was higher than that of *A. flavus*. The presence of both *Aspergillus* species in the same grain is very common (Figure 2). Previous studies had shown that *A. niger* frequently co-occurred with *A. flavus* in the field (Klich, 2007). Hill et al. (1983) had noticed that when the ratio of grain colonised by *A. flavus* compared with *A. niger* was >19:1, there aflatoxin contamination occurred, but there was no contamination with this mycotoxin if the ratio was <9:1. According to these authors, irrigation caused higher incidence of *A. niger* than drought, while the reverse situation was favourable for *A. flavus*.

Based on the obtained results it can be inferred that the occurrence of *A. flavus* was in epidemic proportions in Serbia in 2012. High frequency and incidence of *Aspergillus* species, particularly of *A. flavus*, which were found in this study in 2012, can be explained as a consequence of specific environmental conditions. Very high temperatures and extreme drought were recorded in the period from maize anthesis to the late dough stage in 2012, which resulted in a very low precipitation factor (0.27 to 1.45) or arid conditions. Similar environmental conditions that favoured proliferation of *A. flavus* had been described in Italy (Moretti et al., 2004), Kenya (Muthomi et al., 2012), the USA (Abbas et al., 2006) and other countries around the world. Stress conditions that reduce yield may play a role in predisposing maize infection by *A. flavus* or may increase aflatoxins production once infection has occurred (Jones et al., 1981). Similar conditions, and extreme drought stress, occurred in 2012 when the yield of maize was halved in Serbia. Prolonged drought and high temperatures during the growing season favoured the development of *A. flavus*, while limited competition of other fungi prevented normal pollination of maize plants (Stack and Carlson, 2003).

The ECB maximum occurrence in July was another factor influencing the emergence of *A. flavus* on maize ears under the environmental conditions that existed in 2012. Infections by *A. flavus* mainly on grain damaged by ECB were visible at harvest. The importance of the ECB and other insects in the aetiology of aspergillosis of maize ear was confirmed by other literature data (Lillehoj et al., 1976; McMillian et al., 1985; Windham et al., 1999; Abbas et al., 2006). The correlation coefficient between precipitation factors for 2010 and 2012 indicates that environmental conditions or arid climate during the growing season of maize were similar. In 2010, there was 30 and 2.4 times lower incidence of the ECB in July and August, respectively, than in 2012. As a result, the incidence of *A. flavus* was lower in 2010 than in 2012. A similar interpretation can be applied to data for ear maize infection in 2011. Comparing 2012 to the earlier period, a greater abundance of ECB moths (total 8,347), especially of the second generation (7,978), was recorded only in 1987 (Bača et al., 2007).

There are no data available on characteristics and toxicological potentials of *A. flavus* and *A. niger* populations in Serbia. According to literature data, *A. flavus* can be easily divided into two major subdivisions known as S and L strains. Strain L is very common around the world, while strain S has established in the USA (Nelson et al., 1996), China (Gao et al., 2007) and Kenya (Probst et al., 2007). Strain S isolates consistently produce large amounts of aflatoxin B_1 (>98%), whereas strain L isolates are more variable, with some producing no aflatoxins at all (Nelson et al., 1996). Because L isolates of *A. flavus* do not produce aflatoxin B_1, or if they do its concentrations are low, those isolates are known as atoxigenic isolates. Characterisation of the causal agents is an important initial step for development of management procedures (Probst et al., 2007). This is especially important for improving management in cases where the life cycles of potential causal agents vary (Jaime-Garcia and Cotty, 2006). The existence of *A. flavus* atoxigenic strains may be important in biological control of preharvest aflatoxins contamination of crops by their application to soil where they competitively exclude natural toxigenic strains of *A. flavus* (Razzaghi-Abyanehet al., 2006; Yin et al., 2008). For competitive exclusion to be effective, the biocontrol atoxigenic strains must be predominant in agricultural environments in which crops are susceptible to be infected by toxigenic strains (Yin et al., 2008). It has been proved that native atoxigenic strains adapted to maize growing areas may have a greater value than exotic strains as biocontrol agents (Donner et al., 2009). Competitive exclusion of the S strain by L strain may result in reduced overwintering by S strain isolates and lower toxicity resulting from sclerotial metabolites (Garber and Cotty, 1997).

There are a number of measures that can significantly reduce the development of *A. flavus* and biosynthesis of aflatoxins. Preharvest interventions should include resistant crops (Strosnider et al., 2006), crop rotation (Jaime-Garcia and Cotty, 2004; Razzaghi-Abyaneh et al., 2006; Accinelli et al., 2008), early sowing, growing early maturity hybrids (Jones et al., 1981; Bruns, 2003), proper plant nutrition (especially with nitrogen), optimum plant populations, irrigation and early harvesting (Bruns, 2003; Jaime-Garcia et al., 2006). Integration of these approaches in a cost-effective manner may be the greatest challenge we will face in providing safer food and feed production in the 21st century (Abbas et al., 2009).

In conclusion, it is necessary to study comprehensively the populations of *A. flavus* based on morphological (sclerotia size) and mycotoxicological properties (the ability of synthesis of aflatoxins) and to determine the types of isolates (S and L) present in Serbia. Furthermore, there is a need to characterise the population dynamics in soil and maize in particular regions and to examine maize contamination by aflatoxins across Serbia. In order to reduce the occurrence of *Aspergillus* species it is necessary to make changes in maize management practices, particularly from the standpoint of maize genotypes resistant or tolerant to *A. flavus*, insects and drought, followed by crop rotation, crop irrigation, herbicide application and use of other good agricultural practices. It is necessary to develop and augment the constant surveillance of *Aspergillus* species in the field and in warehouses, to monitor food contamination by aflatoxins, as well as laboratory and public health response in the affected regions. Growers, wholesalers and retailers need to be educated about measures that can be applied to reduce *Aspergillus* species occurrence and aflatoxin production in order to produce and put on the market safe food and feed.

ACKNOWLEDGMENT

The study was part of the project No. TR-31023 funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Intenzivna pojava vrsta roda *Aspergillus* kao odgovor na uslove spoljašnje sredine u Srbiji

REZIME

U ovom radu su analizirani učestalost i intenzitet napada *A. flavus* i *A. niger* na 180 uzoraka zrna ječma, 1138 kukuruza, 283 soje, 89 suncokreta i 79 pšenice, brojnost leptira kukuruzovog plamenca (*Ostrinia nubilalis*), kao i njihova interakcija u zavisnosti od meteoroloških uslova u periodu 2008-2012. U agroekološkim uslovima u Srbiji vrsta *A . niger* je učestalija od vrste *A. flavus*, a u zavisnosti od biljne vrste pojavljuje se najčešće na zrnu suncokreta, zatim soje, kukuruza, ječma i pšenice. *A. flavus* je izuzetno bila dominantna na svim biljnim vrstama u 2012, kako po učestalosti, tako i intenzitetu napada. Ova vrsta je u 2012. godini utvrđena kod svih uzoraka soje (100%), nešto manje na zrnu kukuruza (95,3%), zatim na ječmu (65,2%) i suncokretu (57,1%) i najmanje na zrnu pšenice (45,8%). Intenzitet napada *A. flavus* je, također, bila veća u 2012. u poređenju sa prethodnim godinama. Neuobičajeno visoka učestalost i intenzitet napada *A. flavus* na zrnu kukuruza u 2012. godini bila je uslovljena izuzetno stresnim agrometeorološkim uslovima, visokim temperaturama i sušom, od cvetanja do voštane zrelosti kukuruza. Kišni faktor (*Kf = suma padavina / prosečna mesečna temperatura*) ukazuje da je te godine bila izuzetno aridna klima u junu (Kf = 0,57), julu (Kf = 1,45), avgustu (Kf = 0,15) i septembru (Kf = 1,42). Kukuruzov plamenac je drugi činilac koji je uslovio intenzivnu pojavu *A. flavus* na zrnu kukuruza u 2012. godini. Maksimalni let leptira ovog insekta utvrđen je veoma rano, posebno let druge generacije, već u julu 2012. (5.149 jediniki), a kao posledica toga u vreme berbe su utvrđena i brojna oštećenja na kojima je bio vidljiv razvoj maslinasto-zelenih praškastih kolonija koje su tipične za *A. flavus*. U hronologiji pojave *A. flavus* ovo su prvi podaci o njenoj jako visokoj učestalosti i intenzitetu napada u agroekološkim uslovima u Srbiji. Intenzivna pojava *A. flavus* je bila retka u proteklih 50 godina zbog čega je bio i nizak nivo aflatoksina u zrnu kukuruzu.

Ključne reči: Žita; suncokret; soja; *Aspergillus flavus*; *A. niger*; *Ostrinia nubilalis*; uslovi spoljašnje sredine