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ОДЕЉЕЊЕ ЗА ПРИРОДНЕ НАУКЕ  
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## CONTAMINATION OF DURUM WHEAT LINES KERNELS WITH *Fusarium* SPECIES AND DEOXYNIVALENOL

**ABSTRACT:** *Fusarium* infection and deoxynivalenol (DON) contamination in seven durum wheat lines kernel (six domestic durum lines ZP 16, ZP 34, ZP 41, ZP 74, ZP 120, ZP DSP 66, and one international durum line Cimmyt 7817) during two harvest seasons (2015–2016) has been studied. The four *Fusarium* species, *F. graminearum*, *F. proliferatum*, *F. sporotrichioides*, and *F. verticillioides*, were identified in 2015. A different structure of the *Fusarium* population, which in addition to *F. graminearum*, *F. sporotrichioides* and *F. verticillioides*, also comprised *F. poae*, *F. semitectum*, and *F. subglutinans*, was identified in 2016. *F. graminearum* was the predominant species in the durum wheat lines kernels and the potential producer of DON. The other *Fusarium* spp. were isolated sporadically and with a low incidence in the kernels. The incidence of *F. graminearum* and DON levels were significantly affected by the wheat genotypes and studied years and these parameters were negatively correlated. The incidence of *F. graminearum* was significantly higher in 2015 (75.86%) than in 2016 (63.43%), while the level of DON was significantly higher in 2016 (3.636 mg kg<sup>-1</sup>) compared to 2015 (1.126 mg kg<sup>-1</sup>). Statistically, there was a significantly higher incidence of *F. graminearum* in ZP DSP 66 (73.00%) and ZP 120 (72.75%) durum wheat lines than in the other durum genotypes. DON level was the highest in durum wheat line ZP 120 (3.854 mg kg<sup>-1</sup>). Considering all treatments tested, the mean DON level was 2.381 mg kg<sup>-1</sup>, while the mean incidence of *F. graminearum* was 69.64%. Tested durum wheat lines showed susceptibility to *F. graminearum*, resulting in high DON levels in kernels. The results obtained suggest the importance of using the lines with improved resistance to *Fusarium* head blight in the breeding programs for new durum wheat cultivars.

**KEYWORDS:** *Fusarium* spp., deoxynivalenol, durum wheat lines

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## INTRODUCTION

In Europe, durum wheat (*Triticum turgidum* var. *durum*) is grown on 3 million hectares, mainly in the Mediterranean countries (Italy, Greece, Spain, and France), with an average yield of around 5 t/ha to 6 t/ha. Other European countries have low productivity and quality of durum wheat crops, and they intend to improve breeding processes by focusing on increasing yield and quality features (Gorczyca et al., 2018). In Serbia, an average yield of 2.2 t/ha was reported, which is 30% of the total average yield of bread wheat (*Triticum aestivum* L.) (Đurić et al., 2019).

Fungal pathogens from the genus *Fusarium* cause Fusarium head blight (FHB), one of the most severe diseases of wheat worldwide. Several *Fusarium* spp. participate in pathogenesis FHB of which *F. graminearum* is the most predominant. *F. graminearum* is a member of the *F. graminearum* species complex (FGSC) also called *F. graminearum sensu lato*, which includes at least 16 phylogenetic distinct species (van der Lee et al., 2015). It is a monophyletic species complex in which species are localized in different geographical regions, for example, *F. asiaticum* is responsible for FHB in some parts of Asia. However, the term *F. graminearum sensu stricto* is retained for the species most commonly associated with FHB worldwide (Tóth et al., 2005). In Serbia, besides *F. graminearum* strains, one strain of *F. vorosii* was isolated as a member of FGSC and showed pathogenicity on wheat spikes (Obradović et al., 2022). In general, *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. sporotrichioides*, *F. langsethiae*, and *F. poae* are the most common FHB pathogens (Pancaldi et al., 2010; Karlsson et al., 2021). *F. graminearum* and *F. poae* appear to cause FHB in humid, warm and dry environments, while *F. culmorum*, *F. avenaceum*, *F. sporotrichioides*, and *F. langsethiae* are mostly found in cool and wet agroecological conditions. The distribution of FHB is affected by both the genetic diversity of the pathogens and environmental factors (Nielsen et al., 2011; Zhang et al., 2011).

*F. graminearum* overwinters as a saprobe on the crop residues. The primary inoculum consists of ascospores and macroconidia. Ascospores (sexual spores) develop from perithecia during the flowering wheat stage. Macroconidia (asexual spores) develop from mycelia on the plant infected with *F. graminearum* and can be water splashed onto the adjacent plants and their developing spikes. In general, the most susceptible wheat stages for *Fusarium* infection are from anthesis to soft dough phenophase. Durum wheat is extremely susceptible to FHB and it is very difficult to make resistant cultivars by breeding because of the lack of resistance sources. On the contrary, in bread wheat, there is a range of resistance sources from “exotic” and “native” wheat germplasms and large genetic variation for FHB resistance (Haile et al., 2019).

Direct economic losses of FHB disease are reduced yields and quality of wheat kernels while indirect ones are the consequence of mycotoxin contamination. *F. graminearum* produces trichothecene mycotoxins (the most important are type A and type B) and estrogenic mycotoxin zearalenone. Trichothecenes are sesquiterpenoids that inhibit eukaryotic protein synthesis and cause



numerous human and animal mycotoxicoses. They also exhibit phytotoxicity virulence on sensitive hosts, causing necrosis and proliferation of the pathogen (Starkey et al., 2007; Valverde-Bogantes et al., 2020). The most important B-trichothecenes include deoxynivalenol (DON) and its derivatives [3-acetyl and 15-acetyl deoxynivalenol (3-ADON and 15-ADON)], nivalenol (NIV) and NIV derivative (4-acetyl-nivalenol). *F. graminearum* strains are also capable of producing type A trichothecenes named NX-2. It is a separate population of *F. graminearum*, named NA3 (Valverde-Bogantes et al., 2020), while NA1 and NA2 populations produce 15-ADON and 3-ADON, respectively (Foroud et al., 2019).

In favourable climatic conditions (precipitation and temperature), particular during wheat anthesis, *Fusarium* species can develop and cause FHB. The infection of kernels primarily depends on wheater conditions and the wheat genotype. There are preventive measures to limit FHB development such as agricultural, chemical, and biological control and choice of resistant cultivars. Durum wheat is more susceptible to FHB and DON accumulation than common wheat. Thus, resistance to FHB also includes resistance to DON (Scala et al., 2016). The best timing for fungicide application during the flowering wheat stage has a crucial role in reducing FHB and DON contamination in conducive climatic conditions (Balducci et al., 2022).

*Fusarium* infection and deoxynivalenol contamination of wheat are the two main constraints for wheat safety worldwide. *Fusarium* species as causers of FHB represent the most dangerous wheat pathogens. Consequently, there is a high risk of DON accumulation in wheat production. Since there is almost no data about natural *Fusarium* and deoxynivalenol occurrence in durum wheat kernels in Serbia, the main object of this study was to determine the spectrum and incidence of *Fusarium* spp. and DON levels on kernels in domestic and international durum wheat lines during two harvest seasons (2015–2016). Data for weather conditions in the years of the study were also analyzed because they have a significant role in *Fusarium* infection and DON production.

## MATERIALS AND METHODS

Samples of wheat kernels from six domestic lines (ZP 16, ZP 34, ZP 41, ZP 74, ZP 120, ZP DSP 66) and one international line (Cimmyt 7817) of durum wheat were collected during the harvesting period in 2015 and 2016. The sample size of each line was about 1 kg. Prior to mycological analyses, samples were kept at 4 °C. For determining the moisture content, kernels were first milled in an analytic mill (IKA A11, Germany) and then examined on a moisture analyzer (OHAUS MB35, USA).

In the mycological examination, sub-samples of 200 kernels were disinfected in 1% sodium hypochlorite solution (NaOCl) for about 3–5 min and rinsed twice in sterile water. After drying on sterile filter paper, kernels were plated on Ø90 mm Petri plates (10 kernels per Petri plate) that contained potato dextrose agar with 1.8% salt (18 g NaCl in a 1-litre agar medium) and incubated

for seven days or longer at room temperature. Each sample was done in three replicates. The species of fungi were identified using mycological keys by Leslie and Summerell (2006) and Watanabe et al. (2002). Kernel infection by single fungal species was calculated as the ratio of the number of kernels from which this species was isolated to the total number of analysed kernels, expressed as a percentage of incidence. The base index (BI) was calculated using the formula:  $BI (\%) = Y_i / Y_o \times 100$ , where  $Y_o$  is the lowest incidence of *F. graminearum*, i.e. the lowest level of DON, and  $Y_i$  is the incidence of *F. graminearum*, i.e. level of DON.

The mycotoxicological examinations were performed using the Enzyme-Linked Immunosorbent Assay (ELISA). Before analyses, durum wheat kernel samples were dried for 72 h at 60 °C and then milled in an analytic mill (IKA A11, Germany). By an assay procedure of the ELISA test kit *Celer* DON (Tecna, Italy), the quantitative detection of DON in samples was determined on the ELISA reader (Biotek EL x 800TM, USA) at a wavelength of 450 nm. Each sample was assayed in three replicates. The limit of detection for DON in cereals (durum wheat) was 0.12 mg kg<sup>-1</sup>.

Statistical analysis of variance (ANOVA) (IBM SPSS Statistic 20) was used for testing variables. Tukey's test was used to compare means at a significance level of 5%. Correlation analyses were performed by Pearson's test.

Data for weather conditions (total monthly rainfall and mean monthly temperature) in the years of study were obtained by the Republic Hydrometeorological Service of Serbia for the Belgrade-Surčin area.

## RESULTS

Data for weather conditions during vegetation in 2015 and 2016 are shown in Figure 1. The year 2016 was wetter with total rainfall of 481.2 mm in the period from January to July related to 2015 (362.3 mm). Total rainfall and mean temperatures were favourable for *Fusarium* infection during flowering wheat stages in May, but these factors were higher in May 2015 (90.7 mm and 18.5 °C) than in May 2016 (63.4 mm and 17 °C). However, during the maturity stage, in June 2016 total rainfall was very high (156 mm), five and a half times higher than in June 2015 (28.3 mm). In July, the harvest period was also wetter in 2016 (34.7 mm) than in July 2015 (6.5 mm). In both years, the mean temperatures in June and July were above 20 °C.

The mean moisture content of tested durum wheat kernel samples at harvest was 10.44% in 2015 and 14.43% in 2016. By mycological examinations, four *Fusarium* species, *F. graminearum*, *F. semitectum*, *F. sporotrichioides*, and *F. verticillioides* were isolated in 2015. The six *Fusarium* species, *F. graminearum*, *F. poae*, *F. semitectum*, *F. sporotrichioides*, *F. subglutinans*, and *F. verticillioides* were identified in 2016. In both years, the incidence of *F. graminearum* was the highest. Other *Fusarium* spp. were isolated sporadically with low incidence in the kernels. Among other fungi, species from the genera

*Alternaria*, *Aspergillus*, *Chaetomium*, *Epicoccum*, *Nigrospora*, and *Penicillium* have also been identified, the most frequent being *Alternaria* spp. (data not presented).

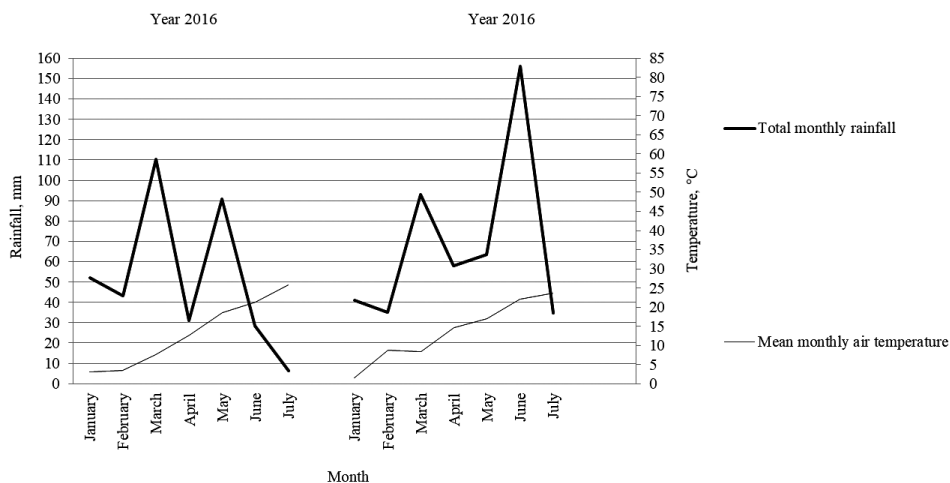


Figure 1. Total monthly rainfall (mm) and mean monthly temperature (°C) during the period January–July in 2015 and 2016 (Belgrade-Surčin area).

The significance of the year and durum wheat line effect on the incidence of *F. graminearum* and DON levels are shown in Table 1 along with the base index values. There was a significant ( $P \leq 0.01$ ) effect of year and durum wheat lines on the incidence of *F. graminearum* and levels of DON. The incidence of *F. graminearum* was significantly higher in 2015 (75.86%) than in 2016 (63.43%) being an increase of 16.39% expressed by the base index. There were no significant differences between durum wheat lines ZP 120 (72.75%) and ZP DSP 66 (73%) and they had a significantly higher incidence of *F. graminearum* than the other studied lines. Line ZP 34 had a 13.18% and 12.79% lower incidence of *F. graminearum* compared to ZP DSP 66 and ZP 120, respectively. On average, in all treatments tested, the incidence of *F. graminearum* was 69.64%. The level of DON was significantly higher in 2016 ( $3.636 \text{ mg kg}^{-1}$ ) compared to 2015 ( $1.126 \text{ mg kg}^{-1}$ ). The ZP 120 line had the highest ( $3.854 \text{ mg kg}^{-1}$ ) and ZP 41 line had the lowest DON level ( $1.658 \text{ mg kg}^{-1}$ ) being an increase of 132.45% expressed by the base index. Among the ZP 34, ZP 41, ZP 74, and ZP DSP 66 lines were no significant differences in DON level. On average, in all treatments tested, the mean DON level was  $2.381 \text{ mg kg}^{-1}$  and was above the maximum limit of  $1.750 \text{ mg kg}^{-1}$  for unprocessed durum wheat prescribed by the European Regulation 1881/2006/EC. A statistically significant ( $P \leq 0.05$ ) negative correlation was determined between the incidence of *F. graminearum* and DON level ( $r = -0.31$ ).

Table 1. Effect of year and wheat durum line on the incidence of *F. graminearum* and DON level

Factor	Incidence of <i>F. graminearum</i> (%)	Index (%)	DON (mg kg <sup>-1</sup> )	Index (%)
Year effect (Y)				
2015	75.86 <sup>a</sup>	83.61	1.126 <sup>b</sup>	100
2016	63.43 <sup>b</sup>	100	3.636 <sup>a</sup>	322.91
F-test	**	-	**	-
Durum wheat lines effects (DWL)				
ZP 16	69.25 <sup>b</sup>	107.36	2.484 <sup>b</sup>	149.82
ZP 34	64.50 <sup>b</sup>	100	1.784 <sup>d</sup>	107.60
ZP 41	72 <sup>b</sup>	111.63	1.658 <sup>d</sup>	100
ZP 74	67.75 <sup>b</sup>	105.04	2.255 <sup>d</sup>	136
ZP 120	72.75 <sup>a</sup>	112.79	3.854 <sup>a</sup>	232.45
ZP DSP 66	73.00 <sup>a</sup>	113.18	2.141 <sup>d</sup>	129.13
Cimmyt 7817	68.25 <sup>b</sup>	105.81	2.491 <sup>c</sup>	150.24
F-test	*	-	**	-
Interactions (F test)				
Y×DWL	**	-	**	-
Means	69.64	-	2.381	-

Means followed by the same letter within a column are not significantly different by the Tukey's test at the  $P \leq 0.05$  level.

ns, not significant; \*significant at the 0.05 level of probability; \*\*significant at the 0.01 level of probability.

## DISCUSSION

This study represents the first report on the occurrence and contamination of durum wheat with *Fusarium* spp. and DON in Serbia during the two harvesting periods (2015–2016). FHB populations of four (*F. graminearum*, *F. semitectum*, *F. sporotrichioides*, and *F. verticillioides*) and six *Fusarium* spp. (*F. graminearum*, *F. poae*, *F. semitectum*, *F. sporotrichioides*, *F. subglutinans*, and *F. verticillioides*) were identified in 2015 and 2016, respectively, with *F. graminearum* as the most prevalent species. This is in agreement with the results of a survey of the main species of the FHB complex on durum wheat kernels in Italy and Poland, in which *F. graminearum* was predominant (Shah et al., 2005; Pancaldi et al., 2010; Covarelli et al., 2015; Gorczyca et al., 2018). In Serbia, according to the previous examination of mycobiota on the bread wheat kernels, *F. graminearum* was also the most isolated while *F. arthrosporioides*, *F. avenaceum*, *F. equiseti*, *F. oxysporum*, *F. poae*, *F. proliferatum*, *F. semitectum*, *F. sporotrichioides*, *F. subglutinans*, *F. tricinctum* and *F. verticillioides* were sporadically isolated (Stanković et al., 2007; Lević et al., 2008; Krnjaja et al., 2008; 2011a,b; 2014, 2015). Furthermore, Lazzaro et al. (2015) have isolated *F. graminearum* and *F. poae* as the main FHB bread wheat pathogens, where

*F. graminearum* was predominant in organic wheat and *F. poae* in conventional one. However, the dominant incidence of these species changed depending on the vegetation season, which was influenced by differences in weather conditions during the flowering and anthesis stages in wheat (Lazzaro et al., 2015). Other fungi identified in tested durum wheat line kernels were species from *Alternaria*, *Aspergillus*, *Chaetomium*, *Epicoccum*, *Nigrospora*, and *Penicillium* genera. Depending on the applied agro-technical measures, cultivars, agro-ecological conditions of localities where wheat is grown and the examined years, Stanković et al. (2007), Krnjaja et al. (2008, 2011a,b, 2014, 2015), and Beccari et al. (2018) with *Fusarium* species, *Acremoniella* spp., *Acremonium* spp., *Alternaria* spp., *Arthrinium* spp., *Aspergillus* spp., *Bipolaris* spp., *Cladosporium* spp., *Chaetomium* spp., *Dreschlera* spp., *Epicoccum* spp., *Mucor* spp., *Nigrospora* spp., *Penicillium* spp., *Phoma* spp., *Ramichloridium* spp., *Rhizopus* spp., *Stemphylium* spp., and *Trichoderma* spp. were also isolated from bread wheat kernels.

The *Fusarium* spectrum species associated with FHB on wheat kernels are determined by weather conditions, especially during the wheat anthesis stage (Francesconi et al., 2019). Gorczyca et al. (2018) have reported that low water content during some tested growing seasons affects the fewer *Fusarium* spectrum species. We have also isolated fewer *Fusarium* spp. in a drier season in 2015 than in 2016. However, the incidence of *F. graminearum* was significantly higher in 2015 than in 2016. The reason for that could be favourable weather conditions such as the total rainfall and mean air temperatures, which were higher during the flowering wheat stage in May 2015 (90.7 mm and 18.5 °C) than in May 2016 (63.4 mm and 17 °C). In addition, Beccari et al. (2018) have emphasized that the incidence of *F. graminearum* decreased compared to *F. avenaceum* incidence due to unfavourable climatic conditions during the flowering stage for FHB infection. Balmas et al. (2015) have isolated more *F. culmorum* strains than *F. graminearum* strains in the durum wheat kernel samples from Sardinia indicating the importance of the influence of the growing region on the spectrum of *Fusarium* spp. Depending on localities in Italy, Infantino et al. (2012) have reported a low infestation (of below 2%) of bread and durum organic wheat seed with a few *Fusarium* spp. of FHB complex, *F. avenaceum*, *F. graminearum*, *F. poae*, and *F. verticillioides* of which *F. poae* had the most incidence. Considering co-infection by *Fusarium* spp. on wheat, Pancaldi et al. (2010) have also noticed that *F. poae* is becoming more common in the FHB species complex in different European countries.

All tested lines showed susceptibility to *F. graminearum*, with ZP 120 and ZP DSP 66 having the highest incidence. It was in agreement with the results of Haidukowski et al. (2005), Covarelli et al. (2015), and Gorczyca et al. (2018), reporting susceptibility of different durum wheat cultivars to *Fusarium* spp. of FHB complex. Research results by Bentivenga et al. (2021), from 35 Italian durum wheat cultivars showed that only three cultivars were evaluated as moderately susceptible to FHB, while almost all of the other cultivars were susceptible or very susceptible. FHB resistance is a complex genetic trait controlled by multiple genes and depends on environmental conditions, which leads to the

conclusion that there are almost no completely resistant durum wheat cultivars to FHB. Besides, there are not enough breeding programs for durum wheat, probably due to growing in smaller acreage (Haile et al., 2019).

In this study, the year and durum wheat genotype effects were significant on DON levels. DON level was about three times higher in 2016 ( $3.636 \text{ mg kg}^{-1}$ ) than in 2015 ( $1.126 \text{ mg kg}^{-1}$ ), which is an increase of 222.91% expressed by the base index (Table 1). This result can be hypothetically explained by abundant rainfall during June 2016 and no positive correlation between *F. graminearum* incidence and DON levels. The average DON level in all treatments ( $2.381 \text{ mg kg}^{-1}$ ) was above the allowed limit ( $1.750 \text{ mg kg}^{-1}$ ) for unprocessed durum wheat determined by the European Commission (1886/2006/EC). Line ZP 120 had the highest DON level ( $3.854 \text{ mg kg}^{-1}$ ). Similarly, Gorczyca et al. (2018) reported that DON synthesis in durum wheat kernels is affected by both the year and cultivars. Scala et al. (2016) have also found higher DON levels in the growing season with more rainfalls. These authors pointed out that DON levels were significantly affected by year and location but not by the cultivars, with low DON levels in all treatments. Shah et al. (2005) and Covarelli et al. (2015) have established that durum wheat cultivars were more susceptible to FHB and DON than bread wheat cultivars. Similarly, Krnjaja et al. (2015) found that DON levels in bread wheat kernels were significantly affected by the cultivars. Balducci et al. (2022) have established the strongest positive correlation between FHB symptoms and DON levels in durum wheat during the wetter growing season. However, in our study, the relationship between *F. graminearum* incidence and DON level was not positively correlated.

## CONCLUSION

*Fusarium* infection and DON contamination were high primarily due to favourable weather conditions in both studied growing seasons and the susceptibility of durum wheat lines to FHB pathogens. Among *Fusarium* species, *F. graminearum* was the most frequently isolated in the kernel of all tested durum wheat lines. This species was also a predominant FHB pathogen. There was a higher incidence of *F. graminearum* in wheat kernels in 2015 than in 2016. On the contrary, DON levels were higher in 2016 than in 2015. The relationship between the incidence of *F. graminearum* and DON level was negatively correlated. All tested durum wheat lines were susceptible to these contaminants. Obtained results indicate the importance of improving and increasing the number of durum wheat breeding programmes. Besides chemical control, the most important and effective agricultural measure in FHB management is sowing less susceptible or resistant wheat cultivars. Moreover, integral strategies can enhance FHB control in durum wheat, including crop rotation, crop residue management, resistant cultivar, fungicide treatments and forecasting.

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ОРИГИНАЛНИ НАУЧНИ РАД

КОНТАМИНАЦИЈА ЗРНА ЛИНИЈА ДУРУМ ПШЕНИЦЕ СА  
*Fusarium* ВРСТАМА И ДЕОКСИНИВАЛЕНОЛОМ

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РЕЗИМЕ: Проучавана је *Fusarium* инфекција и деоксиниваленол (ДОН) контаминација зрна седам линија дурум пшенице (шест домаћих ZP 16, ZP 34, ZP 41, ZP 74, ZP 120, ZP DSP 66 и једна међународна дурум линија Cimmut 7817) у току две жетвене сезоне (2015–2016). Идентификоване су четири *Fusarium* врсте, *F. graminearum*, *F. proliferatum*, *F. sporotrichioides*, и *F. verticillioides* у 2015. години. Другачија структура *Fusarium* популације била је у 2016. години, јер је поред *F. graminearum*, *F. sporotrichioides*, и *F. verticillioides*, обухватала и врсте *F. poae*, *F. semitectum* и *F. subglutinans*. *F. graminearum* је била најучесталија врста на зрну дурум линија пшенице и потенцијални продуцент ДОН-а. Друге *Fusarium* spp. изоловане су спорадично и у ниској учесталости на зрну испитиваних линија. Учесталост *F. graminearum* и садржај ДОН-а били су под значајним утицајем генотипова дурум пшенице и проучаваних година и ови параметри били су у негативној корелацији. Учесталост *F. graminearum* била је већа (75,86%) у 2015. него у 2016. години (63,43%), док је садржај ДОН-а био виши (3,636 mg kg<sup>-1</sup>) у 2016. у поређењу са 2015. годином (1,126 mg kg<sup>-1</sup>). Утврђена је статистички значајно већа учесталост *F. graminearum* на зрну линија ZP DSP 66 (73,00%) и ZP 120 (72,75%) у односу на друге линије дурум пшенице. Највећи садржај ДОН-а детектован је у зрну линије ZP 120 (3,854 mg kg<sup>-1</sup>). Разматрајући све испитиване третмане, просечан садржај ДОН-а био је 2,381 mg kg<sup>-1</sup>, док је просечна учесталост

*F. graminearum* била 69,64%. Испитиване линије дурум пшенице биле су осетљиве према *F. graminearum* а резултат тога био је и висок садржај ДОН-а у зрну. Добијени резултати указују на значај коришћења линија са добром отпорношћу према фузариози класа у селекционим програмима за стварање нових сорти дурум пшенице.

КЉУЧНЕ РЕЧИ: *Fusarium* spp., деоксиниваленол, дурум линије пшенице



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