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RESEARCH ARTICLE

Fusarium spp. infection, mycotoxin contamination, and some agronomic traits in winter barley as affected by N fertilization under Serbia conditions

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ABSTRACT

Fusarium head blight (FHB), caused by different *Fusarium* species, is the most devastating disease of small cereal grains, including barley (*Hordeum vulgare* L.) This study aimed to investigate the influence of N fertilization rates (0 kg N ha^{-1} - N_0, 50 kg N ha^{-1} - N_{50}, and 100 kg N ha^{-1} - N_{100}) on *Fusarium* and mycotoxin (deoxynivalenol - DON and zearalenone - ZEA) contamination and some agronomic traits (plant height - PH, spike length - SL and thousand kernel weight - TKW) in two barley cultivars, NS 565 and Etincel, harvested in 2019 and 2020. Climatic conditions during two successive seasons were favourable for *Fusarium* infection, providing a high incidence of FHB-causing species, of which *F. graminearum* species complex (FGSC) strains were the most frequent (on average > 34% per treatment). The N rates and barley cultivars had nonsignificant effects on the incidence of FGSC strains. However, N rates significantly influenced mycotoxin levels in 2019, with the highest DON at N_{100} (5209.67 µg kg^{-1}) and ZEA levels at N_{50} (47.11 µg kg^{-1}). In 2020, there were nonsignificant differences between N rates for DON levels, while ZEA was not detected. In both years, the six-row barley 'Etincel' had significantly higher DON and ZEA levels than the two-row barley 'NS 565'. Agronomic traits, PH and SL, were affected by barley cultivars, and TKW was affected by N rates and barley cultivars. The highest TKW was at N_{100}, followed by N_{50} and N_0.

Key words: Barley, deoxynivalenol, *Fusarium* spp., *Hordeum vulgare*, nitrogen rates, zearalenone.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cereals cultivated by man and the fourth most important cereal globally after wheat, maize, and rice. Unlike wheat, it requires fertile soil for growth and development processes and is very sensitive to floods and soil acidity (Shiferaw and Tadele, 2022). It is estimated that 70% of the barley produced worldwide is used in animal feed, as green forage, hay, silage or used with kernels included for the production of feeds. It can also be used for the production of compost bedding and in the paper industry (Vargas et al., 2015). In addition, barley is the most widely used cereal in malt production for breweries and human consumption (breakfast cereals, grain-based muesli bars, flour, cookies, bread, coffee substitutes, etc.). The interest in barley has increased in recent decades, mainly due to its high soluble fibre content, especially β-glucans, which have been associated with a reduction in the risk of coronary heart disease and diabetes by regulating cholesterol and blood sugar levels (Schlörmann and Glei, 2017; Hughes and Grafenauer, 2021). Carbohydrates, essential amino acids, and proteins, along with gluten, lipids, minerals (Se, P, Fe, Zn, Cu, Mg), and secondary metabolites such as phenolic compounds and vitamins are the most important nutrients in barley grains (Geng et al., 2022). In Serbia, barley is grown more for animal feed than for malt production, with a production yield of 90 034, 106 315 and 129 025 t (feed barley) and 61 545, 65 494 and 34 022 t (malting barley) in 2020, 2021 and 2022, respectively (Statistical Yearbook of Serbia, 2021, 2022, 2023).

During pre- and post-harvest, barley kernels are susceptible to infection by fungal species, the most often from the genera *Alternaria*, *Fusarium* and *Penicillium*, with *Fusarium* spp. as the biggest threat causing Fusarium head blight (FHB), the most devastating disease of barley and other small cereal grains (Iwase et al., 2020). Several *Fusarium* species can cause FHB, with *F. culmorum* (W.G.Sm.) Sacc. 1895, and members of *F. graminearum* species complex (FGSC), as primary ones. *Fusarium culmorum* is the most frequent in warm and humid regions, while *F. graminearum sensu stricto* (*F. graminearum s.s.*) dominates in cool, wet, and high-humidity regions. *F. graminearum s.s*. is one of at least 16 distinct phylogenetic species in FGSC and is the most prevalent FHB pathogen in small cereal grains worldwide (Ferrigo et al., 2016; Senatore et al., 2023). Other members of FGSC can also cause FHB, but their distribution and frequency are affected by regions and climate conditions. Furthermore, *F. avenaceum* and *F. poae* are FHB causative agents, belonging to other *Fusarium* species complexes than FGSC (Vaughan et al., 2016). The FHB pathogens reduce the yield and quality of small cereal grains. Warm and humid conditions are convenient for *Fusarium* sporulation in the flowering and anthesis stages. Typically, the first FHB symptoms appear on a few spikelets that can turn either bleached, which is more prevalent in wheat, or necrotic, which is more common in barley with spreading the infection to the entire spike, forming *Fusarium*-damaged kernels, also called tombstone or scabby kernels. Infected kernels are lightweight, shrivelled and chalky (Blandino et al., 2012; Hoheneder et al., 2022; Singh et al., 2023).

Many FHB pathogens produce secondary metabolites (mycotoxins), causing diseases in animals and humans (mycotoxicoses). Trichothecenes and zearalenone are *Fusarium* mycotoxins of the biggest concern for small cereal grain production (Karlsson et al., 2021), with *F. graminearum s.s*. as a primary producer worldwide (Leslie et al., 2021). There are four groups of trichothecenes, A, B, C, and D, of which A and B types are the most common in small cereal grains, including barley. In contaminated kernels, B-type trichothecenes are present at higher levels than A-type trichothecenes. The T-2 and HT-2 toxins belong to A-type trichothecenes, while deoxynivalenol (DON) and nivalenol (NIV) with their acetyl derivatives, belong to B-type trichothecenes. In cereals and cereal-based products, DON is the most common naturally occurring *Fusarium* toxin contributing to feed and food safety hazards, followed by zearalenone (ZEA). The association of DON and ZEA is a frequent occurrence in temperature climates when coinciding cool temperatures and high humidity during flowering and the beginning of the kernel-filling stages of cereals (Haidukowski et al., 2022; Błaszczyk et al., 2023). The co-occurrence of more *Fusarium* mycotoxins may provide synergistic actions and increase detrimental effects in animals and humans. In mammals, DON inhibits protein synthesis, suppressing immunity and causing acute (food refusal, emesis, vomiting) and chronic (anorexia, teratogenicity, neurotoxicity) disease symptoms (Yue et al., 2021). The ZEA has immunotoxic, reproductive, genotoxic and hepatotoxic effects. Primary ZEA metabolites are α-zearalenol and β-zearalenol produced in mammary intestinal cells. ZEA is an estrogenic mycotoxin that causes disorders in the reproductive systems of domestic animals, especially in pigs (Prodanov-Radulović et al., 2014; Ropejko and Twarużek, 2021). In 2006, the European Commission set guidance for the maximum levels of DON and ZEA in foodstuffs (2006/1881/EC) and feedingstuffs (2006/576/EC). Maximum DON and ZEA levels in foodstuffs as unprocessed cereals other than maize are 1250 and 100 μg kg^{-1}, respectively. The maximum limits of DON and ZEA are 8000 and 2000 μg kg^{-1}, respectively, for cereals and cereal products except for maize by-products for animal feeding.

The agricultural measures such as sowing tolerant genotypes, early sowing, crop rotation and fungicide treatments, could significantly reduce *Fusarium* infection and mycotoxin contamination of barley kernels. Hence, integrated agrotechnical practices in FHB and mycotoxin management control are recommended to mitigate these contaminants in barley production (Choo et al., 2014; McKee et al., 2019). The N fertilization is one of the usual and unavoidable agrotechnical measures for achieving high crop yields. However, there is little information about N fertilization’s influence on *Fusarium* and mycotoxin occurrence in barley. For that reason, this research aimed to determine the effect of N rates on the incidence of *Fusarium* species, especially causative agents of FHB and their mycotoxins, DON and ZEA, on the kernels of two barley cultivars in two successive seasons (2019 and 2020) and on some of their agronomic traits.

MATERIALS AND METHODS

Field trial design

A field experiment was conducted at the Institute for Animal Husbandry, Belgrade-Zemun (44°84' N, 20°40' E; 88 m a.s.l.), Serbia, under dry growing conditions in response to natural rainfalls. During two harvest seasons, 2019 and 2020, the incidence of *Fusarium* spp., levels of mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) in the kernels and some agronomic traits of two barley (*Hordeum vulgare* L.) cultivars, NS 565 and Etincel, were analysed. The 'NS 565' is the two-row Serbian winter barley cultivar used for animal feed and malt production. The 'Etincel' is a six-row French winter barley cultivar grown only for animal feed. The sowing dates were in the middle of October in both sowing years, 2019 and 2020. Barley cultivars were sown in carbonate Chernozem soil type (International Union of Soil Sciences – IUSS, Rome, Italy), with maize as the previous crop. The field experiment was arranged in a split-split plot randomized block design in three replicates. The two barley cultivars were the main plots while three N rates, 0 (N_0), 50 (N_{50}) and 100 kg N ha^{-1} (N_{100}) were subplots. The plot size was 10 m^2 (5 m × 2 m). The N fertilizers ammonium nitrate (33.4% N) at the rate of 149.7 (N_{50}) and 299.4 (N_{100}) kg ha^{-1} in phenological barley stages, BBCH 23-24, was applied. Chemical treatments were performed at the tillering barley stages BBCH 29-31 to suppress weeds by aminopyralide + florasulam (Lancelot 440 WG) and insects by gamma-cyhalothrin (Vantex 60 SC). The harvested dates were at the end of June in both years, 2019 and 2020.

Fusarium identification and mycotoxin analyses

A total of 30 barley spikes from each subplot were trashed with a laboratory thresher (Wintersteiger LD 180, Ried, Austria). Before the analyses, barley kernel samples were stored at 4 °C. Kernel-ground moisture was determined by a laboratory moisture analyser (MB35, OHAUS, Parsippany, New Jersey, USA). Subsamples of 50 randomized chosen kernels were surface-disinfected in 1% sodium-hypochlorite, twice rinsed in sterile distilled water, dried on Whatman filter paper, and plated on 1.8% salted potato dextrose agar in Petri plates (10 kernels per plate) and incubated for 10-14 d at 20 ± 2 °C. Based on macroscopic and microscopic characteristics, *Fusarium* species were identified using the fungal keys of Leslie and Summerell (2006). The percentage of *Fusarium* incidence was calculated by dividing the number of kernels infested by *Fusarium* spp. and the total number of examined kernels, multiplied by 100.

Barley kernel subplot samples were dried for 72 h at 60 °C, then ground by an analytical mill (A11, IKA, Staufen, Germany) and analysed by competitive direct enzyme-linked immunosorbent assay (ELISA) in the detection of DON and ZEA. The assay procedure was performed according to the instructions of the manufacturer of the Celer DON and ZEA ELISA kits (Eurofins Technology, Budapest, Hungary). Sodium chloride and 70% methanol were added to the ground cereal sample and shaken in an orbital shaker (3015, GFL, Burgwedel, Germany) for 3 min. Then, the extracted sample was transferred to a premix microwell containing enzyme-conjugated investigated mycotoxin, mixed, and placed in microwells coated with an anti-mycotoxin antibody. These microwells were incubated for 10 min in the dark at room temperature. During incubation, the mycotoxin from the extracted sample and the enzyme-conjugated mycotoxin competed for binding with the anti-mycotoxin antibody coated on the microwells. Subsequently, the microwells were washed three times with buffer to remove unbound conjugate and non-specific reactants. The addition of the chromogen to the washed antibody wells triggered an enzymatic reaction between the chromogen and bound conjugate, resulting in the appearance of the blue colour of the chromogen. The mycotoxin level is indirectly proportional to the intensity of the blue colour. If the concentration of mycotoxin in the sample increases, the intensity of the blue colour decreases. Finally, to stop these enzymatic reactions, a sulfuric acid solution was added so that the colour of the chromogen changed from blue to yellow. The absorbance values of the samples were measured optically at a wavelength of 450 nm using an ELISA reader (EL×800TM, BioTek Instruments, Winooski, Vermont, USA). The detection limits for cereals were 40 and 10 µg kg^{-1} for DON and ZEA, respectively.

In the stage of full maturity, with 30 plants per replicate, plant height (PH, cm), spike length (SL, cm) and thousand kernel weight (TKW, g) were analysed in each subplot of two winter barley cultivars, NS 565 and Etincel.

Climate data
Climate data (mean monthly temperatures and relative humidity, and the sum of monthly rainfalls) for the period March-June 2019 and 2020 in the Belgrade area (Surčin) were provided by the Republic Hydrometeorological Service of Serbia (Figure 1).

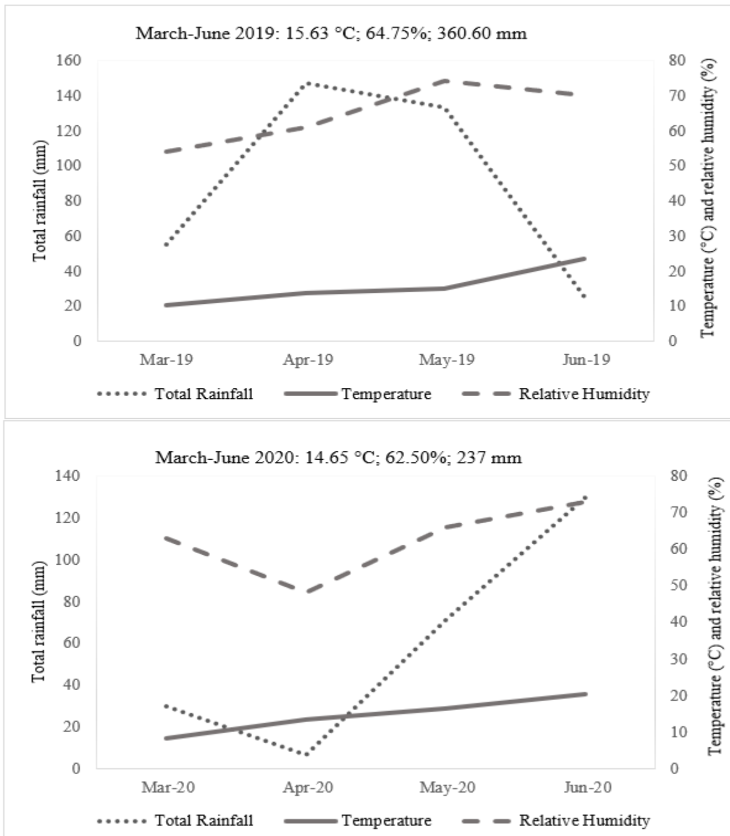

Figure 1. Mean monthly temperature, mean monthly relative humidity and total monthly rainfalls in the period from March to June in 2019 and 2020 in the Surčin area (Belgrade, Serbia).

Statistical data analyses
The effects of two barley cultivars and three N rates on the incidence of the *Fusarium* spp., DON and ZEA levels and agronomic traits (PH, SL, and TKW) in two growing seasons, 2019 and 2020 were analysed using a general linear model (multivariate analysis of variance, MANOVA) with SPSS software (SPSS Statistic 20; IBM, Armonk, New York, USA). Data normality was tested by Levene's test. Comparing treatment means at $P \leq 0.05$ and $P \leq 0.01$ significance levels determined by the Tukey's test. Pearson's correlation coefficients were calculated between tested variables, with values of detection limits for treatments with non-detected mycotoxins.

RESULTS

Fusarium and mycotoxin contamination of barley kernels
Among identified *Fusarium* species, the most frequently isolated strains from barley kernels belonged to the *F. graminearum* species complex (FGSC) in both years. There were nonsignificant differences between N rates and barley cultivars for the incidence of FGSC strains. Across all treatments, the low and sporadic incidence was evaluated for members of *F. fujikuroi* species complex (FFSC) (0%-12% in 2019 and 0%-10% in 2020), *F. poae*

(0%-4% in 2019 and 0%-2% in 2020) and *F. incarnatum* (synonym: *F. semitectum*) (0%-2% in both years) (data not presented). The occurrence of mycotoxins DON and ZEA were observed in all investigated treatments in both years, except ZEA occurrence in 2020 (Table 1). On average, for cultivars, the effect of N rates was significant ($P \leq 0.01$) on DON and ZEA levels in 2019, with the highest DON at N_{100} and ZEA levels at N_{50} treatment. In 2020, there was a nonsignificant N rate influence on DON, while mycotoxin ZEA was not detected. On average, for N rates, barley cultivars differed for DON levels in both years, while for ZEA levels only in 2019. Higher DON and ZEA levels were observed in 'Etincel' compared to 'NS 565'. Interaction Barley cultivar × N rate was significant for DON ($P \leq 0.05$) and ZEA ($P \leq 0.01$) levels in 2019. The highest DON and ZEA levels were found at N_{100} and N_{50} in 'Etincel', respectively (Table 2).

Table 1. Cultivar and N rate effects on the incidence of *Fusarium graminearum* species complex (FGSC) strains and deoxynivalenol (DON) and zearalenone (ZEA) levels in barley kernels in 2019 and 2020. Means followed by the same letter within a column are not significantly different by Tukey's test at $P \leq 0.05$ level. *, **Significant at the 0.05; and 0.01 level of probability, respectively. ^{ns}Nonsignificant.

	2019			2020		
Factor	Incidence of FGSC strains	DON	ZEA	Incidence of FGSC strains	DON	ZEA
	%	µg kg^{-1}	µg kg^{-1}	%	µg kg^{-1}	µg kg^{-1}
Cultivar effects (A)						
Etincel	46.44	5274.22^{a}	40.76^{a}	34.89	2159.44^{a}	not detected
NS 565	40.44	1796.33^{b}	15.13^{b}	42.44	1130.56^{b}	not detected
F-test	^{ns}	**	**	^{ns}	*	
N rate effects (B)						
0 kg ha^{-1}	43.33	2647.83^{b}	16.46^{c}	42	1293.00	not detected
50 kg ha^{-1}	43.33	2748.33^{b}	47.11^{a}	38.67	1967.83	not detected
100 kg ha^{-1}	43.67	5209.67^{a}	20.27^{b}	35.33	1674.17	not detected
F-test	^{ns}	**	**	^{ns}	^{ns}	
Interaction (F-test)						
A × B	^{ns}	*	**	^{ns}	^{ns}	-

Table 2. Effect of barley cultivar (Etincel and NS 565) and N rate on deoxynivalenol (DON) and zearalenone (ZEA) levels in 2019. Means followed by the same letter within a column are not significantly different by Tukey's test at $P \leq 0.05$ level. N_0: 0 kg N ha^{-1}; N_{50}: 50 kg N ha^{-1}; N_{100}: 100 kg N ha^{-1}.

2019	DON	ZEA
Barley cultivar × N rate	µg kg^{-1}	µg kg^{-1}
Etincel × N_0	3873.33^{bc}	11.58^{d}
Etincel × N_{50}	4037.67^{b}	81.25^{a}
Etincel × N_{100}	7911.67^{a}	29.45^{b}
NS 565 × N_0	1422.33^{c}	21.35^{c}
NS 565 × N_{50}	1459.00^{c}	12.96^{d}
NS 565 × N_{100}	2507.67^{bc}	11.09^{d}

Evaluation of the agronomic traits

The PH and SL were not affected by N rates. However, the effect of the N rate was highly significant (P ≤ 0.01) for TKW in both years, with the highest TKW at N_{100}, followed by N_{50} and N_0 (Table 3). There was a significant difference between the two barley cultivars for all evaluated agronomic traits in both years. 'Etincel' had a higher PH and lower SL in both years and higher TKW in 2019 compared to 'NS 565'. Interaction Barley cultivar × N rate was significant (P ≤ 0.05) for TKW in both years. The highest TKW in the first year was at N_{100} in both cultivars, and it did not differ only from the TKW of 'Etincel' at N_{50}. In 2020, the highest TKW was at N_{100} in 'NS 565' (Figures 2 and 3).

Table 3. Cultivar (Etincel and NS 565) and N rate effects on plant height (PH), spike length (SL) and thousand kernel weight (TKW) in winter barley in 2019 and 2020. Means followed by the same letter within a column are not significantly different by Tukey's test at P ≤ 0.05 level. *, **Significant at the 0.05 and 0.01 level of probability, respectively. ^{ns}: Nonsignificant.

Factor	2019			2020		
	PH	SL	TKW	PH	SL	TKW
	cm	cm	g	cm	cm	g
Cultivar effects (A)						
Etincel	79.55^{a}	5.46^{b}	51.43^{a}	80.72^{a}	5.36^{b}	38.62^{b}
NS 565	71.28^{b}	9.14^{a}	48.60^{b}	75.23^{b}	7.34^{a}	41.89^{a}
F-test	**	**	*	**	**	**
N rate effects (B)						
0 kg ha^{-1}	75.58	7.12	43.35^{c}	78.96	6.26	35.03^{c}
50 kg ha^{-1}	72.57	7.12	49.55^{b}	76.95	6.53	39.47^{b}
100 kg ha^{-1}	78.10	7.67	57.15^{a}	78.03	6.27	46.27^{a}
F-test	ns	ns	**	ns	ns	**
Interaction (F-test)						
A × B	ns	ns	*	ns	ns	*

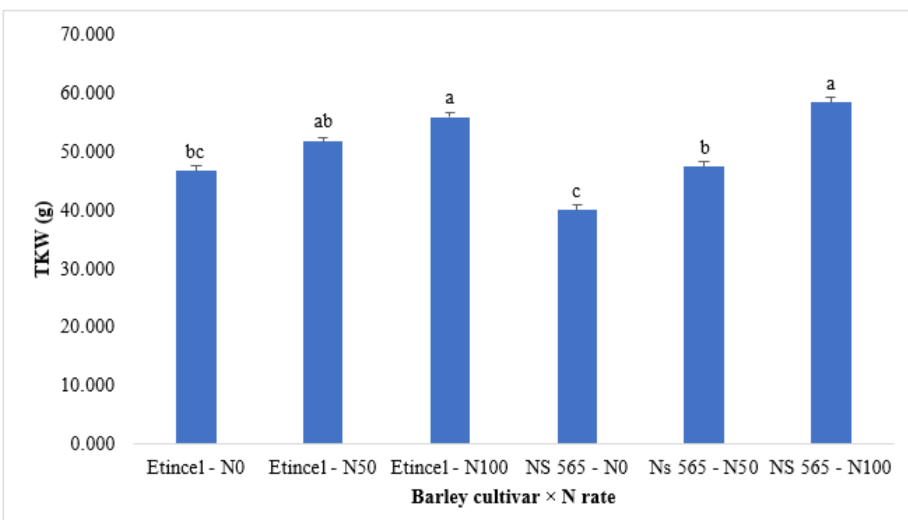

Figure 2. Effect of barley cultivar (Etincel and NS 565) and N rate on thousand kernel weight (TKW) in 2019. Means followed by the same letter on the bars are not significantly different by Tukey's test at P ≤ 0.05 level. Vertical bars correspond to standard error. N_0: 0 kg N ha^{-1}; N_{50}: 50 kg N ha^{-1}; N_{100}: 100 kg N ha^{-1}.

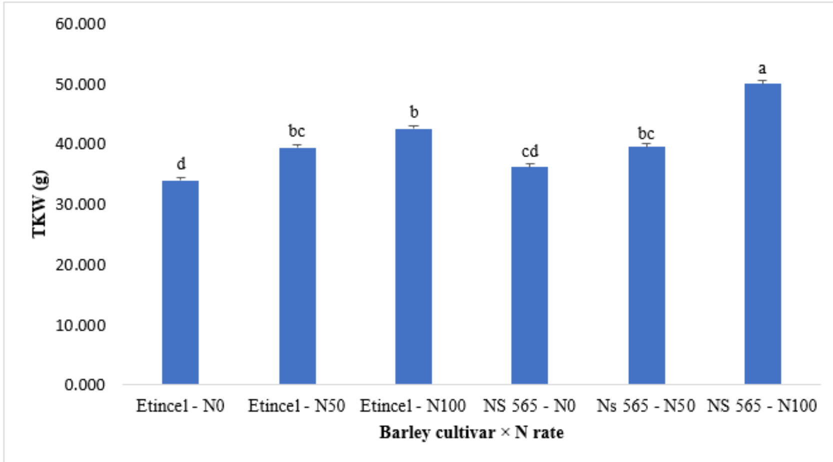

Figure 3. Effect of barley cultivar (Etincel and NS 565) and N rate on thousand kernel weight (TKW) in 2020. Means followed by the same letter on the bars are not significantly different by Tukey's test at $P \leq 0.05$ level. Vertical bars correspond to standard error. N_0: 0 kg N ha^{-1}; N_{50}: 50 kg N ha^{-1}; N_{100}: 100 kg N ha^{-1}.

Correlation analyses

There was a significant positive correlation between the incidence of FGSC strains with DON (r = 0.353*), between DON with ZEA (r = 0.404*) and TKW (r = 0.498**) and between ZEA and TKW (r = 0.357*), an insignificant positive correlation between the incidence of FGSC strains with ZEA level (r = 0.275), PH (r = 0.017), SL (0.061) and TKW (r = 0.156) and between TKW and SL (r = 0.214). There were significant negative correlations between DON and SL (r = -0.376**) and PH and SL (r = -0.637**) (Table 4).

Table 4. Correlation coefficients between investigated variables. *, **Significant at the 0.05 and 0.01 level of probability, respectively. nsNonsignificant. FGSC: *Fusarium graminearum* species complex; DON: deoxynivalenol; ZEA: zearalenone; PH: plant height; SL: spike length; TKW: thousand kernel weight.

Two-year period (2019-2020)	Incidence of FGSC strains	DON	ZEA	PH	SL	TKW
	%	µg kg^{-1}	µg kg^{-1}	cm	cm	g
Incidence of FGSC strains, %	1					
DON, µg kg^{-1}	0.353*	1				
ZEA, µg kg^{-1}	0.275ns	0.404*	1			
PH, cm	0.017ns	0.431**	0.00ns	1		
SL, cm	0.061ns	-0.376**	-0.245ns	-0.637**	1	
TKW, g	0.156ns	0.498**	0.357*	-0.082ns	0.214ns	1

Weather conditions

Mean monthly temperatures and RH, and the sum of rainfall for the period from March to June was higher in 2019 (15.63 °C, 64.75% and 360.60 mm) than in 2020 (14.65 °C, 62.50% and 237 mm) (Figure 1). In both years, weather conditions were convenient for *Fusarium* infection, giving a high mean incidence of FGSC strains in all treatments (> 34%) (Table 1). However, DON and ZEA levels, SL and TKW showed variability depending on investigated treatments and years. Their values were higher in wetter 2019 than in 2020.

DISCUSSION

In barley, as in wheat, FHB is an unavoidable fungal disease worldwide, causing high economic losses in kernel quality and yield. This study provides results on the influence of three N rates (N_0, N_{50} and N_{100}) and two barley cultivars on the incidence of *Fusarium* species, especially FHB causative agents and the occurrence of DON and ZEA as the most common *Fusarium* mycotoxins. Among the identified FHB-causing species, FGSC strains were predominant, and *F. poae* was identified in a low or sporadic incidence as in research by Linkmeyer et al. (2016) in Germany, Schöneberg et al. (2016) and Drakopoulos et al. (2021) in Switzerland. However, *F. poae* was the most commonly isolated species from barley kernels in the UK (Nielsen et al., 2014) and Slovakia (Hudec and Roháčik, 2009). In both investigated years (2019 and 2020) across all treatments, there was no significance for the incidence of FGSC strains. The effect of N rates on mycotoxin levels was significant in 2019, with the highest DON at N_{100} and ZEA levels at N_{50}. There was no significance for DON levels, while ZEA was not detected in the barley kernel samples in 2020. Scientific data on the influence of N rates on *Fusarium* and mycotoxin contamination of small cereal grains are conflicting. According to Hofer et al. (2016), N inputs did not affect the *Fusarium* infection of barley kernels under natural conditions, which is contrary to the conclusion of Yang et al. (2010). Then, Akk et al. (2017) established a significant effect of N rates and barley-pea intercropping on the incidence of microfungi in the barley kernels, with the highest fungal incidence at N_{40}. Similarly, Lemmens et al. (2004) concluded that N fertilization affected FHB development in wheat crops and that DON contamination increased with an increasing N rate of up to N_{80}. However, van der Burgt et al. (2011) determined inconsistent influences of N rates on DON levels in wheat kernels. According to Krnjaja et al. (2015), the inoculum pressure, wheat cultivars and weather conditions influenced *Fusarium*, DON and ZEA kernel contamination more than N rates. In this study, the effect of N rates on DON contamination of barley kernels was not consistent in the two successive harvest seasons, which could be primarily explained by variable weather conditions, especially during the flowering stage in May, similar to Wegulo (2012). Unlike May 2020, in May 2019, there was a higher sum of rainfall (133 mm), mean relative air humidity (74%), and lower mean air temperature (15 °C), causing a higher effect of applied N rates on the levels of mycotoxins in kernels. Thapa et al. (2021) have also established that low temperatures, high air humidity, and abundant rainfall are prerequisites for high levels of DON and ZEA. Furthermore, Salgado et al. (2017) stated the significant effect of N inputs on fungal disease variables and DON accumulation depending on variations in the pathosystems, the N form and the time of N application in small cereal grains. In general, the effect of N rates on *Fusarium* and DON contamination of barley should not be considered individually but in conjunction with other factors, primarily environmental conditions. In support of that, Martínez et al. (2022) have evaluated those warm nights significantly favoured FGSC development and increased DON levels in barley and wheat kernels.

The effect of two barley cultivars, two-row NS 565 and six-row Etincel, was significant on DON and ZEA levels in 2019 and DON levels in 2020. The general conclusion is that six-row barley genotypes are more susceptible to FHB than two-row barley genotypes (Yoshida et al., 2004; Buerstmayr et al., 2004). Although barley cultivars had nonsignificant effect on the incidence of FGSC strains on the kernels, the six-rowed 'Etincel' had higher DON in 2019 and 2020 and ZEA levels in 2019 than the two-rowed 'NS 656'. It is in line with the results of Khanal et al. (2021), who reported that most six-rowed barley cultivars had more DON than two-rowed barley cultivars.

In most investigated treatments, levels of DON mycotoxins exceeded the maximum limit of 1250 µg kg^{-1} in foodstuffs, while all treatments had ZEA levels below the maximum limits in unprocessed cereals for humans (2006/1881/EC) and animal nutrition (2006/576/EC). Similarly, high DON levels and a high percentage (62%) of barley kernel samples with DON levels above the EU maximum limit were reported by Tabuc et al. (2009) in Romania. In Brazil, Piacentini et al. (2018) also found high levels of DON ranging from 1700 to 7500 µg kg^{-1} and levels of ZEA ranging from 300 to 630 µg kg^{-1}. In addition, mean DON levels of 2360 µg kg^{-1} in malting barley kernel samples and a high percentage (44%) of samples with DON levels above the maximum limit reported by Nogueira et al. (2018) in Argentina. On the other hand, DON levels below EU maximum limits in unprocessed barley were reported by Jajić et al. (2014) in Serbia and Beccari et al. (2017) in Italy.

In this study, the N rate and interaction of barley cultivar and N rate effects were significant on TKW. The highest TKW was at N_{100}, followed by N_{50} and N_0. Similarly, Moreno et al. (2003) established the highest TKW at N_{100} and the highest kernel yield of barley (kg ha^{-1}) at N_{100} and N_{150}. Janković et al. (2011) recommended N rates of 70, 90, and 110 kg N ha^{-1} to achieve the highest yield of winter barley kernels. In our study, the N rate effect had no significance for agronomic traits PH and SL. However, Tadesse et al. (2021) and Adhikari and Singh (2022) reported that increasing N rates significantly increased the PH and SL. Then, Drakopoulos et al. (2021) established that shorter barley plants had increased *F. poae* infection and mycotoxins in barley kernels. In this study, 'NS 565' with shorter plants had lower DON than 'Etincel' with taller plants, while the incidence of FGSC strains did not differ among the barley cultivars tested. Plant responses to pathogen infection depend on environmental conditions and other abiotic and biotic factors. In general, *Fusarium* contamination and mycotoxin production in small cereal grains is cultivar-specific and influenced by years, plant stress, some agrotechnical factors and environmental conditions such as water activity, temperature, pH and nutrient substrates, indicating the importance of the interaction of these factors (Thapa et al., 2021; Župunski et al., 2021). In some cases, infectious pathogen pressure can influence the level of susceptibility of small cereal grain cultivars (Hoheneder et al., 2022).

Correlation analyses showed positive relationships between the incidence of FGSC strains with DON and ZEA levels. Similar results were reported by Gil-Serna et al. (2023) and Schöneberg et al. (2016). Disease variables (the incidence of FGSC strains, DON, and ZEA levels) were also positively correlated with yield parameter TKW, as reported by Janssen et al. (2018). There were also positive correlations between disease variables and PH, which is in contrast to the results of Hofer et al. (2016). Buerstmayr et al. (2004) established inconsistent correlations between PH and FHB severity, with most short spring barley lines and high levels of FHB severity. There were negative correlations between PH with SL and TKW that are similar to the results of Vitrakoti et al. (2017).

CONCLUSIONS

Among Fusarium head blight (FHB) causative agents, *Fusarium graminearum* species complex (FGSC) strains were the most frequent in both years. Barley cultivars and N rates had no significant influence on the incidence of FGSC strains. Considering two investigation harvest seasons (2019 and 2020), the effect of N rates on deoxynivalenol (DON) accumulation in barley kernels was inconsistent. Zearalenone (ZEA) was found only in 2019 and affected by N rates. The two barley 'NS 656' and 'Etincel' showed susceptibility to FGSC species, DON and ZEA, especially in a wetter growing season in 2019 than in 2020. The thousand kernel weight (TKW) increases with increasing N rate. Therefore, N application can be considered good agrotechnical practice for achieving high yields of barley crops. The TKW is a yield component with a significant and direct effect on kernel yield. Generally, barley is usually contaminated with *Fusarium* spp. and multiple mycotoxins simultaneously, which this study confirms. As a result, there is a constant search for new barley cultivars and cultivation techniques that can better resist *Fusarium* infection and limit contamination with mycotoxins, especially DON.

Author contribution

Conceptualization: V.K., V.M. Methodology: V.K., V.M. Investigation: V.K., V.M., M.L., A.O., N.M. Writing-original draft: V.K., V.M., T.P. Writing-review & editing: T.P., S.S. All co-authors reviewed the final version and approved the manuscript before submission.

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