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FUSARIUM SPP. OCCURRENCE IN GRAINS OF ANCIENT WHEAT SPECIES

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ABSTRACT

***Fusarium* spp. infestation was studied in different species of genetic resources of spring wheat – einkorn (*Triticum monococcum* L.), emmer [*Triticum diccocum* (Schrank) Schuebl], spelt (*Triticum spelta* L.) and intermediate forms of bread wheat (*Triticum aestivum* L.). The study aimed at the comparison of grain contamination rates by *Fusarium* spp. in various wheat species being grown in organic farming systems. The trials were established on certified organic plots in two different localities in the Czech Republic between 2009 and 2012. The PCR method and specific primers were used to detect *Fusarium* spp. The occurrence of *Fusarium* was influenced by the growth wheat species. The occurrence of *Fusarium poae* in grains was influenced by a reduced resistance of the crop to lodging. The occurrence of *Fusarium poae* species was the weakest one. Grains were less contaminated with *Fusarium culmorum*, whereas the contamination rate was dominantly influenced by the year - einkorn and emmer wheat were the least infested wheat species. *Fusarium graminearum* provoked the strongest and most serious contamination of grains. The contamination of grain by *Fusarium graminearum* influenced DON content in grain. Spring spelt wheat varieties were the least infested ones. On the other hand, landraces of bread wheat (*Triticum aestivum* L.) and both control bread wheat varieties were the most contaminated ones. Grain contamination with the *Fusarium* species must be carefully taken into account in organic farming systems, as these work with less common wheat species and varieties.**

Key words: organic farming, PCR, *Fusarium*, hulled wheat.

INTRODUCTION

A complex of *Fusarium* spp. causes Fusarium head blight (FHB) emerging on wheat plants (Nedělník et al., 2007). Infections can result in yield losses, but more important in grain contamination with mycotoxins produced by pathogens (Köhl et al., 2007). Harvested products are contaminated due to the accumulation of toxins such as deoxynivalenol (DON) produced by *Fusarium* spp. (Nedělník et al., 2007).

Occurrence of *Fusarium* head blight in cereals is strongly influenced by cultivation practices, such as crop rotation or tillage (Vogelgsang et al., 2007). Crop rotation including corn, wheat and a small proportion of other crops, together with a limited soil preparation and working, high doses of nitrogen and favourable weather provoke a significant occurrence of *Fusarium* on spikes (Ittu et al., 2010). Diversified and “colourful” crop rotations, intensive preparation of stubble and a reasonable application of nitrogenous fertilizers are, therefore, important measures to prevent the damage from spike *Fusarium* (Vogelgsang et al., 2007). This approach often has to compete with economic aspects.

The best way to prevent or reduce *Fusarium* infection is the growth of cultivars with high level of disease resistance (Scholten et al., 2007). Bread wheat (*Triticum aestivum* L.) is the most frequent cereal species grown within the Czech organic farming system. Because in the Czech Republic only varieties bred in conventional breeding programmes are available, organic farmers work with a wider diversity of crops (Konvalina et al., 2014). Knowledge of response of ancient wheat species to *Fusarium* sp. infection is very

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important for organic farming. *Triticum monococcum L., Triticum dicoccum* (Schrank) Schuebl. and *Triticum spelta* L., also known as emmer wheat, spelt wheat and einkorn, respectively, were among the earliest *Triticeae* domesticated by a man (Suchowilska et al., 2009). Wheat genetic resources are also used in breeding to create new varieties more resistant to wheat diseases (Tyriskin et al., 2006). There is no enough information on the resistance of particular varieties to Fusarium head blight, as the range of varieties and species are very wide. Therefore, the resistance of wheat genetic resources to Fusarium head blight is an important question as it could assure safety of food products.

This study aimed to analyse the occurrence of spike *Fusarium* in various organic hulled wheat species (einkorn, emmer wheat, spelt wheat) and bread wheat varieties (landraces, intermediate forms, control varieties) by the PCR method.

MATERIAL AND METHODS

Used varieties

The studied and assessed varieties (Table 1) came from the Gene Bank of the Research Institute of Crop Production in Prague-Ruzyne from the collection of genetics resources of wheat.

Table 1. List of varieties

Wheat species	Variety	Origin[1]
T. monococcum L.	Schwedisches Einkorn	SW
	No. 8910	DK
*T. diccocum (*Schrank) Schuebl	Rudico	CZ
	Weisser Sommer	D
T. spelta L.	Spalda bila jarni	CZ
	VIR St. Petersburg	CZ
T. aestivum L. - intermediate	Cervena perla	CZ
	Kasticka presivka	CZ
T. aestivum L. - check varieties	Jara	CZ
	SW Kadrilj	SW

[1]Abbreviations of countries comply with ISO 3166-1 alpha-3.

Description of the trials

Varieties were sown in a randomized, complete block design on organic certified experimental plots in Prague (Prague) and Ceske Budejovice (CB) during 2009-2012. The seeding rate was adjusted for a density of 350 germinating grains per m^2. Rows were 125 mm wide. The trial crop stands were treated in compliance with the European legislation (European Council Regulation No. 834/2007, European Commission Regulation No. 889/2008).

Characteristics of the trial localities: University of South Bohemia in Ceske Budejovice (CB): mild warm climate, soil type – pseudogley cambisols, kind of soil – loamy sand soil, altitude of 388 m. University of Life Sciences – Research station Prague – Uhrineves (Prague): warm and mid-dry climate, soil type – brown soil, kind of soil – loamy clay soil, altitude of 295 m.

Laboratory analysis

Evaluation of occurence of the spike *Fusarium* by the PCR method - DNA extracting and determination of *Fusarium* species by the DNA markers and PCR method was described in paper Konvalina et al. (2011).

Deoxynivalenol (DON): At first, toxin was extracted from the sample (deionizied water was used as a solvent). 100 µl of the extract was diluted in 1 ml of buffer. 300 µl of the diluted extract was applied on the strip (ROSA®-DON Quantitative test). Incubation of the strip was done 10 minutes in the temperature of 45°C (ROSA®-M Incubator). Assessment of the test was done - by ROSA®-M Reader (results in ppb).

Statistical data processing

Basic analyses and Statistica 9.0 (StatSoft. Inc., USA) program provided the statistical data processing. Regression and correlation analyses provided the evaluation of interdependence. Comparison of varieties and their division into statistically different categories were provided by the *LSD* test.

RESULTS AND DISCUSSION

All tested wheat species were the least affected by *Fusarium culmorum*. The infestation degree was classified as “slight infestation” in all cases (Table 2). Grain infestation with *F. poae* was influenced (p<0.01) by the year factor (Table 4). Two einkorn varieties (Schwedisches einkorn, No. 8910) were not affected by *F. culmorum* at all (Table 4). Emmer wheat varieties, just as control bread wheat varieties, were affected by *F. culmorum* to the same degree (Table 2). Špalda bílá jarní was the most resistant spelt wheat variety. Low contamination of grain by DON was found in emmer varieties (only 34 resp. 39 ppb) – it was eight times less than in case of check varieties of *Triticum aestivum* L. (Table 2). Wiwart et al. (2004) reported the response of spelt to spike infection by *F. culmorum* being slightly stronger than that of common wheat. However, we did not confirm this finding by our research trials. We found a slight correlation between the grain infestation with *F. culmorum* and the percentage of DON (Konvalina et al., 2011). Bernhfof et al. (2010) found and presented similar results (the slight correlation between these two features) too. On the other hand, *F. culmorum* is supposed to be one of the most frequent producers of DON (Langseth et al., 1999).

Table 2. Influence of variety factor on *Fusarium* infestation rate

Wheat species	Variety	*Fusarium*			DON (ppb)
		poae	*graminearum*	*culmorum*	
T. monococcum L.	Schwedisches Einkorn	0.75[a]	1.00[ab]	0.13[a]	81.87[ab]
	No. 8910	0.75[a]	1.00[ab]	0.25[a]	109.25[abc]
T. diccocum (Schrank) Schuebl	Rudico	0.75[a]	1.25[a]	0.88[a]	34.12[a]
	Weisser Sommer	0.75[a]	0.63[ab]	0.75[a]	39.13[a]
T. spelta L.	Spalda bila jarni	0.75[a]	0.38[b]	0.50[a]	115.63[abc]
	VIR St. Petersburg	0.75[a]	0.50[ab]	0.88[a]	74.87[ab]
T. aestivum L.- intermadiate	Cervena perla	0.75[a]	1.25[a]	0.63[a]	194.62[bc]
	Kasticka presivka	0.75[a]	1.13[a]	0.63[a]	94.25[abc]
T. aestivum L. - check varieties	Jara	0.75[a]	1.00[ab]	0.88[a]	264.13[c]
	SW Kadrilj	0.88[a]	1.25[a]	0.75[a]	228.75[c]

Different letters document statistical differences between varieties for LSD test, p<0.05*;*
0 = no occurrence; 1 = weak occurrence; 2 = middle occurrence; 3 = massive occurrence.

Tested grains were the most affected by *F. graminearum* (Table 2). In 2009, we noticed "strong infestation" of several varieties. Grain infestation degree was influenced (Table 5) by the year run (p<0.01) and the locality (p<0.05). Spring spelt varieties (Špalda bílá jarní and VIR St. Petersburg) were the least affected by *F. graminearum*. On the other hand, land races of bread wheat – intermediate forms (Červená perla and Kaštická přesívka), as well as both control varieties of bread wheat (Jara and SW Kadrilj) were the most affected by *F. graminearum.* Previous research trials proved (p<0.01) a positive correlation (r=0.69) between the grain infestation with *F. graminearum* and the final percentage of DON in grains (Konvalina et al., 2011). Bernhof et al. (2010), for example, found out similar correlation (r=0.61) too. In our current study (table 5) we found correlation of 0.56).

F. poae affected the least grains at Prague research station in 2009 and 2012 (Table 3). Latest studies proved the augmenting occurrence of *F. poae* in the Czech Republic (Remešová et al., 2006). Minimum difference was noticed between the tested varieties (see Table 4). Grains of SW Kadrilj, a control bread wheat variety, were the most infested with *F. poae* (Table 2). *F. poae* does not produce DON (Suchowilska et al., 2009). The degree of infestation with *F. poae* showed a correlation to the nivalenol percentage (r=0.55) (Bernhof et al., 2010), MON percentage and enniatins (ENs) percentage in grains (Vogelgsang et al., 2008).

Table 3. Influence of experimental factors on *Fusarium* infestation rate

Experimental factor		*Fusarium*			DON (ppb)
		poae	*graminearum*	*culmorum*	
Year	2009	0.05^{b}	1.75^{b}	1.40^{b}	281.75^{b}
	2010	1.15^{a}	0.80^{a}	0.40^{a}	52.50^{a}
	2011	1.30^{a}	0.60^{a}	0.40^{a}	72.50^{a}
	2012	0.55^{c}	0.60^{a}	0.30^{a}	87.90^{a}
Locality	CB	1.13^{b}	0.93^{a}	0.60^{a}	173.58^{b}
	Prague	0.40^{a}	0.95^{a}	0.65^{a}	73.75^{a}
Species	Einkorn	0.75^{a}	1.00^{a}	0.19^{b}	95.56^{a}
	Emmer	0.75^{a}	0.94^{ab}	0.81^{a}	36.62^{a}
	Spelt	0.75^{a}	0.44^{b}	0.69^{ab}	95.25^{a}
	Bread wheat (intermediate)	0.75^{a}	1.16^{a}	0.63^{ab}	114.43^{ab}
	Bread wheat (check varieties)	0.81^{a}	1.13^{a}	0.81^{a}	246.44^{b}

Remark: Different letters document statistical differences between varieties for LSD test, p<0.05.

Table 4. Effect of trial factors on *Fusarium* infestation rate

Factor	df	*Fusarium*						DON	
		poae		*graminearum*		*culmorum*			
		MS	%TV	MS	%TV	MS	%TV	MS	%TV
Variety (1)	9	0.0^{ns}	0	2.1^{*}	24	0.6^{ns}	9	4.9^{*}	7
Locality (2)	1	10.5^{**}	61	0.0^{ns}	0	0.1^{ns}	2	19.9^{**}	29
Year (3)	3	6.6^{**}	38	6.1^{**}	71	5.4^{**}	83	22.6^{**}	33
Error	66	0.2	1	0.4	5	0.4	6	21.6	46

Remark: *p<0.05; **p<0.01; ^{ns}not significant.

Obvious competitive relations have been detected between the tested species of *Fusarium* spp. (Table 5). The tested grains were the most affected by *F. graminearum*, *F. graminearum* infestation degree was in a positive correlation to *F. culmorum* infestation degree (r=0.51). On the other hand, *F. poae* infestation degree was in a negative correlation to *F. graminearum* (r=-0.25) and *F. culmorum* (r=-0.27) infestation degrees.

Table 5. Results of the analysis of correlation (two localities; two years)

Parameter		Mean	SD	1	2	3	4
Fusarium poae	1	0.76	0,77	1.00			
Fusarium graminearum	2	0.94	0.82	-0.25^{*}	1.00		
Fusarium culmorum	3	0.63	0,80	-0.27^{*}	0.51^{**}	1.00	
DON (ppb)	4	123.66	186.66	-0.25^{*}	0.56^{**}	0.37^{**}	1.00

Remark: *p<0.05; **p<0.01; ^{ns} not significant.

Table 5 also shows a common trend in *Fusarium* infestation being obvious in the Czech Republic (*F. graminearum* has been a dominant species and *F. poae* has become more frequent species there); such trend was described by many authors. *F. graminearum* is considered the main Fusarium agent attacking spikes in dry and warm regions in particular, whereas *F. culmorum* dominates in wet and cooler areas (Parry et al., 1995). *F. graminearum* has become the prevailing *Fusarium* species in the Czech Republic, whereas *F. culmorum* used to be dominant here several years before (Chrpová et al., 2004).

CONCLUSIONS

The degree of infestation with *Fusarium* species was influenced by the year and the environmental conditions in the particular locality. Last but not least, individual varieties proved to be differently resistant to diseases. The tested einkorn varieties were the least affected by *F. culmorum*. Spring spelt wheat varieties were the most resistant to *F. graminearum*. There was not any difference in *F. poae* infestation degree between the tested varieties. Considering the obvious relation between the degree of infestation with *F. culmorum* and *F. graminearum* and the percentage of DON in grain, high attention ought to be paid to this issue. Hulled wheat species may be processed at farms, but the processing must be very careful. As the research results have shown, hulls protect grains to certain, but not to the absolute extent.

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