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Original scientific paper

Correlation of deoxynivalenol and zearalenone production by *Fusarium* species originating from wheat and maize grain

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SUMMARY

A total of 39 *Fusarium graminearum*, *F. sporotrichioides*, *F. semitectum* and *F. equiseti* isolates, originating from wheat and maize samples collected at 10 locations in Serbia, were analyzed by ELISA method for their potential of deoxynivalenol (DON) and zearalenone (ZEA) production under optimal laboratory conditions. *Fusarium graminearum* isolates with the highest intraspecies variability were the best producers of both deoxynivalenol and zearalenone. In contrast, *F. equiseti* isolates were the weakest producers of these two toxins. Considering the plant origin of the isolates, wheat-originating *F. sporotrichioides* isolates were better deoxynivalenol producers, while the maize-originating isolates produced more zearalenone. There was no clear difference in ZEA production between wheat- and maize-originating isolates of *F. graminearum*, while higher average DON concentrations were produced by *F. graminearum* wheat-originating isolates.

Negative correlation was detected between the production of deoxynivalenol and zearalenone by various *Fusarium* spp.

Keywords: Mycotoxins; *Fusarium*; Wheat; Maize

INTRODUCTION

Fusarium species are cosmopolitan pathogens of many plant species, as well as endophytes and soil inhabitants. More than 80 of 101 economically important plants have at least one *Fusarium*-associated disease (Leslie & Summerell, 2006). Besides their economic importance in terms of yield reduction, these species are able to produce mycotoxins in pre-harvest infected plants or in stored grains. The main group of mycotoxins produced by *Fusarium* species consists of more than 170 different chemical structures named the trichothecenes (Yazar & Omurtag, 2008). Deoxynivalenol (DON) is the most frequently detected trichothecene in wheat grain, while the highest toxicity has been declared for the T-2 toxin (Preluskey et al., 1994). Directly or indirectly, mycotoxins get into human and animal food chains by contamination of plants or plant products. Regarding

mycotoxin transfers through food chains and their cumulative effects, toxins can cause diseases in human and animal organisms known as mycotoxicoses. Biological activity of mycotoxins includes acute and chronic toxicity, teratogenic, mutagenic and cancerogenic effects (Yazar & Omurtag, 2008).

The toxicological profile of a contaminated crop depends not only on *Fusarium* species but also on opportunistic species in the fungal complex with *Fusarium* (Logrieco et al. 2003). Mycotoxin concentrations are not always proportional to the intensity of *Fusarium*-caused diseases. Also, the profile of *Fusarium* species present in a crop, as well as the composition of toxins that will be biosynthesized may vary drastically if insects have caused plant damage. Low temperatures, high air humidity and moist can also contribute to mycotoxin production increase (Meronuck & Concibido, 1996).

Considering their toxicological characters, *Fusarium* species are highly variable but at the same time they can be very similar, even more than in morphological characters. One *Fusarium* species can produce a number of mycotoxins - for example *F. graminearum* can produce 17 different toxins: zearalenone (ZEA); zearalenole (ZOL); trichothecenes type B: deoxynivalenol (DON), nivalenole (NIV), 3-Acetyldeoxynivalenol (3-AcDON), etc.; trichothecenes type A, such as diacetoxyscirpenol (DAS); aurofusarin; culmorins; fusarin C; fusarochromanone and steroides (Lević et al., 2004; Marić, 2002; Leslie & Summerell, 2006). On the other side, the same toxin can be synthesized by several *Fusarium* species – for example ZEA is most commonly synthesized by *F. graminearum*, but it can also be synthesized by *F. culmorum, F. crookwellense, F. equiseti, F. heterosporum, F. sambucinum, F. semitectum* and *F. sporotrichioides* (Logrieco et al., 2002; Leslie & Summerell, 2006; De Nijs et al., 1997).

Deoxynivalenol concentrations produced by different *Fusarium* species worldwide vary from 0.01 up to 160 μg/g (Tomczak et al., 2002; Ittu et al., 2004), while ZEA concentrations vary from 0.004 up to 18 μg/g (Moretti et al., 2002; Varga et al., 2002). In Serbia, DON has been confirmed to vary from 0.25 up to 45.56 μg/g (Stanković et al., 2008) and ZEA from 0.03 up to 12.8 μg/g (Bočarov-Stančić et al., 2000; Stanković et al., 2007). Some strains of *F. graminearum* may even produce up to 60 000 μg/g of ZEA and they are used for comercial ZEA production, which is then chemically modified and sold as a cattle growth promotant (Leslie & Summerell, 2006).

The aim of this study was to reveal the potential of tested pathogenic *Fusarium* spp. isolates for DON and ZEA production under optimal conditions, and to confirm if there is any correlation in production between these two mycotoxins.

MATERIAL AND METHODS

Plant samples and isolation of Fusaria

Samples of maize and wheat grain were collected from 10 locations in Serbia (Bačka Topola, Kikinda, Kovin, Novi Sad, Sombor, Sremska Mitrovica, Kraljevo, Loznica, Niš and Šabac) in 2005, 2006 and 2007. Each wheat sample consisted of several small subsamples collected from different parts of the grain storage one month after harvest in 2005 and 2006. Thirty-two kernels from each subsample (four subsamples per location) were incubated in water agar (WA) in order to analyze the diversity of *Fusarium* species. Ten maize ears with *Fusarium* symptoms, collected diagonally from each field in 10 locations during 2006 and 2007, were considered as the basic sample. Ten kernels were taken as subsamples from each ear (100 kernels per sample). For fungal identification, 128 wheat kernels (32 per replication) and 100 maize kernels (25 per replication) of each sample were incubated for seven days in water agar (WA) and potato dextrose agar (PDA), respectively. *Fusarium* species were identified according to Leslie & Summerell (2006). All isolated *Fusarium* cultures were refined to monosporous isolates, and 39 isolates were selected for further toxicological investigation analyses: 24 *F. graminearum* isolates (14 wheat-originating and 10 maize-originating), 8 isolates of *F. sporotrichioides* (2 wheat- and 6 maize-originating), 5 *F. semitectum* isolates (1 wheat- and 4 maize-originating) and 2 isolates of *F. equiseti* (both wheat-originating). The obtained pure cultures of *Fusarium* spp. were maintained in the fungal collection of the Maize Research Institute, Zemun Polje, and were marked as MRIZP.

Sample preparation for mycotoxin assessment

Fifty grams of maize grain were soaked in sterile distilled water to reach 40% moisture. After 24 h, maize grain were autoclaved for 30 min. Sterilized maize grain were inoculated with 3-5 plugs (5 × 5 mm) of single-spore cultures of *F. graminearum*, *F. sporotrichioides*, *F. semitectum* and *F. equiseti* isolates previously cultivated for seven days on PDA at 25°C in the dark. The inoculated grain were incubated for 2-3 weeks at 27°C in the dark,

and occasionally shaken to prevent sample conglomeration and nonuniform mycelia growth that could reduce mycotoxin production. After incubation, the infected maize grain was dried at 50°C for 3 days and then milled to powder, which was kept in plastic bags at 4°C until further extraction.

Mycotoxin assessment

The Enzyme-Linked Immunosorbent Assay (ELISA) method encompassed three phases: sample preparation, ELISA assay procedure and ELISA DON and ZEA detection. The ELISA assay procedure was performed according to CELER® Techna (code MZ370) and AgraQuant® Romer (COKAQ4000) Test KitManual. The ELISA DON and ZEA detection was performed at 450 nm (Labsystems MultiScan® MCC/340).

Statistical analyses

The interrelations of DON and ZEA mycotoxins were determined by T-test for dependent variables, while analyses of the influence of isolate origin on mycotoxin production were conducted by T-test for independent samples by group in the Statistica software (StatSoft 12).

RESULTS

All tested isolates of four *Fusarium* species produced both mycotoxins, DON and ZEA, but they were mainly better ZEA producers. The exceptions were 14 of a total of 39 isolates which were better producers of DON (Tables 1, 2 and 3).

Table 1. Deoxynivalenol and zearalenone concentrations produced by *Fusarium graminearum* isolates originating from wheat and maize grain from Serbia

MRIZP isolate	Host plant	Year	Location	DON (μg/g)	ZEA (μg/g)
1490	Wheat	2005	Sombor	8.7	68.87
799	Wheat	2005	Šabac	2.4	42.92
687*	Wheat	2005	Kikinda	22.3	9.52
891	Wheat	2005	Sr. Mitrovica	14.7	33.27
1390	Wheat	2005	Kraljevo	3.8	40.02
770	Wheat	2005	Novi Sad	14.7	40.57
750*	Wheat	2005	Niš	3.2	2.44
1482	Wheat	2006	Sombor	2.6	97.52
1418*	Wheat	2006	Šabac	22.3	15.80
1339*	Wheat	2006	Kikinda	0.75	0.16
1338*	Wheat	2006	Kovin	45.3	41.13
1217	Wheat	2006	Kraljevo	3.8	4.29
1351	Wheat	2006	Novi Sad	3.8	14.73
1486	Wheat	2006	Niš	14.7	16.69
Average				11.6	30.57
1277	Maize	2006	Sombor	2.8	9.90
1282	Maize	2006	B. Topola	3.8	29.14
1367	Maize	2006	Šabac	3.8	18.49
1254	Maize	2006	Loznica	8.7	27.42
1213*	Maize	2006	Kikinda	0.7	0.11
1257	Maize	2006	Kovin	4.3	45.56
1424	Maize	2006	Kraljevo	4.3	49.58
943	Maize	2006	Novi Sad	3.5	84.61
1648	Maize	2007	Loznica	1.1	19.08
1554	Maize	2007	Novi Sad	45.3	22.80
Average				7.8	30.67
Total average				10.1	30.61

*Isolates marked with asterisk were better producers of DON than ZEA

Deoxynivalenol production by all tested isolates (regardless of species) varied from 0.13 μg/g up to 45.3 μg/g, while ZEA production varied among all tested isolates from 0.049 to 97.5 μg/g (Tables 1, 2 and 3). Considering all tested species, *F. graminearum* isolates were the best producers of DON and ZEA, while *F. equiseti* isolates were the weakest producers of both toxins. The tested *F. sporotrichioides* isolates had better DON production potential than *F. semitectum* isolates, while the situation with ZEA production potential was reverse (Tables 1, 2 and 3).

Table 2. Deoxynivalenol and zearalenone concentrations produced by *Fusarium sporotrichioides* isolates originating from wheat and maize grain from Serbia

MRIZP isolate	Host plant	Year	Location	DON (μg/g)	ZEA (μg/g)
885*	Wheat	2005	Kovin	2.14	0.06
1222*	Wheat	2006	Kraljevo	0.19	0.17
Average				1.16	0.12
1281*	Maize	2006	B. Topola	0.13	0.06
1250*	Maize	2006	Loznica	0.49	0.19
918	Maize	2006	Kikinda	0.45	2.25
961*	Maize	2006	Novi Sad	0.51	0.05
1611	Maize	2007	Kovin	0.75	1.52
1656	Maize	2007	Kraljevo	0.51	13.29
Average				0.47	2.89
Total average				0.65	2.19

*Isolates marked with asterisk were better producers of DON than ZEA

F. graminearum isolates produced the widest range of concentrations of both toxins. *F. semitectum* isolates had a wider range of ZEA than DON production. The findings for *F. sporotrichioides* and *F. equiseti* isolates were identical (Tables 2 and 3).

Table 3. Deoxynivalenol and zearalenone concentrations produced by *Fusarium semitectum* and *Fusarium equiseti* isolates originating from wheat and maize grain from Serbia

MRIZP isolate	Host plant	Year	Location	DON (μg/g)	ZEA (μg/g)
			F. semitectum		
689	Wheat	2005	Sombor	0.20	19.87
1256*	Maize	2006	Kovin	0.39	0.07
1273	Maize	2006	Sr. Mitrovica	0.25	43.55
945*	Maize	2006	Novi Sad	0.14	0.11
1562	Maize	2007	Novi Sad	0.17	0.79
Average (maize)				0.24	11.13
			F. equiseti		
1428	Wheat	2006	B. Topola	0.30	0.52
1440*	Wheat	2006	Novi Sad	0.16	0.08
Average				0.23	0.29
Total average				0.23	12.88

*Isolates marked with asterisk were better producers of DON than ZEA

Considering the host plant origin of isolates, the isolates of *F. graminearum* originating from wheat produced higher average DON concentrations with a wider range than the isolates originating from maize (Figure 1). On the other hand, there was no significant difference between the two average concentrations and the range of ZEA concentrations produced by *F. graminearum* isolates originating from wheat and maize (Figure 1). The wheat-originating isolates produced almost equal average concentrations of ZEA as the isolates originating from maize within the same range of concentrations.

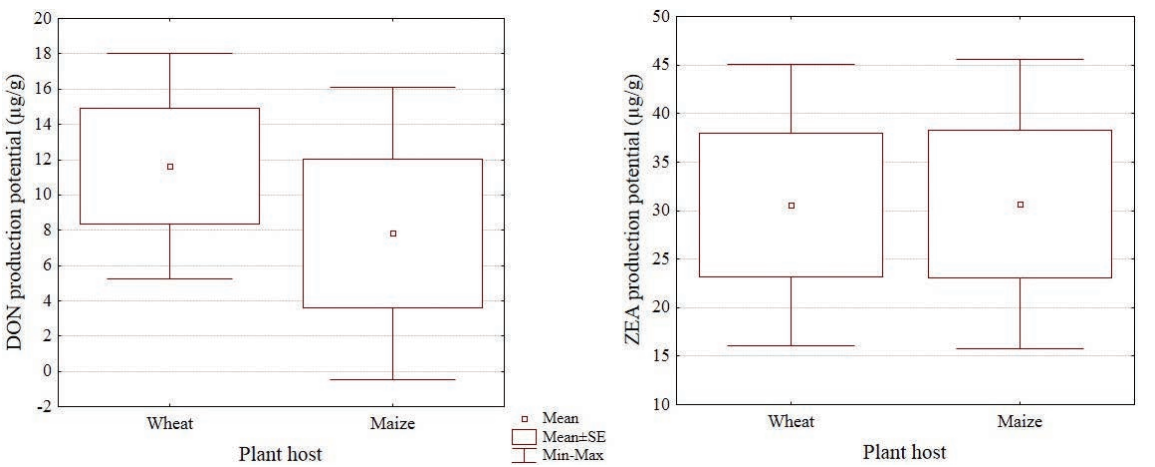

Figure 1. Deoxynivalenol and zearalenone production potential of *Fusarium graminearum* isolates considering their plant host origin

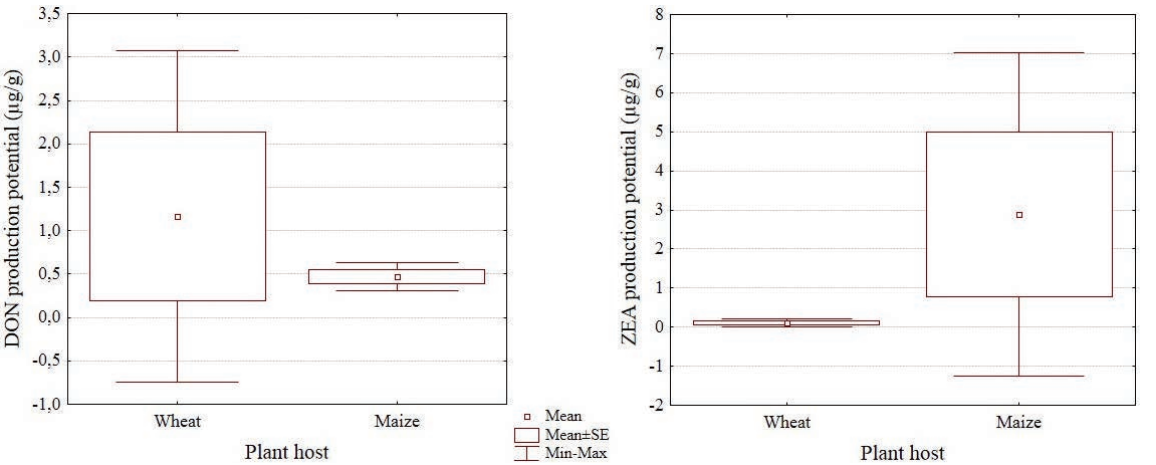

Figure 2. Deoxynivalenol and zearalenone production potential of *Fusarium sporotrichioides* isolates considering their plant host origin

Figure 2 shows the opposite situation with ZEA and DON production, i.e. wheat-originating *F. sporotrichioides* isolates were better producers of DON, while maize-originating isolates were better producers of ZEA (Figure 2).

F. semitectum and *F. equiseti* isolates were not included in our analyses of DON and ZEA production considering their host plant origin because only one isolate of the former species originated from wheat and none of the latter.

According to T-test results for dependent isolates, significant negative correlation between DON and ZEA production was registered in all 39 isolates ($r = -0.0061$; $p = 0.0008$). Significant negative correlation between DON and ZEA production was also detected in *F. graminearum* isolates originating from wheat ($r = -0.0064$; $p = 0.002$) and maize ($r = -0.0083$; $p = 0.002$).

DISCUSSION

Concentrations of DON produced under field conditions in Serbia have been reported by Jajić et al. (2008) to vary from 0.057 to 0.423 µg/g and from 0.027 to 2.210 µg/g in wheat and maize grain, respectively. Those are ten times lower DON concentrations than the average DON concentrations synthesized under laboratory conditions in our present study. Under the same laboratory conditions, Logrieco et al. (1990) detected 17.5 µg/g as the average DON concentration produced by *F. graminearum* isolates. Much higher DON concentrations, up to 465.9 µg/g and even up to 1302 µg/g, were reported by Jajić et al. (2007) and Harris et al. (1999), respectively. Considering this information, *F. graminearum* isolates in this research can be declared medium DON producers. The isolates of *F. sporotrichioides*, *F. semitectum* and *F. equiseti* tested in this research showed low potentials for DON production considering their average concentrations synthesized. Jajić et al. (2007) tested DON production potential of *F. sporotrichioides* isolates under laboratory conditions as well, but their isolates did not produce detectable concentrations after 35 days.

ZEA concentrations produced by the Serbian isolates of *F. graminearum* in this research were close to concentrations obtained by Logrieco et al. (1990), who also studied isolates originating from the former Yugoslavia. This may indicate a similarity in toxicological profiles of isolates originating from close geographical regions. Also, the variability of ZEA production found in this research is consistent with the ranges reported worldwide: 5-60 µg/g (Logrieco et al., 1990), 60-180 µg/g (Cvetnić et al., 2005), and even up to 1500 µg/g (Logrieco et al., 2003). Jajić et al. (2007) found *F. graminearum* isolates with a much lower potential for ZEA production (4.416 µg/g), which were similar to some isolates tested in this research. Considering the highest concentration of ZEA produced in this research, we inferred that the tested isolates displayed an average ZEA productivity, compared to worldwide reports. The isolates of *F. sporotrichioides*, *F. semitectum* and *F. equiseti* had much lower ZEA production potentials than *F. graminearum* isolates. So far, only a few studies have examined the ZEA production potentials of these species. Logrieco et al. (2003) and Jajić et al. (2007) observed no ZEA production by *F. sporotrichioides* isolates within

a range of detection (<0.037 μg/g), while the isolates tested in this research confirmed ZEA production capability but mainly low concentrations of below 2.5 μg/g. ZEA production by *F. semitectum* under field conditions has been reported in concentrations of 0.17 μg/g and 0.04-0.21 μg/g (Wilson et al., 2005; Furlong et al., 1995), which is close to the concentrations produced in our research but under optimal conditions in the laboratory. This leads to a conclusion that *F. semitectum* isolates tested in this research had a low potential to synthesize ZEA. Low concentrations of ZEA produced by *F. equiseti* had been earlier reported by Logrieco et al. (1990; 2003) but concentrations of ZEA produced by the two tested *F. equiseti* isolates in our research were much lower.

Perkowski et al. (1995) analysed infected rye kernels by HPTLC and liquid chromatography for DON and ZEA, respectively, and found no significant correlation between these two toxins (r = 0.230). The situation was reverse in our study, in which a negative correlation between DON and ZEA production potentials (r = -0.0061) was detected. Stanković et al. (2012) reported moderate positive correlations between the occurrence of fumonisin B_1 with DON (r = 0.56) and ZEA (r = 0.48) in wheat grain samples.

In conclusion, the 39 tested isolates of four *Fusarium* species – *F. graminearum*, *F. sporotrichioides*, *F. semitectum* and *F. equiseti* – were able to produce both ZEA and DON. *F. graminearum* isolates were the best producers of both ZEA and DON and had the highest intraspecies variability for both toxins production, while *F. equiseti* isolates were the weakest producers of these two toxins with the lowest intraspecies variability. A negative correlation between DON and ZEA production was confirmed. Considering the isolate plant origin, wheat-originating *F. sporotrichioides* isolates had higher DON than ZEA production (regarding their average concentrations and range of production), while its maize-originating isolates had higher ZEA production. Considering *F. graminearum* isolates, there was no clear distinction in ZEA production between wheat- and maize-originating isolates, while higher average DON concentrations were produced by wheat-originating isolates.

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Korelacija sinteze deoksinivalenola i zearalenola od strane *Fusarium* izolata poreklom sa zrna pšenice i kukuruza

REZIME

Potencijal za stvaranje deoksinivalenola (DON) i zearalenona (ZEA) ukupno 39 izolata vrsta *Fusarium graminearum*, *F. sporotrichioides*, *F. semitectum* i *F. Equiseti*, poreklom iz zrna pšenice i kukuruza sa 10 različitih lokaliteta u Srbiji, analiziran je u optimalnim laboratorijskim uslovima ELISA testom. Izolati vrste *F. graminearum* su imali najvišu intraspecijsku varijabilnost i ujedno su bili najveći proizvođači i deoksinivalenola i zearalenona. Nasuprot njima, izolati *F. equiseti* su bili najslabiji proizvođači ova dva mikotoksina. S obzirom na poreklo izolata, *F. sporotrichioides* izolati poreklom sa pšenice su bili bolji proizvođači deoksinivalenola, dok su izolati ove vrste poreklom sa kukuruza stvarali više koncentracije zearalenona. Među *F. graminearum* izolatima nije bilo jasne razlike u sintetisanim koncentracijama ZEA zavisno od porekla izolata, dok je pri sintezi DON-a uočena viša prosečna koncentracija sintetisana od strane *F. graminearum* izolata poreklom sa pšenice.

Negativna korelacija je registrovana između sinteze deoksinivalenola i zearalenona od strane *Fusarium* spp.

Ključne reči: Mikotoksini; *Fusarium*; Pšenica; Kukuruz