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Nitrogen Fertilization Influence on *Fusarium* and Deoxynivalenol Contamination of Wheat

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ABSTRACT

Fusarium head blight (FHB) is the most devastating disease of small cereal grains worldwide caused by several *Fusarium* species. *F. graminearum* sensu stricto is the most frequently identified FHB pathogen within the *F. graminearum* species complex (FGSC). This pathogen produces the mycotoxin deoxynivalenol, which reduces grain quality and poses a threat to human and animal health. In this study, the effect of nitrogen (N) fertilizer rates (0, 50, and 100 kg N ha^{-1}) on the incidence of *Fusarium* spp., levels of deoxynivalenol (DON), agronomic traits (plant height – PH, spike length – SL, and thousand-grain weight – TGW) and grain yield (GY) in two winter wheat cultivars (Nogal and NS Ilina) over two growing seasons (2016–2017 and 2017–2018) was investigated. Among *Fusarium* species, FGSC strains were the most frequently isolated in both years. The incidence of FGSC strains (20.22%), DON levels (323.59 µg kg^{-1}), PH (83.21 cm), TGW (38.68 g), and GY (8.88 t ha^{-1}) were higher in 2017 than in 2018 (9.66%, 97.72 µg kg^{-1}, 71.05 cm, 34.35 g, and 5.11 t ha^{-1}, respectively). Cultivar NS Ilina had significantly higher FGSC incidence (20.11%), DON levels (302 µg kg^{-1}), PH (85.57 cm), and SL (9.11 cm) than cultivar Nogal (9.77%, 104.71 µg kg^{-1}, 68.68 cm, and 7.65 cm, respectively). The N rate significantly increased PH, but did not affect the other investigated parameters. Although both wheat cultivars tested were susceptible to *Fusarium* pathogens and DON, Nogal displayed less susceptibility than NS Ilina. Therefore, adopting integrated disease management that focuses on FHB-resistant or less susceptible wheat cultivars can reduce the risk of pathogen presence and DON.

1. INTRODUCTION

Bread wheat (*Triticum aestivum* L.), also known as common wheat, is widely cultivated in temperate climates and is one of the most important staple foods in the human diet. Wheat grain contains carbohydrates (~70%), proteins (up to 15%), lipids, dietary fibres, minerals, B vitamins, and other nutrients (Kim & Kim, 2021). It is also used for animal feed and industrial processing (biofuel, bioplastics) (Jiménez-Rosado et al., 2019; Hammond & McCann, 2023). Hexaploid bread wheat is mainly cultivated worldwide (95%), while tetraploid durum wheat is grown in the arid Mediterranean regions (Khalid et al., 2023). Cereal crops are susceptible to pathogenic fungi from the genus *Fusarium* and their secondary metabolites (mycotoxins). *Fusarium* and mycotoxin contamination reduce wheat quality and yield, causing economic losses in wheat production. Dietary exposure to mycotoxins can pose health risks to humans and animals (Pestka, 2010; Miedaner et al., 2017). The yield and qualitative characteristics of wheat crops are also affected by climate factors, soil type, agricultural practices, and their interactions. Priority strategies for sustainable wheat production include reducing excessive use of mineral fertilizers and chemical crop protection agents, which have adverse effects on human and animal health and can damage ecosystems (Mitura et al., 2023). After maize, wheat is the second most important cereal crop in Serbia. According to FAOSTAT data, in Serbia, wheat was grown on 682246 ha, with an average grain yield of 5054.9 kg ha^{-1} and a total production of 3448700 tons in 2023, while worldwide it was grown on 220407070 ha, with an average grain yield of 3625 kg ha^{-1} and a total production of 798975306 tons in 2023 (FAO, 2025).

Fusarium head blight (FHB) is one of the most destructive fungal diseases of wheat. Several *Fusarium* species are involved in the pathogenesis of FHB, with *F. graminearum* sensu lato Schwabe (sexual stage – *Gibberella zeae* (Schwein.) Petch) being the most predominant (Wegulo et al., 2011). *F. graminearum* sensu stricto, the most frequently isolated FHB pathogen worldwide, belongs to *F. graminearum* sensu lato, also referred to as the *F. graminearum* species complex (FGSC), which comprises 16 phylogenetically distinct species (Leslie et al., 2021). In addition to the FGSC species, the other highly virulent and toxigenic *Fusarium* FHB-causing species are *F. culmorum* and *F. avenaceum*, whereas *F. sporotrichioides, F. langsethiae, F. tricinctum,* and *F. poae* are less pathogenic (Al-Hashimi et al., 2025). *F. graminearum* s.s. has two reproductive stages in its life cycle, producing asexual (macroconidia) and sexual spores (ascospores). It overwinters as a saprobe in the soil and maize and small cereal grain residues. At the beginning of the vegetation season, macroconidia (asexual reproductive spores) are produced on crop residues. Ascospores are formed in perithecia (sexual reproductive structures) and released into the air by the rupture of perithecia. During the flowering stage, macroconidia and ascospores are transferred to wheat spikes by wind or rain. Humid weather encourages spore germination and the spread of *Fusarium* infection in wheat spikes, resulting in blight symptoms after a few days. FHB symptoms appear on a few spikelets or the entire spike, which may become bleached or whitish in color. Pink to orange sporodochia containing masses of macroconidia can develop on infected spikelets, and black perithecia may also form. Grains colonized by *Fusarium* spp. are characterized by lightweight, shrunken, wrinkled, and chalky, often referred to as scabby or tombstone (Wegulo, 2012; Dweba et al., 2017).

Warm weather and high humidity, accompanied by heavy or frequent rainfall from the flowering to the soft dough wheat stage, favor *Fusarium* infection and FHB (McMullen et al., 2012). FHB epidemics are associated with increased mycotoxin contamination, usually deoxynivalenol (DON) and less commonly zearalenone (ZEA) produced by *F. graminearum* s.s. However, high mycotoxin levels do not always correspond to the severity of an FHB outbreak but depend on climatic conditions during the wheat maturity stage and before harvest (Leslie et al., 2021). Mycotoxins can be detrimental to human and animal health, causing mycotoxicosis. Deoxynivalenol (DON) belongs to the B-type of trichothecenes and represents the most frequently detected mycotoxin in wheat grains worldwide (Wegulo, 2012; Tian et al., 2016), causing acute (vomiting) and chronic (anorexia, growth retardation, immunotoxicity, and reproductive disorders) diseases in animals and humans (Pestka, 2010). DON chemotypes/genotypes produce acetylated derivatives of DON, 3-ADON, or 15-ADON, which are also grain contaminants (Foroud et al., 2019). In Serbia, the predominant genotype in *F. graminearum* s.s. is 15-ADON (Krnjaja et al., 2018; Obradović et al., 2025). FHB symptoms can be exacerbated by the presence of DON, which may act as a virulent factor in FHB pathogenesis (Tian et al., 2016). According to the recently adopted Commission Regulation (EU) 2024/1022, the maximum permissible limit of DON is 1000 µg kg^{-1} for unprocessed cereals, except for unprocessed durum wheat and maize grains (1500 µg kg^{-1}) and oat grains with inedible husk (1750 µg kg^{-1}).

To suppress FHB development and reduce mycotoxin levels in wheat crops, FHB management strategies include preventive agrotechnical practices such as tillage, crop rotation, sowing less susceptible cultivars, and chemical or biological control (Palazzini et al., 2007; McMullen et al., 2012). Although nitrogen (N) fertilization is a crucial agrotechnical practice for achieving high yields in wheat crops, its role in FHB development and DON synthesis is unclear, with inconsistent and variable results. The exact cause of these contradictory reports remains unclear and is likely due to different N forms, pathogen strains, and timing of fertilizer application. There is a

report of a significant effect of low N rates (up to 80 kg N ha^{-1}) on increased FHB development and DON levels in wheat grains (Lemmens et al., 2004) and a report that weather conditions affect FHB severity and DON synthesis more than increasing N rates (Yoshida et al., 2008). However, Dordas (2008) stated that the use of fertilizers can affect the rate of plant growth and microclimate in crops, as well as infection and sporulation of pathogens, decreasing *Fusarium* severity with high N supply. Likewise, Akgul & Erkilic (2016) reported that fertilizer treatments reduced the severity of *Fusarium* foot rot in wheat more than non-fertilizer treatments.

Good agricultural practice (GAP) in sustainable wheat production requires integrated agrotechnology to control FHB and DON. First and foremost, the development of wheat cultivars adapted to climatic conditions and stress factors, as well as resistant to FHB, can improve high-quality wheat production. Although N fertilization is an essential aspect of the GAP, promoting rapid plant growth and ensuring grain quality with high protein content and yield, rational N application is recommended (Tabak et al., 2020). Understanding the response of cereal yields to nitrogen (N) fertilizer is crucial for enhancing nutrient management strategies and developing policies that ensure both the economic viability of crops and food security while minimizing nitrogen pollution (van Grinsven et al., 2022). Given the importance of implementing and enhancing integrated agrotechnical approaches in the management of FHB, including N fertilization as a key factor for high-yielding and quality grain production, as well as scientific reports indicating that *Fusarium* and DON contamination can be affected by N rates, this research aims to evaluate the impact of three N rates (0, 50 and 100 kg N ha^{-1}) on *Fusarium* incidence, DON levels, selected agronomic traits, and grain yield in two wheat cultivars with different breeding origins.

2. MATERIALS AND METHODS

The field experimental design

Field experiments were carried out in rainfed conditions in the Belgrade district in Serbia at the experimental field of the Institute for Animal Husbandry, Belgrade-Zemun (44°84' N, 20°40' E; 88 m a.s.l.). Two winter wheat cultivars, the French cultivar Nogal and the Serbian cultivar NS Ilina (Table 1), were grown during the 2016-2017 and 2017-2018 seasons at three N fertilization rates (0, 50, and 100 kg N ha^{-1}) under natural infection conditions. In both seasons, the preceding crop was maize (*Zea mays* L.). Wheat cultivars were sown on October 10, 2016, and October 12, 2017. The sowing density was 500 germinated grains m^{-2}. The field experiments were arranged in a split-plot randomized block design with three replicates. The two wheat cultivars were the main plots, and three N rates, 0 (N_0), 50 (N_{50}), and 100 kg N ha^{-1} (N_{100}), were subplots. The plot size was 5 m^2 (5 m × 1 m). During the wheat growth stages, BBCH 23-24, ammonium nitrate fertilizer containing 33.4% nitrogen was applied at the rate of 149.7 (N_{50}) and 299.4 (N_{100}) kg ha^{-1}. Chemical treatments to suppress weeds by Lancelot 440 WG (a.i. aminopyralide + florasulam) and insects by Vantex 60 SC (a.i. gamma-cyhalothrin) were performed at the tillering wheat stages BBCH 29-31. The harvested dates were in the first week of July 2017 and 2018 at the ripening wheat stage (BBCH 92).

Table 1. Characteristics of two winter wheat cultivars, Nogal (awned) and NS Ilina (awnless)

Item	Wheat cultivars	
	Nogal	NS Ilina
Origin	French	Serbian
Maturity	Early	Medium-late
Sowing rate	450-500 germinating grains m^{-2}	500 germinating grains m^{-2}
Optimum sowing time	October 10-15	October 15 - early November
Height	Short to medium-height	Tall (85-90 cm)
Hectoliter (kg hl^{-1})	>80	80-84
Protein content (%)	12-15	12-14
Moist gluten (%)	30-40	25-30
Yield potential (t ha^{-1})	9-12	8-10
Farinogram	A2	B1
Source	https://www.axereal.hr/sites/default/files/2024-04/Nogal-ENG.pdf	https://nsseme.com/en/products/small-grains/wheat/

Soil type properties and meteorological data of the field experiment location

The soil type was carbonate Chernozem (International Union of Soil Sciences – IUSS, Rome, Italy) with neutral pH in H_2O, organic matter > 6%, and humus >4%. Nitrate nitrogen (NO_3-N) and ammonium (NH_4-N) are balanced. P_2O_5 and K_2O had low and medium content, respectively.

According to climate data from November to June, the 2017–2018 season had higher total rainfall (387.1 mm), mean relative humidity (73%), and average temperature (10.6°C) than the 2016–2017 season (272.3 mm, 70.8%, and 8.9°C, respectively). During the wheat flowering stage in May, weather conditions promote fungal activity. In May, total rainfall and mean RH were higher in 2017 (82.1 mm and 68%) than in 2018 (42.6 mm and 62%), while mean temperature was lower in 2017 (17.8°C) than in 2018 (20.8°C). During the grain-filling stage in June, weather conditions influence the yield components and yield. In June, total rainfall and mean RH were higher in 2018 (78.7 mm and 70%) than in 2017 (35.8 mm and 59%), while mean temperature was lower in 2018 (21.8°C) than in 2017 (23.9°C) (Table 2).

Table 2. Mean monthly temperature, relative humidity (RH), and total rainfall from November to June for the wheat growing seasons 2016–2017 and 2017–2018 in the Belgrade district (location Surčin)

Growing season	Month	Temperature (ºC)	Rainfall (mm)	RH (%)
2016–2017	November	7	66.5	76
	December	0.0	2.2	80
	January	-4.4	18.8	83
	February	4.8	21.0	73
	March	10.5	26.6	65
	April	12.3	45.6	62
	May	17.8	82.1	68
	June	23.9	35.8	59
	Seasonal mean temperature	8.9		
	Total season rainfall		272.3	
	Seasonal mean RH			70.8
2017–2018	November	7.7	33.1	79
	December	4.4	47.8	80
	January	4.4	35.7	76
	February	1.8	55.0	81
	March	6.2	54.5	74
	April	17.4	39.7	62
	May	20.8	42.6	62
	June	21.8	78.7	70
	Seasonal mean temperature	10.6		
	Total season rainfall		387.1	
	Seasonal mean RH			73

Evaluation of Fusarium incidence, deoxynivalenol (DON) levels, some agronomic traits, and grain yield

A total of 30 wheat spikes were collected from each subplot, thrashed using a laboratory thresher (Wintersteiger LD 180, Ried, Austria), packed in paper bags, and stored in the refrigerator at 4°C. After grinding the grain samples, the moisture content was calculated using an analyzer (MB35, OHAUS, Parsippany, New Jersey, USA). A total of 50 grains per wheat subsample were disinfected in 1% sodium-hypochlorite for 2-3 min, rinsed in sterile distilled water twice, dried on filter paper, and plated on 1.8% salted potato dextrose agar in Petri plates (10 grains per Petri plate) and incubated for 10–15 days at a temperature of 20 ± 2°C. Based on macroscopic (colony appearance, pigmentation) and microscopic (absence or presence of microconidia, macroconidia, chlamydospores, etc.) characteristics, *Fusarium* species were identified using the fungal keys of Leslie & Summerell (2006). The percentage values of *Fusarium* incidence were determined by dividing the number of grains infested with *Fusarium* spp. by the total number of examined grains, multiplying by 100.

Before mycotoxin analyses, wheat grain samples were dried for 3 days at 60°C and ground using an analytical mill (A11, IKA, Staufen, Germany). By performing an enzyme-linked immunosorbent assay (ELISA), the DON was determined in wheat samples, using the Celery DON ELISA kit (Eurofins Technology, Budapest). According to the manufacturer's instructions, ground wheat samples were mixed with sodium chloride and 70% methanol in Erlenmeyer flasks and shaken vigorously for 3 minutes in an orbital shaker (3015, GFL, Burgwedel, Germany). After sample extraction, 50 µl of the supernatant was added to a microwell containing enzyme-conjugated DON,

mixed, and transferred to microwells coated with an anti-DON antibody. Then, it was incubated in the dark at room temperature for 10 minutes. During incubation, the DON from the supernatant and the enzyme-conjugated DON competed for binding to the anti-DON antibody coated on the microwells. After incubation, 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 the bound conjugate, resulting in the appearance of a blue color. The DON level is inversely proportional to the intensity of the blue color. If the concentration of DON in the sample increases, the intensity of the blue color decreases. Finally, to stop these enzymatic reactions, sulfuric acid solution was added, changing the chromogen color from blue to yellow. The absorbance of the samples was measured at 450 nm wavelength using an ELISA reader (EL×800TM, BioTek Instruments, Winooski, Vermont, USA). The detection limit of this ELISA test for wheat was 40 µg DON kg^{-1}.

A total of 30 plants per subplot was selected to evaluate plant height (PH, cm), spike length (SL, cm), and thousand-grain weight (TGW, g) of two winter wheat cultivars, Nogal and NS Ilina, at the maturity stage. Grain yield was harvested at the ripening stage from each subplot, converted to t ha^{-1}, and expressed at 14% moisture content.

Statistical data analyses

The effects of two growing seasons, two winter wheat cultivars, and three N rates on the incidence of the *Fusarium* spp., DON levels, agronomic traits (PH, SL, and TGW), and GY were analysed using a general linear model (multivariate analysis of variance, MANOVA) with SPSS software (SPSS Statistics 20; IBM, Armonk, New York, USA). The homogeneity of treatment variances was evaluated by Levene's test. Mean DON levels were log-transformed to normalize the distribution. When significant differences were detected ($P \leq 0.05$), means were separated using Tukey's HSD post hoc test. Correlations between the tested variables were determined by Pearson statistical analyses, including detection limits for treatments with non-detected deoxynivalenol.

3. RESULTS

Fusarium incidence, deoxynivalenol levels, agronomic traits, and grain yield affected by nitrogen fertilization

Microscopic observations showed that among *Fusarium* species, *F. graminearum* species complex (FGSC) strains were the most frequently isolated in both investigated years, while *F. poae*, *F. incarnatum* (synonym: *F. semitectum*), and members of the *Fusarium fujikuroi* species complex (FFSC) had a low and sporadic incidence. In addition, fungal species from the genera *Alternaria*, *Acremonium*, *Aspergillus*, *Epicoccum*, *Nigrospora,* and *Penicillium* were identified, with a high incidence of *Alternaria* (>60%) in both years, and other species had minor and sporadic occurrences. The mean moisture content of harvested wheat grain samples was under 14% in both years (data not shown).

According to the MANOVA analyses, the growing season (year) factor had a highly significant effect ($P \leq 0.01$) on all investigated variables (Table 3). The incidence of FGSC strains (20.22%), DON levels (323.59 µg kg^{-1}), PH (83.21 cm), TGW (38.68 g), and GY (8.88 t ha^{-1}), were higher in 2017 than in 2018 (9.66%, 97.72 µg kg^{-1}, 71.05 cm, 34.35 g, and 5.11 t ha^{-1}, respectively). On the contrary, SL was higher in 2018 (9.15 cm) than in 2017 (7.61 cm). The effect of cultivar was highly significant on all investigated parameters, except TGW and GY. Cultivar Nogal had a lower incidence of FGSC strains (9.77%), DON levels (104.71 µg kg^{-1}), PH (68.68 cm), and SL (7.65 cm) compared to NS Ilina (20.11%, 302 µg kg^{-1}, 85.57 cm, and 9.11 cm, respectively). The N rates (50 and 100 kg ha^{-1}) significantly influenced PH compared to the control. However, these N rates did not differ for PH.

The interaction effects on the investigated variables

A significant year × wheat cultivar interaction was observed for all variables investigated. Year × N rate interaction had a statistically significant influence ($P \leq 0.01$) on the incidence of FGSC strains and PH. *F* values for the interaction between wheat cultivar and N rate were statistically significant for the incidence of FGSC strains ($P \leq 0.05$), PH ($P \leq 0.01$), and SL ($P \leq 0.05$). The year × wheat cultivar × N rate interaction significantly influenced log-transformed DON levels ($P \leq 0.05$), PH ($P \leq 0.01$), and SL ($P \leq 0.05$) (Table 3).

Table 3. Year, wheat cultivar, and nitrogen rate effects on the incidence of *F. graminearum* species complex (FGSC) strains, the log-transformed deoxynivalenol (DON) levels, plant height (PH), spike length (SL), thousand-grain weight (TGW), and grain yield (GY)

Factor	FGSC incidence (%)	Log 10 DON (µg kg^{-1}) [a]	PH (cm)	SL (cm)	TGW (g)	GY (t ha^{-1})
	Year effects (A)					
2017	20.22[a]	2.51 (323.59)[a]	83.21[a]	7.61[b]	38.68[a]	8.88[a]
2018	9.66[b]	1.99 (97.72)[b]	71.05[b]	9.15[a]	34.35[b]	5.11[b]
F-test	**	**	**	**	**	**
	Wheat cultivar effects (B)					
Nogal	9.77[b]	2.02 (104.71)[b]	68.68[b]	7.65[b]	36.45	6.69
NS Ilina	20.11[a]	2.48 (302.00)[a]	85.57[a]	9.11[a]	36.59	7.30
F-test	**	**	**	**	ns	ns
	Nitrogen rate effects (C)					
0 kg ha^{-1}	14.66	2.11 (128.82)	70.11[b]	8.32	36.62	6.77
50 kg ha^{-1}	13.16	2.26 (181.97)	81.65[a]	8.25	36.38	7.41
100 kg ha^{-1}	17.00	2.38 (239.88)	79.62[a]	8.57	36.55	6.81
F-test	ns	ns	**	ns	ns	ns
	Interactions					
A × B	**	*	**	**	**	*
A × C	**	ns	**	ns	ns	ns
B × C	*	ns	**	*	ns	ns
A × B × C	ns	*	**	*	ns	ns
Means	14.94	2.25 (177.83)	77.13	8.38	36.52	7.00

Means followed by the same letter within a column are not significantly different by *Tukey's test* at the P ≤ 0.05 level. ns, not statistically significant; *significant at the 0.05 level of probability; **significant at the 0.01 level of probability.
[a] log 10 transformed data, back-transformed means are shown in parentheses

In 2017, the highest incidence of FGSC strains (27.78%) and DON levels (724.44 µg kg^{-1}) was found in the cultivar NS Ilina (Table 4). The lowest FGSC incidence was at N_{50} in 2018 (Figure 1) in the cultivar Nogal (Figure 2). The highest DON levels were in the cultivar NS Ilina at N_0, N_{50}, and N_{100}, and in the cultivar Nogal at N_{50} and N_{100} in 2017, as well as in the cultivar NS Ilina at N_{50} and N_{100} in 2018, with insignificant differences. There were no significant differences in PH at N_0, N_{50}, and N_{100} in 2017, and at N_{50} in 2018, at the cultivar NS Ilina. SL was the highest in the cultivar NS Ilina in 2018, with no statistically significant differences between N_0, N_{50}, and N_{100} (Table 5).

PH and SL were the highest in 2017 and 2018, respectively, at NS Ilina. TGW was higher in 2017 than in 2018, with no significant differences among the investigated wheat cultivars. In 2017, there were no statistical differences between wheat cultivars for GY, while in 2018, NS Ilina had higher GY than Nogal (Table 6). PH showed no statistical differences between N_0, N_{50}, and N_{100} in 2017. PH was the lowest at N_0 in 2018 (Figure 3). In the NS Ilina cultivar, the highest PH did not differ between N_{50} and N_{100}, whereas the highest SL did not differ among N_0, N_{50}, and N_{100} (Table 7).

Table 4. Effect of year and wheat cultivar on the means of the incidence of FGSC strains and log-transformed deoxynivalenol (DON) levels

Year	Wheat cultivar	The incidence of FGSC strains (%)	Log 10 DON (µg kg^{-1})[a]
2017	Nogal	12.67[b]	2.17 (147.91)[b]
2017	NS Ilina	27.78[a]	2.86 (724.44)[a]
2018	Nogal	6.89[b]	1.86 (72.44)[b]
2018	NS Ilina	12.44[b]	2.11 (128.82)[b]

Means followed by the same letter within a column are not significantly different by *Tukey's test* at the P ≤ 0.05 level.
[a] log 10 transformed data, back-transformed means are shown in parentheses

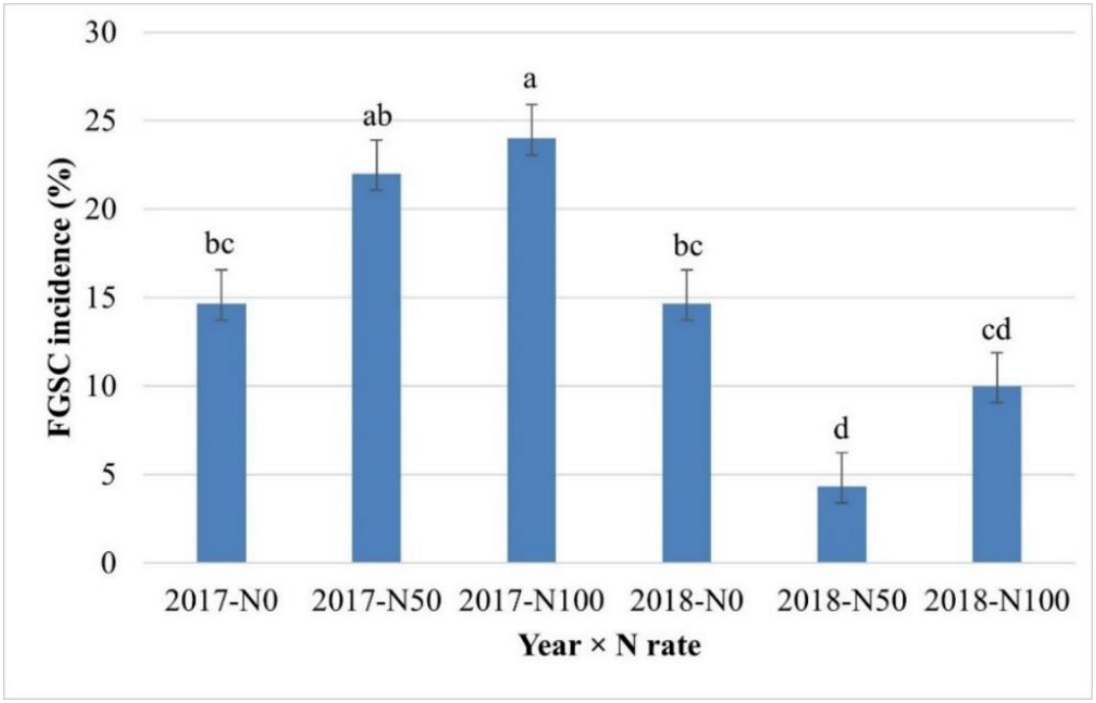

Figure 1. Effect of year and nitrogen (N) rate on FGSC incidence in wheat crops in 2017 and 2018. Means followed by the same letter within a column are not significantly different by *Tukey's test* at the $P \leq 0.05$ level. Vertical bars correspond to standard error. N_0, N_{50}, and N_{100} – N rates of 0, 50, and 100 kg N ha^{-1}, respectively; FGSC – *Fusarium graminearum* species complex.

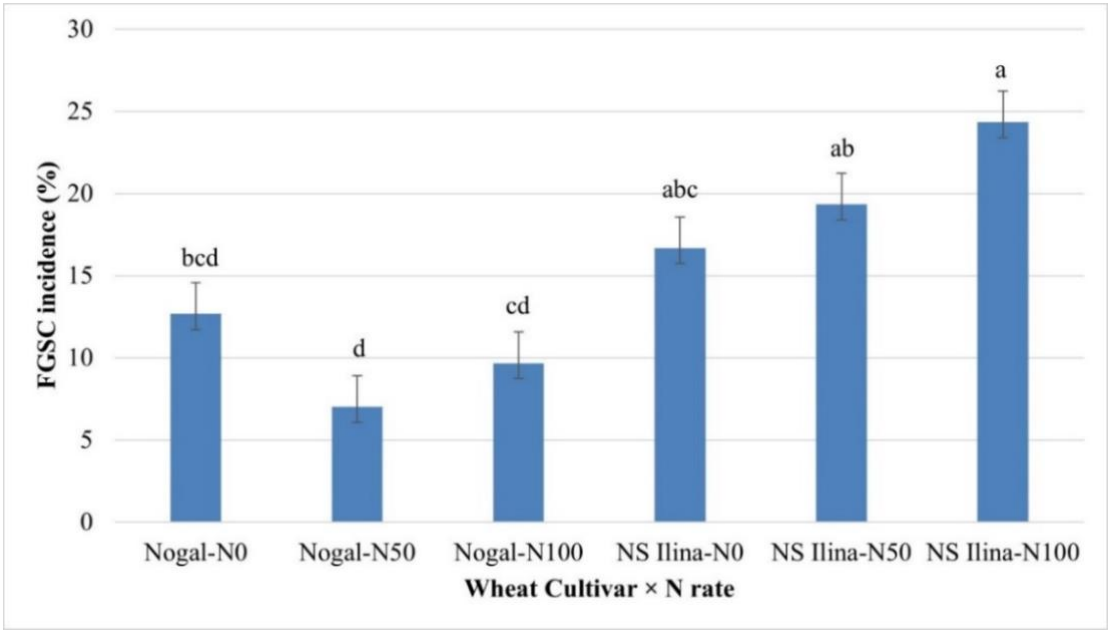

Figure 2. Effect of wheat cultivar and nitrogen (N) rate on FGSC incidence. Means followed by the same letter within a column are not significantly different by *Tukey's test* at the $P \leq 0.05$ level. Vertical bars correspond to standard error. Nogal and NS Ilina – wheat cultivars; N_0, N_{50}, and N_{100} – N rates of 0, 50, and 100 kg N ha^{-1}, respectively; FGSC – *Fusarium graminearum* species complex.

Table 5. Effect of year, wheat cultivar, and nitrogen (N) rate on the log-transformed deoxynivalenol (DON) levels, plant height (PH), and spike length (SL)

Year	Wheat cultivar	N rate (kg N ha^{-1})	Log 10 DON (µg kg^{-1})[a]	PH (cm)	SL (cm)
2017	Nogal	0	1.65 (44.67)^{d}	70.44^{cd}	7.57^{bc}
2017	Nogal	50	2.31 (204.17)^{abcd}	75.05^{c}	6.91^{c}
2017	Nogal	100	2.53 (338.84)^{abc}	72.77^{cd}	7.58^{bc}
2017	NS Ilina	0	2.88 (758.58)^{a}	90.97^{ab}	7.56^{bc}
2017	NS Ilina	50	2.89 (776.25)^{a}	94.47^{a}	8.10^{b}
2017	NS Ilina	100	2.79 (616.60)^{ab}	95.53^{a}	7.95^{bc}
2018	Nogal	0	1.91 (81.28)^{cd}	59.67^{e}	8.09^{bc}
2018	Nogal	50	1.74 (54.95)^{cd}	67.57^{cde}	7.96^{bc}
2018	Nogal	100	1.94 (87.10)^{bcd}	66.60^{d}	7.77^{bc}
2018	NS Ilina	0	1.98 (95.50)^{bcd}	59.35^{e}	10.06^{a}
2018	NS Ilina	50	2.10 (125.89)^{abcd}	89.50^{ab}	10.01^{a}
2018	NS Ilina	100	2.25 (177.83)^{abcd}	83.60^{b}	11.00^{a}

Means followed by the same letter within a column are not significantly different by *Tukey's test* at the P ≤ 0.05 level.
[a] log 10 transformed data, back-transformed means are shown in parentheses

Table 6. Effect of year and wheat cultivar on the means of agronomic traits and grain yield

Year	Wheat cultivar	PH (cm)	SL (cm)	TGW (g)	GY (t ha^{-1})
2017	Nogal	72.75^{c}	7.35^{c}	37.89^{a}	8.99^{a}
2017	NS Ilina	93.66^{a}	7.87^{bc}	39.47^{a}	8.77^{a}
2018	Nogal	64.61^{d}	7.94^{b}	35.00^{b}	4.40^{c}
2018	NS Ilina	77.48^{b}	10.36^{a}	33.70^{b}	5.83^{b}

Means followed by the same letter within a column are not significantly different by *Tukey's test* at the P ≤ 0.05 level. PH – plant height; SL – spike length; TGW – thousand-grain weight; GY – grain yield.

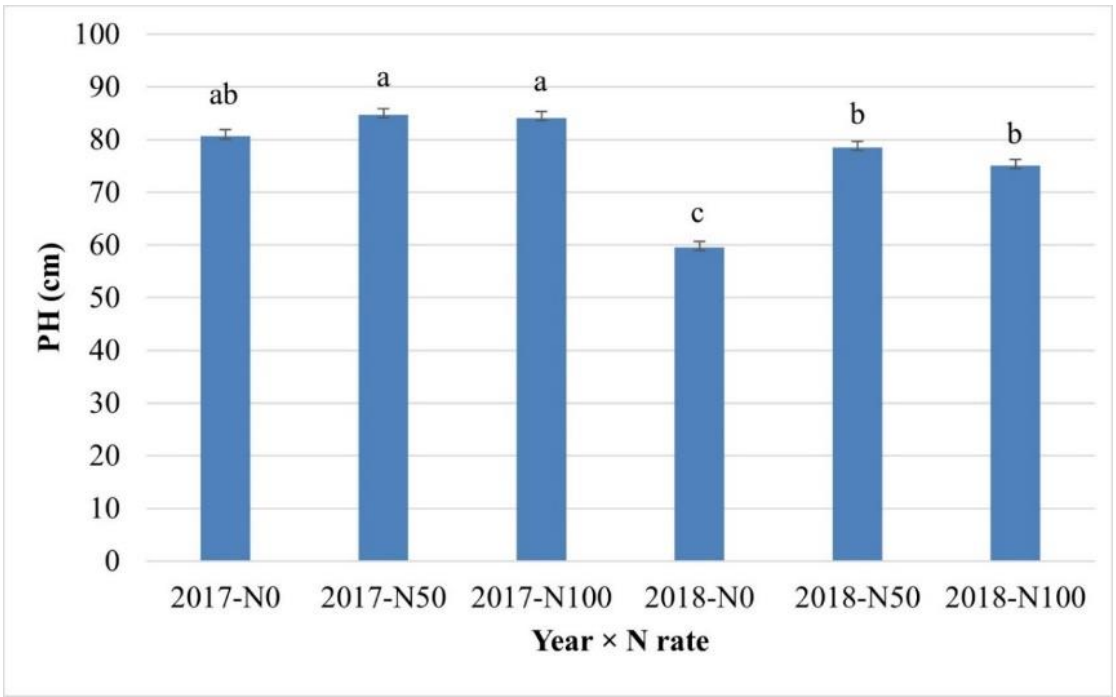

Figure 3. Effect of year and nitrogen (N) rate on plant height (PH) in 2017 and 2018. Means followed by the same letter within a column are not significantly different by *Tukey's test* at the P ≤ 0.05 level. Vertical bars correspond to standard error. N_0, N_{50}, and N_{100} – N rates of 0, 50, and 100 kg N ha^{-1}, respectively.

Table 7. Effect of wheat cultivar and nitrogen (N) rate on the means of plant height (PH) and spike length (SL)

Wheat cultivar	N rate (kg N ha^{-1})	PH (cm)	SL (cm)
Nogal	0	65.06^{d}	7.83^{b}
Nogal	50	71.31^{bc}	7.44^{b}
Nogal	100	69.68^{cd}	7.67^{b}
NS Ilina	0	75.16^{b}	8.81^{a}
NS Ilina	50	91.99^{a}	9.06^{a}
NS Ilina	100	89.57^{a}	9.48^{a}

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

Correlation relationship between the investigated variables

Correlation analyses revealed moderately positive correlations between the incidence of FGSC strains with log-transformed DON levels (r = 0.629**), PH (r = 0.545**), TGW (r = 0.455**), and GY (r = 0.582**), between log10 DON levels with PH (r = 0.657**), TGW (r = 0.528**), and GY (r = 0.464**), and between PH with TGW (r = 0.436**) and GY (r = 0.491**) (Table 8). There was a weak positive correlation between PH and SL (r = 0.058). Relationship SL with FGSC incidence (r = -0.045), DON (r = -0.139), TGW (r = -0.624**), and GY (r = -0.377*) were negatively correlated.

Table 8. Correlation analysis between investigated variables

Two-year period (2017–2018)	The incidence of FGSC strains (%)	Log 10 DON (µg kg^{-1})	PH (cm)	SL (cm)	TGW (g)
Log 10 DON (µg kg^{-1})	0.629**				
PH (cm)	0.545**	0.657**			
SL (cm)	-0.045^{ns}	-0.139^{ns}	0.058^{ns}		
TGW (g)	0.455**	0.528**	0.436**	-0.624**	
GY (t ha^{-1})	0.582**	0.464**	0.491**	-0.377*	0.648**

**significant at the 0.01 level of probability; ns, not statistically significant; FGSC – *Fusarium graminearum* species complex; DON – deoxynivalenol; PH – plant height; SL – spike length; TGW – thousand-grain weight; GY – grain yield.

4. DISCUSSION

Fusarium species are the most important pathogens of FHB worldwide, jeopardising the yield and quality of small cereal grains. In this study, FGSC strains were the most frequently isolated from wheat grains in both harvest seasons. *F. poae, F. incarnatum*, and FFSC strains were isolated sporadically at low incidence, consistent with previous results by Krnjaja et al. (2015, 2022) in common and durum wheat. Additionally, surveys of FHB species in wheat crops found *F. graminearum* s.s. to be predominant in Italy (Covarelli et al., 2015) and Poland (Bilska et al., 2018). Among other fungal species, *Alternaria* spp. was the most frequent (>60%) in wheat grains harvested in both years, consistent with the results of Jevtić et al. (2019).

Results showed that increased N rates only increased PH. Similarly, Salgado et al. (2017) found that N rates ranging from 34 to 180 kg ha^{-1} had no significant effect on any FHB parameters and DON levels in wheat crops over three successive years. Oldenburg et al. (2007) also did not determine a statistically significant effect of N fertilization on DON levels in wheat crops in the four-year study. They stated that the main factors contributing to DON contamination were the amount of *Fusarium* inoculum, wheat cultivar susceptibility to FHB, and weather conditions. Conversely, Lemmens et al. (2004) found that FHB severity and DON levels in spring wheat increased with increasing N rates from 0 to 80 kg N ha^{-1}, while there was no increase at higher N rates, of 120 and 160 kg N ha^{-1}. Likewise, Heier et al. (2005) and Podolska et al. (2017) reported that excessive N fertilization can significantly increase DON contamination in wheat crops. However, N fertilization rates can have inconsistent effects on FHB parameters and DON levels, with the impact of the growing season being more critical than N rates (Van der Burgt et al., 2011).

N rates influence agronomic traits. In our study, N rates of 50 and 100 kg ha^{-1} affected PH. Luo et al. (2021) also observed increased wheat plant height at higher N rates (90–270 kg N ha^{-1}), as well as increased numbers of leaves and greater spike leaf length and width. Ullah et al. (2018), Abera et al. (2024), and Alaamer et al. (2024) found the maximum PH of wheat at 203, 69, and 100 kg N ha^{-1}, respectively. These authors concluded that N rates increased yield components, such as SL and TGW. In addition, Litke et al. (2018) reported that the N rate of 180 kg N ha^{-1} significantly increased winter wheat yield. However, Zheng et al. (2021) found that late sowing dates and excessive N rates reduced yield parameters such as TGW and grain yield.

Although both investigated wheat cultivars were susceptible to FGSC and DON contamination, the French cultivar Nogal showed significantly lower susceptibility to FGSC and DON than the Serbian cultivar NS Ilina. According to Shah et al. (2018), morphological traits such as plant height, awn presence, and the percentage of flower opening in wheat crops can influence FHB. Our study showed that the shorter wheat cultivar had lower FGSC incidence and lower DON levels, indicating a positive correlation between plant height and FHB susceptibility. This result aligns with the strategies proposed by DePauw et al. (2011) and Cuthbert et al. (2017), which demonstrated that combining shorter, earlier flowering genotypes with moderate resistance can shift the negative relationship between plant height and FHB symptoms, resulting in crops with reduced FHB symptoms. On the other hand, there were no significant differences in TGW between the less susceptible cultivar Nogal and the more susceptible cultivar NS Ilina, similar to the findings of Jevtić et al. (2022). There are five types of FHB resistance in wheat cultivars: I – resistance to initial infection, II – resistance to spread of *Fusarium* species within the spike, III – resistance to seed infection, IV – tolerance to FHB and DON, and V – resistance to DON accumulation (Mesterházy, 1995). Although FHB disease resistance is genetically controlled, *Fusarium* infection and DON synthesis in wheat crops are also influenced by climatic conditions, cultivation areas, abiotic and biotic factors, plant stress, agrotechnical practices, and their interaction (Beccari et al., 2022; Župunski et al., 2022). Growing wheat cultivars that are resistant or less susceptible can reduce *Fusarium* and mycotoxin contamination and is recommended as a preventive, economical, and environmentally friendly strategy in FHB management. The combination of superior agronomic and qualitative parameters with resistance presents an additional challenge for FHB resistance, because rare segregants that combine the most important traits can be found within the indigenous wheat population when the populations are large enough (Miedaner et al., 2017).

The incidence of FGSC strains, DON levels, PH, TGW, and GY were significantly higher in 2017 than in 2018. Examining the climatic factors from November to June, mean monthly temperatures, total rainfall, and mean RH were lower in the 2016–2017 season (8.9°C, 272.3 mm, and 70.8%) than in the 2017–2018 season (10.6°C, 387.1 mm, and 73%). However, in May, during the flowering and post-flowering stages of wheat, which are crucial for *Fusarium* infection and wheat development, total rainfall was higher in 2017 (82.10 mm) than in 2018 (42.60 mm) (Table 2). Cowger et al. (2009) and Andersen et al. (2015) also established the positive effect of extended post-anthesis or post-flowering moisture on FHB and DON contamination in wheat crops. Moreover, Nguyen et al. (2024) found a positive correlation between *Fusarium* and trichothecene contamination and rainfall during the flowering stage of wheat. They identified a higher incidence of *F. graminearum* than *F. poae* in the season with more rainfall at the flowering wheat stage. In addition, maize, as a preceding crop, provides more *Fusarium* inoculum under favorable climatic conditions (Landschoot et al., 2013).

DON levels varied between years and wheat cultivars, with higher levels observed in 2017 and NS Ilina grains. There was no significant effect of N rate on DON levels, consistent with the findings of El Chami et al. (2022). In all treatments investigated, DON levels did not exceed the maximum limits of 1000 µg kg^{-1} for unprocessed cereals (2024/1022/EC). Low mean DON levels in winter wheat samples under natural infection of grains were reported by Jajić et al. (2008) in Serbia, Covarelli et al. (2015) in Central Italy, Okorski et al. (2022) in Poland, and Miedaner et al. (2024) in Germany. Miedaner et al. (2024) stated that DON levels are variable and influenced by year and the number of rainy days during flowering, reporting a high percentage of DON-positive samples (69-100%) and individual wheat grain samples with high DON levels (> 3000 µg kg^{-1}). Similarly, Palazzini et al. (2015) found DON in 79.7% of DON-positive wheat grain samples in Argentina with levels ranging from 400 to 8450 µg kg^{-1}.

Our results showed that the interaction between year and N rate influenced FGSC incidence and PH, and the interaction between year and wheat cultivar affected PH, SL, TGW, and GY. In addition, the interaction between year and wheat cultivar was also significant for FGSC incidence and DON levels, similar to the results of Cowger et al. (2009). Similarly, Ma et al. (2004) demonstrated through biplot analyses that the interaction between N rates and the environment increased FHB intensity in spring wheat in some environments, while the interaction between year and cultivar was significant for grain yield. In contrast, Heier et al. (2005) reported that the interaction between nitrogen and wheat protection treatments did not significantly affect FHB severity or DON levels. However, Van der Burgt et al. (2009) found inconsistent data on the interaction effect between topdressing and year, which was significant for DON levels but not significant for FHB-infected grains under natural infection in wheat crops.

Correlation coefficients between FGSC incidence and log-transformed DON levels, PH, TGW, and GY were significant and moderately positive, as well as between log-transformed DON levels and PH, TGW, and GY, and between PH values and TGW and GY. Accordingly, not all short genotypes should be considered at risk for FHB, but some forms of the *Rht* gene that reduce plant height may carry a higher risk (Miedaner et al., 2022). A weak positive correlation was observed between SL and PH. SL was negatively correlated with FGSC incidence, DON levels, TGW, and GY. Similar to our results, Jevtić et al. (2022) reported a significantly positive correlation between disease variables such as the FHB index and *Fusarium*-damaged grains (FDG), while the relationship between FHB traits and TGW was not straightforward and depended on the degree of wheat cultivar susceptibility. In general, TGW was more strongly correlated with FDG and wheat cultivar susceptibility. There were no

significant yield variations per growing season in moderately resistant wheat cultivars compared to susceptible and moderately susceptible cultivars, suggesting that yield responses to FHB depend mainly on the FHB index and FDG. In this study, the absence of artificial spike inoculation with *Fusarium* pathogens at the flowering stage may explain the positive correlation between the incidence of FGSC and DON with TGW and GY, since the number of symptomless wheat grains was higher than the number of FDG. Similarly, Tekauz et al. (2000) reported that in wheat, due to the low weight of individual grains, a large amount of FDG is lost, which further affects the preserved yield. There is a positive correlation between DON levels and *F. graminearum* biomass and between FHB infection and DON levels in common wheat (Wegulo, 2012), durum wheat (Berraies et al., 2024), and spelt wheat (Chrpová et al., 2021). In contrast to our results, Chrpová et al. (2021) found a weak negative correlation between PH and FHB parameters as well as between PH and DON levels. In addition, Younis et al. (2023) found a significant positive correlation between most of the investigated agronomic traits, including PH and TGW. Generally, differences in strength across studies indicate that trait interactions depend on both environmental conditions and the genetic background of the breeding material.

5. CONCLUSION

By evaluating the incidence of *Fusarium* spp. in harvested wheat grains under natural conditions, FGSC strains were predominant in both growing seasons (2017 and 2018). Year and wheat cultivar had significant effects on the incidence of FGSC strains, levels of DON, and agronomic traits (PH and SL), while the N rate had a highly significant influence only on PH. The French cultivar Nogal was less susceptible to FGSC and DON than the Serbian cultivar NS Ilina, although PH and SL were higher in NS Ilina. There were no significant differences between PH at the N_{50} and N_{100} rates, whereas PH was lowest at N_0. TGW and GY were affected only by the growing season, with higher TGW and GY in 2017. These results suggest that agricultural practices such as selecting resistant or less susceptible cultivars are more important than nitrogen fertilization for improving and implementing integrated approaches to FHB management in wheat production. Generally, an optimal amount of nitrogen fertilizers is necessary as an essential agronomic practice for achieving high-yielding wheat crops.

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Conflicts of Interest

The authors declare no conflict of interest.

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