



Fusarium head blight and mycotoxin concentrations in a moderately resistant winter wheat cultivar under different nutrient regimes

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ABSTRACT

Winter wheat cv. Boomer was grown in a field-plot experiment in Tomaszkowo near Olsztyn. During the growing season, the severity of Fusarium head blight (FHB) was evaluated on a 5-point scale. The quantitative and qualitative composition of *Fusarium* fungi colonizing wheat grain was evaluated in a laboratory. The content of *Fusarium* mycotoxins (deoxynivalenol, DON, nivalenol, NIV, zearalenone, ZEA, fumonisins FB1 and FB2) and ergosterol (ERG) in grain was determined by high-performance liquid chromatography (HPLC). The relationships between the severity of FHB and mycotoxin concentrations in grain were determined by calculating Pearson's correlation coefficient r in the CORR SAS procedure. The effect of microelement fertilizers on the severity of FHB, the species composition of *Fusarium* fungi colonizing winter wheat grains and mycotoxin concentrations in grain were determined.

Analyses of winter wheat spikes revealed that FHB was less severe in 2012 (healthy ears in the NPK+Mn treatment and the lowest value of the infection index 1% was noted in the absolute control treatment) than in 2013 (the most evident symptoms of FHB in the NPK+Nano-Gro treatment – infection index of approx. 12%). Mineral fertilization, i.e. NPK, NPK with microelements (Cu, Zn, Mn) and NPK with the Nano-Gro® organic growth stimulator, reduced the production of trichothecenes, ZEA and fumonisins B1 and B2 in both years of the study. The highest levels of DON and NIV were noted in winter wheat grain in 2012 in control, control/NPK, NPK+Cu and NPK+Mn treatments. Toxin-producing fungi: *Fusarium culmorum*, *F. poae*, *Gibberella avenacea*, *G. zeae* were isolated most frequently from winter wheat grain in the above treatments. The severity of FHB was not significantly correlated with the concentrations of ERG, FB1, FB2 and ZEA in grain. A negative correlation was observed between the severity of FHB vs. DON and NIV levels in grain.

Indexing terms/Keywords

Triticum aestivum L., microelement fertilizers, *Fusarium* spp., secondary metabolites.

Academic Discipline And Sub-Disciplines

Agronomy; Plant diseases

SUBJECT CLASSIFICATION

Phytopathology

TYPE (METHOD/APPROACH)

Research article

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is the most widely cultivated cereal in the world with a high number of varieties grown in different climate zones. Its popularity can be attributed to the high nutritional value of wheat grain. Fusarium head blight (FHB), a disease caused by fungi of the genus *Fusarium*, decreases the quality and quantity of grain yield. *Fusarium* species produce mycotoxins that are toxic for both humans and animals, including trichothecenes, zearalenone and fumonisins (Jestoi, 2008; Abbas et al., 2013). Fusarium head blight poses a particular threat for wheat which is highly susceptible to the disease (more susceptible than barley) and covers vast areas of cultivated land (Langevin et al., 2004). The severity of FHB is determined by the complex of *Fusarium* species colonizing wheat spikes and weather conditions, in particular during flowering (Goliński et al., 2010; Landschoot et al., 2013). *F. graminearum*, *F. culmorum* and *F. avenaceum* are the main causal agents of FHB around the world (Bottalico and Perrone, 2002; Chakraborty et al., 2006). The grain of winter wheat grown in Lithuania was colonized mostly by *F. poae*, followed by *F. avenaceum* and *F. sporotrichioides* (Supronienė et al., 2012). According to Stenglein (2009), *F. poae* plays an important role in the development of FHB, and this species is regarded as the main cause of the disease in Great Britain and Ireland (Xu et al., 2005). In Poland, the main causal agents of FHB are *F. poae*, *F. tricinatum*, *F. avenaceum*, *F. culmorum* and *F. graminearum* (Goliński et al., 2010; Filoda and Wickiel, 2009; Chełkowski et al. 2012).

The severity of FHB and mycotoxin concentrations in grain are also influenced by agronomic practices, variety/cultivar and protective treatments (Oldenburg et al., 2008). In a study by Lori et al. (2009), the concentration of deoxynivalenol (DON) was twice higher in the grain of direct-seeded wheat (no-till) than in the grain of wheat plants grown in a conventional tillage system. Bernhoft et al. (2012) reported that mycotoxin levels were significantly lower when cereals were grown after non-cereal crops. Mycotoxin concentrations are also much lower in wheat varieties resistant to FHB (Anderson, 2007). The cited authors demonstrated that DON biosynthesis was nearly completely inhibited in the grain of wheat cultivars



characterized by increased resistance to FHB. In a study by McMullen et al. (2008), the use of triazole fungicides contributed to a two-fold reduction in DON accumulation in grain, but only in treatments where crops were grown after oilseed rape and not wheat. According to Willyerd et al. (2012), a combination of fungicides and moderately-resistant wheat cultivars offered the most effective protection against FHB. In an experiment conducted by Remža et al. (2016), mycotoxin concentrations (DON, ZEA) in wheat grain were lower in the organic farming system than in the conventional farming system. According to Brandt and Mølgaard (2006), rapid and excess supply of nutrients in conventional farming systems can inhibit the induction of natural defense mechanisms in plants. Blandino et al. (2012) described the synergistic effects of the following factors: susceptibility to infections, forecrop, cultivation system (conventional tillage, direct seeding – no-till), fungicide treatments to minimize the risk of *Fusarium* infections, and the level of DON accumulation in grain. Schaafsma et al. (2001) demonstrated that the accumulation of mycotoxins in wheat grain was far more likely to be influenced by the rates of nitrogen fertilization than by weather conditions during the growing season. Manganese plays an important role in many metabolic processes in plants – it participates in enzymatic reactions and the biosynthesis of lignins and suberins (Marschner et al., 2003). Thompson and Huber (2007) reported that manganese has a toxic effect on many pathogens. Several compounds containing copper, an element that is essential for plant growth, are used as fungicides in plant production due to their fungicidal effects (Grzebisz et al., 2010). According to Sharma et al. (2011), zinc compounds have a fungistatic effect on *Pythium debaryanum*. In a study by He et al. (2011), zinc compounds completely inhibited the growth of *Botrytis cinerea* and *Penicillium expansum*.

The aim of this study was to evaluate the severity of FHB during the growing season and to determine the species composition of *Fusarium* fungi colonizing winter wheat grain under different fertilization regimes (N-90, P-79, K-100 and foliar microelement fertilizers), with and without the use of the Nano-Gro growth stimulator. Changes in the concentrations of *Fusarium* mycotoxins in wheat grain were also analyzed.

MATERIALS AND METHODS

Winter wheat (*Triticum aestivum* L.) cv. Boomer was grown in a field-plot experiment in Tomaszkowo near Olsztyn (53°72' N; 20°42' E). The experiment had a randomized block design with three replications. Plot sown area and harvested area was 6.25 m² and 4.0 m², respectively. The experiment was established on class IIIb podzolic soil (complex 4 in the Polish classification system of agricultural land suitability) with pH 4.62 (in 1 M solution of KCl) with the following composition: C_{org.} – 7.93 g/kg, N_{total} – 0.95 g/kg, plant-available P – 58.9 mg/kg, K – 203.4 mg/kg, Mg – 8.1 mg/kg, Cu – 2.5 mg/kg, Fe – 1800 mg/kg, Zn – 7.9 mg/kg and Mn – 189 mg/kg. The experiment consisted of 7 treatments:

I – absolute control treatment (without fertilizers or the growth stimulator);

II – control/NPK: 90 kg N/ha, including 54 kg N/ha applied to the soil (46% urea in the tillering stage, BBCH 22-23) (Meier, 2001) and 36 kg N/ha applied to the leaves (10% urea in the stem elongation stage, BBCH 30-31); 70 kg/ha P₂O₅ as preplant fertilizer (triple superphosphate, 46%) at a rate equivalent to 30.2 kg P/ha; 100 kg/ha K₂O as preplant fertilizer (potash salt, 56%) at a rate of 83.1 kg K/ha;

III – NPK fertilizer, as above, and 0.2 kg Cu/ha applied to the leaves (foliar application of microelement fertilizer) at the stem elongation stage, BBCH 30-31 (1% solution of CuSO₄);

IV – NPK fertilizer, as above, and 0.2 kg Zn/ha (1% solution of ZnSO₄);

V – NPK fertilizer, as above, and 0.2 kg Mn/ha (0.5% solution of MnSO₄);

VI – NPK fertilizer, as above, and 0.2 kg Cu/ha + 0.2 kg Zn/ha + 0.2 kg Mn/ha;

VII – NPK fertilizer, as above, and the Nano-Gro® organic growth stimulator (oligosaccharide pellets containing Fe, Co, Al, Mg, Mn, Ni and Ag sulfates in a concentration of 10⁻⁹ mol). The experiment was performed under natural infection conditions.

Winter wheat was sown on 14 September 2012 (thousand grain weight – 46.9 g, 88% germination rate, seeding rate - 293 kg/ha) and 17 September 2013 (thousand grain weight – 45.2 g, 90% germination rate, seeding rate - 276 kg/ha) after winter triticale. Plant density was 550 plants m² in both years of the study. All operations (sowing, plant protection, fertilization, harvest) were conducted in accordance with the agronomic requirements of each species (Institute of Soil Science and Plant Cultivation - National Research Institute in Puławy). Weeds were eliminated with the use of herbicides. Grain was harvested on 31 July in both years of the experiment.

The severity of FHB (*Fusarium* spp.) was estimated based on an evaluation of 50 spikes harvested in the medium milk stage (BBCH 75). The evaluation was conducted on a 5-point scale, where 0 points denotes the absence of disease symptoms and 5 points indicates that more than 50% of spike area has been infected. The infestation index (Ii) was expressed as percentage.

$$I_i = \frac{\sum(a \times b) \times 100\%}{N \times I}$$

where $\sum(a \times b)$ is the sum of the products resulting from the multiplication of the number of plants (a) with points on the five-point scale (b); N is the total number of plants; and I is the highest number of points on the scale.



The quantitative and qualitative composition of *Fusarium* fungi colonizing harvested grain was determined in a laboratory. The analyses were performed on 100 randomly sampled grains which were disinfected in 50% ethylene for 1 minute, 0.1% sodium hypochlorite for 1 minute and rinsed three times in sterile water. Fungi were cultured on potato-dextrose-agar (PDA) for 7 days at 22°C. After incubation, fungal colonies were transferred to agar slants and identified under a microscope.

Mycotoxin analysis

Standards and chemical reagents. Fumonisin B₁ and B₂, zearalenone, deoxynivalenol, nivalenol and ergosterol analytical standards were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium dihydrophosphate, potassium hydroxide, sodium hydroxide, potassium chloride, acetic acid, hydrochloric acid and o-phosphoric acid were purchased from POCh (Gliwice, Poland). Organic solvents (HPLC grade), disodium tetraborate, *n*-pentane, 2-mercaptoethanol, sodium acetate and the remaining chemicals were also purchased from Sigma-Aldrich (Steinheim, Germany). Water for the HPLC mobile phase was purified in the Milli-Q system (Millipore, Bedford, MA, USA).

Extraction and purification procedure. Homogenized samples of 10 g of winter wheat grain were analyzed. All mycotoxins (FB₁, FB₂, ZEA, DON, NIV) and ergosterol (ERG) were extracted and purified according to a previously described procedure (Goliński et al., 2010; Waśkiewicz et al., 2013). The elute was evaporated to dryness at 40°C under a stream of nitrogen. Dry residue was stored at -20°C until HPLC analyses.

HPLC analysis. Chromatographic analyses were performed in the Waters 2695 high-performance liquid chromatography system (Waters, Milford, USA) with the following detectors:

- Waters 2475 Multi λ Fluorescence Detector ($\lambda_{\text{ex}}=335$ nm, $\lambda_{\text{em}}=440$ nm) with an XBridge column (3.0 \times 100 mm) for FB₁ and FB₂ analysis,
- Waters 2996 Photodiode Array Detector with a Nova Pak C-18 column (150 \times 3.9 mm) for ERG ($\lambda_{\text{max}}=282$ nm) analysis and a Nova Pak C-18 column (300 \times 3.9 mm) for DON and NIV analysis ($\lambda_{\text{max}}=224$ nm),
- Waters 2475 Multi λ Fluorescence Detector ($\lambda_{\text{ex}}=274$ nm, $\lambda_{\text{em}}=440$ nm) and Waters 2996 Photodiode Array Detector with a Nova Pak C-18 column (150 \times 3.9 mm) for ZEA analysis.

Mycotoxins were quantified by measuring peak areas for retention times according to the relevant calibration curve. The limits of detection were: 1 ng/g for ZEA, 20 ng/g for FB₁ and FB₂, and 10 ng/g for DON, NIV and ERG.

Statistical analysis

The results were analyzed statistically in the Statistica 10.0 program (StatSoft Poland, by the analysis of variance (ANOVA). Mean values were compared in Duncan's test ($p=0.05$). The relationships between the severity of FHB and mycotoxin concentrations in grain were determined by calculating Pearson's correlation coefficient r in the CORR SAS procedure.

Weather conditions

In both growing seasons, total precipitation levels in May were similar and somewhat below the long-term average (Table 1). In 10-day periods of May 2013, temperatures were higher than in the corresponding month in 2012. The flag leaf stage (BBCH 39) was achieved at a faster rate in the first year of the study, but from the second half of May onwards, winter wheat plants completed successive growth stages on the same dates in both years. Weather conditions between flowering (BBCH 61-69) and milk ripe (BBCH 73) stages differed in the experimental years. In June 2012, temperatures were moderate with a monthly average of 15°C and total precipitation of 103.2 mm which exceeded the long-term average. In June 2013, average monthly temperature was 17.5°C, whereas total precipitation reached 61.2 mm and was unevenly distributed in the analyzed month (drought between June 10 and June 20). In July of 2012 and 2013, significant fluctuations in temperature and uneven distribution of rainfall did not contribute to the gradual ripening of grain.



Table 1: Weather conditions (Meteorological Station in Tomaszkowo).

Month	10 days	2012				Temp °C x1961-2000	Rainfall mm Σ 1961-2000	2013			
		Phenological growth stages	BBCH-scale	Temperature °C	Rainfall mm			Phenological growth stages	BBCH-scale	Temperature °C	Rainfall mm
May	I	05.05/First node at least 1cm above tillering node 08.05/Node 2 at least 2 cm above node 1 08.05-10.05/Stem elongation	31 32 33-38	13.1	0.8	13,5	58,5	06.05/Beginning of stem elongation 10.05/First node at least 1 cm above tillering node 15.05/ Node 2 at least 2 cm above node 1	30 31 32	14.7	6.5
	II	18.05/Flag leaf fully unrolled	39	12.0	48.6			20.05/ : Node 3 at least 2 cm above node 2 - flag leaf fully unrolled	33-39	15.8	20.5
	III	21.05/Early boot stage- First awns visible 24.05/Beginning of heading 28.05/End of heading	41-49 51 55	14.8	2.3			25.05/Early boot stage- First awns visible 27.05/Beginning of heading 30.05/End of heading	41-49 51 55	13.8	27.5
	x/Σ			13.4	51.7					14.8	54.5
June	I	01.06/Beginning of flowering 05.06/Full flowering 10.06/End of flowering	61 65 69	12.2	33.8	16,1	80,4	03.06/Beginning of flowering 06.06/Full flowering - 12.06. End of flowering	61 65-69	16.3	26.4
	II	Watery ripe	71	16.5	18.5			Watery ripe	71	18.2	0
	III	Early milk	73	16.4	50.9			Early milk	73	18.1	34.8
	x/Σ			15.0	103.2					17.5	61.2
July	I	Early dough	83	21.6	76.7	18,7	74,2	Early dough	83	18.2	16.9
	II	Hard dough: grain content solid. Fingernail impression held	87	15.5	32.1			Hard dough: grain content solid. Fingernail impression held	87	16.6	100.9
	III	31.07/Harvest/Over-ripe: grain very hard, cannot be dented by thumbnail	91	19.9	12.2			31.07/Harvest/Over-ripe: grain very hard, cannot be dented by thumbnail	91	19.1	4.1
	x/Σ			19.0	121.0					18.0	121.9

RESULTS AND DISCUSSION

Winter wheat (*Triticum aestivum* L.) cv. Boomer is moderately resistant to Fusarium head blight FHB (Góral et al. 2015). In the growing season of 2012, symptoms of FHB were observed only sporadically, on 4.5% of wheat plants fertilized with NPK+Cu+Zn+Mn. The disease did not affect wheat plants in the NPK+Mn treatment (Fig. 1). The symptoms of FHB were more pronounced in the growing season of 2013, and the highest infection index (12%) was noted in wheat plants treated with the Nano-Gro growth stimulator, where the observed difference was statistically significant in comparison with the remaining treatments. The severity of FHB symptoms varied in response to fertilization with NPK and microelements. Wheat is susceptible to infections caused by *Fusarium* spp. between heading and ripening stages (Dill-Macky, 2010), and high rainfall, humidity and high temperatures during flowering contribute to the spread of FHB (Edwards, 2004). According to published data, N fertilization exerts varied effects on the severity of FHB and mycotoxin accumulation in grain. Aufhammer et al. (2000) demonstrated that N fertilization had no significant influence on FHB severity or mycotoxin biosynthesis. Schaafsma et al. (2001) and Oldenburg et al. (2007) reported a significant increase in the severity of FHB under the influence of N fertilization before heading, and they attributed those effects to changes in agronomic practices and a specific microclimate. Lacko-Bartošová and Kobida (2011) noted the highest rate of wheat grain infection with *Fusarium* in a conventional farming system with mineral fertilization but no fungicides, a lower infection rate in a conventional system with both mineral fertilization and fungicides, and the lowest infection rate in an organic system. Microelements essential for plant development, such as Zn, Cu and Fe, inhibit the growth of selected fungal species (Hartikainen et al., 2012), including fungi of the genus *Fusarium*.

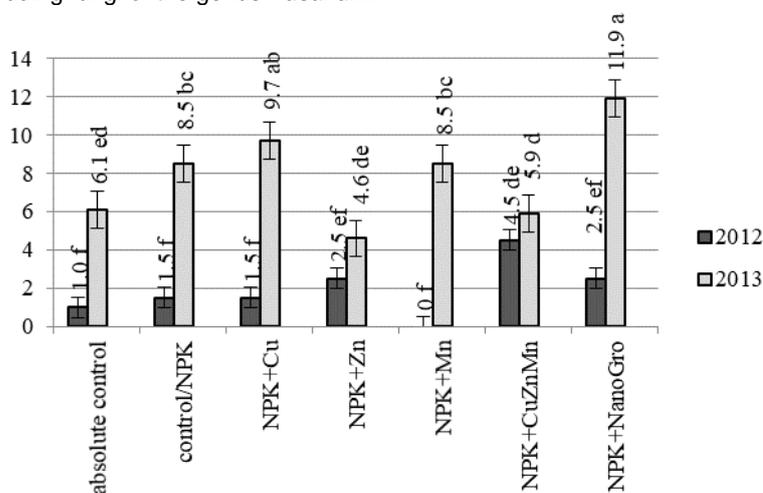


Fig 1: Winter wheat infections caused by *Fusarium* spp. fungi (infection index Ii %).

Fusarium head blight, caused mainly by *Fusarium graminearum*, is the most destructive fungal disease in wheat production (Santos et al., 2013). In Poland, the main causal agents of FHB in wheat are *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. poae*, *F. sporotrichioides* and *F. tricinctum*. The prevalence of the above pathogens is influenced by weather conditions, and the first two species are characterized by the highest pathogenicity (Filoda and Wickiel, 2009). According to Lenc (2011), the severity of FHB is not positively correlated with grain colonization by *Fusarium* spp. In this study, FHB was caused by six toxin-producing species: *F. culmorum*, *Gibberella avenacea* (teleomorph of *F. avenaceum*), *F. poae*, *G. tricincta* (teleomorph of *F. tricinctum*), *F. sporotrichioides* and *G. zeae* (teleomorph of *F. graminearum*) as well as *F. chlamyosporum*, *F. sacchari* and *Gibberella intricans* (teleomorph of *F. equiseti*) (Table 2). Grain colonization by fungi of the genus *Fusarium*, including toxin-producing species, was higher in 2012 than in 2013 (excluding NPK+Zn and NPK+Nano-Gro treatments), whereas the severity of FHB was higher in 2013 than in 2012. In selected treatments, the percentage of *Fusarium* species in the total number of fungal isolates was high in both years of the study (approx. 40% to more than 50%). It should be stressed that *F. graminearum* was isolated from all treatments in 2013. This ubiquitous species is highly resistant to diverse weather conditions (Jestoi et al., 2009), and it is the main causal agent of FHB in Africa (Sydenham et al., 1989), Europe (Bottalico and Perrone, 2002), Asia (Puri et al., 2012), America (McMullen et al., 2012), and south-western Australia (Tan et al., 2012). According to Bottalico and Perrone (2002), unlike *F. tricinctum* which is the main cause of FHB in northern and north-eastern Europe and Russia, *F. acuminatum* is far less frequently associated with the disease in Europe.

The quality of grain is determined by mycotoxin accumulation levels (Chelkowski, 2007, 2012). Cromei et al. (2001) found a correlation between the concentrations of *Fusarium* mycotoxins and grain colonization by pathogens. According to the cited authors, fungicides reduce the severity of FHB, protect grain against colonization by *Fusarium* spp. and decrease mycotoxin concentrations in grain. Filoda and Wickiel (2009) observed that mycotoxin production was limited in grain harvested from wheat plants characterized by slight symptoms of FHB. Cultivation of resistant cultivars combined with fungicide treatment reduce the severity of FHB and grain contamination with DON and NIV. In a two-year study of wheat plants artificially inoculated with *F. graminearum*, N fertilization (75 and 150 kg/ha) did not increase the severity of FHB (Krnjaja et al., 2015). The accumulation of DON and ZEA in wheat grain increased under the influence of N fertilization in only one year of the study, and the highest mycotoxin levels were noted in the treatment fertilized with 150 kg N/ha. In the



above study, artificial inoculation significantly increased the severity of FHB, *F. graminearum* accumulation levels and grain contamination with mycotoxins, mainly DON.

Table 2: Fungi of the genus *Fusarium* isolated from winter wheat grain.

Species	Absolute control	Control/ NPK	NPK+Cu	NPK+Zn	NPK+Mn	NPK+ CuZnMn	NPK+ NanoGro	
2012								
toxin-producing	<i>Fusarium culmorum</i>	4.2	6.5		3.6		5.3	
	<i>F. poae</i>	8.3	8.0	24.6	1.8	3.6		
	<i>F. sporotrichioides</i>	8.3	25.8		3.6	25.0	14.5	
	<i>Gibberella avenacea</i>	4.2	3.2			31.6	5.3	4.6
	<i>G. tricineta</i>	2.1	4.8		7.3	10.5	3.6	2.0
	<i>G. zeae</i>			17.5	3.6			
	total	27.1	48.3	42.1	20.0	42.1	37.5	26.3
others <i>F. chlamydosporum</i>						17.9		
total of fungi isolates/ number	96	124	114	110	114	112	152	
2013								
toxin-producing	<i>Fusarium culmorum</i>	4.0	4.9	4.7	4.9		4.3	
	<i>F. poae</i>	2.0	2.4	4.6			2.1	
	<i>F. sporotrichioides</i>				13.1	4.7	11.2	21.3
	<i>G. avenacea</i>			2.3	2.5		4.3	6.4
	<i>G. tricineta</i>				9.0		3.4	2.1
	<i>G. zeae</i>	5.1	2.4	4.7	5.7	6.3	9.5	10.5
	total	11.1	9.7	16.3	35.2	11.0	28.4	46.7
others	<i>F. chlamydosporum</i>	2.0	26.8	9.3	15.5	23.4	21.1	4.3
	<i>F. sacchari</i>				1.6	1.6		
	<i>G. intricans</i>						1.7	
total of fungi isolates/ number	98	82	86	122	128	116	94	

The quality of grain is determined by mycotoxin accumulation levels (Chetkowski, 2007, 2012). Cromey et al. (2001) found a correlation between the concentrations of *Fusarium* mycotoxins and grain colonization by pathogens. According to the cited authors, fungicides reduce the severity of FHB, protect grain against colonization by *Fusarium* spp. and decrease mycotoxin concentrations in grain. Filoda and Wickiel (2009) observed that mycotoxin production was limited in grain harvested from wheat plants characterized by slight symptoms of FHB. Cultivation of resistant cultivars combined with fungicide treatment reduce the severity of FHB and grain contamination with DON and NIV. In a two-year study of wheat plants artificially inoculated with *F. graminearum*, N fertilization (75 and 150 kg/ha) did not increase the severity of FHB (Krnjaja et al., 2015). The accumulation of DON and ZEA in wheat grain increased under the influence of N fertilization in only one year of the study, and the highest mycotoxin levels were noted in the treatment fertilized with 150 kg N/ha. In the above study, artificial inoculation significantly increased the severity of FHB, *F. graminearum* accumulation levels and grain contamination with mycotoxins, mainly DON.

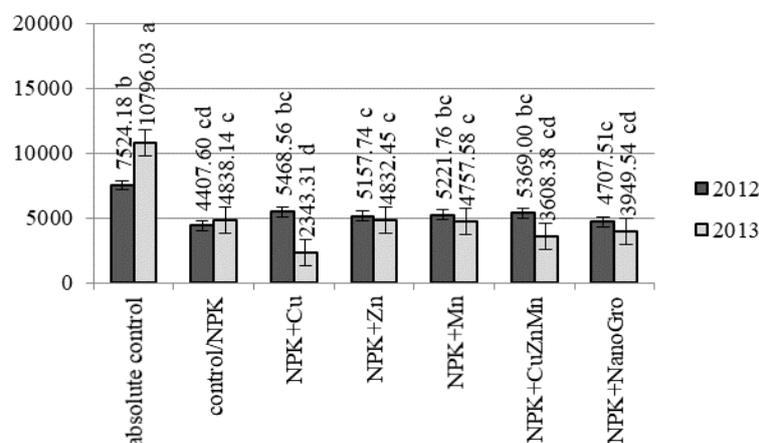
In the present study, ergosterol levels – as a selective fungal bioindicator - in grain were significantly higher in the absolute control treatment at 7,524.18 ng/g in 2012 and 10,796.03 ng/g in 2013 (Fig. 2a). Ergosterol accumulation was reduced in the grain of wheat plants fertilized with NPK and microelements and treated with the growth stimulator. In those treatments, ERG concentrations in grain were similar in both years of the study, excluding the NPK+Cu treatment where ERG levels were twice higher in 2012. Trichothecenes, mostly deoxynivalenol, are the major mycotoxins associated with FHB (Santos et al., 2013). In this study, the concentrations of DON (excluding the NPK+Nano-Gro treatment), NIV and ZEA (excluding control/NPK and NPK+Cu treatments) were higher in grain harvested in 2012 than in 2013. In both years of the experiment, DON and NIV concentrations were highest in the absolute control treatment (without fertilization), in control/NPK, NPK+Cu and NPK+Mn treatments (in the NPK+Mn treatment only in 2012). Maximum permissible DON levels (1,500-1,855 ng/g) were exceeded in grain from the above treatments in 2012. In the remaining treatments, DON concentrations in grain were significantly lower in both years (excluding NPK+Zn and NPK+Cu+Zn+Mn treatments), and the lowest DON levels (216 ng/g) were noted in 2012 in the NPK+Nano-Gro treatment (Fig. 2b). In a study of naturally infected winter wheat, DON was identified in 82.4% of grain samples at a concentration of 68-1,572 mg/kg (Jajić et al., 2011). In the work of Supronienė et al. (2012), DON concentrations in winter wheat grain were determined at 100-182.0 µg/kg. Wu et al. (2015) reported that DON levels in grain did not change with an increase in FHB severity and remained relatively low in most wheat varieties susceptible to the disease. Heier et al. (2005) observed that N fertilization at 190 kg/ha led to a significant increase in DON accumulation in winter wheat grain after harvest. In contrast, Oldenburg et al. (2007) did not report changes in DON concentration in wheat grain in response to N fertilization rates of up to 240 kg/ha. In a study by Lacko-Bartošová and Kobida (2011), DON concentrations were 46% lower in the grain of organically grown wheat than in the integrated farming system with NPK fertilization.



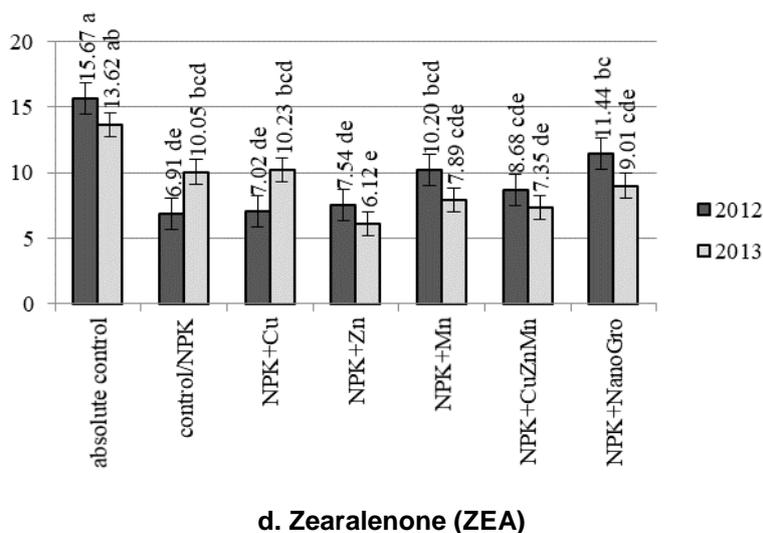
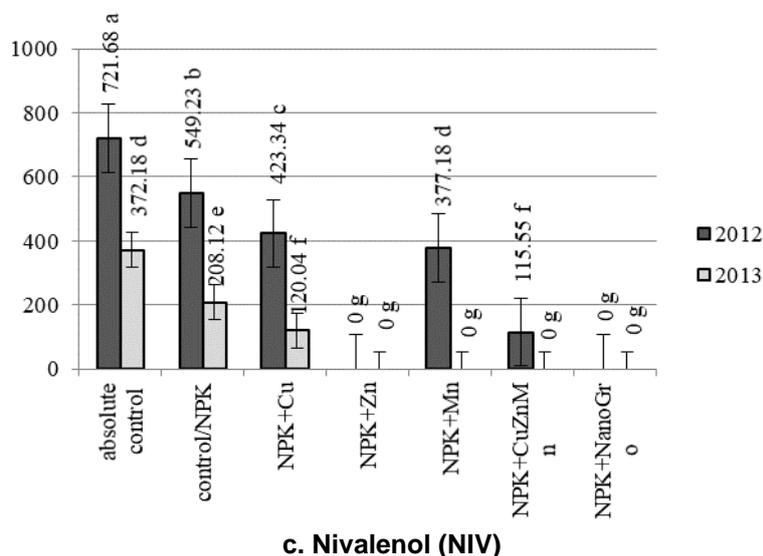
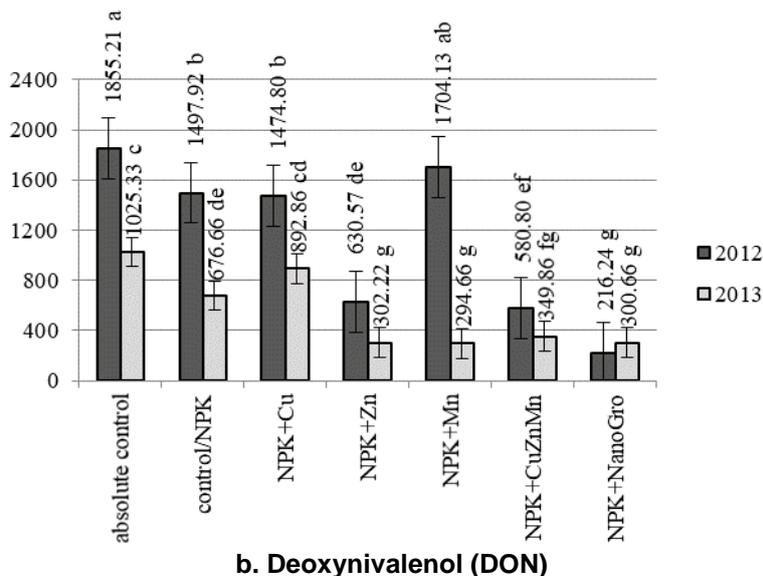
In our study, NIV concentrations in wheat grain were lowest (below LOD) in NPK+Zn and NPK-Nano-Gro treatments in both years and in NPK+Mn and NPK+Cu+Zn+Mn treatments in 2013 (Fig. 2c). In both growing seasons, NIV contamination was significantly higher in the absolute control treatment than in the remaining treatments. A mycological analysis of grain harvested in 2012 revealed high levels of colonization by toxin-producing *Fusarium* species such as *F. culmorum*, *F. poae*, *G. avenacea* and *G. zeae* in the absolute control treatment and in control/NPK (approx. 20%), NPK+Mn (30%) and NPK+Cu (42%) treatments. In the remaining treatments, the above *Fusarium* species represented up to 10% of the identified mycotoxin-producing fungi. In grain harvested in 2013, the percentage of the above pathogens did not exceed 20% in all treatments (excluding NPK+Nano-Gro treatment), and the lowest levels of contamination were noted in the NPK+Mn treatment. *F. graminearum* and *F. culmorum* are dangerous wheat pathogens (Edwards, 2004) and important producers of DON and NIV (Goliński et al., 2009; 2010; Gale et al., 2011; Lacko-Bartošova and Kobida, 2011). *F. culmorum* produces mycotoxins under specific weather conditions, whereas *F. poae* is not toxic for cereals, but may pose a health hazard for humans and animals due to its toxigenic properties (Bottalico and Perrone, 2002; Chełkowski et al., 2007). Microelements essential for plant growth, such as Zn, Cu and Fe, inhibit the growth of selected fungal species, including fungi of the genus *Fusarium* (Hartikainen et al., 2012). In an *in vitro* study, ZnSO₄ and Zn(ClO₄)₂ inhibited the growth of *F. graminearum* (Savi et al., 2013). In a field experiment, ZnSO₄, in particular ZnO-NP (nanoparticles), effectively decreased the severity of FHB in wheat (Savi et al., 2015). The growth of *F. graminearum* and DON synthesis in wheat grain were effectively inhibited by ZnO-NP.

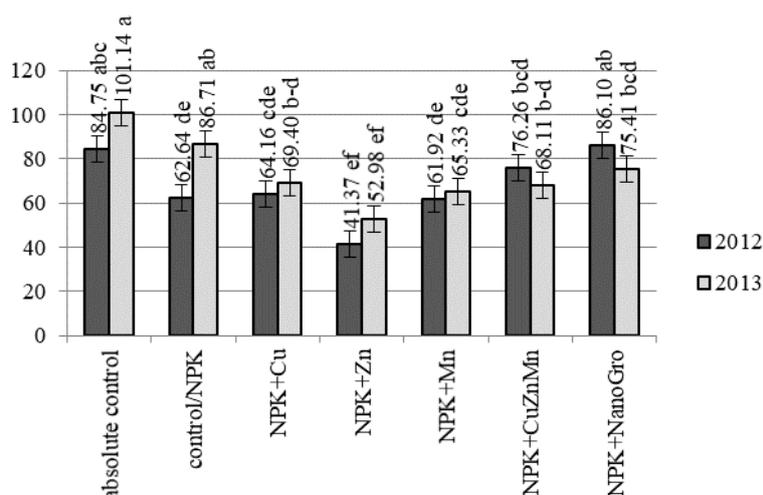
Zearalenone is often identified in cereal grain together with trichothecenes (Stanković et al., 2012). In this study, ZEA concentrations in grain harvested in both growing seasons were within the safe range. In both 2012 and 2013, ZEA levels were significantly higher in grain harvested in absolute control treatment (15.67 ng/g and 13.62 ng/g, respectively) than in the remaining treatments (excluding control/NPK and NPK+Cu treatments in 2013). In the remaining treatments, ZEA concentrations in grain ranged from 6.12 ng/g in the NPK+Zn treatment in 2013 to 11.44 ng/g in the NPK+Nano-Gro treatment in 2012 (Fig. 2d). In an experiment by Supronienė et al. (2012), winter wheat grain accumulated 0-43.6 ng/g of ZEA. In a Polish study, open-pollinated varieties of winter wheat differed significantly in ZEA accumulation levels, and the concentration of this mycotoxin in grain varied widely from 29.66 to 734.45 ng/g (Waśkiewicz et al., 2012). According to Gromadzka et al. (2008) and Goliński et al. (2010), ZEA is most readily synthesized at relative humidity of around 16% and ambient temperature below 25°C. The severity of FHB symptoms on spikes considerably influences mycotoxin accumulation in grain. In a study by Chełkowski et al. (2012), ZEA levels in uninfected grain were 20-fold lower than in infected grains. DON and ZEA are often found together in cereal grain because they are produced by the same *Fusarium* species, namely *F. graminearum* and *F. culmorum* (Kosawang et al., 2014). Zearalenone is also synthesized by *F. cerealis*, *F. equiseti* and *F. semitectum* (Glenn, 2007).

In both years of the study, similar amounts (non-significant differences) of fumonisins B₁ and B₂ were accumulated in the corresponding treatments (excluding control/NPK treatment). Fumonisin concentrations were higher in 2013 than 2012, excluding NPK+Cu+Zn+Mn and NPK+Nano-Gro treatments. In treatments with NPK and microelement fertilizers and the Nano-Gro growth stimulator, mycotoxin levels in grain were significantly reduced only in 2013, excluding the NPK treatment. In 2012, fertilizers and the growth stimulator exerted varied effects on mycotoxin concentrations in grain. In both years, the lowest contamination levels were noted in grain harvested in the NPK+Zn treatment (Fig. 2e). According to Waśkiewicz et al. (2012, 2013) and Czembor et al. (2015), fumonisins (FBs) are produced mainly by *F. verticillioides* and *F. proliferatum*. The optimal weather conditions for the key producers of DON (*F. culmorum* and *F. graminearum*) and FBs (*F. verticillioides*) differ from the optimal conditions for the synthesis of those mycotoxins (Medina et al., 2013). In an *in vitro* study by Savi et al. (2013), zinc compounds (zinc sulfate ZnSO₄ and zinc oxide nanoparticles ZnO-NP) had a fungistatic effect on *F. verticillioides* and inhibited FBs accumulation.



a. Ergosterol (ERG)





e. sum of fumonisin B₁ and B₂

Fig 2: Mycotoxin concentrations (ng/g) in the grain of winter wheat fertilized with microelements

A statistical analysis revealed a negative correlation between the severity of FHB and the concentrations of DON and NIV in winter wheat grain (Table 3). The values of the correlation coefficient r ranged from -0.555 to -0.509. The severity of FHB was not significantly correlated with the accumulation levels of ERG, FB₁, FB₂ and ZEA in grain. Different types of correlation between FHB severity and the concentrations of *Fusarium* toxins in grain have been observed in previous studies. Deoxynivalenol concentrations in grain are not always correlated with the severity of FHB. Mesterhazy et al. (1999) demonstrated that varieties susceptible to FHB, where disease severity was high, were characterized by moderate or low levels of DON accumulation, whereas high DON concentrations were noted in resistant genotypes. Liu et al. (1997) and Edwards et al. (2001) reported no significant correlations between FHB severity and the DON content of grain under natural field conditions; the percentage of *Fusarium*-damaged kernels (FDK) was a more reliable indicator of DON accumulation. Champeil et al. (2004) found no relationships between FHB severity and the levels of DON, NIV and ZEA in grain. The cited authors observed a positive correlation between the biomass of *Fusarium* fungi – trichothecene producers and DON concentrations in harvested grain. Paul et al. (2005) also suggested that DON accumulation in grain could be correlated with the percentage of FDK. Shaner and Buechley (2004) observed a relationship between DON accumulation in grain and *Fusarium* infections in the analyzed wheat varieties, but the coefficient of correlation was low. According to the above authors, differences in DON levels between wheat varieties resulted from differences in their genetic susceptibility to FHB rather than from weather conditions during flowering. Miedaner et al. (2003) found a correlation between FHB severity and DON concentrations in grain ($r=0.8$). Other studies demonstrated a positive correlation between the severity of FHB and the percentage of FDK with DON levels (Wegulo et al., 2011), and with DON and NIV levels (Góral et al., 2015).

Table 3: Coefficients of correlation between the severity of FHB and mycotoxin concentrations in winter wheat grain.

Mycotoxin	FHB
Ergosterol	-0.255
Fumonisin B	0.276
Zearalenone	-0.060
Deoxynivalenol	-0.555*
Nivalenol	-0.509*

*- correlation coefficients are statistically significant at $p < 0.05$

CONCLUSION

Mineral fertilization, i.e. control/NPK, NPK with microelements (Cu, Zn, Mn) and NPK with the Nano-Gro® organic growth stimulator, reduced the production of trichothecenes, ZEA and fumonisins B₁ and B₂. The highest levels of trichothecenes, DON and NIV, were noted in winter wheat grain in 2012 in control, control/NPK, NPK+Cu and NPK+Mn treatments. Toxin-producing fungi of the genus *Fusarium* (*Fusarium culmorum*, *F. poae*, *F. avenaceum* teleomorph *Gibberella avenacea*, *F. graminearum* teleomorph *G. zeae*) were isolated most frequently from winter wheat grain in the above treatments.

Foliar application of microelements could increase the effectiveness of integrated strategies for managing infections caused by toxin-producing fungi of the genus *Fusarium* and reducing the accumulation of mycotoxins in cereal grain.



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