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OCCURRENCE OF *Fusarium* spp. IN HULLS AND GRAINS OF DIFFERENT WHEAT SPECIES

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ABSTRACT

The occurrence of selected *Fusarium* species was studied in wheat sample collections that involved common wheat, einkorn, emmer and spelt genotypes. The study using PCR (Polymerase Chain Reaction) assays aimed to identify the *Fusarium* species occurring in hulls and grains of evaluated wheat samples separately and to assess the role of hull in *Fusarium* spp. contamination of the wheat grain. The obtained results suggested that hulls play the role of a protective barrier against *Fusarium* spp. contamination and reduce the incidence of these fungi in wheat grain. The incidence of *Fusarium* spp. in grains decreased by 44.54% in *F. avenaceum*, 45.15% in *F. graminearum,* 54.83% in *F. sporotrichoides*, 55.22% in *F. culmorum*, 57.49% in *F. poae* and 85.07% in *F. equiseti*, compared to incidence of *Fusarium* spp. in hulls of hulled wheat species. In the case of common wheat, the incidence of *Fusarium* spp. in grains decreased by 21.43% in *F. avenaceum*, 29.16% in *F. graminearum*, 32.14% in *F. poae*, 39.13% in *F. sporotrichoides*, 40.91% in *F. culmorum* and 49.99% in *F. equiseti*. A lower *Fusarium* spp. occurrence in hulled wheats grain seems to be connected especially with morphology of hulled wheat spikelets with tight and hard hulls, which could be able to reduce spread of fungal hyphae in the spikelet tissue. At the same time, it is possible to suppose that narrow opening of hulled wheats flowers reduces entry of spores to flowers. Genotypes with high resistance to FHB (emmer wheat genotype Rudico, spelt wheat genotypes Rubiota, Alkor, Tauro) were found in hulled wheat species and since their high grain quality parameters and satisfying yields are already known, they could be good alternative to common wheat, mainly in organic cropping systems.

Keywords: common wheat, einkorn, emmer, spelt, PCR assay, *Fusarium* spp. occurrence, organic farming.

INTRODUCTION

Fusarium head blight (FHB) is an important disease of small-grain cereals in various areas of the world and under various environmental conditions (McMullen et al., 2012). Damage from head blight involves shrunken and discoloured kernels and causes reductions in yield and seed quality (Lori et al., 2009). FHB also leads to the accumulation of very stable low-molecular secondary metabolites called mycotoxins, which are very harmful to both humans and animals (Kuzdraliński et al., 2013). In Europe, FHB is caused predominantly by several *Fusarium* species such as *F. culmorum, F. graminearum, F. pseudograminearum, F. poae* and *F. avenaceum*, while *F. equiseti, F. langsethiae* and *F. sporotrichoides* belong to the less commonly identified species (Vogelgsang and Sulyok, 2008).

High attention has been given to FHB in common wheat, since common wheat (*Triticum aestivum* L.) is one of the main cereals cultivated in Europe. The best way to prevent or reduce *Fusarium* infection would be to grow cultivars with high levels of disease resistance, but a high degree of FHB resistance has not yet been obtained in commercially grown European wheat cultivars (Chrpová et al., 2013).

Current trends towards organic and low-input agriculture, as well as an increase in the utilization of organic food products provide wider possibilities of using some

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other wheat species (Brandolini et al., 2008). Hulled wheat species *T. dicoccum* (Schrank) Schuebl, *T. monococcum* L. and *T. spelta* L., also known as emmer, einkorn and spelt, respectively, were among the earliest *Triticeae* domesticated by man (Chrpová et al., 2013). These wheat species could be an alternative next to common wheat in organic farming with a wider diversity of crops. They are cultivated on many organic farms in Europe not only because they are believed to have a higher nutritive value in comparison with common wheat, but also due to their higher resistance to unfavourable environmental conditions, as well as lower fertilization and soil demands (Konvalina et al., 2014; Zaharieva et al., 2010; Finckh, 2008).

However, information as to the response of hulled wheat species to *Fusarium* spp. infection is still scarce (Chrpová et al., 2013), although this knowledge is especially important both for breeding resistance into cereals and for organic farming (Suchowilska et al., 2010).

At the same time, there is a lack of data regarding the occurrence of the same *Fusarium* species on hulls and grains. The classical model for *F. graminearum* colonization of wheat spikes indicates that infection is initiated on dead anthers, followed by hyphal penetration of the ovary and eventual infection of the floral bracts including glumes, palea and lemma (Essau, 1965). It was claimed that anthers were a nutritious substrate for *Fusarium,* and the sites of initial infection (Strange et al., 1974). However, direct fungal penetration through the outer faces of floral bracts has been reported, too. *Fusarium* spores germinate on the adaxial surface of the glumes and giving rise to unbranched hyphae that frequently come in contact with stomata (Wanjiru et al., 2002). Despite not producing an appressorium, *F. graminearum* has been reported to penetrate the adaxial surface and the stomatal opening of the floral brackets such as the glumes, lemma and palea (Pritsch et al., 2000). After single wheat spikelet inoculation, the infection process of *Fusarium culmorum* and spread of fungal hyphae in the spike tissues were studied by scanning and transmission electron microscopy. While hyphal growth on outer surfaces of the spike was scanty and no successful penetration was observed, the fungus developed a dense mycelium on the inner surfaces and effectively invaded the lemma, glume, palea and ovary by penetration pegs (Kang and Buchenauer, 2002).

The aim of this work was: (1) to evaluate the occurrence of selected *Fusarium* species on hulls and grains of hulled wheat (einkorn, emmer, spelt) and common wheat samples using the PCR assays; (2) to determine the differences in *Fusarium* spp. occurrence between common wheat and other wheat species (einkorn, emmer, spelt); (3) to assess the role of hull in *Fusarium* spp. contamination in wheat grain.

MATERIAL AND METHODS

Samples collection

The collection of 27 wheat genotypes was obtained from exact field plot trials, carried out during the 2015/2016 and 2016/2017 growing seasons at the experimental station of the Czech University of Life Sciences in Prague-Uhříněves (central part of Bohemia, 295 m above sea level, average annual temperature 8.4°C, average sum of precipitation 575 mm). The collection involved 5 spring common wheat (*Triticum aestivum* L.), 6 spring einkorn wheat (*T. monococcum* L.), 5 spring emmer wheat (*T. dicoccum* Schrank), 6 spring spelt wheat and 5 winter spelt wheat (*T. spelta* L.) genotypes (both present cultivars, old landraces and other genetic resources, obtained from the Gene Bank of the Crop Research Institute Prague). The examined spring spelt, einkorn and emmer genotypes were evaluated using criteria valuable for growing under organic farming conditions (canopy shape in early stage of development, length of upper internodium, resistance to lodging, special grain quality) within the grant project "The Use of Participatory Breeding Method in the Wheat Breeding for Organic Farming" supported by the

Ministry of Agriculture of the Czech Republic (2014-2017). The evaluation of *Fusarium* spp. occurrence was a part of this complex evaluation.

The field trials with evaluated wheat genotypes were established using random blocks, in 3 replicates, with an experimental plot average area of 10 m^2. The trials were carried out under organic cropping system. Red clover was used as a preceding crop to the wheat. Treatment of the wheat stands by weeding harrows was used during the vegetation; no fertilizers or pesticides were applied. Only natural *Fusarium* spp. contamination was evaluated - any artificial inoculation was not performed.

In the case of hulled wheat species (einkorn, emmer and spelt), samples of about 1 kg of hulled spikelets were taken after field experiment harvests from each replicate, mixed together to obtain an average sample and dehulled using the laboratory dehulling machine. Ear samples of common wheat (60 ears from each replicate) were taken randomly from the experimental plots immediately prior to harvest and mixed together. Then hulls and grains were separated using the laboratory thresher. All grain and hull samples (all floral bracts - glumes, lemma, palea were included under the term "hulls") were ground using the laboratory grinder to pass through a 0.5 mm screen and homogenized well. The obtained meal was used for the PCR assay. Three replicated processes were carried out in all of the following analyses.

DNA extraction

DNA both from a mycelium of all tested fungi and from grain and hulls samples was extracted using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer instructions. The quality and concentration of the extracted DNA were verified electrophoretically in 0.8% agarose gel. DNA was visualized by ethidium-bromide and detected under a UV lamp. As a size and concentration standard Lambda DNA/HindIII Marker (Fermentas, Lithuania) was used. The DNA extracted from samples was diluted to a concentration of 50 $ng.\mu l^{-1}$ using a GeneQuantPro spectrophotometer (Amersham, Cambridge, UK).

***Fusarium* species-specific amplification**

The markers specific to the species: *F. culmorum* (*Fc*), *F. graminearum* (*Fg*), *F. poae* (*Fp*), *F. sporotrichoides* (*Fsp*), *F. equiseti* (*Fe*), *F. pseudograminearum* (*Fpse*) and *F. avenaceum* (*Fa*) were borrowed from the literature (Parry and Nicholson, 1996; Doohan et al., 1998; Aoki and O´Donnell, 1999; Leišová et al., 2006). PCR reactions were performed in a 15 µl reaction mixture (0.3 µM of each primer, 170 µM dNTP, 1 × PCR buffer, 2 mM $MgCl_2$, 1U Tth DNA polymerase Biotools (DYNEX) and 50 ng of template DNA) in the cycler SensoQuest (Goettingen, Germany). The amplification products were separated in 1.6% agarose gel, stained with ethidium bromide and visualised under UV light. The size of the product was verified by comparison with the size standard GeneRuler^{TM} 100 bp DNA Ladder (Fermentas, Lithuania). The estimation of the infection level was done using image analysis by evaluating the intensity of bands visualized after an electrophoretic separation compared to the band of positive standards. As it is not a precise method, four categories were proposed, where number 3 means strong (maximum) infection grade, 2 medium infection, 1 slight infection and 0 no infection. An example of electrophoretic spectra is shown at Figure 1. The results obtained were statistically processed by ANOVA (Tables 1-3). The results, expressed in percentage, were a source of Figures 2 and 3, too.

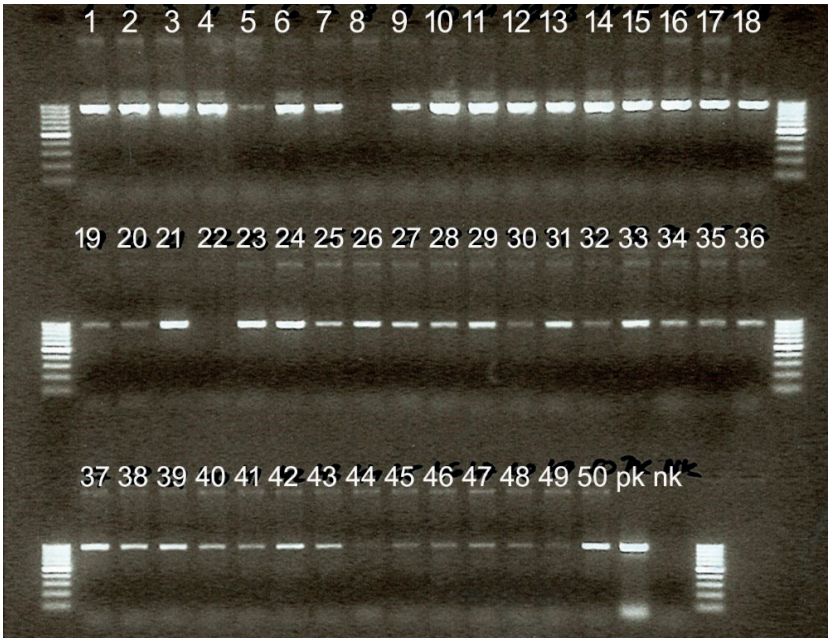

Figure 1. Example of electrophoretic spectra
[pk – positive control (DNA extracted from pure *F. avenaceae* mycelium),
nk – negative control (without *Fusarium* DNA).
Size standard is a 100 bp ladder by Fermentas (ThermoFisher Scientific).
Infection grades 3, 2, 1 and 0 represent, for example, sample numbers 4, 9, 5 and 8 respectively.]

Real-time PCR quantification

Selected *Fusarium* species – *F. culmorum* and *F. graminearum* were used for real-time PCR quantification. *F. culmorum* assay was used from the previous project [27]. *F. graminearum* specific primers for real-time PCR were designed on the base of sequences for the elongation factor obtained from public databases using the Primer Express for Windows NT 1.5 software (Applied Biosystems, Foster City, CA, USA). In case of *F. graminearum* the assay contained also a MGB (minor groove binder) probe. The specificity of primers and probes was verified *in silico* by blast analysis. After optimisations, PCR reactions were carried out in 25 µl volume consisting of either 1xTaqMan Universal PCR Master Mix (Life Technologies, Foster City, CA, USA; in case the MGB probe was used), 0.3 µM of each primer, 0.3 µM Taq Man MGB probe and 250 ng of template DNA. Real-time quantitative PCR was performed using the cycler ABI PRISM 7900 (Life Technologies, Foster City, CA, USA) in MicroAmp optical 96-well plates. The reaction consisted of 2 min at 50°C, 10 min at 95°C and 40 cycles of 95°C for 15 s and 60°C for 1 min. The Sequence Detection Software (Life Technologies, Foster City, USA) collected data for the reported dye every 7 seconds from each well, generating a fluorescence profile for each amplification. The threshold cycle (Ct) was recorded for each dye as the cycle at which a fluorescent signal, associated with an exponential growth of PCR product, exceeded the background fluorescence. The dilution series of *F. culmorum* and *F. graminearum* isolates - DNA (from 0.1 pg to 100 ng) was included in triplicate as standard in every real-time PCR experiment. Standard curves for assayed fungi were generated by plotting the known DNA amounts against the Ct values calculated by the SDS software. Unknown samples were quantified from measured Ct values by interpolation using the regression equation derived from standard curves. The final results of fungal DNA amount in samples were expressed in micrograms per 100 mg of meal.

Statistical analysis

Obtained results were statistically analysed by the analysis of variance (ANOVA) method. The differences between mean values were evaluated by the Tukey´s HSD test in the program SAS, version 9.4 (SAS Institute, USA) at the level of significance $p = 0.05$.

RESULTS AND DISCUSSION

Detection of *Fusarium* spp. using species-specific primers

Fusarium graminearum, F. culmorum, F. avenaceum, F. poae and *F. pseudograminearum* are the most frequent *Fusarium* species caused FHB in the central European conditions (Suchowilska et al., 2010; Ostrowska-Kolodziejczak et al., 2016), while *F. equiseti* and *F. sporotrichoides* belong to the less commonly identified species (Vogelgsang and Sulyok, 2008). In our experiments, we identified 6 of these 7 *Fusarium* species – *F. avenaceum*, *F. poae*, *F. culmorum*, *F. graminearum*, *F. equiseti* and *F. sporotrichoides* - in all of evaluated wheat species, while *F. pseudograminearum* was not detected in any sample (Tables 1 and 2). The most influential factor in relation to *Fusarium* contamination was the part of seed (the highest F-values), except *F. culmorum*, where the year had the greatest impact. The effect of wheat species was statistically significant, but less than that of parts of seed. Some factor interactions have also been detected as influential.

Table 1. The effect of wheat species, part of seeds, years and their interaction on *Fusarium* spp. infection grade (3 factor ANOVA with interactions)

Source of variation	*F. avenaceum*			*F. culmorum*		
	df	MS	*F*	df	MS	*F*
Wheat species	4	1.68	4.42**	4	1.80	5.06**
Part of seed (hulls, grains)	1	33.33	87.82**	1	22.23	62.30**
Year	1	0.33	0.88	1	30.08	84.31**
Wheat species x Part of seed	4	0.65	1.70	4	0.60	1.68
Wheat species x Year	4	0.61	1.61	4	2.07	5.80**
Part of seed x Year	1	0.15	0.39	1	0.01	0.03
	F. equiseti			*F. graminearum*		
	df	MS	*F*	df	MS	*F*
Wheat species	4	0.92	2.72*	4	3.51	7.99**
Part of seed (hulls, grains)	1	39.12	115.01**	1	22.23	50.60**
Year	1	0.01	0.03	1	0.23	0.53
Wheat species x Part of seed	4	0.51	1.49	4	0.28	0.63
Wheat species x Year	4	0.44	1.30	4	0.98	2.22
Part of seed x Year	1	0.01	0.03	1	0.01	0.03
	F. poae			*F. sporotrichoides*		
	df	MS	*F*	df	MS	*F*
Wheat species	4	1.94	9.72**	4	1.67	4.58**
Part of seed (hulls, grains)	1	56.33	282.74**	1	33.33	91.57**
Year	1	0.04	0.19	1	3.00	8.24**
Wheat species x Part of seed	4	0.76	3.83**	4	0.71	1.94
Wheat species x Year	4	0.17	0.85	4	1.39	3.82**
Part of seed x Year	1	0.04	0.19	1	0.04	0.10

[df: degree of freedom; MS: Mean Square; *F*: Fisher´s *F* value; p< 0.05*; p< 0.01**.
The full 3 factor ANOVA model was used for computation, but the triple interaction is not shown to improve the comprehensibility of the presented results.]

Crop rotations and crop residues management play an important role in the control of primary *Fusarium* inoculums. For example, maize crop residues increase the risk of FHB occurrence in following crops (Reis et al., 2011), while some other crops like alfalfa or mustard have been already successfully used to reduce the survival of plant pathogens (Friberg et al., 2009). We used red clover as a preceding crop of wheat in our experiments. Red clover could also be included in the preceding crops´ ability to reduce the pathogens' survival. The *Fusarium* spp. infection grade in our samples was relatively high. It varied in most of the evaluated wheat species between medium and strong infection in the case of *F. avenaceum* and *F. poae*, between the slight and medium infection in case of *F. graminearum*, *F. sporotrichoides* and *F. culmorum* and between no infection and slight infection in *F. equiseti* (Table 2). Maize stands close to our experimental field and their crop residues were probably a source of *Fusarium* conidia or ascospores and their dispersal by rain splashing or wind dispersal to our wheat stands.

Fusarium species present on common wheat (*T. aestivum* L.) have already been analysed in many studies using PCR assays (Kuzdraliński et al., 2017; Karlsson et al., 2017). However, there is a lack of information regarding the *Fusarium* spp. contamination in other wheat species. Our results showed that the common wheat *Fusarium* infection grade was generally higher in comparison with evaluated hulled wheat species, although the differences in infection grade between the common wheat and hulled wheats were not statistically significant in many cases (Table 2).

Our results also showed that winter spelt was the least infected wheat of all evaluated wheat species. In spring spelt, infection grade was higher and achieved similar level as in the others hulled wheat species – einkorn and emmer. According to Konvalina et al. (2011), common wheat was the most contaminated wheat species while the spring spelt was the least infected one. Similarly, results of other authors showed the weakest response to *F. culmorum* infection in *T. spelta* and the strongest response in *T. polonicum* (Wiwart et al., 2016). The susceptibility of *T. polonicum* to *Fusarium* pathogens maybe relates to the fact, that it is a threshable species with naked grain, similar to common wheat. On the other hand, spelt relatively low susceptibility to infection caused by FHB pathogens could result from long culms and loose spikes that contribute to the lower moisture content in spikelets (Wiwart et al., 2016).

Table 2. Fusarium spp. infection grade in the wheat species, years, hulls and grains (Tukey´s HSD test)

Factor	*Fa*	*Fc*	*Fe*	*Fg*	*Fp*	*Fsp*
Common wheat	2.5a	1.8a	1.2a	2.1a	2.4a	1.9a
Einkorn	2.4a	1.2b	0.8ab	2.0a	2.2ab	1.6ab
Spring spelt	2.2ab	1.1b	1.0ab	1.8a	2.0ab	1.9a
Emmer	2.0ab	1.3ab	1.1ab	1.7a	1.9bc	1.3b
Winter spelt	1.8b	0.9b	0.7b	1.0b	1.6c	1.3b
$HSD_{0.05}$	0.52	0.51	0.50	0.56	0.38	0.51
Hulls	2.7a	1.7a	1.5a	2.2a	2.7a	2.2a
Grains	1.6b	0.8b	0.3b	1.3b	1.3b	1.0b
$HSD_{0.05}$	0.24	0.23	0.22	0.25	0.17	0.23
2016	2.1a	0.7b	0.9a	1.7a	2.0a	1.4b
2017	2.2a	1.8a	0.9a	1.8a	2.0a	1.8a
$HSD_{0.05}$	0.24	0.23	0.22	0.25	0.17	0.23

[HSD: honestly significant difference; *Fa = F. avenaceum*, *Fc = F. culmorum*, *Fe = F. equiseti*, *Fg = F. graminearum*, *Fp = F. poae*, *Fsp = F. sporotrichoides*; infection grade: 3 = strong (maximum) infection, 2 = medium infection, 1 = slight infection, 0 = no infection; values in table represent mean values of a categories (wheat species, years, hulls and grains).]

Response of 35 spring wheat cultivars of four *Triticum* species to spray inoculation with *F. culmorum* was evaluated in field experiments. Data of DON content were complemented by symptom scores and determination of the percentage of *Fusarium* damaged kernels. Resistance to FHB was variable in all the examined genotype groups. DON accumulation was significantly higher in the modern common wheat cultivars than in the other *Triticum* species (Chrpová et al., 2013). *Fusarium* occurrence and DON content were studied in genetic resources of spring wheat species (einkorn, emmer, spelt and bread wheat). The study aimed at the comparison of grain contamination rates in various wheat species being grown in organic farming system. The strongest contamination rate was identified in grains of the common wheat cultivars (Konvalina et al., 2011).

Our results of the *Fusarium* species total infection grade in individual evaluated wheat genotypes are arranged in Table 3. It is evident from this table that, in accordance with the findings of Goral et al. (2008), there were some differences in the genotype response to *Fusarium* spp. infection. Nevertheless, the differences between genotypes were not statistically significant in most of cases. In almost every species of interest it was possible to find a genotype that was more sensitive either overall or specifically to some species of *Fusarium*. The winter spelt cultivar Tauro followed by another winter spelt cultivar Rubiota, the emmer wheat cultivar Rudico, winter spelt cultivars Alkor and Samir demonstrated the lowest *Fusarium* spp. infection grade of all the evaluated genotypes. Common spring wheat cultivars SW Kadrilj, Jara and Granny, the spring spelt genotype Špalda bílá jarní and the einkorn genotype *T. monococcum* (ALB) represented the genotypes with the highest *Fusarium* spp. infection grade.

Studied winter spelt cultivars are already known for their high grain-quality parameters and satisfying yields even in organic farming conditions. However, the emmer wheat cultivar Rudico, for which a certificate of legal protection was obtained (Stehno et al., 2010), can also be recommended for growing in organic farming system. It was documented that emmer and spelt wheat genotypes with higher resistance to FHB (Rudico, *T. spelta* Tabor, *T. spelta* Ruzyně) have some outstanding grain-quality parameters (e.g. very high protein content) and can be utilized in breeding wheat for alternative use and growing in organic farming (Chrpová et al., 2013).

Wheat hulls function as an efficient barrier against the contamination of grain (Suchowilska et al., 2010; Kuzdraliński et al., 2017). Our data analysis (ANOVA, *F.* values) reveals that the effect of the studied part of the seed (hulls, grains) on the *Fusarium* infection grade was significantly higher than the effect of the wheat species and years in all of the evaluated *Fusarium* species (Table 1). It is evident also from the Tukey´s test values – significantly higher infection grade in hulls was observed in all of the evaluated *Fusarium* species (Table 2).

Figure 1 compares the percentage of *Fusarium* spp. incidence in hulls and grains in hulled wheat species; the percentage of *Fusarium* spp. incidence in hulls and grains in common wheat is shown in Figure 2. Percentage incidence in hulls and grain was expressed as a ratio from maximum possible incidence (infection grade 3) in sum of tested samples. Data analysis suggests, in accordance with the findings of Kuzdraliński et al. (2017), that the presence of *Fusarium* species in hulls was inversely proportional to the percentage reduction of *Fusarium* spp. in grain in hulled wheats, just as in common wheat. However, there were some differences between the common wheat and hulled wheat species.

When we assume the incidence of *Fusarium* spp. in hulls of hulled wheats (Figure 1) as 100%, the incidence of *Fusarium* spp. in grains decreased by 44.54% in *F. avenaceum*, 45.15% in *F. graminearum,* 54.83% in *F. sporotrichoides*, 55.22% in *F. culmorum*, 57.49% in *F. poae* and 85.07% in *F. equiseti*. In the case of common wheat (Figure 2), the incidence of *Fusarium* spp. in grains decreased by 21.43% in *F. avenaceum*, 29.16% in *F. graminearum*, 32.14% in *F. poae*, 39.13% in *F. sporotrichoides*, 40.91% in *F. culmorum* and 49.99% in *F. equiseti*.

Table 2. Fusarium spp. total infection grade in evaluated wheat genotypes (Tukey´s HSD test)

Genotype	Country origin	*Fa*	*Fc*	*Fe*	*Fg*	*Fp*	*Fsp*
T. monococcum	ALB	2.8a	1.0bc	0.8b	2.8a	2.5a	1.5abc
T. monococcum	GEO	2.5ab	1.0bc	0.8b	1.8abcd	2.0ab	1.5abc
Schwedisches Einkorn	SWE	2.3ab	1.3bc	0.8b	1.8abcd	2.3ab	2.0abc
T. monococcum No.8910	DNK	2.3ab	1.3bc	0.8b	2.3abc	2.0ab	1.3abc
Malonty	unknown	2.3ab	1.0bc	1.0ab	2.0abcd	2.5a	1.8abc
Probio	unknown	2.3ab	1.8ab	0.5b	1.3bcd	2.0ab	1.5abc
T. dicoccum Dagestan	RUS	2.5ab	1.0bc	1.0ab	2.5ab	2.0ab	1.5abc
T. dicoccum Palestine	ISR	2.3ab	1.0bc	1.3ab	1.5abcd	2.3ab	1.0bc
May-Emmer	CHE	2.3ab	1.8ab	1.5ab	1.5abcd	1.8abc	1.8abc
Weisser Sommer	DEU	1.8abc	1.5abc	0.8b	1.8abcd	2.0ab	1.3abc
Rudico	CZE	1.3bc	1.0bc	0.8b	1.3bcd	1.5abc	1.0bc
T. spelta Kew	GBR	2.5ab	0.8c	1.0ab	2.3abc	2.0ab	2.3ab
Špalda bílá jarní	CZE	2.3ab	1.8ab	0.8b	2.3abc	2.3ab	2.0abc
T. spelta Tabor	CZE	2.3ab	1.0bc	1.0ab	1.5abcd	2.0ab	1.8abc
T. spelta No.8930	DNK	2.3ab	1.0bc	1.0ab	2.0abcd	2.0ab	1.5abc
VIR St. Petersburg	CZE	2.0abc	1.0bc	1.0ab	1.6abcd	2.0ab	1.5abc
T. spelta Ruzyně	CZE	2.0abc	1.3bc	1.3ab	1.3bcd	2.0ab	2.3ab
Titan	CHE	2.5ab	1.0bc	1.0ab	1.3bcd	1.8abc	1.8abc
Alkor	CHE	2.0abc	0.8c	0.5b	0.8d	2.3ab	1.0bc
Samir	CHE	2.0abc	1.3bc	0.5b	1.3bcd	0.8c	2.0abc
Rubiota	CZE	1.0c	1.0bc	1.0ab	1.0bcd	1.3bc	1.0bc
Tauro	CHE	1.3bc	0.8c	0.5b	0.8d	1.8abc	0.8c
Jara	CZE	2.5ab	2.3a	0.8b	2.5ab	2.5a	1.5abc
Granny	CZE	2.3ab	1.5abc	2.0a	2.5ab	1.8abc	2.0abc
Izzy	CZE	2.5ab	1.5abc	1.3ab	1.8abcd	2.5a	1.5abc
Astrid	CZE	2.5ab	1.5abc	0.5b	1.5abcd	2.5a	1.8abc
SW Kadrilj	SWE	2.5ab	2.0ab	1.5ab	2.0abcd	2.5a	2.5a
$HSD_{0.05}$		1.18	0.96	1.09	1.33	1.06	1.31

[HSD: honestly significant difference*; Fa = F. avenaceum, Fc = F. culmorum, Fe = F. equiseti, Fg = F. graminearum*, *Fp = F. poae, Fsp = F. sporotrichoides*; Infection grade: 3 = strong infection, 2 = medium infection, 1 = slight infection, 0 = no infection (values in table represent mean values of wheat genotypes).]

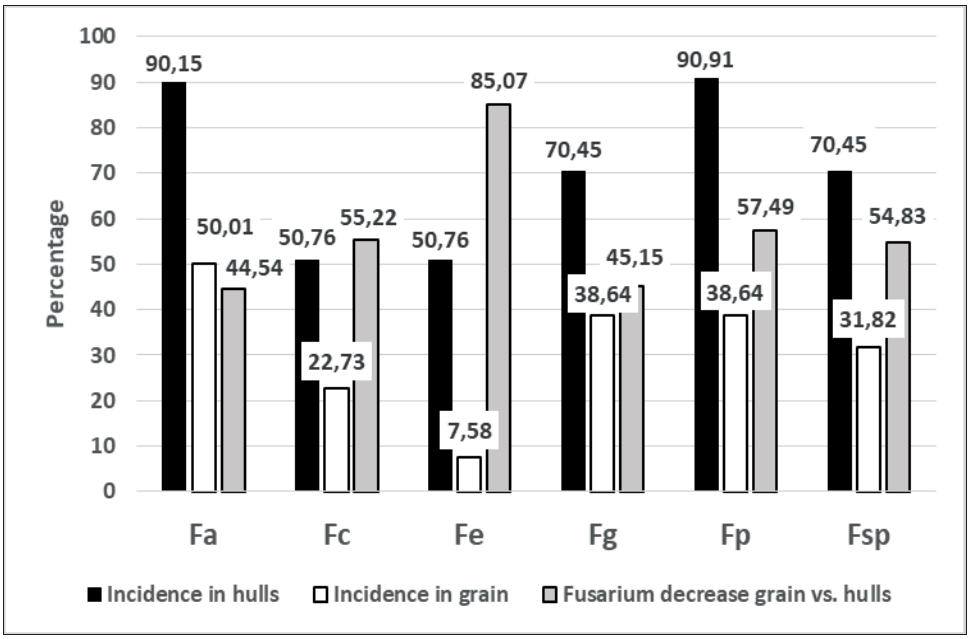

Figure 2. Reduction of *Fusarium* spp. incidence in grain vs. hulls, as compared to *Fusarium* spp. incidence in hulls (100%) (average of hulled wheat species; 2016-2017)
[The decrease is expressed as the rest of the quotient of *Fusarium* incidence in grain vs. incidence in hulls in percentage.]

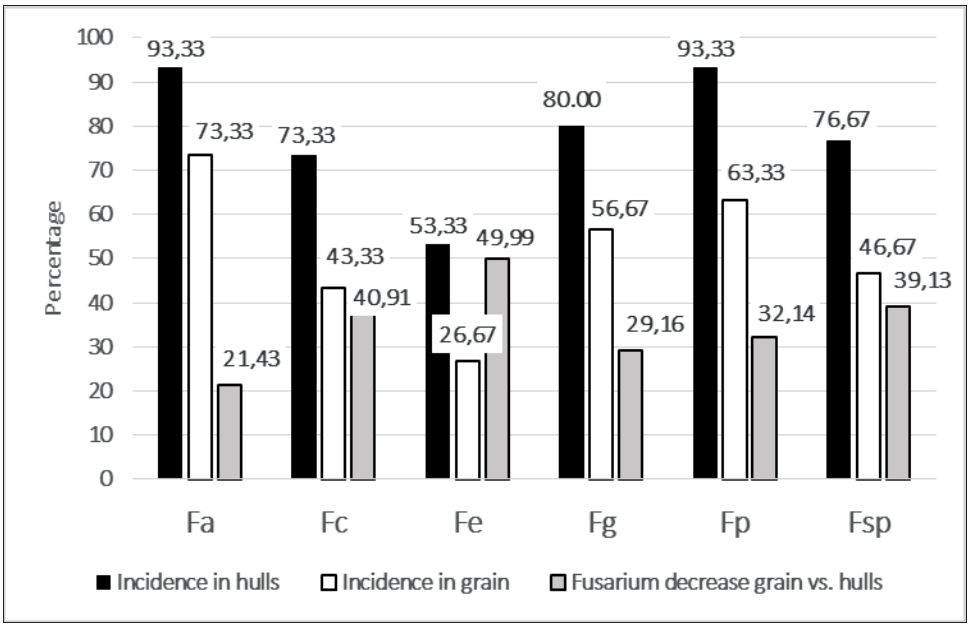

Figure 3. Reduction of *Fusarium* spp. incidence in grain vs. hulls, as compared to *Fusarium* spp. incidence in hulls (100%) (common wheat; 2016-2017)
[The reduction is expressed as the rest of the quotient of *Fusarium* incidence in grain vs. incidence in hulls in percentage.]

These results confirm that hulls form a barrier for *Fusarium* and reduce the incidence of these fungi in grain. It is also evident from the results, that the protective effect of hulls was higher in hulled wheat species compared to common wheat. Hard hulls that cling tightly to the kernel make up a very effective barrier to mycelia filaments (Suchowilska

et al., 2010). Thus, the generally lower *Fusarium* spp. infection grade of hulled wheats grain seems to be connected especially with morphology of hulled wheat spikelets with the tight and hard hulls (including glumes, lemma, palea), which could be able to reduce spread of fungal hyphae in the spikelet tissue. At the same time, it is possible to suppose that narrow opening of the hulled wheat flowers reduces entry of spores to flowers.

This theory is supported by results suggesting that narrow flower opening prevents entry of spores and wherefore *F. graminearum* infection (Gilsinger et al., 2005). Comparing of *Fusarium culmorum* infection in hulled and hulless barley genotypes showed that hulless lines were more susceptible to infection than hulled ones and suggested hulls protective effect against penetration of pathogen to the seed (Warzecha et al., 2010). Other published results also indicated that certain physical barriers can limit the advance of fungal infection between the hulls and grain (Hope et al., 2005). Similarly, other authors found that hulls function as an efficient barrier against *Fusarium* contamination of grain (Kuzdraliński et al., 2017). This barrier seems to be stronger in hulled cereals (Suchowilska et al., 2010; Warzecha et al., 2010). On the other hand, hulls could be an inoculum reservoir for wheat head infections during the growth season (Trenholm et al., 2007).

Real-time PCR quantification

Real-time PCR quantification gives very exact information about the fungal DNA amount in evaluated samples. Although *F. avenaceum* and *F. poae* prevailed on samples evaluated in this study, the main *Fusarium* species which caused FHB in central and central-eastern Europe were *F. culmorum* and *F. graminearum* for many years (Chrpová et al., 2013; Wagacha and Muthomi, 2007). At the same time, strong attention was given to these *Fusarium* species as producers of mycotoxins such as deoxynivalenol (DON) (Faltusová et al., 2015). Therefore, *F. graminearum* and *F. culmorum* assays used in our former research were also used in this work.

The results (Table 4) confirmed findings obtained in the previous part of this work. Winter spelt was the least contaminated wheat species, while common wheat was the most contaminated one. The *F. culmorum* and *F. graminearum* DNA amount was significantly higher in hulls in comparison with grain. The year effect is also evident from the results – while the level of *F. graminearum* infection was similar in both of the evaluated years, *F. culmorum* occurrence was significantly higher in 2017 compared to the 2016 harvest year.

Table 4. Quantitative evaluation of *F. culmorum* and *F. graminearum* DNA amount in wheat species, years, hulls and grains (Tukey´s HSD test)

Factor	*F. culmorum* DNA amount	*F. graminearum* DNA amount
	µg.100 mg^{-1} of meal	
Einkorn	0.206a	0.261a
Emmer	0.235a	0.262a
Spring spelt	0.206a	0.220ab
Winter spelt	0.063b	0.069b
Common wheat	0.319a	0.337a
$HSD_{0.05}$	0.136	0.169
Hulls	0.312a	0.355a
Grains	0.099b	0.104b
$HSD_{0.05}$	0.061	0.077
2016	0.069b	0.211a
2017	0.343a	0.248a
$HSD_{0.05}$	0.061	0.077

[HSD: honestly significant difference]

Quantitative evaluation of *F. culmorum* and *F. graminearum* DNA amount in the evaluated wheat genotypes is arranged in Table 5. These results confirmed the findings obtained in the previous part of the work. *Fusarium culmorum* and *F. graminearum* DNA amounts in evaluated winter spelt cultivars were the lowest, while in common wheat cultivars such as Jara, SW Kadrilj and Granny and spring spelt genotype Špalda bílá jarní were the highest of all the evaluated genotypes. At the same time, very good results were confirmed, in accordance with the previous part, in the emmer wheat genotype Rudico.

Table 5. Quantitative evaluation of *F. culmorum* and *F. graminearum* DNA amount in evaluated wheat genotypes (Tukey´s HSD test)

Genotype and its origin	*F. culmorum* DNA amount	*F. graminearum* DNA amount
	μg.100 mg^{-1} of meal	
T. monococcum (ALB)	0.141efg	0.352abc
T. monococcum (GEO)	0.119efg	0.257abc
Schwedisches Einkorn (SWE)	0.265bcdefg	0.227abc
T. monococcum No. 8910 (DNK)	0.196cdefg	0.336abc
Malonty (unknown origin)	0.177defg	0.332abc
Probio (unknown origin)	0.338bcdefg	0.059bc
T. dicoccum Dagestan (RUS)	0.166defg	0.632a
T. dicoccum Palestine (ISR)	0.159defg	0.187bc
May-Emmer (CHE)	0.494ab	0.226abc
Weisser Sommer (DEU)	0.267bcdefg	0.242abc
Rudico (CZE)	0.087fg	0.023c
T. spelta Kew (GBR)	0.109efg	0.339abc
Špalda bílá jarní (CZE)	0.409abcd	0.388abc
T. spelta Tabor (CZE)	0.162defg	0.163bc
T. spelta No. 8930 (DNK)	0.103efg	0.171bc
VIR St. Petersburg (CZE)	0.163defg	0.093bc
T. spelta Ruzyně (CZE)	0.292abcdef	0.166bc
Titan (CHE)	0.078fg	0.114bc
Alkor (CHE)	0.048fg	0.004c
Samir (CHE)	0.136efg	0.155bc
Rubiota (CZE)	0.024g	0.033c
Tauro (CHE)	0.031g	0.038bc
Jara (CZE)	0.529a	0.468ab
Granny (CZE)	0.257bcdefg	0.321abc
Izzy (CZE)	0.219cdefg	0.271abc
Astrid (CZE)	0.239cdefg	0.251abc
SW Kadrilj (SWE)	0.354abcde	0.375abc
$HSD_{0.05}$	0.253	0.432

[HSD: honestly significant difference]

CONCLUSIONS

The results reported in this work demonstrate the occurrence of selected *Fusarium* species in hulls and grains of all evaluated wheat species. The results also demonstrate differences in *Fusarium* spp. infection grade between the evaluated wheat species and genotypes. Especially in hulled wheat species, genotypes with high resistance to FHB were found, and since their high grain quality parameters are already known, they could be an alternative to common wheat mainly in organic cropping systems.

The results also suggest that hulls can be classified as an important factor of passive resistance against *Fusarium* spp. infection under natural infection conditions and reduce the incidence of these fungi in the wheat grain; at the same time, the protective effect of hulls seems to be higher in hulled wheat species compared to common wheat. In addition, presented data indicate the importance of *Fusarium* spp. analysis not only in wheat grain, but also in hulls in pathological studies.

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