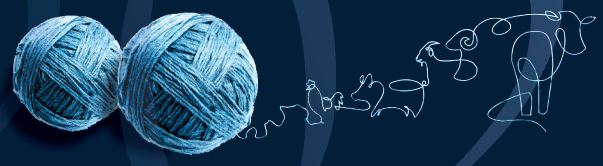
13th INTERNATIONAL SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

2021



PROCEEDINGS

6 - 8 October 2021, Belgrade, Serbia

Institute for Animal Husbandry
Belgrade - Zemun, SERBIA

13th
INTERNATIONAL
SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

2021

PROCEEDINGS

6 -8 October 2021, Belgrade, Serbia

PATRON

Ministry of Education, Science and Technological Development of the Republic of Serbia

ORGANIZER

Institute for Animal Husbandry
Autoput 16, P. Box. 23,
11080, Belgrade-Zemun, Serbia
Tel: +381 11 2691 611; +381 11 2670 121; +381 11 2670 541;
Fax: + 381 11 2670 164;

PUBLISHER
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Editor-in-Chief
Čedomir Radović, PhD, Senior Research associate
Director of the Institute for Animal Husbandry, Belgrade-Zemun

Editor
Zdenka Škrbić, PhD, Principal Research Fellow
Institute for Animal Husbandry, Belgrade-Zemun

The Proceedings is printed by the Institute for Animal Husbandry, Belgrade, 2021

biotechnology.izs@gmail.com
www.istocar.bg.ac.rs
Circulation 100 copies.

ISBN 978-86-82431-77-0

INTERNATIONAL SCIENTIFIC COMMITTEE

CHAIRMAN

Prof. Dr. **Giacomo Biagi,**
Department of Veterinary Medical Sciences, University of Bologna, Italy

SECRETARY

Dr. **Čedomir Radović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Dr. **Zdenka Škrbić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Veselin Petričević,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Marjeta Čandek-Potokar,**
Agricultural Institute of Slovenia, Slovenia
Dr. **Giuseppe Bee,**
Agroscope Posieux, Posieux, Switzerland
Prof. Dr. **Elena Kistanova,**
Institute of Biology and Immunology of Reproduction „Kiril Bratanov“, Sofia, Bulgaria
Prof. Dr. **Stayka Laleva ,**
Agricultural Institute, Stara Zagora, Bulgaria
Prof. Dr. **Galia Zamaratskaia,**
Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden
Dr. **Miloš Lukić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Vlada Pantelić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Maya Ignatova,**
Institute of Animal Science, Kostinbrod, Bulgaria
Dr. **Dragana Ružić-Muslić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Marina I. Selionova,**
FSBSI-All-Russian Scientific Research Institute of Sheep and Goat Breeding, Stavropol, Russia
Prof. Dr. **Radica Đedović,**
University of Belgrade, Faculty of Agriculture, Serbia

Prof. Dr. **Slavča Hristov,**
University of Belgrade Faculty of Agriculture, Serbia
Dr. **Dušica Ostojić Andrić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Lidija Perić,**
University of Novi Sad, Faculty of Agriculture Novi Sad, Serbia
Prof. Dr. **Wladyslav Migdal,**
Department of Animal Product Technology,
University of Agriculture in Kraków, Poland
Prof. Dr. **Danijela Kirovski,**
University of Belgrade, Faculty of Veterinary Medicine, Serbia
Prof. Dr. **Yalçin Bozkurt,**
Isparta University of Applied Science,
Department of Animal Science, Isparta, Turkey
Dr. **Snežana Mladenović Drinić,**
Maize Research Institute „Zemun Polje", Zemun Polje, Serbia
Prof. Dr. **Randelin Dmitry Alexandrovich,**
Faculty of Biotechnology and Veterinary Medicine,
Volgograd State Agricultural University, Russia
Assoc. Prof. **Itskovich Aleksandr Yuryevich,**
Faculty of Biotechnology and Veterinary Medicine,
Volgograd State Agricultural University, Russia
Prof. Dr. **Dragan Radojković,**
University of Belgrade Faculty of Agriculture, Serbia
Prof. Dr. **Milun Petrović,**
University of Kragujevac Faculty of Agronomy, Serbia
Prof. Dr. **Dragan Glamočić,**
University of Novi Sad, Faculty of Agriculture, Serbia
Prof. Dr. **Snežana Trivunović,**
University of Novi Sad, Faculty of Agriculture, Serbia
Prof. Dr. **Predrag Perišić,**
University of Belgrade, Faculty of Agriculture, Serbia
Prof. Dr. **Vladan Bogdanović,**
University of Belgrade, Faculty of Agriculture, Serbia
Dr. **Violeta Caro Petrović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Zoran Ilić,**
University of Pristina, Faculty of Agricultural Sciences, Lešak, Serbia

Dr. **Nevena Maksimović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Ivan Pavlović,**
Scientific Veterinary Institute of Serbia, Serbia
Dr. **Aleksandar Stanojković,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Nenad Đorđević,**
University of Belgrade, Faculty of Agriculture, Serbia
Dr. **Zorica Bijelić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Violeta Mandić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Yusup A. Yuldashbaev,**
Russian State Agrarian University,
Moscow Timiryazev Agricultural Academy,
Faculty of Animal Science and Biology, Russia
Prof. Dr. **Pero Mijić,**
Josip Juraj Strossmayer University of Osijek,
Faculty of Agrobiotechnical Sciences, Osijek, Croatia
Prof. Dr. **Zoran Luković,**
University of Zagreb Faculty of Agriculture,
Department of Animal Science and Technology, Croatia Asist.
Asist. Prof. **Dubravko Škorput,**
University of Zagreb Faculty of Agriculture,
Department of Animal Science and Technology, Croatia
Prof. Dr. **Ivan Radović,**
University of Novi Sad, Faculty of Agriculture, Serbia
Prof. Dr. **Aleksandar Simić,**
University of Belgrade, Faculty of Agriculture, Serbia
Prof. Dr. **Nikola Pacinovski,**
Ss Cyril and Methodius University in Skopje,
Institute of Animal Science, North Macedonia
Prof. Dr. **Yessenbay Islamov,**
Kazakh National Agrarian University, Kazakhstan
Prof. Dr. **Ricmar P. Aquino,**
University President, Isabela State University, Philippines
Prof. Dr. **Rosa Nieto,**
Departament of Physiology and Biochemistry of Animal Nutrition Estacion
Experimental del Zaidín, CSIC Armilla, Granada, Spain

ORGANIZING COMMITTEE

CHAIRMAN

Dr. **Čedomir Radović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

SECRETARY

Dr. **Dragan Nikšić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Dr. **Maja Petričević,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Marina Lazarević,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Nikola Delić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Ljiljana Samolovac,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Marija Gogić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

SYMPOSIUM SECRETARIAT

CHAIRMAN

Dr. **Dragan Nikšić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Slavko Maletić, grad. econ.
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Olga Devečerski, grad. prof.
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Nenad Mićić, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Bogdan Cekić, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Miloš Marinković, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Vladimir Živković, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Nenad Stojiljković, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Ivan Ćosić, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Tamara Stamenić, BSc,
Institute for Animal Husbandry, Belgrade-Zemun

Address:

Institute for Animal Husbandry,
Autoput 16, P. Box 23,
11080, Belgrade-Zemun, Serbia

Tel:
+381 11 2691 611
+381 11 2670 121
+381 11 2670 541
Fax:
+381 11 2670 164

E-mail: biotechnology.izs@gmail.com
www.istocar.bg.ac.rs

CONTENTS

INVITED PAPERS

ORAL PRESENTATION

POSTER PRESENTATION

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

FUSARIUM SPP. AND DEOXYNIVALENOL CONTAMINATION OF RYEGRASS SEEDS

Vesna Krnjaja[1], Violeta Mandić[1], Zorica Bijelić[1], Slavica Stanković[2], Milica Nikolić[2], Tanja Vasić[3], Nikola Delić[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia
[3]University of Niš, Faculty of Agriculture, Kosančićeva 4, 37000, Kruševac, Serbia
Corresponding author: Vesna Krnjaja, vesnakrnjaja.izs@gmail.com

Abstract: The aim of this study was to evaluate fungal infection, with a special focus on *Fusarium* spp. and deoxynivalenol (DON) presence, as a participant in Fusarium head blight (FHB) pathogenesis in two cultivars of Italian ryegrass K-13 and K-29. A total of 24 seed samples were collected during harvest in 2019.

By mycological analyses, *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. and non-sporulating species (Mycelia sterilia) were isolated on the seeds of both cultivars of Italian ryegrass. Among *Fusarium* spp., four species, *F. graminearum*, *F. poae*, *F. proliferatum* and *F. subglutinans* were identified in cultivar K-13 and three species, *F. graminearum*, *F. poae* and *F. subglutinans*, in cultivar K-29. *F. graminearum* and *F. poae* were identified as FHB pathogens, of which *F. graminearum* was dominant in both cultivars with 20.5% (cultivar K-13) and 32% (cultivar K-29) compared to *F. poae* which was present in both cultivars with a frequency of 0.5%. The frequency of DON positive samples was 100%. A statistically significant difference in DON level was found between the two tested cultivars, with a higher DON level in cultivar K-29 (5334.33 μg kg^{-1}) compared to cultivar K-13 (4738.58 μg kg^{-1}).

The obtained results indicate that two *Fusarium* species, *F. graminearum* and *F. poae*, were FHB pathogens, with *F. graminearum* as the predominant species in both cultivars of Italian ryegrass. High DON levels (>3000 μg kg^{-1}) in the tested seed of Italian ryegrass indicate on potentially significant participation of DON in FHB pathogenesis, as well as a potential risk for the quality seed production, feed safety and the food chain in general. In Serbia, this is the first report about *Fusarium* infection and DON presence in ryegrass seed.

Key words: *Fusarium* spp., deoxynivalenol, Italian ryegrass

Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is annual and also biennial or short-lived perennial bunchgrass. It is a high-yielding and high-quality fodder crop native to southern Europe. It is an integral part of pasture for hay and silage production. Its high nutrition, digestibility, and palatability, conducives to the diet of ruminants (dairy, sheep) (*Simić et al., 2005; 2012*). This crop grows best between 20 and 25°C and requires a cool climate with high rainfall and well-drained soils. It has the fastest growth and regeneration and produces a lot of forage in a short time (*Delić et al., 2012; Kostrzewska et al., 2020*). It is sensitive to strong frosts and quite sensitive to drought, and longer droughts can bring it very low yields (*Simić et al., 2005*).

In recently year, due to global warming, the occurrence of pathogenic and potentially toxigenic fungi from *Fusarium*, *Aspergillus* and *Penicillium* genera has become more frequent on cereal grains and other grasses (*Silva et al., 2014; Torres et al., 2019*). In addition, agro-ecological and environmental conditions in Serbia are favorable to growth of these fungal species on cereal grains, with *Fusarium* species as predominant (*Lević, 2008).* Members of *Fusarium graminearum* species complex (FGSC) cause Fusarium head blight (FHB) disease of small grains and several grasses, including annual ryegrass (*Machado et al., 2015*). *F. graminearum* is the most common pathogen which cause FHB of small grains. The first symptoms of FHB appear after flowering at anthesis. Spikelets are bleached, and with development of mycelium, the disease spreads throughout the head (spike), causing shrivelled seeds (*Francesconi et al., 2019*). *F. graminearum*-infected seeds have poor germination with slow emergence and can cause seedling blight disease (*Wiewióra, 2012*). *F. graminearum* is a hemibiotroph, with the biotrophic (short last from 24 to 32 h after infection) and necrotrophic phase (the pathogen grows and extends within the head through the vascular bundles and parenchyma) (*Bai et al., 2002; Goswami and Kistler, 2004; Trail, 2009*).

In addition to causing FHB disease, *Fusarium* species produce a wide range of toxic secondary metabolites (mycotoxins), which are serious constraints for feed/food production (*Richardson et al., 1996*). The occurrence of these fungal metabolites in food/feed poses healt risks to the consumers (*Haidukowski et al., 2005*). Deoxynivalenol (DON), primarily produced by *F. graminearum*, is the most associated with FHB. It belongs sesquiterpene epoxides, namely trichothecenes. The most commonly DON found in grain/seed from FHB-diseased cereal and grass heads (*Gunupuru et al., 2017*). Negative effects of DON ingestion by experimental animals are manifested as acute (emesis) and chronic poisoning (anorexia, growth retard, neurotoxicity, immunotoxicity as well as reproduction disorders. Likewise, DON can be associated with human gastroenteritis (*Pestka, 2010*). Reffering to

participation of DON in FHB pathogenesis, it also has the ability to be virulent and facilitates the spread of fusariosis within the head. Like FHB, DON may cause premature bleaching of head and leaves. DON-producing *F. graminearum* strains can supress programmed cell death (apoptosis) in the leaves and support biotrophic and necrotrophic phases of *Fusarium* infection (*Diamond et al., 2013*). According to *Bushnell et al. (2010),* DON-producing strains inhibited the formation of the wall thickenings, allowing the pathogen to spread in the head.

According to European Regulations EC No. 1126/2007 amended the Regulation (EC) No. 1881/2006 and EC No. 576/2006, the maximum limits of DON level are 1250 μg kg^{-1} in unprocessed cereal other than durum wheat, oats and maize and 8000 μg kg^{-1} in unprocessed cereal and cereal products except in maize by-products intended for animal feeding, respectively. Quality and healthy seeds are a prerequisite for high yields of ryegrass as well as for safe feed production. The high yield of seed and fodders is correlated with the genetic potential of the selected varieties (*Velijević et al., 2016*). Grass (cereal) cultivars have different types of resistance to FHB (from I to V resistance types), there is no unsusceptible genotype. Types I and II are the most described. Type I resistance is resistance to initial infection, type II is resistance to spread of pathogen within the head. Other types include resistance to seed infection (type III), tolerance to FHB and DON (type IV), and resistance to DON accumulation (Type V) (*Mesterházy, 1995; Boutigny et al., 2008*). In addition to growing resistant cultivars, crop rotation, fertilization, tillage, and the application of fungicides and biological agents can also significantly reduce *Fusarium* seed infestation (*Shah et al., 2018*).

Since the quality and healthy seed is important for ryegrass, livestock and feed production, the main aim of this work was to identify and quantify *Fusarium* spp. associated with ryegrass seeds, as well as to determine DON levels in seed samples of two ryegrass cultivars and to assess its potential risk in FHB pathogenesis.

Materials and Methods

A total of 24 seed samples of two commercial Italian ryegrass cultivars (12 samples of cultivar K-13 and 12 samples of cultivar K-29) were analyzed by mycological and toxicity tests. About 1 kg of ryegrass intended for seed production were randomly taken during the 2019 harvest season. The area of tested ryegrass cultivars was on about one hectare and located on the experimental fields of Institute for Animal Husbandry, Belgrade-Zemun in Serbia.

The each of 24 samples was divided into two sub-samples, so, it was 12 sub-samples per cultivar. In mycological analyses, per each cultivar, the first 12 seed sub-samples were pooled in one representative sample. To evaluate *Fusarium*

spp., 600 seeds per cultivar from representative samples were analyzed. First, the seeds were surface-disinfected for 3 minutes with 1% sodium hypochlorite, washed twice with sterilized distilled water and dried on filter paper. Then, the seeds were plated, 10 seeds per 90 mm Petri-dishes with Potato Dextrose Agar (PDA), and incubated at room temperature during 7-10 days. Based on microscopic observations, *Fusarium* species were identified using fungal keys of *Burgess et al. (1994)* and *Leslie and Summerell (2006)*. Incidence of fungal species was presented as percentage values in pooled seed samples.

For mycotoxicological analyses, a total of 24 samples, 12 sub-samples per cultivar, were ground in an analytical mill (IKA A11, Staufen, Germany). Using the moisture analyzer (OHAUS MB35, USA), the moisture content of tested samples was determined. ELISA assay for determining DON levels was done according to the manufacturer's instructions Celer Tecna® ELISA kits. The limit of detection for DON was 40 μg kg^{-1}.

Data were statistically analyzed using the independent-samples T-test (IBM SPSS Statistic 20). Pearson's correlation coefficients between DON levels and moisture contents were determined.

Results and Discussions

The results of mycobiota isolated from the seed of two Italian ryegrass cultivars, K-13 and K-29, are shown in Table 1. The fungal species isolated were *Acremonium* spp., *Alternaria* spp., *F. graminearum, F. poae, F. proliferatum, F. subglutinans* and non-sporulating fungi (Mycelia sterilia) on the seed of both cultivars, except on seed of cultivar K-29, in which *F. proliferatum* was not identified. Among isolated species, *Alternaria* spp. was the most present with 70% and 61.67% on the seed of K-13 and K-29 cultivar, respectively. Considering incidence of *Fusarium* species, *F. graminearum* was the most common at both cultivars, with higher incidence on the seed of cultivar K-29 (32%) than cultivar K-13 (20.50%). *F. graminearum* is followed by *F. subglutinans* which was isolated in a higher percent on the seed of cultivar K-13 (7.50%) compared to cultivar K-29 (2%). A low incidence of *F. poae* (0.50%) was found on the seed of both cultivars, as well as the incidence of *F. proliferatum* on the seed of cultivar K-13 (0.17%). In a similar study, *Wiewióra (2012)* has established *Alternaria alternata* as the most common species on the seed of perennial ryegrass and identified 10 *Fusarium* species of which *F. avenaceum* and *F. solani* were the most frequent. Further, the same autor stated that the most species such as *Alternaria* spp., *Epicoccum* spp., *Septonema* spp. and *Penicillium* spp. were saprophytes or weak parasites, while *Drechslera* spp., *Fusarium* spp., *Phoma* spp., *Curvularia* spp. and *Bipolaris* spp. were presented as pathogenic species. Similar results were reported by *Varga and*

Fischl (2005) on the ryegrass seeds and *Pathak and Zaidi (2013)* on wheat seeds. According to reported data of *Torres et al. (2019)*, the major FHB pathogens include FGSC members and related species such as *F. avenaceum*, *F. culmorum* and *F. poae*. Similarly, in this study, *F. graminearum* and *F. poae* identified as FHB pathogens from which *F. graminearum* was more presence than *F. poae* on the seed of both tested cultivars. *Machado et al. (2015)* have identified three FGSC species, *F. graminearum*, *F. asiaticum* and *F. cortaderie* from diseased ryegrass spikes and confirmed their pathogenicity to ryegrass. It was the first report of FGSC members as head blight pathogens of ryegrass (*L. multiflorum* L.) in Brazil. The results of this study were also the first report of *Fusarium* spp. incidence on the seed of two Italian ryegrass cultivars (K-13 and K-29) in Serbia.

Table 1. Incidence of fungal species on the seed of two tested ryegrass cultivars

Fungal species	Incidence (%)	
	Cultivar of ryegrass	
	K-13	K-29
Acremonium spp.	0.50	0.83
Alternaria spp.	70	61.67
Fusarium graminearum	20.5	32
Fusarium poae	0.50	0.50
Fusarium proliferatum	0.17	0
Fusarium subglutinans	7.50	2
Mycelia sterilia	0.83	3

By mycotoxicological assays, DON was found in all tested seed samples of two ryegrass cultivars, respectively the incidence of DON positive samples was 100%. It has been established the statistically significant higher mean level of DON on the seed of cultivar K-29 (5334.33 μg kg^{-1}) compared to cultivar K-13 (4738.58 μg kg^{-1}). There were no statistically significant differences between means of moisture contents of seeds in tested cultivars (Table 2). By investigating the participation of DON in FHB pathogenesis, *Bai et al. (2002)* have stated that DON production had a significant role in the spread of FHB within a spike. According to the reports of *Diamond et al. (2013)*, relatively low DON levels might inhibite plant programmed cell death (PCD), while high DON levels might induce cell death. So, DON-producing *F. graminearum* strains with low and 10,000 μg kg^{-1} DON levels might contribute to biotrophic and necrotrophic phases development in *Fusarium* infection leading to FHB disease symptoms (*Gunupuru et al., 2017*). In Brazil, the first report of DON-producing *F. graminearum* isolated from ryegrass spikes, as well as their pathogenicity on annual ryegrass reported by *Machado et al. (2015)*. In this study, high DON levels in all seed samples of both

cultivars indicating that there was potentially risk of isolated *F. graminearum* strains in FHB pathogenesis.

Table 2. Means of DON level and moisture content in tested seed samples of two ryegrass cultivars

	DON level (μg kg^{-1})		Moisture content (%)
Item	Mean ± S.D.	Range	Mean ± S.D.
Cultivar K-13	4738.58 ± 244.17^{a}	3157 – 5586	11.74 ± 0.32
Cultivar K-29	5334.33 ± 147.26^{b}	4382 – 5886	11.30 ± 0.53
Level of significance	*	-	ns

Means followed by the same letter within a column are not significantly different at P ≤ 0.05 level; *, ** - significant at the 0.05 and 0.01 probability levels; ns - not significant.

Temperature, moisture and relative humidity are the most important environmental factors influencing the development of FHB and therefore DON accumulation in cereal grains *(Wegulo, 2012)*. Weak positive correlations were found for levels of DON with moisture contents on seeds of tested cultivars K-13 (r = 0.348) and K-29 (r = 0.380) (data not presented). Similarly, positive correlations between moisture-related variables with biological variables such as DON accumulation in wheat cultivars were established by *Cowger et al. (2009), Kriss et al. (2010)* and *Hernandez Nopsa et al. (2012)*.

Conclusion

This study presents a natural occurrence of fungal infection and DON mycotoxin in the seed of two ryegrass cultivars, K-13 and K-29, as a participant in FHB pathogenesis. *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. and non-sporulating fungal species (Mycelia sterilia) were isolated at both cultivars. Among *Fusarium* spp., *F. graminearum*, *F. poae*, *F. proliferatum*, and *F. subglutinans* and *F. graminearum*, *F. poae*, and *F. subglutinans* were identified on the seed of cultivars K-13 and K-29, respectively. *F. graminearum* and *F. poae* were identified as FHB pathogens, with *F. graminearum* as predominant in both cultivars. All tested seed samples were DON positive. DON level in cultivar K-29 was statistically significant higher (5334.33 μg kg^{-1}) than in K-13 (4738.58 μg kg^{-1}). High DON levels (>3000 μg kg^{-1}) in tested seed samples were indicated on potential significant participation of DON in FHB pathogenesis. Both ryegrass cultivars were susceptible to FHB pathogens. These results indicate on importance of health of seeds, especially for ryegrass seeds production as well as forage production. Ryegrass as the weed may also be potentially a source of inoculum for FHB epidemics in cereal crops.

Fusarium spp. i deoksinivalenol kontaminacija semena italijanskog ljulja

Vesna Krnjaja, Violeta Mandić, Zorica Bijelić, Slavica Stanković, Milica Nikolić, Tanja Vasić, Nikola Delić

Rezime

Cilj rada bio je da se oceni gljivična infekcija sa specijalnim fokusom na *Fusarium* spp. i prisustvo deoksinivalenola (DON) kao učesnika u patogenezi fuzarioze klasa (FHB) kod dve sorte italijanskog ljulja, K-13 i K-29. Ukupno 24 uzoraka semena sakupljeno je tokom žetve u 2019. godini. Mikološkim analizama izolovane su *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. i nesporulišuće vrste (Mycelia sterilia) na semenu obe ispitivane sorte italijanskog ljulja. Među *Fusarium* spp., identifikovane su četiri vrste, *F. graminearum*, *F. poae*, *F. proliferatum* i *F. subglutinans*, kod sorte K-13 i tri vrste, *F. graminearum*, *F. poae* i *F. subglutinans*, kod sorte K-29. *F. graminearum* i *F. poae* su identifikovane kao FHB patogeni, od kojih *F. graminearum* je dominantnija kod obe ispitivane sorte sa 20,5% (sorta K-13) i 32% (sorta K-29) u odnosu na *F. poae* koja je bila prisutna kod obe sorte sa učestalošću od 0,5%. Učestalost DON pozitivnih uzoraka bila je 100%. Utvrđena je statistički značajna razlika u koncentraciji DON između dve ispitivane sorte, s tim da je utvrđena veća koncentracija DON kod sorte K-29 (5334,33 μg kg^{-1}) u odnosu na sortu K-13 (4738,58 μg kg^{-1}). Dobijeni rezultati ukazuju da su dve *Fusarium* vrste, *F. graminearum* i *F. poae*, FHB patogeni, s tim da je *F. graminearum* preovladjujuća vrsta kod obe ispitivane sorte italijanskog ljulja. Visoke koncentracije DON mikotoksina (>3000 μg kg^{-1}) u ispitivanim uzorcima semena italijanskog ljulja ukazuju na potencijalno značajno učešće DON u FHB patogenezi, kao i na potencijalni rizik za proizvodnju kvalitetnog semena, bezbednost hrane za životinje i lanca ishrane uopšte. U Srbiji, ovo je prva objava o *Fusarium* infekciji i prisustvu DON u semenu italijanskog ljulja.

Ključne reči: *Fusarium* spp., deoksinivalenol, italijanski ljulj

Acknowledgment

Research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, Agreement on the realization and financing of scientific research work of SRO no. 451-03-9/2021-14/200022.

References

BAI G.H., DESJARDINS A.E., PLATTNER R.D. (2002): Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia, 153, 91-98.

BOUTIGNY A.L., RICHARD FORGET F., BARREAU C. (2008): Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes. European Journal of Plant Pathology, 121, 411-423.

BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory manual for *Fusarium* research. Third edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney, pp. 133.

BUSHNELL W.R., PERKINS-VEAZIE P., RUSSO, V.M., COLLINS J., SEELAND T.M. (2010): Effects of deoxynivalenol on content of chloroplast pigments in barley leaf tissues. Phytopathology, 100, 33-41.

COWGER C., PATTON-ÖZKURT J., BROWN-GUEDIRA G., PERUGINI L. (2009): Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99, 320-327.

DELIĆ D., STAJKOVIĆ-SRBINOVIĆ O., ŽIVKOVIĆ S., PROTIĆ N., RASULIĆ N., KUZMANOVIĆ DJ., SIMIĆ A. (2012): Growth promotion of Italian ryegrass (*Lolium multiflorum* Lam.) by application of plant growth promoting rhizobacteria. Plant Protection, 63, 280, 93-99.

DIAMOND M., REAPE T.J., ROCHA O., DOYLE S.M., KACPRZYK J., DOOHAN F.M., MCCABE P.F. (2013): The *Fusarium* mycotoxin deoxynivalenol can inhibit plant apoptosis-like programmed cell death. PloS One, 8, 7, e69542.

FRANCESCONI S., MAZZAGLIA A., BALESTRA G.M. (2019): Different inoculation methods affect components of Fusarium head blight resistance in wheat. Phytopathologia Mediterranea, 58, 3, 679-691.

GOSWAMI R.S., KISTLER H.C. (2004): Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology, 5, 515-525.

GUNUPURU L.R., PEROCHON A., DOOHAN F.M. (2017): Deoxynivalenol resistance as a component of FHB resistance. Tropical Plant Pathology, 42, 175-183.

HAIDUKOWSKI M., PASCALE M., PERRONE G., PANCALDI D., CAMPAGNA C., VISCONTI A. (2005): Effect of fungicides on the development of Fusarium head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. Journal of the Science of Food and Agriculture, 85, 191-198.

HERNANDEZ NOPSA J., BAENZIGER P.S., ESKRIDGE K.M., PEIRIS K.H.S., DOWELL F.E., HARRIS S.D., WEGULO S.N. (2012): Differential accumulation

of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions. Canadian Journal of Plant Pathology, 34, 380-389.
KOSTRZEWSKA M.K., JASTRZĘBSKA M., TREDER K., WANIC M. (2020): Phosphorous in spring barley and Italian rye-grass biomass as an effect of inter-species interactions under water deficit. Agriculture, 10, 329.
KRISS A.B., PAUL P.A., MADDEN L.V. (2010): Relationship between yearly fluctuations in Fusarium head blight intensity and environmental variables: A window-pane analysis. Phytopathology 100, 784-797.
LESLIE J.F., SUMMERELL B.A., (2006): The *Fusari*um Laboratory Manual. Blackwell Publishing Ltd, Oxford, pp. 388.
LEVIĆ J. (2008): Vrste roda *Fusarium* u oblasti poljoprivrede, veterinarske i humane medicine. Cicero, Beograd. pp. 1226.
MACHADO F.J., MÖLLER P.A., NICOLLI C.P., Del PONTE E.M. (2015): First report of *Fusarium graminearum*, *F. asiaticum*, and *F. cortaderiae* as head blight pathogens of annual ryegrass in Brazil. Plant Disease, 99, 12, 1859-1859.
MESTERHÁZY A. (1995): Types and components of resistance to Fusarium head blight of wheat. Plant Breeding, 114, 377-386.
PATHAK N., ZAIDI R. (2013): Fungi associated with wheat seed discolouration and abnormalities in *in-vitro* study. Agricultural Sciences, 4, 9, 516-520.
RICHARDSON M.J. (1996): Seed mycology. Mycological Research 100, 4, 385-392.
SHAH L., ALI A., YAHYA M., ZHU Y., WANG S., SI H., RAHMAN H., MA C. (2018): Integrated control of fusarium head blight and deoxynivalenol mycotoxin in wheat. Plant Pathology, 67, 532-548.
SILVA A.E.L., REIS E.M., TONIN R.F.B., DANELLI A.L.D., AVOZANI A. (2014): Identification and quantification of fungi associated with seeds of ryegrass (*Lolium multiflorum* Lam.). Summa Phytopathologica, 40, 2, 156-162.
SIMIĆ A., VUČKOVIĆ S., SABOVLJEVIĆ R. (2005): Seed yield and quality of Italian ryegrass (*Lolium italicum*) containing different ploidy produced in the first harvest year. Plant Breeding and Seed Production, XI, 1-4, 19-24.
SIMIĆ A., VUČKOVIĆ S., SOKOLOVIĆ D., STANISAVLJEVIĆ R., DURONIĆ G. (2012): Response of Italian ryegrass seed crop to spring nitrogen application in the first harvest year. African Journal of Biotechnology, 11, 26, 6826-6831.
TORRES A.M., PALACIOS S.A., YERKOVICH N., PALAZZINI J.M., BATTILANI P., LESLIE J.F., LOGRIECO A.F., CHULZE S.N. (2019): *Fusarium* head blight and mycotoxins in wheat: prevention and control strategies across the food chain. World Mycotoxin Journal, 12, 4, 333-355.
TRAIL F. (2009): For blighted waves of grains: *Fusarium graminearum* in the postgenomics era. Plant Physiology, 149, 103.110.

VARGA Z., FISCHL G. (2005): Infection rates of perennial ryegrass seeds (*Lolium perenne* L.) with different fungi. Communications in Agricultural and Applied Biological Sciences, 70, 195-198.
VELIJEVIĆ N., SIMIĆ A., VUČKOVIĆ S., ĐUKANOVIĆ L., POŠTIĆ D., ŠTRBANOVIĆ R., STANISAVLJEVIĆ R. (2016): Varijabilnost dormantnosti, klijavosti semena i vigora klijanaca sorti crvene deteline i italijanskog ljulja. XXI Savetovanje o biotehnologiji, 11. – 12. mart 2016. godine, Čačak, Srbija. Zbornik radova, 21, 23, 73-79.
WEGULO S.N. (2012): Influencing deoxynivalenol accumulation in small grain cereals. Toxins, 4, 1157-1180.
WIEWIÓRA B. (2012): The effect of seed health of perennial ryegrass (*Lolium perenne* L.) on germination capacity. Plant Breeding and Seed Science, 65, 51-62.

==========

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

636/638(082)(0.034.2)
631/635(082)(0.034.2)

INTERNATIONAL Symposium Modern Trends in Livestock Production (13 ; 2021 ; Beograd)
Proceedings [Elektronski izvor] / 13th International Symposium Modern Trends in Livestock Production, 6 -8 October 2021, Belgrade, Serbia ; [organizer] Institute for Animal Husbandry, Belgrade - Zemun ; [editor Zdenka Škrbić]. - Belgrade : Institute for Animal Husbandry, 2021 (Belgrade : Institute for Animal Husbandry). - 1 USB fleš memorija ; 1 x 3 x 6 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 100. - Bibliografija uz svaki rad.

ISBN 978-86-82431-77-0

а) Сточарство -- Зборници б) Пољопривреда -- Зборници

COBISS.SR-ID 46411785

==========

13th INTERNATIONAL SYMPOSIUM MODERN TRENDS IN LIVESTOCK PRODUCTION

6 - 8 October 2021 - Belgrade, Serbia

PROCEEDINGS

INSTITUTE FOR ANIMAL HUSBANDRY
Autoput 16, P. Box 23, 11080, Belgrade - Zemun, Serbia
www.istocar.bg.ac.rs

ISBN 978-86-82431-77-0

2021