

14th
INTERNATIONAL
SYMPOSIUM

MODERN
TRENDS
IN LIVESTOCK
PRODUCTION



P R O C E E D I N G S

4 - 6 October 2023, Belgrade, Serbia

Institute for Animal Husbandry
Belgrade - Zemun, SERBIA

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INTERNATIONAL
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and Innovation of the Republic of Serbia

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FUSARIUM AND DEOXYNIVALENOL CONTAMINATION OF WINTER WHEAT DEPENDING ON GROWING SEASON AND CULTIVAR

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Abstract: Wheat is the most important staple food in the world and the main source of carbohydrates, fibre, proteins, vitamins, minerals and phytochemicals for human consumption. The nutrients from wheat kernels can also be used as livestock feed. Fusarium head blight (FHB), caused by fungal species of the *Fusarium* genus, is one of the most important wheat diseases worldwide. The lack of FHB management strategies results in significant economic losses in yield and quality of wheat kernels. In this study, the influence of growing season and wheat cultivar on some FHB and yield component traits, as well as on the trichothecene mycotoxin deoxynivalenol (DON) levels during the harvest period in 2014 and 2015, was investigated. Significant influence of growing season (year) and wheat cultivar on disease (FHB index – FHBI, incidence of *Fusarium*-damaged kernels – FDK and levels of DON) and yield parameters (spike weight – SW, kernel weight per spike – KWS, and 100-kernel weight) was found. FHBI, FDK, and DON were higher in 2014, while SW, KWS, and 100-kernel weight were lower in 2014 than in 2015. The mid-early wheat cultivar Simonida had lower FHBI, FDK, and levels of DON and significantly higher SW, KWS, and 100-kernel weight than the mid-late cultivar NS 40S. There was a significant effect of year × cultivar interaction on FHBI, FDK and 100-kernel weight.

Key words: Fusarium head blight, *Fusarium* and deoxynivalenol contamination, yield component traits, winter wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. In Serbia, wheat is grown on about 600,000 ha, with a total production of 3.4 million tonnes and an average yield of 5.7 t ha⁻¹ in 2021 (*Statistical Yearbook of the Republic of Serbia, 2022*). It is used in the food industry to produce bread, pasta, cakes, crackers, cookies, pastries, starch, alcohol, flour, etc. The nutrient composition of wheat kernels varies depending on genotypic characteristics, yields, climate and soil. Wheat grown in dry climates is usually a durum type with a protein content of 11-15% and a strong structure of protein gluten (elastic protein). The durum type provides the most suitable flour for bread making. Wheat from humid areas is softer, with a protein content of about 8-10% and a weak gluten structure, ideal for cakes, cookies, pastries, etc. (*Lafiandra et al., 2022*). Due to the high proteins (on average 10-12%), carbohydrates (75%) and fibre (13.4%) contents, wheat is also used for animal feed, in feeding ruminants, pigs and poultry, especially in developed countries (*Grote et al., 2021; Tóth et al., 2022; Kartseva et al., 2023*). Fusarium head blight (FHB) is a devastating fungal disease in wheat crops that causes yield loss with destructive effects on grains, shrivelled and discoloured kernels, and the formation of the trichothecene mycotoxin deoxynivalenol (DON) (*Jones, 2000; Wegulo et al., 2011*). *Fusarium graminearum* sensu lato (teleomorph: *Gibberella zeae* (Schwein.) Petch.), also called *F. graminearum* species complex (FGSC), is the major causal agent of FHB. The FGSC includes more than 16 phylogenetically distinct species (*Sarver et al., 2011*), of which *F. graminearum* sensu stricto (s.s.) is the most widespread. In Serbia, in addition to *F. graminearum* s.s., *F. vorosii* was recently identified as a member of the FGSC (*Obradović et al., 2022*). Several *Fusarium* species, *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. poae*, are considered the predominant species causing FHB. Among them, *F. graminearum* and *F. culmorum* are the most aggressive to winter wheat and the most important DON-producing fungal species (*Bottalico and Perrone, 2002; Tan et al., 2021; Balducci et al., 2022*).

Wheat straws and chaffs are used in pig and turkey production and can be contaminated with secondary metabolites of fungi (mycotoxins) as forage of wheat kernels for cattle feed. Mycotoxins have toxic effects on animal and human health, causing acute and chronic diseases (mycotoxicoses). Animal toxicoses occur through mycotoxin-contaminated feed and bedding (*Cowger and Arellano, 2013*). Intoxication of animals through DON-contaminated feed can cause anorexia, emesis, and feed refusal. DON has also neurotoxicity and immunotoxicity effects (*Haidukowski et al., 2005*).

FHB and DON contamination are influenced by many factors such as climatic, agroecological and environmental conditions, especially during flowering stages, which is the most sensitive host stage for *Fusarium* infection, and by agrotechnical measures. Agricultural practices such as tillage, crop rotation, crop residue management, irrigation management, resistant cultivars, chemical and biological control, and disease forecasting are preharvest strategies against FHB and to reduce DON accumulation in wheat kernels (Yuen and Schoneweis, 2007; Blandino et al., 2012; Wegulo et al., 2015). Since of the economic importance of FHB in wheat crops, the main objective of this study was to evaluate the effect of the growing season (year) and wheat cultivar on *Fusarium* and DON contamination during harvest in 2014 and 2015 in Serbia, taking into account the infection with *Fusarium* spp. under natural conditions.

Material and Methods

Field trials. A field trial was conducted at the Institute of Animal Husbandry, Belgrade-Zemun, Serbia (44°84' N, 20°40' E; 88 m a.s.l.) under dry farming conditions in response to natural precipitation. During the two harvest seasons, 2014 and 2015, FHB and yield traits and DON levels of two Serbian wheat cultivars, Simonida and NS 40S, were evaluated. These cultivars were selected due to widespread cultivation around the experimental area in Serbia. The sowing dates were in the third decade of October, with seven days between the sowing of mid-early and mid-late cultivars. The soil type was carbonate chernozem. The previous crop was maize. The field trials were arranged as split-plot randomized block designs with four replications. The size of the subplot was 5 m². Fertilization with ammonium nitrate was applied at the end of February at the rate of 250 kg ha⁻¹ (33.4% N). At the tillering wheat stage, weeds, fungal leaf diseases and insects were controlled by adequate chemical treatments. Wheat kernels were harvested in the first decade of July in both investigated years.

Disease and wheat yield component traits. FHB severity of naturally infected plants was estimated on 20 randomly selected wheat spikes within each subplot (80 wheat spikes per treatment tested) at the end of the flowering stage (GS 69). The evaluation was based on the scale of Blandino et al. (2012) with seven evaluation classes (1 = 0 to 5%, 2 = 5 to 15%, 3 = 15 to 30%, 4 = 30 to 50%, 5 = 50 to 75%, 6 = 75 to 90%, 7 = 90 to 100% spike area infected). Following the methods of Wegulo et al. (2011), the FHB index (FHBi) was calculated using the formula = (incidence (%) × severity (%))/100, where FHB incidence was the percentage of infected spikes and FHB severity was the percentage of infected spikelets, while the incidence of *Fusarium*-damaged kernels (FDK) was visually evaluated in wheat-harvested subsamples of 10 grams.

Wheat kernel subsamples were ground by an analytical mill (IKA A11, Germany) and analysed by competitive direct enzyme-linked immunosorbent assay (ELISA) for the detection of deoxynivalenol (DON). According to the instructions of the manufacturer of the Celer DON ELISA kits (Tecna, Italy), the assay procedure was performed. The absorbance values of the samples were measured optically at a wavelength of 450 nm using an ELISA reader (Biotek EL x 800TM, USA). The detection limit for DON in wheat was 0.04 mg kg⁻¹.

Spike weight (SW), kernel weight per spike (KWS) and 100-kernel weight as yield components were also determined.

Meteorological data. Weather data for the Surčin area (Belgrade) were provided by the Republic Hydrometeorological Service of Serbia (Table 1).

Table 1. Meteorological data (mean monthly temperature, total precipitation and mean monthly relative humidity (RH)) in two growing seasons (October 2013 – July 2014) and (October 2014 – July 2015) in the Surčin area (Belgrade)

Growing season	Month	Temperature (°C)	Precipitation (mm)	RH (%)
2013–2014	October	14.6	45.8	72
	November	9.4	28.7	80
	December	2.1	6.7	85
	January	4.6	24.6	80
	February	7.0	12.9	72
	March	9.9	44.5	69
	April	13.1	86.9	73
	May	16.8	233.4	72
	June	20.8	85.6	68
	July	22.4	181.0	71
	Seasonal mean temperature	12.07		
	Total season precipitation		750.1	
	Seasonal mean RH			74.2

Table 1. (continue)

Growing season	Month	Temperature (°C)	Precipitation (mm)	RH (%)
2014–2015	October	13.6	57.1	76
	November	9.2	9.9	78
	December	3.8	61.4	82
	January	2.9	48.3	78
	February	3.2	50.0	77
	March	7.0	102.0	73
	April	11.8	30.9	62
	May	17.7	58.9	70
	June	20.6	84.2	70
	July	25.1	1.7	58
Seasonal mean temperature		11.49		
Total season precipitation			504.4	
Seasonal mean RH				72.4

Data analyses. The effects of two growing seasons on FHB variables (FHBI and FDK), DON levels and yield component traits (SW, KWS, and 100-kernel weight) in two wheat cultivars were analysed using a general linear model (Multivariate Analysis of Variance) with SPSS software (IBM SPSS Statistic 20). Tukey's test was used to compare treatment means at $P \leq 0.05$ and $P \leq 0.01$ significance levels. Linear relationships between tested variables were calculated using Pearson's correlations.

Results

FHB traits and deoxynivalenol levels. *F* values of the year and cultivar and their interaction are shown in Table 2. The year and cultivar had a highly significant ($P \leq 0.01$) effect on FHBI, FDK, and DON levels. FHBI, FDK and DON were higher in 2014 than in 2015. The mid-late cultivar NS 40S had a statistically significantly higher ($P \leq 0.01$) FHBI, FDK and DON than the mid-early cultivar Simonida. High significance ($P \leq 0.01$) showed by year \times cultivar interaction for FHB variables, FHBI and FDK (Table 2).

Yield component traits. The influence of year and cultivar treatments was significant ($P \leq 0.05$) for SW and KWS and highly significant ($P \leq 0.01$) for 100-kernel weight. The *F* values of the year \times cultivar interaction were highly significant ($P \leq 0.01$) for 100-kernel weight. SW, KWS and 100-kernel weight were

statistically significantly lower in 2014 and in the mid-late cultivar NS 40S compared to 2015 and the mid-early cultivar Simonida (Table 3).

Table 2. Year and cultivar effects on FHB index (FHBI), the incidence of *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) levels in wheat kernels

Factor	FHBI (%)	FDK (%)	DON (mg kg ⁻¹)
Year effects (Y)			
2014	27.67 ^a	17.46 ^a	3.238 ^a
2015	17.08 ^b	7.28 ^b	1.916 ^b
F-test	**	**	**
Cultivar effects (C)			
Simonida	20.47 ^b	5.94 ^b	1.640 ^b
NS 40S	24.28 ^a	18.80 ^a	3.514 ^a
F-test	**	**	**
Interactions (F-test)			
Y × C	**	**	ns
Means	22.38	12.37	2.58

Y, Year; C, Cultivar;

Means followed by the same letter within a column are not significantly different by *Tukey's test* at $P \leq 0.05$ level. ns, not statistically significant; **significant at the 0.01 level of probability.

Table 3. Year and cultivar effects on wheat yield component traits, spike weight (SW), kernel weight per spike (KWS) and 100-kernel weight

Factor	SW (g)	KWS (g)	100-kernel weight (g)
Year effects (Y)			
2014	1.79 ^b	1.42 ^b	3.63 ^b
2015	2.09 ^a	1.72 ^a	4.37 ^a
F-test	*	*	**
Cultivar effects (C)			
Simonida	2.10 ^a	1.70 ^a	4.43 ^a
NS 40S	1.78 ^b	1.45 ^b	3.57 ^b
F-test	*	*	**
Interactions (F-test)			
Y × C	ns	ns	**
Means	1.94	1.57	4.00

Y, Year; C, Cultivar;

Means followed by the same letter within a column are not significantly different by *Tukey's test* at $P \leq 0.05$ level. ns, not statistically significant; **significant at the 0.01 level of probability.

Correlations between tested variables. In correlation analyses, there were highly significant ($P \leq 0.01$) positive correlations between FHBI with FDK ($r = 0.84$) and DON levels ($r = 0.80$), SW with KWS ($r = 0.99$) and 100-kernel weight ($r = 0.65$), and KWS with 100-kernel weight (0.70). FDK had a highly significant

($P \leq 0.01$) positive correlation with DON levels ($r = 0.90$). Significant negative correlation coefficients were also obtained for the relationships between disease variables (FHBI, FDK, and DON level) and yield component traits (SW, KWS, and 100-kernel weight) (Table 4).

Table 4. The correlation coefficients (r) between tested variables

	FHBI	FDK	DON	SW	KWS
FDK	0.84**				
DON	0.80**	0.90**			
SW	-0.61**	-0.51**	-0.68*		
KWS	-0.69**	-0.57**	-0.72*	0.99**	
100-kernel weight	-0.85**	-0.95**	-0.92**	0.65**	0.70**

FHBI, FHB index; FDK, the incidence of *Fusarium*-damaged kernels; DON, deoxynivalenol; SW, spike weight; KWS, kernel weight per spike. *significant at the 0.05 level of probability; **significant at the 0.01 level of probability

Discussion

This study evaluated year and cultivar effects on wheat kernel quality and yield. During the observation period, the 2013-14 growing season was wetter, with total precipitation of 750.1 mm compared to 2014-15 (504.4 mm). Mean temperatures and RH were similar in both investigated growing seasons, October 2013 – July 2014 (12.07 °C and 74.2%) and October 2014 – July 2015 (11.49 °C and 72.4%) (Table 1). During the most susceptible stage for *Fusarium* infection (flowering), in May, total precipitation was higher in 2014 (233.4 mm) than in 2015 (58.9 mm), with mean monthly temperatures of 16.8 °C and 17.7 °C, respectively. In June, at the milk and maturity wheat stages, total precipitation and mean temperatures were similar in both years (2014 – 85.6 mm and 20.8°C, and 2015 – 84.2 mm and 20.6°C). In May and June 2014, the mean relative humidity (RH) was 72 and 68%, respectively, and in May and June 2015, they were similar (70%).

Considering the meteorological data during flowering in both investigated growing seasons, the weather conditions for FHB development on wheat spikes were more favourable in 2014 than in 2015. FHBI, FDK, and DON were significantly higher by 1.6, 2.4, and 1.7 times, respectively, in 2014, while SW, KWS, and 100-kernel weight were significantly lower by 1.2, 3.2, and 2.1 times, respectively, in 2014 than in 2015 (Tables 2 and 3). In similar studies in Serbia, *Jevtić et al.* (2021, 2022) reported higher FHBI, FDK, and yield losses in 2014 than in 2015, emphasizing to some extent the influence of climatic factors during wheat anthesis in May. According to the results of *Cowger et al.* (2009), prolonged fog in

the post-flowering period affected FHB severity, FDK, and DON in winter wheat. *Kriss et al. (2010)* also found that year-to-year variation in FHB intensity was related to environmental conditions (moisture and wetness), especially two months before wheat maturity and indicated a strong influence of wheat cultivar resistance, fungicide control, and agronomic practices such as crop rotation and tillage type on FHB epidemics, yield loss, and DON contamination. In this study, levels of DON were higher in treatments with higher FHBI and FDK, which is in agreement with reports by *Jones and Mirocha (1999)*, *Jones (2000)*, *Wegulo et al. (2011)*, and *Mesterházy et al. (2003, 2011, 2015)*. In treatments of years and cultivars, DON levels exceeded the maximum level (MPL) prescribed by the European Commission (1881/2006/ EC) for unprocessed cereals, except for durum wheat, oats, and maize ($1,250 \text{ mg kg}^{-1}$) (Table 2), consistent with previous reports by *Krnjaja et al. (2015)*.

Although resistant cultivars are critical for reducing FHB and DON contamination of wheat, most commercial cultivars are susceptible to FHB in practice. In this study, the mid-late cultivar NS 40S showed higher susceptibility to *Fusarium* and DON contamination than the mid-early cultivar Simonida. *Jevtić et al. (2021)* identified sets of moderately resistant cultivars with FHBI of 25% or less and moderately susceptible or susceptible cultivars with FHBI of 26 to 54% based on cultivar response to FHB in 2014. Since FHBI was 20.47% (Simonida) and 24.28% (NS 40S), both wheat cultivars studied could be classified as moderately resistant.

In correlations between disease variables (FHBI, FDK, DON), FHBI and FDK, FHBI and DON, and FDK and DON were significantly positively correlated (Table 4). According to the reports of *Paul et al. (2005)* and *Wegulo et al. (2011)*, the correlation coefficient between FDK and DON and FHBI and DON was above 0.50, while in this study, it was between FHBI and FDK, FHBI and DON, and FDK and DON. *Jevtić et al. (2021)* found a higher correlation coefficient between FHBI and FDK in moderately resistant wheat cultivars ($r = 0.732$) than in susceptible/moderately susceptible wheat cultivars ($r = 0.362$). *Mesterházy et al. (2015)* reported that FHB levels had a lower effect on DON contamination than FDK in 40 winter wheat cultivars from Hungary (20) and Austria (20). There were also significant negative correlations between yield component traits (SW, KWS, 100-kernel weight) with FHB variables (FHBI and FDK) and DON values (Table 4). These results were in line with those of *Wegulo et al. (2011)*. In addition, using statistical analysis with multiple stepwise regression, *Jevtić et al. (2022)* found a more significant influence of FDK than FHBI on thousand kernel weight (TKW) in moderately resistant cultivars, while both FHBI traits, FHBI and FDK, influenced TKW in moderately susceptible and susceptible cultivars.

Conclusion

It can be concluded that in this study, growing season and cultivar had a significant effect on both disease and yield variables in winter wheat. Contamination of wheat kernels with *Fusarium* and deoxynivalenol was higher in the wetter year of 2014 than in 2015, while yield component traits (SW, KWS and 100-kernel weight) were lower in 2014 than in 2015. The mid-early wheat cultivar Simonida showed lower susceptibility to FHB and DON than the mid-late cultivar NS 40S.

These results indicate the need to improve breeding programs by creating wheat cultivars from an earlier maturing group. Since environmental conditions in Serbia are very convenient for FHB development and DON accumulation in wheat crops, cultivar selection should be recommended as a primary agricultural measure to achieve high and healthy wheat yields.

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