The impact of *Fusarium* infection on the content of selected basic nutrients in the barley grain

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Abstract: Economic quality of agricultural products is considerably influenced by a health status of crops. In the recent past, a greater attention has been paid to infections of cereals caused by pathogens of the genus *Fusarium*, which in addition to grain yield and quality decrease exhibit health risks to consumers, especially due to the presence of mycotoxins, their toxic secondary metabolites. A set of 28 cultivars and genetic resources of spring barley was grown in 2011–2013 at two locations (Kromeriz and Zabcice) under natural infection and inoculation with a selected strain of *Fusarium culmorum*. Barley materials differed in selected morphological traits and chemical characteristics. Contents of basic nutrients (N-substances, starch, beta-glucans, fat in %) and levels of selected mycotoxins (trichothecenes B, trichothecenes A, zearalenone + zearalenols and "emerging" mycotoxins in $\mu g.kg^{-1}$) in grain were determined. The differences due to the infection were evaluated both within the examined groups of barley and on the level of relationships between analysed nutrients and detected mycotoxins.

Key-Words: mycotoxins, genetic resources, spring barley, natural infection, inoculation, *Fusarium culmorum*

Introduction

Cereal diseases associated with infections of the pathogens from genus *Fusarium* are linked to reduced grain yield and quality and can result in substantial economic loss due to contamination of production with mycotoxins [1], their secondary metabolites. In addition, *Fusarium* mycotoxins, which are present in plants and products derived from them might pose significant health risks for humans and farm animals. Most common diseases which can be related to mycotoxicoses are nephropathy, various types of cancer, alimentary toxic aleukia, hepatic diseases, various hemorrhagic syndromes, and immune and neurological disorders [2].

Much attention is paid to the occurrence of these toxicogenic pathogens worldwide, however, prediction of the infection severity and level of mycotoxins in production still remains very difficult [3] because the extent of the occurrence and degree of infection of cereals are affected by a variety of external and internal factors (weather course, preceding crop, choice of cultivars and others). The spectrum of detected mycotoxins and information on their toxicity are broadened owing to the development of detection methods, instrumentation and a size of analysed samples. Until now, tens of mycotoxins have been known but a maximum acceptable level has been regulated by law for a low number of them (deoxynivalenol, zearalenone, ochratoxin A, fumonisins B1 and B2, and aflatoxins B1, B2, G1 and G2; recommendations for monitoring toxins T-2 and HT-2 in cereals and cereal products have been issued).

All these mycotoxins have been investigated for several decades and much information on their occurrence and toxic effects has been available.

Along with the development of instrumentation and detection methods, other *Fusarium* mycotoxins (so-called "emerging" mycotoxins, for example, enniatins, beauvericin, moniliformin and fusaproliferin and others [4]) have begun to occur, but little is known about their toxicity and especially about joint effects with other groups of mycotoxins.

Changes in the spectrum of mycotoxins are also encouraged by climatic changes when the occurrence of early less spread *Fusarium* spp. is recorded [5].

Infection of barley grain by pathogens of the genus *Fusarium* has an impact on the yields. It is also reported that germination ability, 1000-kernel weight, kernel plumpness, the content of N-substances and beta-glucans, and other malting parameters are affected [6, 7].

The objective of the paper was to examine the occurrence of basic groups of mycotoxins and changes in the content of basic nutrients in grain harvested from naturally and artificially infected stands of various cultivars, breeding lines and genetic resources of spring barley.

Material and Methods

Material, crop management practice Selected cultivars and genetic resources of spring barley (a total of 28 – Table 1), registered in the Czech Republic or maintained in the Genebank), with differences in the type of grain, ear and starch were grown in the years 2011–2013 at the locations Kromeriz (49°17‘N, 17°22‘E, 235 m a.s.l.; average annual temperature 8.7 °C, average annual precipitation 559 mm.) and Zabcice (49°01'N, 16°37´E, 184 m a.s.l.; average annual temperature 9.2 °C, average annual precipitation 480 mm) following standard preceding crops (plot area was 2.5-4.5 m^2), under conditions of both natural and artificial infection with *F. culmorum.* The inoculation with *F. culmorum* (W. G. Sm.) Sacc. strain KM16902; DON chemotype) was carried out according to Tvaruzek et al. [8] by spraying at an appropriate growth stage (BBCH 61-64), when 50 % of plants are at the beginning of anthesis (concentration of 0.5 million conidia of *F. culmorum* in 1 ml of inoculum, spray dose of 200 l.ha^{-1}).

Table 1: List of cultivars and their characteristics of the grain (Kromeriz and Zabcice, 2011–2013)

Cultivar	Caryop.[1)]	Typ of ear	Type of starch[2)]	Cultivar	Caryop.[1)]	Typ of ear	Type of starch[2)]
6NDRFG-1	cov	6-row	stand.	KM 2551	n	2-row	waxy
AC Klinck	cov	6-row	stand.	Kompakt	cov	2-row	stand.
AF Lucius	n	2-row	stand.	Krasnodarskij 95	cov	2-row	stand.
Amulet	cov	2-row	stand.	Madeira	cov	2-row	stand.
Annabell	cov	2-row	stand.	Merlin	n	2-row	waxy
Arra	cov	6-row	stand.	Nitran	cov	2-row	stand.
CDC Rattan	n	2-row	waxy	Nordus	cov	2-row	stand.
Cebada Capa	cov	6-row	stand.	Pejas	cov	2-row	stand.
Diplom	cov	2-row	stand.	Primus	cov	2-row	stand.
Druvis	cov	6-row	stand.	Prosa	cov	2-row	stand.
Henrike	cov	2-row	stand.	Ricardo	cov	6-row	stand.
Chevron	cov	6-row	stand.	Rolfi	cov	6-row	stand.
KM 1057	n	2-row	stand.	Taiga	n	2-row	stand.
KM 2460	cov	2-row	waxy	Waggon	cov	2-row	stand.

[1)] - huskinness of caryopsis: cov = covered, n = naked (hulless) ; [2)] - type of starch: stand. = standard starch with ca 25% of amylose and 75% of amylopectin, waxy = starch with low level of amylose

Analysis of quality parameters Moisture content of flours was determined according to Method CSN ISO 712 (2003). Protein content was determined by Method ICC STANDARD No. 167 (2000) using the conversion factor 6.25, starch content by CSN EN ISO 10520 and fat content by Javorsky [9]. Beta-glucans were determined enzymatically using the beta-glucan enzymatic assay kit (Megazyme Ireland International, Ltd., Wicklow, Ireland) following the procedure of Megazyme (ICC Nr. 166).

Analysis of mycotoxins Sample preparation for mycotoxins analysis was performed by means of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure, *i.e.* acetonitrile/water extraction followed by phases partition induced by inorganic salts addition, where

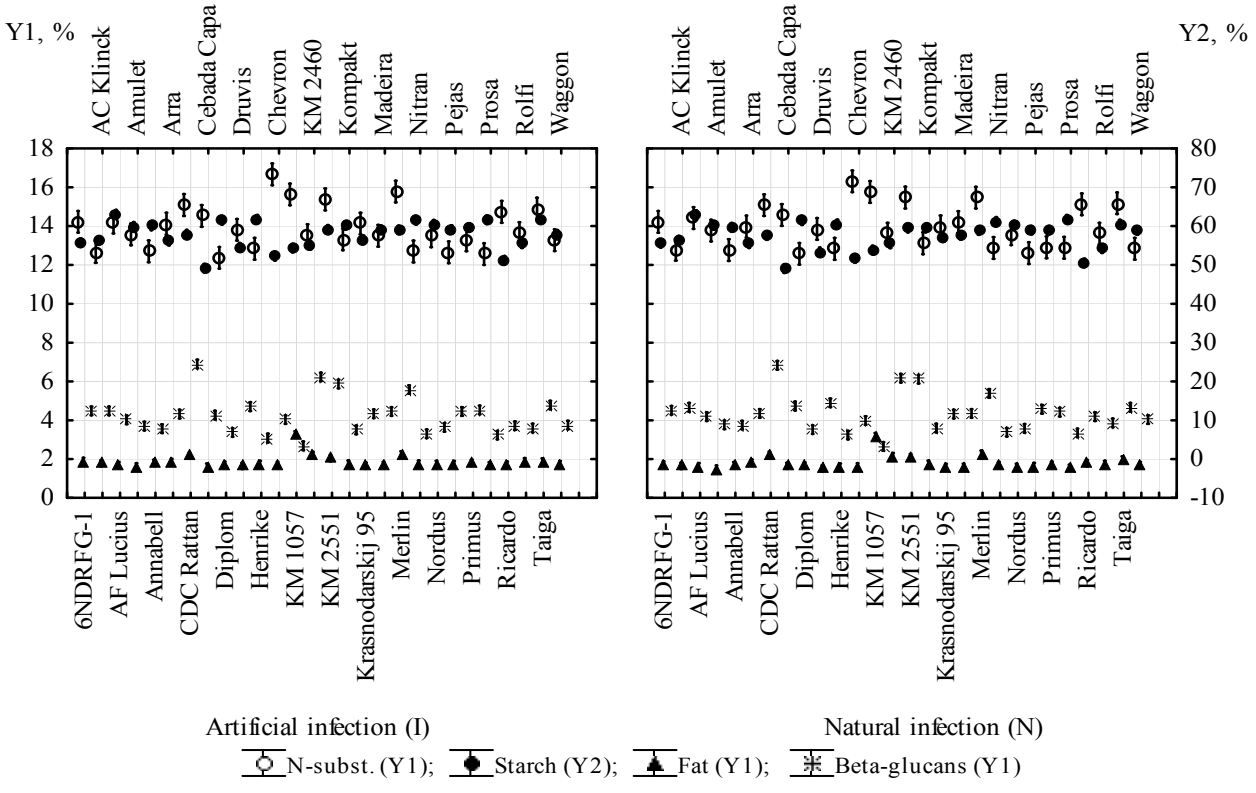
mycotoxins are salted out in the upper acetonitrile phase, and polar matrix co-extracts remain in aqueous phase. For separation and target mycotoxins detection, ultra-high performance liquid chromatography (analytical column HSS T3 - 100 x 2.1 mm; 1.8 µm) coupled with tandem mass spectrometry (QTRAP 5500, AB Sciex) was used. Quantification of mycotoxins was realized by the external matrix-matched calibration method, results were corrected for recovery determined based on spikes. Summary concentrations of four groups of mycotoxins (all in $\mu g.kg^{-1}$) were determined: trichothecenes B: nivalenol, deoxynivalenol, deoxynivalenol-3-glucoside, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon X; trichothecenes A: T-2 toxin, HT-2

Table 2: Summary concentrations of mycotoxin groups ($\mu g.kg^{-1}$) in natural and inoculated variants

Factor /	Trichothecenes B		Trichothecenes A		ZEA + ZOLs		„emerging“	
MXT/infection[1)]	N[1)]	I[1)]	N	I	N	I	N	I
naked grain	204.2 a	8 541.0 a	70.7 a	**96.0 b**	5.5 a	181.6 a	589.5 a	463.4 a
covered grain	193.8 a	**13 678.8 b**	40.8 a	45.5 a	4.2 a	406.6 a	520.9 a	331.8 a
standard starch	191.1 a	13 083.0 a	45.9 a	51.9 a	3.9 a	378.4 a	550.9 a	359.5 a
waxy starch	225.5 a	9 546.9 a	55.1 a	82.7 a	8.0 a	238.2 a	443.7 a	362.8 a
2-row	218.9 a	10 697.2 a	51.2 a	**67.9 b**	5.8 a	249.1 a	518.1 a	343.7 a
6-row	139.0 a	**17 279.5 b**	37.2 a	27.5 a	1.2 a	**631.6 b**	579.3 a	366.5 a
location: Kromeriz	136.5 a	**22 475.7 b**	11.3 a	17.5 a	0.7 a	**713.8 b**	218.2 a	151.2 a
location: Zabcice	255.5 a	2 680.0 a	83.2 a	**95.2 b**	8.3 a	2.9 a	**853.0 b**	**568.8 b**
year: 2011	170.3 a	**19 255.6 c**	16.4 a	18.1 a	12.3 a	**990.1 b**	479.4 a	95.3 a
year: 2012	248.3 a	12 597.5 b	**58.1 b**	**94.5 c**	0.6 a	77.7 a	547.3 a	**483.2 b**
year: 2013	169.5 a	5 880.5 a	**67.2 b**	56.4 b	0.5 a	7.2 a	580.2 a	**501.5 b**

[1)] - N – variant with natural infection, I – variant with F. culmorum inoculation ; [2)] – different letters in the column indicate significant difference at P0.05, bold indicates statistically significant difference

Fig. 1 – Average contents and variability of basic nutrients in spring barley stands after inoculation with *F. culmorum* and under natural infection (Kromeriz and Zabcice, 2011–2013)



toxin, diacetoxyscirpenol, neosolaninol; zearalenone + zearalenols (ZEA+ZOLs): zearalenone, α-zearalenol, β-zearalenol; "emerging" mycotoxins: beauvericin, enniatin A, enniatin A1, enniatin B, enniatin B1.

Data analysis: Experimental data were assessed using STATISTICA software, version 12.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and Discussion

Basic nutrients

Individual cultivars responded to inoculation by their chemical composition in a different way (Fig. 1). In a whole set of genotypes at least one of the examined nutrients decreased, but in naked line KM 2551 with waxy starch the decrease was found in all of them. In contrast, some materials increased the content in the inoculated variant on average of all years. The content of N-substances was increased most (+0.44%) whereas starch content decreased most (-0.97%) in cv. Waggon. In cv. Madeira, however, an opposite effect of inoculation was observed (N-substances -0.71%, starch +1.51%). The content of beta-glucans decreased in most cases due to strong infection pressure. The highest reduction in beta-glucan content was assessed in 6-row cvs. Ricardo and Cebada Capa (both -0.47%).

Concentrations of mycotoxins were different in naturally and artificially infected variants and ranged on average from 0.5 to 1065.6 $\mu g.kg^{-1}$ (natural infection) and from 7.7 to 31144.2 $\mu g.kg^{-1}$ (inoculation with *F. culmorum*). Statistically significant differences were found in concentrations of individual groups of mycotoxins between the natural and inoculated variant (data not shown) and also between groups of barley genotypes, experimental locations and years (Table 2). However, over-limit contents of mycotoxins monitored in foods and feeds were not determined in any sample from the naturally infected variant.

Trichothecenes B

The average values of summary concentrations of all mycotoxin groups are in Table 2. In particular years, the content of trichothecenes B significantly differed only in the inoculated variant. The highest level was assessed in 2011, which considerably differed from the years 2012 and 2013 (19255.6, 12597.5 and 5880.5 $\mu g.kg^{-1}$, respectively). Considerable differences were also recorded between locations; significantly higher content of trichothecenes B was in Kromeriz (22475.7 *vs.* 2680 $\mu g.kg^{-1}$, respectively). In the inoculated variant the highest total contents of trichothecenes B were determined in 6-row cultivars (17279.5 $\mu g.kg^{-1}$), which is consistent with the results by Legzdina, Buerstmayr [10]. An exception was 6-row cv. Chevron which had lower concentrations of all studied groups of mycotoxins in the inoculated variant (data not shown). This cultivar was resistant to *Fusarium* spp. infection already in earlier experiments [10, 11, 12].

Under higher infection pressure, a significantly lower content of trichothecenes B was determined in naked genotypes in comparison with covered ones (8541 $\mu g.kg^{-1}$ *vs.*13678.8 $\mu g.kg^{-1}$). Similar findings are also reported by foreign authors [10, 13 and others] who found that a major portion of trichothecenes was accumuluted in the hulls of barley. Correlations between summary contents of the examined mycotoxin groups and individual nutrients are given in Table 3. Positive and in most cases significant correlations were calculated between the content of trichothecenes B and the content of starch and fat in the inoculated variant in all groups of genotypes (r = 0.16-0.56**). The increased concentration of trichothecenes B reduced mainly the content of N-substances and beta-glucans when the correlations were stronger under inoculation. At the infection of barley kernels, some authors detected higher levels of proteolytic and cytolytic enzymes that are attributed to both defensive reaction of the genotype and higher enzymatic activity of the pathogen [14, 15, 16]. In the naturally infected variant, a significant negative correlation was found between the presence of trichothecenes B (but as well as ZEA + ZOLs and "emerging" mycotoxins) in grain and the content of beta-glucans in the group with waxy starch. Given that the beta-glucans are building blocks of the cell walls, it can be concluded that it is a mechanical barrier to the penetration of the pathogen into the cells.

Trichothecenes A

The toxicity of these *Fusarium* mycotoxins is very high, which is documented by tolerable daily intake (TDI) of 100 $\mu g.kg^{-1}$ b.w. for the sum of T-2 and HT-2 toxins [17]. In Zabcice, the content of trichothecenes A in the inoculated variant was significantly higher than in the naturally infected one (95.2 *vs.*17.5 $\mu g.kg^{-1}$, respectively). Average contents of trichothecenes A were significantly higher in 2-row and naked genotypes and non-significantly higher in genotypes with waxy starch.

Table 3: Correlations between summary contents of the examined mycotoxin groups and nutrients

		Trichoth. B		Trichoth. A		ZEA + ZOLs		„emerging“	
Nutrient	Factor	N[1)]	I[1)]	N	I	N	I	N	I
N-subst.	naked	0.12	-0.25	0.44**	0.50**	-0.09	-0.37*	0.39*	0.42*
	covered	-0.07	-0.25**	0.24**	0.16	-0.18*	-0.22*	0.08	0.31***
	standard	-0.06	-0.28**	0.31***	0.26**	-0.17*	-0.22**	0.20*	0.34***
	waxy	0.15	-0.14	0.43	0.55**	-0.08	-0.37	0.21	0.46*
	2-row	-0.04	-0.25**	0.34***	0.38***	-0.16	-0.26**	0.18	0.36***
	6-row	0.20	-0.42**	0.32*	0.31*	-0.25	-0.32*	0.19	0.35*
Starch	naked	-0.11	0.44**	-0.41*	-0.43**	0.06	0.46**	-0.40*	-0.30
	covered	0.06	0.24**	-0.21*	-0.13	0.12	0.18*	-0.22*	-0.32***
	standard	0.02	0.21**	-0.24**	-0.17*	0.11	0.16	-0.28**	-0.28**
	waxy	0.03	0.56**	-0.25	-0.31	0.16	0.49*	-0.19	-0.28
	2-row	-0.03	0.41***	-0.34***	-0.35***	0.11	0.32***	-0.28**	-0.27**
	6-row	-0.33*	0.46**	-0.39**	-0.48**	0.29*	0.38**	-0.35	-0.55***
BG[2)]	naked	-0.13	-0.01	-0.15	-0.02	-0.18	-0.02	-0.33	-0.06
	covered	-0.07	-0.26**	-0.07	-0.06	-0.10	-0.15	-0.08	-0.03
	standard	-0.07	-0.23**	-0.10	-0.06	-0.09	-0.15	-0.12	0.03
	waxy	-0.50*	-0.35	-0.17	-0.08	-0.67***	-0.21	-0.47*	-0.21
	2-row	-0.08	-0.19*	-0.03	0.03	-0.10	-0.09	-0.19*	-0.02
	6-row	0.04	-0.44**	-0.17	0.05	-0.15	-0.37**	-0.12	0.07
Fat	naked	0.33*	0.28	0.26	0.08	0.18	0.33*	0.34*	-0.15
	covered	0.20*	0.30***	0.13	0.09	0.08	0.27**	0.06	-0.01
	standard	0.16	0.16*	0.28**	0.21*	0.04	0.15	0.25**	-0.02
	waxy	0.46*	0.56**	-0.03	-0.06	0.41*	0.67***	0.18	-0.13
	2-row	0.19*	0.20*	0.27**	0.18*	0.09	0.20*	0.23**	-0.02
	6-row	0.12	0.32*	0.05	-0.27	-0.26	0.31*	0.08	-0.13

[1)] N – variant with natural infection, I – variant with F. culmorum inoculation; [2)] BG – beta-glucans

The high level of mycotoxins of this group in naked barley cv. Merlin was also detected by Malachova et al. [18]. As in the case of trichothecenes B, high resistance to the accumulation of this mycotoxin group was detected in cv. Chevron (data not shown). In contrast to the results obtained with trichothecenes B, there was no significant relationship between the content of beta-glucans and trichothecenes A. Positive correlations were calculated between summary concentration of trichothecenes A and the content of N-substances (r = 0.16-0.55**) and partly fat content, and on the contrary, negative correlations with starch content.

ZEA + ZOLs

This group of mycotoxins in barley grain was present in lowest concentrations but, as reported by Zinedine et al. [19], their toxicity for humans and animals is higher than in trichothecenes B. The highest average levels were measured in the inoculated variant in 2011 (990.1 μg.kg^{-1}) and in Kromeriz (713.8 μg.kg^{-1}), and in 6-row genotypes (631.6 μg.kg^{-1}). Consistent with the results observed for trichothecenes B, in this mycotoxin group there were also significant correlations with starch and fat content and negative with the content of N-substances in the inoculated variant. In the naturally infected variant, these relationships were also found, nevertheless, they were not always significant. No considerable relationships between the content of beta-glucans and ZEA+ZOLs, except genotypes with waxy starch, were found.

"Emerging" mycotoxins

The significantly higher (3.7 to 3.9 times) content of this group of mycotoxins was determined in samples from Zabcice in both infection variants. Average contents of these mycotoxins (especially enniatins B – data not shown) were lower in the inoculated variant where pathogen *F. culmorum* obviously predominated due to inoculation and which, as reported by Stepien [20], produces mainly trichothecenes B and zearalenone. In the variant with a natural spectrum of *Fusarium* pathogens, especially in Zabcice, *F. poae* [21] was prevalent ranking among producers of mycotoxins falling in the group so-called "emerging". The relationships determined between contents of basic nutrients and these mycotoxins were similar to those for trichothecenes A. A significant negative correlation was calculated between the accumulation of "emerging" mycotoxins and the content of beta-glucans in waxy genotypes and 2-row barleys in samples from the naturally infected variant.

Conclusion

In 2011–2013, levels of four groups of *Fusarium* mycotoxins (trichothecenes B, trichothecenes A,

zeralenone+zearalenols and "emerging" mycotoxins) were investigated in different cultivars, breeding lines and genetic resources of spring barley. The genotypes were grown at two locations (Kromeriz and Zabcice) in two variants, natural infection and inoculation of stands with *F. culmorum*, and the content of basic nutrients in samples from both variants was determined. Strong pressure of the pathogen due to the inoculation was most apparent in 2011 and in Kromeriz where the major contaminants were groups of trichothecenes B and ZEA + ZOLs. In 2012 and 2013 and in Zabcice, higher levels of trichothecenes A and "emerging" mycotoxines were detected not only in the inoculated but especially in the naturally infected variant. The inoculation of stands resulted in the decrease in at least one of the examined nutrients, differently depending on a particular cultivar/line or genetic resource and decrease in beta-glucan content in nearly all barley materials. Dividing the set of examined barley genotypes according to kernel, starch or ear row type affected the level and direction of interrelationships between mycotoxin groups and basic grain nutrients.

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