


# *Fusarium* head blight in wheat: Impact of growing season, wheat variety and nitrogen fertilization under natural infection

Elias El Chami\* , Josepha El Chami, Ákos Tarnawa, Katalin Mária Kassai, Zoltán Kende and Márton Jolánkai

Agronomy Institute, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

## RESEARCH ARTICLE

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## ABSTRACT

The fungal genus *Fusarium* encompasses a diverse group of species responsible for synthesizing mycotoxins, particularly deoxynivalenol, fumonisin, and zearalenone and inducing *Fusarium* head blight in wheat. The research was undertaken over a period of two consecutive growing seasons (2020 and 2021) on the premises and facilities of the Hungarian University of Agriculture and Life Sciences (MATE). The objective of this study was to investigate the impact of growing season, nitrogen fertilization, and wheat variety on *Fusarium* infection as well as mycotoxin contamination in wheat kernel. Zearalenone was absent throughout the course of the two growing seasons, whereas deoxynivalenol was found solely in 2020. The findings demonstrate that nitrogen fertilization failed to exhibit a statistically significant impact on both *Fusarium* infection and mycotoxin production. The impact of wheat variety on *Fusarium* infection and deoxynivalenol was not found to be statistically significant. However, it exerted a significant effect on fumonisin production. The growing season exerted a statistically significant impact on the incidence of *Fusarium* infection and the ensuing contamination with mycotoxins, attributable to augmented precipitation levels in 2021 compared to 2020, specifically during the flowering period when the spike of wheat is highly susceptible to *Fusarium* infection.

## KEYWORDS

*Fusarium*, mycotoxin, growing season, nitrogen, wheat

\* Corresponding author. E-mail: El.Chami.Elias@phd.uni-mate.hu, elias-elchami@outlook.com

## INTRODUCTION

*Triticum aestivum* L., commonly known as wheat, holds paramount significance for human nutrition by functioning as a principal source of nutrients. Wheat is mainly used to produce bread, bakery, and confectionery products such as cakes, noodles, pasta, and biscuits. Furthermore, it is used in the production of animal feed, biofuel, and ethanol. The annual global wheat output for 2021 and 2022 was estimated at 775.1 million and 778 million metric tons respectively. *Fusarium* head blight (FHB), caused by *Fusarium* species, is among the leading fungal infections that significantly impact wheat production, jeopardize human and animal health, and pose a substantial risk to the global economy (Gilbert and Tekauz, 2000; Lori et al., 2003; Osborne and Stein, 2007). FHB diminishes the yield and the quality of the wheat harvest due to the production of lightweight, shriveled, and discolored wheat kernels (Molinié et al., 2005). The *Fusarium* species predominantly associated with *Fusarium* head blight (FHB) in wheat all over Europe are *Fusarium graminearum*, *Fusarium avenaceum* and *Fusarium culmorum* and to a lesser extent *Fusarium verticillioides* and *Fusarium proliferatum* (Bottalico and Perrone, 2002). Annually, 25%–50% of the crops harvested globally are found to be infected with mycotoxins (Ricciardi et al., 2013). Mycotoxins are secondary metabolites synthesized by fungi, especially *Fusarium* spp., that cause acute and chronic toxic effects leading to diseases and even mortality (Logrieco et al., 2003; Domijan et al., 2005; Ferrigo et al., 2016). The most frequently encountered *Fusarium* mycotoxins in Europe are deoxynivalenol and zearalenone produced by *F. graminearum* and *F. culmorum*, with the former more common in southern (warmer) and the latter in northern (colder) European areas. Deoxynivalenol, also known as vomitoxin, has been identified as the causative agent of feed refusal in animals (Miller et al., 2001). Zearalenone is an estrogenic mycotoxin that affects the endocrine and the reproductive system of human and animal (Hagler et al., 2001). In addition, it is interesting to note that the occurrence of fumonisin, produced by *F. verticillioides* and *F. proliferatum*, is more common nowadays in wheat even though it used to be limited to maize (Infantino et al., 2001). Fumonisin is a carcinogenic mycotoxin linked with the incidence of esophageal and liver cancer in human and animal (Marin et al., 2013). Thus, the presence of these mycotoxins raises concerns about the safety of wheat products for human and animal consumption (Bennett and Klich, 2003). Therefore, to protect human and animal health, countries have continuously monitored the maximum levels of mycotoxins in foods and other commodities. FHB can be limited by measures that reduce and prevent the spread of *F. spp.*, such as crop rotation, weed control, biological control, and the use of tolerant or resistant varieties (Magan and Aldred, 2007). The present study was designed to investigate the impact of three variables, namely, growing season, nitrogen fertilization, and wheat variety, on *Fusarium* infection and mycotoxin production in wheat kernel.

## MATERIAL AND METHODS

The experiment was carried out during the 2020 and 2021 growing seasons at the experimental field and research facilities of the Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő, Hungary. The experimental field is in a hilly area (47°35'42.5"N 19°22'10.7"E, 210 m above sea level) with a brown forest (Chromic Luvisol) soil type and a climate close to the average of the country. The experimental field was cleaned up, plowed, rototilled, and the seedbed was



made before sowing. Plot machines were used to sow and harvest the plots. The rate of sowing was 450–500 seeds per square meter. Weeds were controlled by herbicides and wheat pests were controlled by pesticides. A split-plot design was used for the experiment with the main plots consisting of several wheat cultivars and the subplots consisting of several nitrogen doses. The area of each subplot was 5 m<sup>2</sup>, the main plots and subplots were spaced 50 cm horizontally and 30 cm vertically apart. Three replications of each treatment were made. The wheat cultivars used were Mv Kolompos, Mv Karěj and Alföld. Nitrogen fertilizer was applied in the form of granular ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) with 34% content of the active ingredient. Nitrogen fertilizer was applied once during the month of April of each growing season in the following doses: 40, 80, and 120 kg N ha<sup>-1</sup>. Nitrogen free plots were used as control. The calculation of the *Fusarium* infection level was done by counting the number of colonies that developed on 100 wheat grains from each treatment disinfected for 2 min with a solution of pentachloronitrobenzene (PCNB) and chloramphenicol (distilled water 1 L, PCNB 1 g, chloramphenicol 100 ppm) and then incubated for 7 days under laboratory conditions (23 °C ± 0.6 °C and 45% RH ± 5% RH) on Nash and Snider *Fusarium* selective media (distilled water 1 L, peptone 15 g, KH<sub>2</sub>PO<sub>4</sub> 1 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, agar 20 g, PCNB 1 g, chloramphenicol 100 ppm). Deoxynivalenol (DON), zearalenone (ZEA), and fumonisin (FUM) mycotoxin concentrations were examined using Charm Sciences' ROSA FAST 5 Quantitative Test (DONQ-FAST5 Test, FUMQ-FAST5 Test, ZEARQ-FAST5 Test). The analysis of variance (ANOVA) module followed by a Tukey's test of IBM SPSS V.21 software was used to evaluate the results statistically at a 5% significant level.

## RESULTS

The study of the influence of growing season, wheat variety, and nitrogen fertilization on *Fusarium* infection and subsequent mycotoxin production in wheat kernel was carried out in 2020 and 2021.

The growing season significantly affected *Fusarium* infection ( $F = 277.89$ ,  $P = 0.000$ ) and subsequent mycotoxin production (DON,  $F = 7.29$ ,  $P = 0.008$ ; FUM,  $F = 3.81$ ,  $P = 0.05$ ) (Table 6). *Fusarium* infection was higher in 2021 (93.56%) than in 2020 (44.33%) (Fig. 5, Table 5). The presence of zearalenone was not detected during the two consecutive growing seasons. Fumonisin concentration (total mean = 24.44 ppb) was higher than that of deoxynivalenol (total mean = 23.89 ppb). Deoxynivalenol was not detected in 2021, its concentration was 47.78 ppb in 2020. Fumonisin concentration was higher in 2021 (32.22 ppb) than in 2020 (16.67 ppb) (Fig. 6, Table 5).

The wheat variety did not significantly affect *Fusarium* infection ( $F = 0.139$ ,  $P = 0.87$ ) and deoxynivalenol production (DON,  $F = 0.551$ ,  $P = 0.579$ ) but it significantly influenced fumonisin production (FUM,  $F = 4.67$ ,  $P = 0.012$ ) (Figs 3–4, Table 4). Fumonisin was the highest in Mv Kolompos (36.67 ppb) and Alföld (28.33 ppb) and the lowest in Mv Karěj (8.33 ppb) (Fig. 4, Table 3). The presence of zearalenone was not detected during the two consecutive growing seasons. The presence of deoxynivalenol could not be detected during the 2021 growing season.

The nitrogen fertilization did not significantly affect *Fusarium* infection ( $F = 0.181$ ,  $P = 0.948$ ) and subsequent mycotoxin production (DON,  $F = 0.914$ ,  $P = 0.460$ ; FUM,  $F = 1.827$ ,  $P = 0.131$ ) (Figs 1–2, Tables 1 and 2).



Table 1. Descriptive statistics of *Fusarium* infection (%), deoxynivalenol (DON) and fumonisin (FUM) concentration (ppb) affected by nitrogen fertilization ( $\text{kg N ha}^{-1}$ )

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	0	18	27.78	117.85	27.78	0	500
	40	18	5.56	16.17	3.81	0	50
	80	18	19.44	48.93	11.53	0	150
	120	18	11.11	47.14	11.11	0	200
	160	18	55.56	138.15	32.56	0	550
	Total	90	23.89	86.84	9.15	0	550
FUM	0	18	16.67	29.70	7.00	0	100
	40	18	16.67	24.25	5.72	0	50
	80	18	13.89	23.04	5.43	0	50
	120	18	36.11	50.89	12.00	0	200
	160	18	38.89	50.16	11.82	0	200
	Total	90	24.44	38.40	4.05	0	200
<i>Fusarium</i>	0	18	69.39	28.17	6.64	12	100
	40	18	66.78	26.33	6.21	24	96
	80	18	71.78	28.79	6.79	22	100
	120	18	65.22	31.50	7.43	12	100
	160	18	71.56	29.65	6.99	20	100
	Total	90	68.94	28.40	2.99	12	100

0: no nitrogen

40: the nitrogen dose was  $40 \text{ kg N ha}^{-1}$ 80: the nitrogen dose was  $80 \text{ kg N ha}^{-1}$ 120: the nitrogen dose was  $120 \text{ kg N ha}^{-1}$ 160: the nitrogen dose was  $160 \text{ kg N ha}^{-1}$ Table 2. Analysis of variance for *Fusarium* infection deoxynivalenol (DON) and fumonisin (FUM) concentration affected by nitrogen fertilization

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	27,666.667	4	6,916.667	0.914	0.460
	Within Groups	643,472.222	85	7,570.261		
	Total	671,138.889	89			
FUM	Between Groups	10,388.889	4	2,597.222	1.827	0.131
	Within Groups	120,833.333	85	1,421.569		
	Total	131,222.222	89			
<i>Fusarium</i>	Between Groups	604.667	4	151.167	0.181	0.948
	Within Groups	71,172.056	85	837.318		
	Total	71,776.722	89			

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

Table 3. Descriptive statistics of *Fusarium* infection (%), deoxynivalenol (DON) and fumonisin (FUM) concentration (ppb) affected by wheat variety

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	Alföld	30	36.67	133.22	24.32	0	550
	Mv Kolompos	30	13.33	39.25	7.17	0	150
	Mv Karéj	30	21.67	59.72	10.90	0	250
	Total	90	23.89	86.84	9.15	0	550
FUM	Alföld	30	28.33	42.92	7.84	0	200
	Mv Kolompos	30	36.67	43.42	7.93	0	200
	Mv Karéj	30	8.33	18.95	3.46	0	50
	Total	90	24.44	38.40	4.05	0	200
<i>Fusarium</i>	Alföld	30	67.90	26.32	4.81	20	100
	Mv Kolompos	30	71.20	27.88	5.09	12	100
	Mv Karéj	30	67.73	31.57	5.76	12	100
	Total	90	68.94	28.40	2.99	12	100

Table 4. Analysis of variance for *Fusarium* infection and deoxynivalenol (DON) and fumonisin (FUM) concentration affected by wheat variety

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	8,388.889	2	4,194.444	0.551	0.579
	Within Groups	662,750.000	87	7,617.816		
	Total	671,138.889	89			
FUM	Between Groups	12,722.222	2	6,361.111	4.670	0.012
	Within Groups	118,500.000	87	1,362.069		
	Total	131,222.222	89			
<i>Fusarium</i>	Between Groups	229.356	2	114.678	0.139	0.870
	Within Groups	71,547.367	87	822.384		
	Total	71,776.722	89			

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

Table 5. Descriptive statistics of *Fusarium* infection (%), deoxynivalenol (DON) and fumonisin (FUM) concentration (ppb) affected by growing season

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	2020	45	47.78	118.68	17.69	0	550
	2021	45	0	0	0	0	0
	Total	90	23.89	86.84	9.15	0	550
FUM	2020	45	16.67	36.93	5.50	0	200
	2021	45	32.22	38.66	5.76	0	200
	Total	90	24.44	38.40	4.05	0	200
<i>Fusarium</i>	2020	45	44.33	19.00	2.83	12	88
	2021	45	93.56	5.61	0.84	80	100
	Total	90	68.94	28.40	2.99	12	100



Table 6. Analysis of variance for *Fusarium* infection and deoxynivalenol (DON) and fumonisin (FUM) concentration affected by growing season

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	51,361.11	1	51,361.11	7.29	0.008
	Within Groups	619,777.78	88	7,042.93		
	Total	671,138.89	89			
FUM	Between Groups	5,444.44	1	5,444.44	3.81	0.05
	Within Groups	125,777.78	88	1,429.29		
	Total	131,222.22	89			
<i>Fusarium</i>	Between Groups	54,513.61	1	54,513.61	277.89	0.000
	Within Groups	17,263.11	88	196.17		
	Total	71,776.72	89			

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

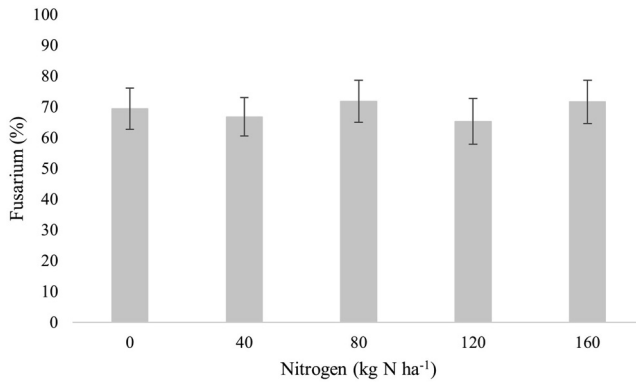


Fig. 1. Effect of nitrogen fertilization (kg N ha<sup>-1</sup>) on *Fusarium* infection (%)

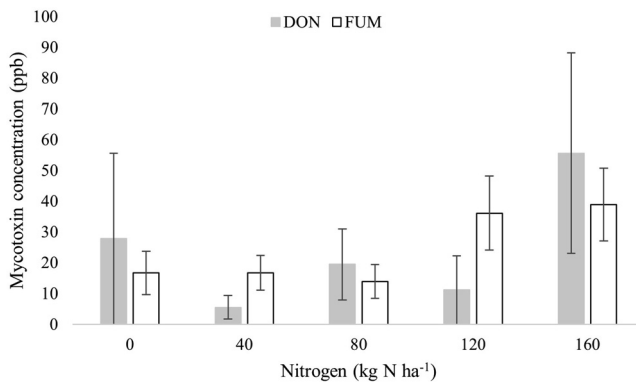


Fig. 2. Effect of nitrogen fertilization (kg N ha<sup>-1</sup>) on mycotoxin concentration (ppb)



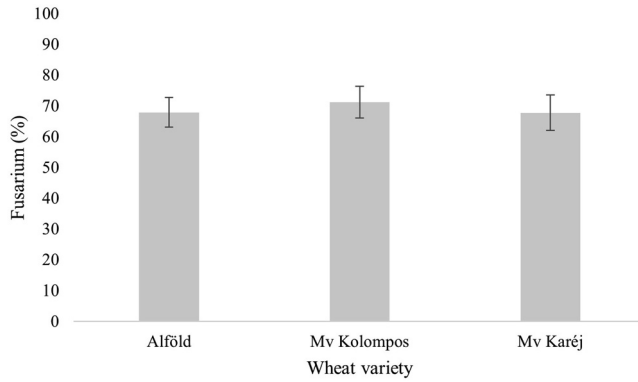


Fig. 3. Effect of wheat variety on *Fusarium* infection (%)

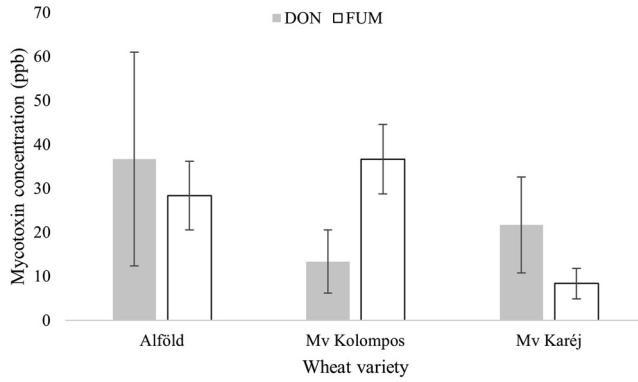


Fig. 4. Effect of wheat variety on mycotoxin concentration (ppb)

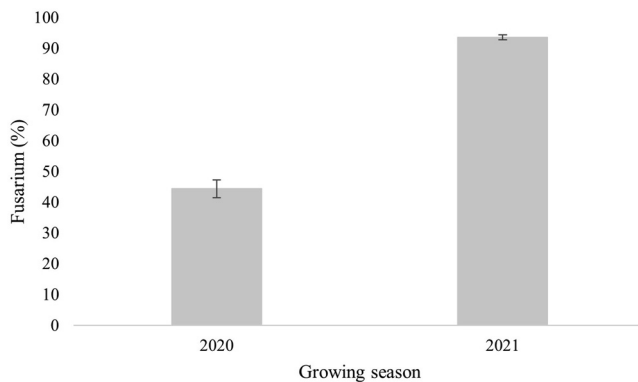


Fig. 5. Effect of growing season on *Fusarium* infection (%)



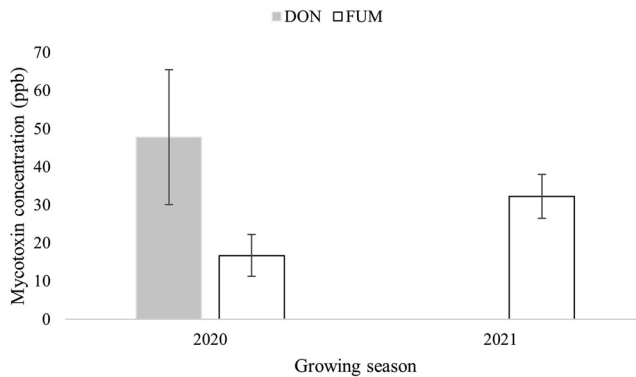


Fig. 6. Effect of growing season on mycotoxin concentration (ppb)

## DISCUSSION

According to our findings, the environmental conditions in 2020/2021 may be the cause of the rise in *Fusarium* infection and fumonisin concentration. Precipitation (mm) during anthesis (May) when wheat is most susceptible to *Fusarium* infection was 88.39 mm in 2021, higher than in 2020 (42.8 mm) according to the World Weather Online<sup>®</sup> meteorological service, this increase in rain could explain the increased *Fusarium* infection and fumonisin concentration. Environmental conditions such as precipitation, temperature and humidity in the atmosphere are major factors modulating *Fusarium* infection. Wheat heads are most susceptible to FHB infection during anthesis, but infection can occur up to the soft dough stage (Lacey et al., 1999; Windels, 2000). Generally, a temperature range between 25 and 30 °C and relative humidity between 88% and 95% are the optimal conditions for *Fusarium* infection (Doohan et al., 2003; Berthiller et al., 2009).

The results of this study are in accordance with the results of Schaafsma et al. (2001), who ascertained from a four year-survey that the climatical and weather conditions are the factors associated with variation in *Fusarium* and mycotoxin levels in wheat grain. Also, Brennan et al. (2003) found that humidity, precipitation, and temperature play an important role in the development of FHB, the production and dispersal of the inoculum and the infection of wheat heads. In addition, Bryła et al. (2016) noted that rainfall has a significant impact on FHB as well as the severity of infection. According to Mesterházy et al. (2012), González et al. (2008), Bernhoft et al. (2012), Czaban et al. (2015), Covarelli et al. (2015) and Kelly et al. (2015) environmental conditions play a significant role in *Fusarium* infection and mycotoxin contamination especially during anthesis. Osborne and Stein (2007) and Zhang et al. (2008) suggest that weather conditions, plant development, and genetic or morphological cultivar characteristics as factors influencing the epidemiology of *F. species* and the risk of FHB in wheat.

The prevention of wheat diseases, especially *Fusarium* head blight, requires the use of agronomic techniques such as tillage, crop rotation, cultivar selection, and chemical or biological management (Wegulo et al., 2015). Fertilization with nitrogen is thought to have an impact on *Fusarium* head blight. Our research indicates that nitrogen dose had no effect on mycotoxin and *Fusarium* contamination. Some studies (Lemmens et al., 2004; Ma et al., 2004; Muhammad et al., 2010) indicated an increasing effect of FHB with higher nitrogen availability, but other studies reported decreasing





nitrogen effects on FHB (Obst et al., 2002; Yang et al., 2010). Several investigations (Teich and Hamilton, 1985; Fauzi and Paulitz, 1994; Aufhammer et al., 2000) failed to find any evidence of nitrogen influence or produced contradictory results (Heier et al., 2005; Subedi et al., 2007). Krnjaja et al. (2015) found that nitrogen fertilization did not increase FHB intensity. Kuzdralinski et al. (2014) reported that nitrogen fertilization did not affect *Fusarium* head blight. Oldenburg et al. (2007) concluded that nitrogen fertilization did not influence *Fusarium* growth and their production of mycotoxins in wheat grains. According to Parry et al. (1995), the impact of nitrogen fertilization on *Fusarium* infestation remains unclear. Aufhammer et al. (2000) found that nitrogen fertilization did not promote *Fusarium* infection or the production of mycotoxin. According to Lemmens et al. (2004) nitrogen fertilization significantly affected *Fusarium* infection and subsequent mycotoxin contamination in wheat, but this can't be attributed exclusively to nitrogen input in crop production. All these findings imply that nitrogen fertilization exerts a limited impact on the establishment of favorable circumstances necessary for the emergence of *F. spp.*

## CONCLUSION

The present study was designed to investigate the impact of three variables, namely, growing season, nitrogen fertilization, and wheat variety, on *Fusarium* infection and mycotoxin production in wheat kernel. The findings demonstrate that nitrogen fertilization failed to exhibit a statistically significant impact on both *Fusarium* infection and mycotoxin production. The impact of wheat variety on *Fusarium* infection and deoxynivalenol production was not found to be statistically significant. However, it exerted a significant effect on fumonisin production. The growing season exerted a statistically significant impact on the incidence of *Fusarium* infection and the ensuing contamination with mycotoxins, attributable to augmented precipitation levels in 2021 compared to 2020, specifically during the flowering phase when the spike of wheat is highly susceptible to *Fusarium* infection.

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