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Influence of growing season, nitrogen fertilisation and wheat variety on *Fusarium* infection and mycotoxin production in wheat kernel

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SHORT COMMUNICATION

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ABSTRACT

Fusarium spp. are phytopathogens causing fusarium head blight in wheat. They produce mycotoxins, mainly fumonisins, deoxynivalenol, and zearalenone. The study was conducted during two growing seasons (2020 and 2021) at the experimental field and laboratories of the Hungarian University of Agriculture and Life Sciences (MATE). The aim of the study was to determine the influence of growing season, nitrogen fertilisation, and wheat variety on *Fusarium* infection and mycotoxin production in wheat kernel. Zearalenone was not detected during the two growing seasons and deoxynivalenol was only detected in 2020. The results indicate that nitrogen fertilisation and wheat variety did not have statistically significant influence on *Fusarium* infection and mycotoxin production. The growing season had statistically significant influence on *Fusarium* infection and fumonisins production due to higher rainfall in 2021 compared to 2020 during the flowering period when the wheat spike is the most vulnerable to *Fusarium* infection.

KEYWORDS

Fusarium, mycotoxin, growing season, nitrogen, wheat

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most cultivated crops around the world. It is grown across a wide range of environments. The primary use of wheat is for bread making. In addition, it is used in the production of bakery and confectionery products, animal feed, and ethanol. The genus *Fusarium* is a plant pathogen of wheat. It causes fusarium head blight (FHB), a major fungal disease in wheat production (Sifuentes dos Santos et al., 2013). Initial symptoms of FHB appear on the spike and grain. FHB reduces the quality of the grain and might decrease the yield up to 70%. Wheat is particularly susceptible to FHB infection during the period of anthesis and the early stages of grain development. Diseased grains are shrivelled, discoloured, and light-weight (Goswami and Kistler, 2004). Under favourable conditions, *Fusarium* species can produce mycotoxins, mainly deoxynivalenol (DON), zearalenone (ZEA), and fumonisins (FUM).

The presence of mycotoxins in food and feed can cause chronic or acute mycotoxicosis in animals and humans (Bottalico and Perrone, 2002). Deoxynivalenol (DON), commonly known as vomitoxin, causes food refusal, diarrhea, alimentary haemorrhaging, and contact dermatitis (Bennett and Klich, 2003). Zearalenone (ZEA) has estrogenic effects and reduces the reproductive capability of domestic animals (Biagi, 2009; Stanković et al., 2012). Fumonisins (FUM) are carcinogenic mycotoxins causing hepatotoxicity and apoptosis of the liver. In humans, it is linked with esophageal cancer (Marasas, 2001).

To minimise the risk of FHB and mycotoxins, some preventive measures should be applied to reduce their occurrence. The application of integral wheat protection measures such as cultivation of resistant cultivars, crop rotation, tillage, and application of appropriate fertilisers and fungicides can significantly reduce wheat infection by *Fusarium* species (Lemmens et al., 2004).

2. MATERIALS AND METHODS

The experiment was conducted during two growing seasons (2020 and 2021) at the experimental field and laboratories of the Hungarian University of Agriculture and Life Sciences (MATE), Crop Production Institute, Gödöllő, Hungary. The experimental site is in a hilly area with a close to average climatic zone of the country (47°35′40.8″N 19°22′08.4″E, 210 m above sea level).

The soil type of the experimental field is brown forest soil (Chromic Luvisol). Prior to sowing, the field was cleared, ploughed, rotor-tilled, and the seedbed was prepared. The plots were sown and harvested with plot machines. The trial design was that of a split-plot with main plots consisting of different wheat varieties and subplots consisting of different nitrogen doses. Main plots and subplots were 50 cm apart horizontally and 30 cm apart vertically, and the area of each subplot was 5 m^2. Each treatment had three replications. The wheat varieties used were: Alföld, Mv Kolompos, and Mv Karéj. Nitrogen fertiliser (NH_4NO_3) was applied twice during the growing season, the first application was done at tillering stage and the second application at heading stage. The doses of nitrogen in the first application were: 40, 80, and 120 kg N ha^{-1}. In the second application 40 kg N ha^{-1} was added only. Plots without nitrogen topdressing were used as control. *Fusarium* percentage was calculated by counting the number of colonies that formed on wheat kernels disinfected with a solution of pentachloronitrobenzene (PCNB) and chloramphenicol (100 kernels from each treatment) incubated for 7 days under laboratory conditions on Nash and Snider *Fusarium* selective medium (distilled water 1 L, peptone 15 g, KH_2PO_4 1 g, $MgSO_47H_2O$ 0.5 g, agar 20 g, PCNB 1 g, chloramphenicol 100 ppm). Mycotoxin concentrations of deoxynivalenol (DON), zearalenone (ZEA), and fumonisins (FUM) were analysed using ROSA FAST 5 Quantitative Test by Charm Sciences.

For the statistical evaluation of the results, analysis of variance (ANOVA) module of the IBM SPSS V.21 software at 5% significance level with subsequent Tukey's test was performed to determine the influence of growing season, nitrogen fertilisation and wheat variety on *Fusarium* infection and mycotoxin production in wheat kernel.

3. RESULTS AND DISCUSSION

The study of the influence of growing season, wheat variety, and nitrogen fertilisation on *Fusarium* infection and subsequent mycotoxin production in wheat kernel was carried out in 2020 and 2021. The growing season significantly affected *Fusarium* infection (F = 187.31, P = 0.000) and fumonisins concentration (F = 4.7, P = 0.03) but did not significantly affect deoxynivalenol concentration (F = 3.61, P = 0.06) (Figs 1 and 2, Table 2). *Fusarium* infection was higher in 2021 (93.1%) than in 2020 (46.9%) (Fig. 1). Zearalenone was not detected throughout the two growing seasons. Fumonisins concentration (total mean = 22.2 ppb) was higher than that of deoxynivalenol (total mean = 15.97 ppb) (Table 1). Deoxynivalenol was not detected in 2021, its concentration was 31.9 ppb in 2020 (Fig. 2). Fumonisins concentration was higher in 2021 (30.6 ppb) than in 2020 (13.9 ppb) (Fig. 2). Rainfall (mm) measurements were collected from the Hungarian National Meteorological Service during the flowering period (May) when wheat is most susceptible to *Fusarium* infection. Rainfall during the flowering period (May) in 2021 was 123.1 mm, higher than in 2020 (39.8 mm), this increase in rainfall could explain the increased *Fusarium* percentage and fumonisins concentration.

The wheat variety did not significantly affect *Fusarium* infection (F = 0.63, P = 0.54) and subsequent mycotoxin production (DON, F = 0.32, P = 0.73; FUM, F = 1.94 P = 0.15) (Table 2).

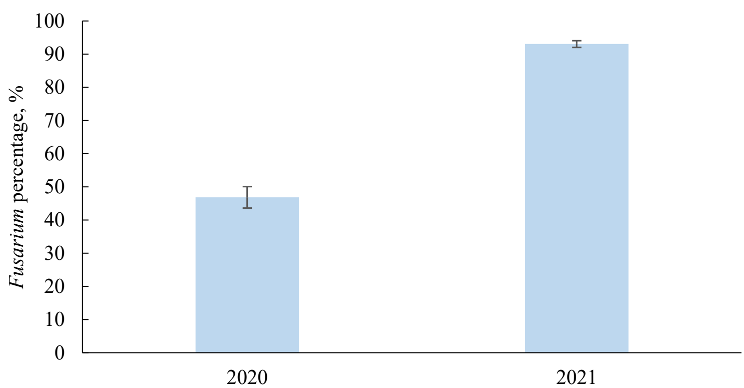

Fig. 1. Effect of growing season on *Fusarium* percentage (%)

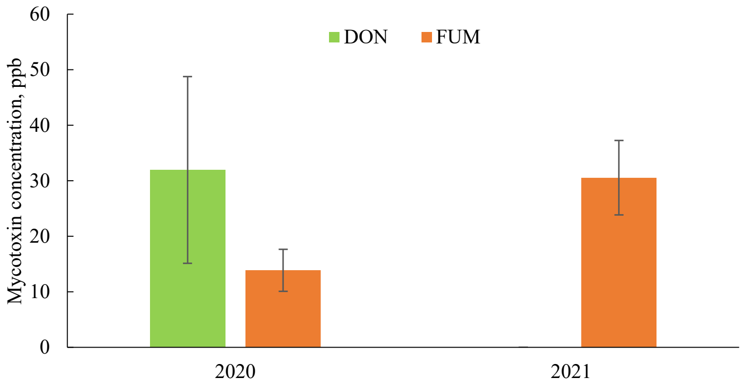

Fig. 2. Effect of growing season on mycotoxin concentration (ppb)

Table 1. Descriptive statistics of *Fusarium* percentage (%), DON and FUM concentration (ppb) affected by growing season, wheat variety, and nitrogen dosage (kg N ha^{-1})

			Mean	Std. deviation	Std. error	Minimum	Maximum
Growing season	*Fusarium*	2020	46.86	19.31	3.22	12	90
		2021	93.06	6.11	1.02	76	100
		Total	69.96	27.26	3.21	12	100
	DON	2020	31.94	100.82	16.8	0	500
		2021	0	0	0	0	0
		Total	15.97	72.59	8.55	0	500
	FUM	2020	13.89	22.71	3.79	0	50
		2021	30.56	40.14	6.69	0	200
		Total	22.22	33.45	3.94	0	200
Wheat variety	*Fusarium*	Alföld	64.88	30.27	6.18	22	100
		Kolompos	72.00	25.19	5.14	22	100
		Karéj	73.00	26.50	5.41	12	100
		Total	69.96	27.26	3.21	12	100
	DON	Alföld	25.00	103.21	21.07	0	500
		Kolompos	8.33	40.82	8.33	0	200
		Karéj	14.58	61.64	12.58	0	300
		Total	15.97	72.59	8.55	0	500
	FUM	Alföld	22.92	25.45	5.19	0	50
		Kolompos	12.50	22.12	4.51	0	50
		Karéj	31.25	46.19	9.43	0	200
		Total	22.22	33.45	3.94	0	200
Nitrogen dosage	*Fusarium*	0	69.39	28.17	6.64	12	100
		40+40	70.33	29.21	6.89	22	100
		80+40	66.44	28.54	6.73	22	100
		120+40	73.67	24.79	5.84	24	96
		Total	69.96	27.26	3.21	12	100
	DON	0	27.78	117.85	27.78	0	500

(continued)

Table 1. Continued

			Mean	Std. deviation	Std. error	Minimum	Maximum
		40+40	16.67	70.71	16.67	0	300
		80+40	16.67	51.45	12.13	0	200
		120+40	2.78	11.79	2.78	0	50
		Total	15.97	72.59	8.55	0	500
	FUM	0	16.67	29.70	7.00	0	100
		40+40	19.44	25.08	5.91	0	50
		80+40	33.33	48.51	11.43	0	200
		120+40	19.44	25.08	5.91	0	50
		Total	22.22	33.45	3.94	0	200

0: no nitrogen application.
40 + 40: the first nitrogen application was 40 kg N ha^{-1} and the second was 40 kg N ha^{-1}.
80 + 40: the first nitrogen application was 80 kg N ha^{-1} and the second was 40 kg N ha^{-1}.
120 + 40: the first nitrogen application was 120 kg N ha^{-1} and the second was 40 kg N ha^{-1}.

Table 2. Analysis of variance for *Fusarium* percentage and DON, FUM concentrations affected by growing season, wheat variety, and nitrogen dosage

		Source of variation	Sum of Squares	df	Mean Square	*F*	Sig.
Growing season	*Fusarium*	Between Groups	38410.68	1	38410.68	187.31	0.00
		Within Groups	14354.19	70	205.06		
		Total	52764.88	71			
	DON	Between Groups	18368.06	1	18368.06	3.61	0.06
		Within Groups	355763.89	70	5082.34		
		Total	374131.94	71			
	FUM	Between Groups	5000.00	1	5000.00	4.7	0.03
		Within Groups	74444.44	70	1063.49		
		Total	79444.44	71			
Wheat variety	*Fusarium*	Between Groups	942.250	2	471.13	0.63	0.54
		Within Groups	51822.625	69	751.05		
		Total	52764.875	71			
	DON	Between Groups	3402.778	2	1701.39	0.32	0.73
		Within Groups	370729.167	69	5372.89		
		Total	374131.944	71			
	FUM	Between Groups	4236.111	2	2118.06	1.94	0.15
		Within Groups	75208.333	69	1089.98		
		Total	79444.444	71			
Nitrogen dosage	*Fusarium*	Between Groups	478.15	3	159.38	0.21	0.89
		Within Groups	52286.72	68	768.92		
		Total	52764.88	71			
	DON	Between Groups	5659.72	3	1886.57	0.35	0.79
		Within Groups	368472.22	68	5418.71		
		Total	374131.94	71			
	FUM	Between Groups	3055.56	3	1018.52	0.91	0.44
		Within Groups	76388.89	68	1123.37		
		Total	79444.44	71			

df: degree of freedom; Sig.: significance; Significance level = $P < 0.05$.

The nitrogen fertilisation did not significantly affect *Fusarium* infection ($F = 0.21$, $P = 0.89$) and subsequent mycotoxin production (DON, $F = 0.35$, $P = 0.79$; FUM, $F = 0.91$, $P = 0.44$) (Table 2).

In our study, the different climatic conditions that prevailed during 2020/2021 could be the reason for the increase in *Fusarium* percentage and fumonisins concentration. According to Brennan et al. (2003), the development of FHB in wheat depends on rainfall. Bryła et al. (2016) also stated that the development, growth, and spread of *Fusarium* fungi and the degree of infection strongly depend on rainfall. Mesterházy et al. (1999) pointed out that climatic conditions may play an important role in *Fusarium* infection of wheat. González et al. (2008) suggested that the relatively high level of natural *Fusarium* contamination could be due to a high rainfall period that occurred during the flowering stage. Risk of FHB in wheat plants depends also on genetically determined resistance of the given wheat cultivar to *Fusarium* spp. (Zhang et al., 2008). According to these authors, the main factors affecting *Fusarium* contamination of wheat were weather conditions and susceptibility of wheat cultivars to *Fusarium* spp.

In our study, nitrogen dosage did not influence *Fusarium* contamination and mycotoxin production. Krnjaja et al. (2015) found that nitrogen fertilisation did not increase FHB intensity. Kuzdraliński et al. (2014) reported that the rate of autumn N fertilisation did not affect the number of *Fusarium* detections.

Bernhoft et al. (2012) concluded that farming system (organic versus conventional) impacted *Fusarium* infestation, and that organic management tended to reduce *Fusarium* contamination and mycotoxins. However, *Fusarium* infestation and mycotoxin concentrations may be influenced by a range of factors such as local topography and local climate. Oldenburg et al. (2007) concluded that nitrogen rates of up to 240 kg N ha^{-1} did not influence *Fusarium* growth and their production of mycotoxins in wheat grains. According to Parry et al. (1995), the impact of nitrogen fertilisation on *Fusarium* infestation remains unclear. Moreover, Aufhammer et al. (2000) concluded that nitrogen fertilisation did not stimulate *Fusarium* infection and mycotoxin production. In addition, Martin et al. (1991) observed that nitrogen rates increasing from 70 to 170 kg N ha^{-1} significantly increased the occurrence of *Fusarium* infected grains in wheat. According to Lemmens et al. (2004), increasing nitrogen fertilisation rates up to 80 kg N ha^{-1} significantly affected *Fusarium* infection and subsequent mycotoxin contamination in wheat. However, Lemmens et al. (2004) concluded that the occurrence of *Fusarium* spp. could not be solely based on the nitrogen input in crop production. All these results suggest that the effect of nitrogen fertilisation can only partially influence the creation of favourable conditions for the occurrence of *Fusarium* spp.

4. CONCLUSIONS

Growing season, nitrogen fertilisation and wheat variety were studied to evaluate *Fusarium* infection and mycotoxin production in wheat kernel. The results indicate that nitrogen fertilisation and wheat variety did not show statistically significant influence on *Fusarium* infection and mycotoxin production. The growing season showed statistically significant influence on *Fusarium* infection and fumonisins production due to higher rainfall in 2021 compared to 2020 during the flowering period when the wheat spike is the most vulnerable to *Fusarium* infection.

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