

*Aleksandra S. Bočarov-Stančić¹, Jelena T. Lević²,
Slavica Ž. Stanković², Vesna S. Krnjaja³,
Tamara M. Kovačević², Sonja L. Tančić²*

¹ Hold. Comp. “Center for Bio-Ecology”, Petra Drapšina 15,
23000 Zrenjanin, Serbia, microb.bec@beotel.net

² Maize Research Institute, Zemun Polje, Slobodana Bajića, 11185 Belgrade, Serbia

³ Institute for Animal Husbandry, Autoput 16, 11081 Belgrade, Serbia

THE TOXIGENIC POTENTIAL OF *FUSARIUM POAE* ORIGINATED FROM WHEAT

ABSTRACT: Eleven isolates of *F. poae*, originated from wheat grain at 9 locations mainly in Vojvodina, were encompassed by the present study. The greatest number of samples was collected in 2005, in which the climatic conditions favoured a more intensive occurrence of *Fusarium* ear blight of wheat. In order to determine toxicological potential of this species, cultures of the selected isolates were grown in liquid media (GPY and SPY) on a rotary shaker (180 revolutions min⁻¹), at room temperature (21—26°C) for three days. Crude toxins were isolated from liquid culture filtrates of isolates by the use of ethyl acetate, while quantification of mycotoxins was done by the thin layer chromatography method. A liquid culture of the isolate GZ-LES (*F. graminearum*) was used as a control for the evaluation of the zearalenone biosynthesis potential. On the other hand, the liquid culture of the isolate KF-38/1 (*F. sporotrichioides*) was used as a control for both type-A trichothecenes (T-2 toxin and diacetoxyscirpenol — DAS).

The obtained results show that *F. poae*, in contrast to *F. graminearum*, has no potential for the zearalenone biosynthesis. The presence of DAS was determined only in one isolate of *F. poae* (MRIZP-666), and in the control isolate of *F. sporotrichioides* (KF-38/1/R), that were grown in the GPY liquid medium. The T-2 toxin was detected in the isolate MRIZP-666, grown in both media, and in the isolates MRIZP-37 and MRIZP-860, cultured in the GPY and SPY liquid medium, respectively. The control culture KF-38/1/R (*F. sporotrichioides*) produced the T-2 toxin at the concentration of 4,000 µg L⁻¹. According to the gained information, it can be concluded that the potential of *F. poae* for the type-A trichothecene biosynthesis was low, as the concentration of DAS or T-2 toxin did not exceed 80 µg L⁻¹ or 240 µg L⁻¹, respectively.

KEY WORDS: diacetoxyscirpenol, *F. poae*, *in vitro* biosynthesis, T-2 toxin, wheat

INTRODUCTION

The occurrence of *Fusarium* head blight of stronger intensity in wheat was recorded not only in Europe, including Serbia (Lević et al., 2004), but worldwide during the last decade of the 20th century. The disease resulted in a

significant economic damage, due to the grain yield reduction, in a quality loss, due to shrunk grain, and in contamination with mycotoxins.

An enormous number of species of the genus *Fusarium*, including *F. poae* (Peck) Wollenw., was isolated from *Fusarium* damaged wheat grain (Waalwijk, 2002). Although a high percentage of *Fusarium poae* was isolated from the grain of wheat, barley and oats in certain years, its role in the aetiology of *Fusarium* head blight had not been yet completely clarified (Kestemont et al., 2002; Kryuchkova et al., 2002; Lew et al., 2001; Hornok and Toth, 2001; Hysek et al., 2000).

It is most often stated that only *F. graminearum* Schwabe and *F. culmorum* (W. G. Smith) Sacc. are important for the aetiology of *Fusarium* head blight (Teich, 1989). Parry et al. (1995) and Waalwijk (2002) have an opinion that *Fusarium* head blight could be caused by four species — two previously stated, *F. avenaceum* (Fr.) Sacc. and *F. poae*. Furthermore, in England, *F. poae* has been very often isolated from chaff spots, although the connection between its occurrence and head blight has not been confirmed (Nicholson et al., 1997).

The species *F. poae* is important from the toxicological aspect as it biosynthesises a great number of mycotoxins, such as: diacetoxyscirpenol (DAS), monoacetoxyscirpenol, nivalenol, fusarenone-X, T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, deoxynivalenol, neosolaniol, beauvericin and enniatines (BEA) (Bottalico, 1998; Torp and Langseth, 1999; Nicholson et al., 2004; Chełkowski et al., 2007). Out of the stated mycotoxins, type-A trichothecenes (T-2 toxin and DAS), produced by these species, are the most important.

In Italy, it was determined that two main *Fusarium* species isolated from wheat, *F. poae* and *F. sporotrichioides* Sherb., biosynthesised the T-2 toxin and DAS in the amount that ranged from 6,200 to 120,000 ppb (Criseo et al., 1989). According to the results obtained by Gagkaeva et al. (2006), *F. poae* synthesises more DAS than *F. sporotrichioides*, 400 ng mL⁻¹ vs. 62 ng mL⁻¹, while *F. sporotrichioides* produces more T-2 toxins (75,860 ng mL⁻¹) than *F. poae* does (158 ng mL⁻¹). Besides the type-A trichothecenes, it was determined that the species *F. poae*, originated from *Fusarium* damaged wheat grain in Canada, produced the type-B trichothecenes (Wong et al., 1995). In Poland, *F. poae* was determined in 35% of wheat samples, while chemical analyses showed no presence of the type-A trichothecene (T-2 toxins) but just the presence of the type-B trichothecenes (Grabarkiewicz-Szczena et al., 2001). Contrary to these authors, Kosiak et al. (2003) found out that the same species in Norway was the best producer of the type-A trichothecenes (T-2 and HT-2 toxins). A somewhat lower frequency of *F. poae* on wheat grain (13.5%) was recorded by Muthomi et al. (2006) in Kenya. These authors determined only the presence of T-2 toxin and zearalenone in the same substrate.

According to the mycological studies carried out in Serbia by Bočarov-Stančić et al. (1991), only the type-A trichothecene (T-2 toxin), in the amount of 310—780 ppb, was present in wheat grain, originated from a macro-trail in Klek (Vojvodina). Škrinjar et al. (1996) detected zearaleno-

ne just in one sample of wheat, harvested during 1994. In both cases, not a single determined *Fusarium* spp. belonged to *F. poae*. Dopuđa and Lević (2004) and Stojanović et al. (2005) determined the presence of *F. poae* in 12% and 7% of the samples, respectively, but the authors did not present data on their toxigenicity. The results of Dopuđa and Lević (2004), obtained on the basis of studies performed on the samples of five wheat cultivars harvested during 2002 and 2003 at four locations, show that *F. poae* occurred more intensively in the year (2003) in which the frequency of *F. graminearum* was lower, and vice versa. Similar results were achieved by Schafsm a (1999) who stated that the presence of *F. poae*, *F. sporotrichioides* and *F. avenaceum* on wheat grain in Canada was reduced during the years with more intensive occurrence of *F. graminearum*.

Our previous studies performed on *Fusarium* isolates originated from wheat (harvested during 1982 and 1984), cultured on the natural solid substrate, showed that 22% of the observed *Fusarium* spp. biosynthesised DAS, and even 44% produced T-2 toxin, although not a single one belonged to the species *F. poae* (Bočarov-Stančić et al., 1986).

The potential of *F. poae* to biosynthesise one group of fusariotoxins — the type-A trichothecenes (T-2 toxin and DAS), and zearalenone (ZEA) — was observed under *in vitro* conditions in the present study. In our country according to the literature data, a little attention was paid, to the study of the toxicological profile of this species, considering its distribution and toxicogenic properties.

MATERIAL AND METHODS

Cultures of *Fusarium poae*. Isolates of the fungus *F. poae* were obtained from grain samples of wheat, collected in harvest at 20 locations in 2003, 2005 and 2006. A total of 103 isolates of this species were isolated and determined. Out of these 103, 11 isolates, designated as MRIZP-32, MRIZP-37, MRIZP-664, MRIZP-665, MRIZP-666, MRIZP-833, MRIZP-834, MRIZP-860, MRIZP-879, MRIZP-890 and MRIZP-897, were selected for toxicological studies.

Each sample of 32 wheat kernels (four replicates) was analysed. Eight surface sterilised kernels were placed on each water agar (WA) in the 10-cm Petri dishes, and incubated under indoor conditions for seven days. Resulting colonies were purified by the procedure of obtaining monosporous cultures that were then used for the identification of *Fusarium* species. Monosporous cultures were subcultured on the potato dextrose agar (PDA), carnation sterilised leaf-fragment agar (CLA), and synthetic nutrient agar (SNA). Cultures on the PDA were incubated in the dark at $25 \pm 1^\circ\text{C}$, while cultures on the CLA and SNA were incubated under fluorescent and near ultraviolet light for 12 hours at $25 \pm 1^\circ\text{C}$, and in the dark for 12 hours at $20 \pm 1^\circ\text{C}$. The identification of the obtained species was done after Nelson et al. (1983) and Burgess et al. (1994). The identified isolates were stored on the PDA, CLA and

SNA slants in ampoules at +4°C, until studying of their toxicological potential in the liquid culture.

Control isolates. The following species for which it was previously determined (Bočarov - Stančić, unpublished data) to have the capacity to biosynthesise fusariotoxins five weeks after the cultivation on wet maize grain at 30°C, were chosen as the control isolates: a) *F. graminearum*, the isolate GZ-LES that synthesises ZEA and DON at the concentration of 4,420 ppb and 465,900 ppb, respectively; b) *F. sporotrichioides*, KF-38/1/R, a re-isolate of the original strain that biosynthesises T-2 toxin and DAS at the concentration of 2,400 ppb and 1,600 ppb, respectively.

Medium and conditions for the toxin production. All isolates of *F. poae* and both control isolates were grown in the glucose-peptone-yeast extract (GPY) liquid medium. Also, three isolates of *F. poae* and the control isolates were grown in sucrose-peptone-yeast extract (SPY) liquid medium. The GPY liquid medium (pH 5.8) contains 5% of glucose, 0.1% of peptone and 0.1% of yeast extract. The SPY liquid medium (pH 6.5) contains 5% of sucrose, 0.1% of peptone and 0.1% of yeast extract.

Media (100 ml each) were poured into 500-ml Erlenmeyer flasks, and cultured with five fragments (5 x 5 mm) of the fungus that were grown on potato dextrose agar (PDA) in the Petri dishes at 27°C, for seven days. In order to obtain submersed cultures, after inoculation of the medium the Erlenmeyer flasks were kept on the rotary shaker (180 rounds min⁻¹) at room temperature (21–26°C) for three days. The pH value was measured after the incubation of the isolate.

Determination of fusariotoxins. After the cultivation on the rotary shaker, liquid cultures were filtered. Qualitative and quantitative ZEA determinations in filtrates of mould cultures were carried out by applying the multitoxin thin layer chromatographic method, developed by Cvetnić et al. (2005). Crude extracts of the type-A trichothecenes (DAS and T-2 toxin) were obtained by the use of ethyl acetate. Each liquid culture (25 ml) was extracted twice with 15 ml of ethyl acetate. Organic extracts were recovered by filtration through the layer of anhydrous sodium sulphate, combined and evaporated almost to dryness, under the rotary evaporator. Further purification was done by the method of Romer et al. (1978). The crude oily extract of trichothecenes was dissolved in the methanol/water (1:1, v/v) extraction solvent, which tends to extract compounds of the polarity of trichothecenes, while it does not extract low polar compounds, such as fats and oils. Afterwards, 30% of aqueous ammonium sulphate was added to remove additional interferences. The further step was selective concentration of the analytes into chloroform, and removal of acidic interferences from chloroform extracts, by washing it with the aqueous potassium hydroxide solution. Thinlayer chromatography was performed according to Pepeļnjak and Babić (1991) with toluole/ethyl acetate/formic acid developing solvent (5:4:1, v/v/v). All analyses were done in three repetitions.

RESULTS AND DISCUSSION

After the incubation period, with the exception of one *F. poae* isolate (MRIZP-890), the decreased pH value was determined in both liquid media, especially in the control isolates (Tables 1 and 2).

The results obtained in mycotoxicological studies show that *F. poae*, in contrast to *F. graminearum* (isolate GZ-LES), did not have the potential for the zearalenone biosynthesis (Tables 1 and 2). These results are in accordance with literature data (Marašas et al., 1984). Generally, there is a very small number of authors stating that *F. poae* biosynthesises zearalenone, among others Kocić-Tanackov (2004). By re-testing numerous toxicogenic isolates of the *Fusarium* species, Marašas et al. (1984) determined that some results obtained on the production of mycotoxins were incorrect as the identification of fungi was not correct.

Tab. 1 — Yields ($\mu\text{g L}^{-1}$) of zearalenone and type-A trichothecene (DAS, T-2 toxin) in GPY liquid cultures of 11 *F. poae* isolates originated from wheat and control isolates of *F. graminearum* (No. 12) and *F. sporotrichioides* (No. 13)

No.	Isolate designation	Origin	pH	Fusariotoxin yields ($\mu\text{g L}^{-1}$)		
				ZEA	DAS	T-2
1.	MRIZP-32	Indija	5.30	n.d.*	n.d.**	n.d.**
2.	MRIZP-37	Erdevik	5.50	n.d.*	n.d.**	240
3.	MRIZP-664	Zemun Polje	5.23	n.d.*	n.d.**	n.d.**
4.	MRIZP-665	Zemun Polje	5.40	n.d.*	n.d.**	n.d.**
5.	MRIZP-666	Zemun Polje	5.41	n.d.*	80	n.d.**
6.	MRIZP-833	Lipnički Šor	5.04	n.d.*	n.d.**	n.d.**
7.	MRIZP-834	Stari Banovci	5.63	n.d.*	n.d.**	n.d.**
8.	MRIZP-860	Sombor	5.48	n.d.*	n.d.**	80
9.	MRIZP-879	Loznica	5.52	n.d.*	n.d.**	n.d.**
10.	MRIZP-890	Pazova	5.87	n.d.*	n.d.**	n.d.**
11.	MRIZP-897	Pirot	5.71	n.d.*	n.d.**	n.d.**
12.	GZ-LES	Leskovac	4.40	37	n.d.**	n.d.**
13.	KF-38/1/R ^a	Poland	4.20	n.d.**	240	4,000

^a — from barley grains; n.d.* — not detected (F^{-1}); n.d.** — not detected (F^{-1})

Tab. 2 — Yields ($\mu\text{g L}^{-1}$) of zearalenone and type-A trichothecene (DAS, T-2 toxin) in SPY liquid cultures of three *F. poae* isolates (No. 1—3) originated from wheat and control isolates of *F. graminearum* (No. 4) and *F. sporotrichioides* (No. 5)

No.	Isolate designation	Origin	pH	Fusariotoxin yields (mg L^{-1})		
				ZEA	DAS	T-2
1.	MRIZP-666	Zemun Polje	6.09	n.d.*	n.d.**	80
2.	MRIZP-860	Sombor	5.88	n.d.*	n.d.**	80
3.	MRIZP-897	Pirot	5.84	n.d.*	n.d.**	n.d.**
4.	GZ-LES	Leskovac	4.73	n.d.*	n.d.**	n.d.**
5.	KF-38/1/R ^a	Poland	4.69	n.d.*	n.d.**	160

^a — from barley seeds; n.d.* — not detected (F^{-1}); n.d.** — not detected (F^{-1})

The presence of DAS was recorded in the isolate MRIZP-666 (*F. poae*) and the control isolate KF-38/1/R (*F. sporotrichioides*) in the glucose-peptone-yeast (GPY) liquid medium at the concentration of 80 $\mu\text{g L}^{-1}$ and 240 $\mu\text{g L}^{-1}$, respectively. In both cases, the DAS yield was low, close to the limit of detection (LOD) of the applied method. Marasas et al. (1984) concluded that some, but not all isolates in *F. poae*, were able to produce DAS, and the ability could be lost rapidly in culture.

The T-2 toxin was detected in the following three *F. poae* isolates: MRIZP-860 (in both liquid cultures — 80 $\mu\text{g L}^{-1}$), and MRZIP-37 (240 $\mu\text{g L}^{-1}$) and MRIZP-666 (80 $\mu\text{g L}^{-1}$) in the GPY and SPY liquid culture, respectively (Table 1). The control culture KF-38/1/R (*F. sporotrichioides*) in the glucose-peptone-yeast extract (GPY) liquid medium, produced this mycotoxin at the concentration of 4,000 $\mu\text{g L}^{-1}$. However, these values were significantly lower than those recorded with the original strain of this species, in which the production of the T-2 toxin at the concentration of 150,000 $\mu\text{g L}^{-1}$ had been recorded (Mašić et al., 1997). The obtained results can be explained by the fact that a long-term passaging of microorganism isolates, even fungi isolates, on the artificial media leads to a gradual loss of their biochemical properties. Although *F. sporotrichioides* KF-38/1/R were reisolated (KF-38/1R) from sterile, wet maize grain, it is obvious that their initial potential for production was not completely recovered. On the other hand, a low T-2 toxin yield in the *F. poae* isolate (Table 1 and 2) can not be interpreted in such a way, considering that the majority of the isolates were from 2005, hence they were subcultivated under laboratory conditions only for a short period of time. Thus, these isolates of *F. poae* can be considered as non-toxic.

The gained results show that under such conditions of cultivation in the liquid media, the isolates of *F. poae* from wheat originated in Serbia express low potential for biosynthesis of the type-A trichothecenes. Similar results were obtained with isolates of other potentially toxigenic *Fusarium* spp. (*F. oxysporum* and *F. proliferatum*) also determined on wheat. A fairly weak potential for the production of fusariotoxins was evaluated when the cultivation was performed in the liquid medium: 250—320 $\mu\text{g L}^{-1}$ ZEA, i.e. 320 $\mu\text{g L}^{-1}$ DAS and 160 $\mu\text{g L}^{-1}$ T-2 toxin (Bočarov-Stanić et al., 2003). According to our previous studies, greater amounts of the T-2 toxin in *Fusarium* spp. cultures originated from wheat grain from Serbia were recorded only in *F. sporotrichioides* and *F. culmorum* (Bočarov-Stanić, 1996).

Unlike low toxigenic potential of the isolates of *Fusarium* spp., originated from wheat from Serbia, the information gained in the countries of Northern Europe show that yields, especially those of T-2 toxin, were significantly higher at the cultivation of *Fusarium* spp. under laboratory conditions. In Norway, Torp and Langseth (1999) determined the biosynthesis of the T-2 toxin in all tested isolates at the concentration of 25,000—400,000 $\mu\text{g L}^{-1}$. A *Fusarium* species resembling *F. poae* (= *F. langsethiae* Torp and Nirenberg) was cultured on the PDA, or in the liquid medium with yeast extract and sucrose. Kroiakova et al. (1989) obtained T-2 toxin yields ranging from 50 to 600,000 ppb, when three isolates of *F. sporotrichioides* v. *poae*, originated from wheat grain, harvested in Moscow region, were *in vitro* cultivated. Hor-

nok and Toth (2001) state that the application of the thinlayer chromatography assay revealed no trichothecene producing strain among *F. poae* isolates originated from Hungary.

Considering the presented results, in order to obtain the final answer to the question on the toxicological profile of the *F. poae* isolates in Serbia, it is necessary to carry out additional studies, not only with new isolates of the coming years, but also under different cultivation conditions, first of all on the sterile natural substrates, such as wheat and maize. Marasas et al. (1984) brought forward examples in which differences in the trichothecene production occurred due to conditions and substrates of the *F. poae* cultivation.

CONCLUSIONS

According to the presented results, it can be concluded that *F. poae* isolates from wheat, in contrast to *F. graminearum*, have no potential for the zearalenone biosynthesis.

In the case of the type-A trichothecenes, the diacetoxyscirpenol, i.e. T-2 toxin production was detected only in one culture of *F. poae*, i.e. 15.38% of the studied isolates, respectively. The potential of *F. poae* from wheat, for the type-A trichothecene production was low, as the concentration of DAS and T-2 toxin did not exceed $80 \mu\text{g L}^{-1}$ and $240 \mu\text{g L}^{-1}$, respectively.

An answer to the question on the toxicological profile of the *F. poae* cannot be made, unless other cultivation conditions are not observed, and unless a greater number of isolates of this species, originated from different harvest years of wheat and other cereals that are hosts of this species, are not studied.

ACKNOWLEDGEMENTS

This paper is a part of the investigations realised within the scope of the project No. TR-6826B, financially supported by the Ministry of Science and Environmental Protection of Serbia.

REFERENCES

- Bočarov-Stančić, A. (1996): *Učestalost Fusarium spp. i njihovih mikotoksina u pšeničnom zrnu*, Monografija „Proizvodnja i prerada žita i brašna, domaći potencijali — svetski kvalitet“, Tehnološki fakultet, Zavod za tehnologiju žita i brašna, Novi Sad, 131—140.
- Bočarov-Stančić, A., Eremić, S., Vukoje, M. (1991): *Ispitivanje mikološke i mikotoksikološke kontaminacije nekoliko sorti pšenice iz makroogleda u Kleku*, Savetovanje „Žito-hleb“, Tehnološki fakultet, Zavod za tehnologiju žita i brašna, Novi Sad, (Apstr.).
- Bočarov-Stančić, A., Laco, D., Tomašević-Čanović, M., Adamović, M., Daković, A. (2003): *Toksigenost izolata Fusarium spp. sa pšenice*

- kontaminirane zearalenonom*, X Simpozijum „Tehnologija stočne hrane”, 19—23. 10. 2003, V. Banja, ZB. radova: 299—305.
- Bočarov-Stančić, A., Muntañola-Cvetković, M., Oberan, Lj. (1986): *Proizvodnja DAS i T-2 toksina kod izolata roda Fusarium sa pšenice*, Poseb. izd. ANBIH, LXXX, Odelj. med. nauka 12: 147—160.
- Bottalico, A. (1998): *Fusarium diseases of cereals: Species complex and related mycotoxin profiles, in Europe*, J. Plant Pathol. 80: 85—103.
- Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P., Backhouse, D. (1994): *Laboratory for Fusarium Research*, Third Edition, Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic.
- Chełkowski, J., Ritieni, A., Wiśniewska, H., Mulè, G., Logrieco, A. (2007): *Occurrence of toxic hexadepsipeptides in preharvested maize ear rot infected by Fusarium poae in Poland*, J. Phytopathol. 155 (1): 8.
- Criseo, G., Guerrisi, R., Medici, M. A., Pernice, I., Urzi, C. (1989): *12,13-epoxy-trichothecenes and zearalenone production by Fusarium isolates from Sicilian cereals*, Microbiologie-Aliments-Nutrition 7: 157—160.
- Cvetnic, Z., Pepeljnjak, S., Sevgic, M. (2005): *Toxicogenic potential of Fusarium species isolated from non-harvested maize*, Arh Hig Rada Toksikol. 56 (3): 275—280.
- Dopuđa, M., Lević, J. (2004): *Sastav mikrobiote (Fusaria) semena pšenice na području Srema*, ZB. str. 112, 5. Kong. z. bilja, Zlatibor, 22—26. novembra 2004. Gardnes, Sydney, 133.
- Gagkaeva, T., Gavrilova, O., Levotin, M., Kononenko, G., Burkin, A. (2006): *Characterization of distribution, cultural characters and T-2 toxin production of F. sporotrichioides, F. poae and F. langsethiae from Russia*, pp. 49, Book of Abstracts, European Fusarium Seminar, Wageningen, The Netherlands, 19—22 September, 2006.
- Grabarkiewicz-Szczesna, J., Kosteckki, M., Golinski, P., Kiecana, I. (2001): *Fusariotoxins in kernels of winter wheat cultivars field samples collected during 1993 in Poland*, Nahrung 45 (1): 28—30.
- Hornok, L., Toth, A. (2001): *Reports on natural occurrence of toxigenic fungi and mycotoxins in Hungary between 1990—1999*, pp. 68, in: Logrieco, A. (ed.), *Occurrence of toxigenic fungi and mycotoxins in plants, food and feed in Europe*, Agriculture and Biotechnology, COST Action 835, EUR 19695, European Commission.
- Hysek, J., Vanova, M., Sychrova, E., Koutecka-Brozova, J., Radová, Z., Hajšlová, J. (2000): *Fusarioses of barley — the spectrum of species and the levels of mycotoxins (trichothecenes)*, pp. 29, in: 6th European Fusarium Seminar and Third COST 835 Workshop of Agriculturally Important Toxigenic Fungi, Berlin (Germany), September 11—16, 2000.
- Kestemont, M. H., Donis, T., Chandelier, A., Cavelier, M. (2002): *Occurrence of head blight agents and deoxynivalenol in Belgian wheat*, pp 98, in: Abstracts, VIIth European Seminar “Fusarium — Mycotoxins, Taxonomy and Pathogenicity” and WG-4 COST 835 Action Workshop (Mycotoxins in Cereals), Poznan, Poland, September 4—7, 2002.

- Kocić-Tanackov, S. (2004): *Rast toksigenih Fusarium vrsta i sinteza zearalena u ječmu namenjenom proizvodnji pivarskog slada*, magistrarska teza, Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, 97.
- Kosiak, B., Torp, M., Skjerve, E., Thrane, U. (2003): *The prevalence and distribution of Fusarium species in Norwegian cereals*, Acta Agriculturae Scandinavica, Section B — Plant Soil Sci. 53 (4): 168—176.
- Kroiakova, E. A., Yang, S. F., Boltianskaia, E. V. (1989): *Detection of toxigenic Fusarium strains, producing T-2 toxin, in wheat grain mycoflora by microbiologic assay*, Vopr Pitan. Mar-Apr (2): 54—57 (in Russian).
- Kryuchkova, L., Dragovoz, I., Yavorska, V., Raichuk, L. (2002): *Fusarium species in wheat grains in the Ukraine*, J. Appl. Genet. 43A: 177—184.
- Lević, J., Stanković, S., Bočarov-Stančić, A., Škrinjar, M., Mašić, Z. (2004): *The overview of toxigenic fungi and mycotoxins in Serbia and Montenegro*, pp. 201—218, in: Logrieco, A., Visconti, A. (eds), *An overview of toxigenic fungi and mycotoxins in Europe*, Kluwer Academic Publishers, Dordrecht, Boston, London.
- Lew, H., Adler, A., Thimm, N., Krska, R., Wiedner, G., Schuh, M. (2001): *Occurrence of toxic fungi and related mycotoxins in plants, food and feed in Austria*, pp. 3—12, in: Logrieco, A. (ed.), *Occurrence of Toxigenic Fungi and Mycotoxins in Plants, Food and Feed in Europe*, Agriculture and Biotechnology, COST Action 835, EUR 19695, European Commission.
- Marasas, W. F. O., Nelson, P. E., Toussoun, T. A. (1984): *Toxigenic Fusarium species. Identity and mycotoxicology*, The Pennsylvania State University Press, University Park and London.
- Mašić, Z., Bočarov-Stančić, A., Pavkov, S., Zurovac-Kuzman, O. (1997): *Gasnohromatografsko određivanje trihotecenskih mikotoksina tipa A u ekstraktima gljivičnih kultura*, Acta Vet. 47 (1): 23—32.
- Muthomi, J. W., Hindorf, H., Ndung'u, J. K., Gathumbi, J. K. (2006): *Occurrence of Fusarium head blight-causing pathogens and mycotoxins in Kenyan wheat*. Available: www.tropentag.de/2006/abstracts/full/608.
- Nelson, P. E., Toussoun, T. A., Marasas, W. F. O. (1983): *Fusarium species. An Illustrated Manual for Identification*, The Pennsylvania State University Press, University Park and London, pp. 193.
- Nicholson, P., Doohan, F., Joyce, D., Rezanoor, H. N., Simpson, D., Smith, P. H., Turner, A., Weston, G. (1997): *Detection and quantification of individual fungal species in Fusarium ear blight by PCR*, pp. 40—46, in: Dubin, H.J., L. Gilchrist, J. Reeves, A. McNab (ed.), *Fusarium Head Scab: Global Status and Future Prospects*. Mexico, D.F.: CIMMYT.
- Nicholson, P., Simpson, D., Wilson, A. H., Chandler, E., Thomsett, M. (2004): *Detection and differentiation of trichothecene and enniatin-producing Fusarium species on small-grain cereals*, Eur. J. Plant Pathol. 110: 503—514.
- Parry, D. W., Jenkinson, P., McLeod, L. (1995): *Fusarium ear blight (scab) in small grain cereals — a review*, Plant Pathol. 44: 207—238.
- Pepeljnjak, S., Babić, A. (1991): *Detekcija trihotecenskih mikotoksina T-2, HT-2, DON i DAS tankoslojnom hromatografijom i biološkim metodama*, Prehrambeno-tehnol. Biotechnol. Rev. 29: 65—70.

- Romer, T. R., Boling, T. M., MacDonald, J. L. (1978): *Gas-liquid chromatographic determination of T-2 toxin and diacetoxyscirpenol in corn and mixed feeds*, J. AOAC 61, 801—808.
- Schaafsma, A. W. (1999): *1996 Epidemic in winter wheat-aftermath*, pp. 31—33, Canadian Workshop on Fusarium Head Blight, Winnipeg, Manitoba, November 28—30, 1999.
- Stojanović, T. V., Škrinjar, M. M., Psodorov, Đ. B. (2005): *Fusarioses of certain wheat grain categories and their mycotoxicological infection*, Proc. Nat. Sci, Matica Srpska, Novi Sad 108: 43—50.
- Škrinjar, M., Dimić, G., Matković, K. (1996): *Prisustvo gljiva i nekih mikotoksina u pšenici*, Monografija „Proizvodnja i prerada žita i brašna, domaći potencijali — svetski kvalitet”, Tehnološki fakultet, Zavod za tehnologiju žita i brašna, Novi Sad, 121—130.
- Teich, A. H. (1989): *Epidemiology of wheat (Triticum aestivum L.) scab caused by Fusarium spp.*, pp. 269—282, in: Chefkowski, J. (ed.), *Fusarium Mycotoxins, Taxonomy and Pathogenicity*, Elsevier, Amsterdam—Oxford—New York—Tokyo, 492.
- Torp, M., Langseth, W. (1999): *Production of T-2 toxin by a Fusarium resembling Fusarium poae*, Mycopathol. 147 (2): 89—96.
- Waalwijk, C. (2002): *Fusarium species on wheat in the Netherlands: inventory and molecular identification*, J. Appl. Genet. 43A: 125—130.
- Wong, L. S. L., Abramson, D., Tekauz, A., Leslie, D., McKenzie, R. H. I. (1995): *Pathogenicity and mycotoxin production of Fusarium species causing head blight of wheat cultivars varying in resistance*, Can. J. Pl. Sci., 75: 262—267.

ТОКСИГЕНИ ПОТЕНЦИЈАЛ ИЗОЛАТА *FUSARIUM POAE* ПОРЕКЛОМ СА ПШЕНИЦЕ

Александра С. Бочаров-Станчић¹, Јелена Т. Левић,² Славица Ж. Станковић²,
Весна С. Крњаја³, Тамара М. Ковачевић², Соња Ј. Танчић²

¹ А.Д. „Био-еколошки центар”, Петра Драпшина 15, 23000 Зрењанин, Србија

² Институт за кукуруз „Земун поље”, Слободана Бајића 1, Београд, Србија

³ Институт за сточарство, Аутопут 16, 11081 Београд, Србија

Резиме

У овом раду је у *in vitro* условима проучена способност изолата *F. poae* за биосинтезу једне групе фузариотоксина — трихотечена типа А (Т-2 токсин и ди-ацетоксисцирпенол-ДАС), као и зearаленона (ЗЕА). Токсиколошки профил ове врсте је код нас недовољно испитан с обзиром на њену заступљеност и токсигена својства према литературним подацима.

Проучавањима је било обухваћено 11 изолата *F. poae*, пореклом са пшенице из 9 локалитета, углавном са подручја Војводине. Највећи број узорак прикупљен је 2005. године, када су климатски услови погодовали интензивнијој појави фузариоза класа ове пољопривредне културе. За одређивање токсиколошког потенцијала *F. poae*, културе одабраних изолата су гајене у течним подлогама (ГПК и СПК) током 3 дана на собној температури (21—26°C) и на ротационој треси-

лици (180 обртаја мин⁻¹). Сирови токсини су изоловани из филтрата течних култура испитаних изолата помоћу етил ацетата, док је квантификација микотоксина извршена методом танкослојне хроматографије. Течна култура изолата ГЗ-ЛЕС (*F. graminearum*) је коришћена као контролна култура за утврђивање потенцијала за биосинтезу зеараленона, а КФ-38/1/Р (*F. sporotrichioides*) за оба трихотечена типа А (Т-2 токсин и ДАС).

Добијени резултати показују да *F. poae*, за разлику од *F. graminearum*, не поседује потенцијал за биосинтезу зеараленона. Присуство ДАС-а је утврђено само код једне културе *F. poae* (МРИЗП-666) и контролног изолата *F. sporotrichioides* (КФ-38/1/Р) који су гајени у течном ГПК медијуму. Т-2 токсин је детектован код изолата МРИЗП-666 при гајењу у обема подлогама, као и изолата МРИЗП-37 у ГПК, односно МРИЗП-860 у СПК медијуму. Контролна култура КФ-38/1/Р (*F. sporotrichioides*) произвођила је Т-2 токсин у концентрацији од 4000 µg L⁻¹.

На основу изнетих података може се закључити да је потенцијал *F. poae* за биосинтезу трихотечена типа А био низак у датим условима с обзиром да концентрација ДАС-а није прелазила 80 µg L⁻¹, односно Т-2 токсина 240 µg L⁻¹.

Имајући у виду приказане резултате, сматрамо да је за добијање коначног одговора на питање о токсиколошком профилу изолата *F. poae* у Србији неопходно предузети додатна испитивања, не само са новим изолатима из година које следе, него и у другим условима култивисања, у првом реду на стерилном природном супстрату као што су пшеница и кукуруз.