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OCCURRENCE OF *FUSARIUM* SPP. ON MALTING BARLEY GRAINS IN THE CZECH REPUBLIC DURING 2011–2013

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Abstract

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During 2011–2013, the frequency of occurrence and species spectrum of *Fusarium* fungi occurring on grains of five malting varieties of spring barley (Aksamit, Bojos, Malz, Radegast, and Kangoo) were monitored at two locations (Kroměříž and Žabčice, Czech Republic). The effect of three fungicide treatment variants on *Fusarium* species suppression was also evaluated. During the monitored period, five species were detected: *F. poae, F. culmorum, F. graminearum, F. avenaceum*, and *F. tricinctum.* The most frequently isolated species was *F. poae.* Radegast had the highest frequency of naturally occurring *Fusarium* fungi, while Kangoo was the least infected variety at both locations. The greatest fungicide effectiveness against *Fusarium* spp. occurrence on ears was recorded after the variant with application of Hutton at BBCH 39 and Prosaro 250 EC at BBCH 65. During the monitored years, Žabčice had a higher rate of infection by *Fusarium* fungi. The rates of barley infection by *Fusarium* pathogens differed among individual years, with the highest rate occurring in 2011 (16–17 %) and the lowest rate in 2012 (1–2 %).

Keywords: spring barley, *Hordeum vulgare, Fusarium* spp., varieties, fungicides, Fusarium head blight

INTRODUCTION

Barley is the third most common crop in the Czech Republic and is grown primarily for human food (beer and food products) and animal feeding purposes. Spring barley is grown on ca 250,000 ha, which is 17.5 % of the total area sown to cereals (1,428,171 ha; Ministry of Agriculture, 2015). Grain yield and quality is affected not only by numerous agro-environmental factors (weather, preceding crop, flowering period) but also by pathogens and pests (Mesterházy, 2003; Paul *et al.*, 2005; Šafránková *et al.* 2010; Kumar *et al.*, 2015). Of these latter, the greatest attention over the long term has been dedicated to fungi from the genus *Fusarium*, and in particular species producing mycotoxins (Semaškienė *et al.*, 2006; Kulík, 2008). Seventeen *Fusarium* species are known to have been recorded worldwide to date on barley (Bottalico and Perrone, 2002; Barreto *et al.*, 2004; Stenglein, 2009; Malachová *et al.*, 2013; Gilbert and Tekauz, 2011; Běláková *et al.*, 2014). Of these, *F. graminearum*, *F. culmorum*, *F. poae*, and *F. avenaceum* are among the mycotoxigenic species most frequently seen in Europe, with *F. langsethiae* and *F. tricinctum* being less common (Nicolaisen *et al.*, 2009; Kmoch *et al.*, 2012; Nielsen *et al.*, 2014). Higher occurrence of *Fusarium* pathogens on ears is usually recorded during warm, humid weather. Under advantageous weather conditions, the onset of ear infection is very rapid and the period suitable for effective protection is therefore considerably shortened (Širučková and Kroutil, 2007; Wegulo *et al.*, 2015).

Demethylation inhibitor (DMI) fungicides (i.e. triazols, including the active ingredients metconazole, tebuconazole, and prothioconazole) are currently considered to be the most effective against ear infection by *Fusarium* spp. (Semaškienė *et al.*, 2006; Yu *et al.*, 2011; Wise, 2015). Their effectiveness is influenced by many factors, in particular the variety's resistance; the fungicide's application date, coverage, and application rate; and the pathogen population's aggressiveness (Simpson *et al.*, 2001; Mesterházy, 2003; Hudec and Muchová, 2010).

The objectives of the present study were to determine over three years (2011–2013) the *Fusarium* species spectrum on the grains of malting barley varieties grown in the Czech Republic, to record at the monitored locations potential changes in the proportions of individual species, and to evaluate the sensitivity of selected malting barley varieties to *Fusarium* pathogens and the effectiveness of fungicides on their suppression.

MATERIALS AND METHODS

A small-plot experiment (10 m^2) with five malting varieties of spring barley (Aksamit, Bojos, Kangoo, Malz, and Radegast) was established during 2011–2013 in two different agricultural production areas in the Czech Republic: Žabčice (49°0′43″N, 16°36′8″E), a maize-growing area, and Kroměříž (49°17′56″N, 17°23′35″E), a beet-growing area. Tab. I presents the mean monthly rainfall and mean temperatures during the development periods of Fusarium head blight in barley (May–June) for 2011–2013.

The experiment included three commercially available fungicides (Hutton, Zantara, and Prosaro 250 EC) manufactured by Bayer CropScience AG (Germany) from which three treatment variants were created (Tab. II) and compared with a control variant receiving no treatment. Fungicides were applied at two sets of dates, with the first set being at BBCH 39 (during stem elongation: ligule of last leaf just visible) and BBCH 65 (full flowering) and the second at BBCH 25–30 (full tillering to stem elongation) and BBCH 65. Each variant was always established in three replications.

Evaluation of Frequency of Natural Occurrence of *Fusarium* spp. on Barley Grain from Variants Not Treated with Fungicide-Control

The roll method was used to evaluate barley grains from the five varieties for the natural occurrence of *Fusarium* pathogens. From 200–300 g samples of each variety not treated with fungicide, 100 grains were randomly selected in three replications. Grains with surfaces disinfected (using a 5 % sodium hypochlorite solution) were fixed with starch adhesive between bands of filter paper and rolled. To suppress accompanying microflora, the rolls were placed vertically into a container with a water solution of iprodione (Rovral FLO fungicide, 200 µl/l) to create a continuous flow of the solution to the germinating grains. After 6 days of incubation (21–23 °C, light cycle 12/12 h), the number of grains with *Fusarium* species colonies growing out of individual grains was counted. The results were processed in Statistica 10 using a multifactor analysis of variance (factors: location, year, variety).

I: *Climatic conditions during fungicide application (according to Czech Hydrometeorological Institute, 2015)*

Location	Year	Average temperature [°C]			Average rainfall [mm]		
		May	June	Year	May	June	Year
Žabčice (South Moravian Region)	2011	14.3	18.4	9.5	55	60	442
	2012	15.8	18.6	9.5	33	99	501
	2013	13.6	17.2	9.2	102	121	601
Kroměříž (Zlín Region)	2011	13.1	17.2	8.6	73	117	630
	2012	14.5	17.6	8.7	49	102	723
	2013	13.0	16.5	8.5	101	119	769

II: *Variants of spring barley fungicide treatment (Meier, 1997)*

Treatment variant	Fungicide	BBCH	Active ingredients	Application rate
1. variant	Hutton	39	prothioconazole, spiroxamine, tebuconazole	0.8 $l.ha^{-1}$
	Zantara	65	tebuconazole, bixafen	1.5 $l.ha^{-1}$
2. variant	Hutton	39	prothioconazole, spiroxamine, tebuconazole	0.8 $l.ha^{-1}$
	Prosaro 250 EC	65	prothioconazole, tebuconazole	0.75 $l.ha^{-1}$
3. variant	Hutton	25–30	prothioconazole, spiroxamine, tebuconazole	0.8 $l.ha^{-1}$
	Prosaro 250 EC	65	prothioconazole, tebuconazole	0.75 $l.ha^{-1}$

III: *Primers used to identify Fusarium pathogens*

Target species	Primer name	Nucleotide Sequences (5′–3′)	Citation
Fusarium avenaceum	JIAf JIAr	GCT AAT TCT TAA CTT ACT AGG GGC C CTG TAA TAG GTT ATT TAC ATG GG	Turner et al., 1998
Fusarium culmorum	OPT18F470 OPT18R470	GAT GCC AGA CCA AGA CGA AG GAT GCC AGA CGC ACT AAG AT	Schilling et al., 1996
Fusarium graminearum	Fg16F Fg16R	CTC CGG ATA TGT TGC GTC AA GGT AGG TAT CCG ACA TGG CAA	Nicholson et al., 1998
Fusarium poae	FP82F FP82R	CAA GCA AAC AGG CTC TTC ACC TGT TCC ACC TCA GTG ACA GGT T	Parry, Nicholson, 1996
Fusarium tricinctum	Ftri573f Ftri630r	TTG GTA TGT TGT CAC TGT CTC ACA CTA T TGA CAG AGA TGT TAG CAT GAT GCA	Nicolaisen et al., 2009

Determining *Fusarium* Fungi on Barley Grains

Determining Fusarium spp. Based on Morphological Characteristics

Infected grains from the rolls (see above) were used to determine the *Fusarium* species spectrum on barley grains from the five varieties not treated with fungicide. Infected grains were transferred to a growth medium (potato dextrose agar [PDA] and agar according to Bilaj [Fassatiová, 1979]) in Petri dishes and cultivated for 2–5 days (20 °C, light cycle 12/12 h). Monosporic isolates were prepared from individual samples and used to detect individual *Fusarium* species based on morphological features (size, shape, and number of septa; shape of the macroconidia's apical and basal cells; shape, size, and number of microconidia cells; chlamydospores), aerial mycelium colouration, and pigmentation (obverse, reverse) of the culture grown on the PDA (Leslie and Summerell, 2006). An Olympus BX41 optical microscope and Olympus SZX12 stereo microscope were used for detection.

Determining Fusarium spp. Based on Molecular Characteristics

The correctness of the determination of individual *Fusarium* species was verified using p*olymerase chain reaction (PCR*). Primers for *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, and *F. tricinctum* were used for detection (Tab. III). DNA was isolated from cultures of monosporic *Fusarium* species isolates using a DNeasy Plant Mini Kit (Qiagen). A Taq Core Kit (Qiagen) was used for PCR.

Effect of Fungicides on *Fusarium* Infection

The roll method (see above) was used to evaluate the effectiveness of individual treatment variants in suppressing *Fusarium* pathogens in barley grain from individual varieties. Individual variants of fungicides and application dates were statistically evaluated using a multifactor analysis of variance (factors: location, year, variety, fungicide treatment) in Statistica 10.

RESULTS

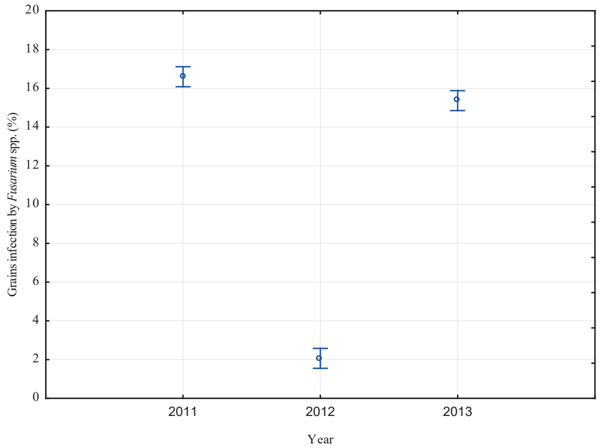
Evaluation of Frequency of Natural Occurrence of *Fusarium* spp. on Barley Grains from Variants Not Treated with Fungicide-Control

Figs. 1–3 present the effects of individual factors (year, location, variety) on the natural occurrence of *Fusarium* spp. on barley grains. Year significantly affected barley's rate of infection by *Fusarium* pathogens (Fig. 1). The highest rate was recorded in 2011, while the barley stands had almost no *Fusarium* infection in 2012. No significant difference was found between Žabčice and Kroměříž in the frequency of naturally occurring *Fusarium* infections of barley varieties (Fig. 2).

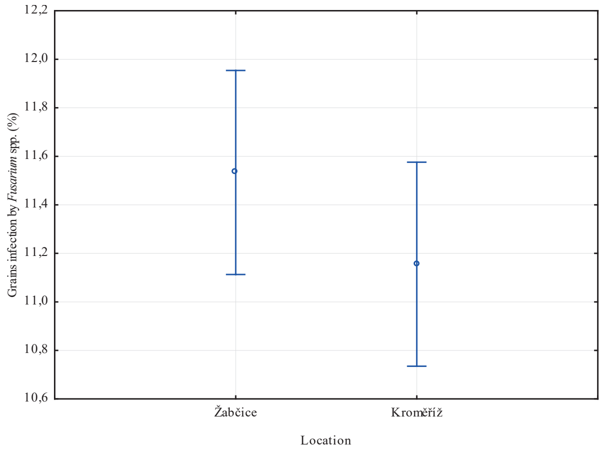
Monitoring the effect of variety on *Fusarium* infection revealed significant differences between Aksamit and Bojos (which did not themselves differ significantly from one another) versus Kangoo, Malz, and Radegast (Fig. 3). During the monitored years (2011–2013), Kangoo had the lowest infection rate of the five malting barley varieties (7–9 %) while Radegast had the highest (16–17 %).

The interaction between variety and year revealed significant differences among individual years (Fig. 4). In all monitored years, Kangoo had the lowest frequency of *Fusarium* occurrence while the highest frequency of occurrence (30 %) was recorded in Radegast only in 2011. In 2012, the average infection rate did not exceed 5 % at both location and individual barley varieties did not differ significantly. In 2013, Kangoo and Malz were the least infected.

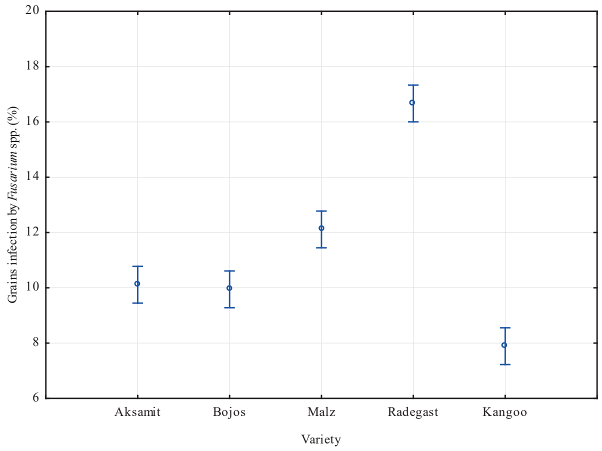
Evaluating statistically the interaction between variety and location revealed very uneven *Fusarium* infection (Fig. 5). At Kroměříž, the infection rate ranged between 9 % and 13 % for all varieties with no significant differences. At Žabčice, infection in Kangoo (4–6 % infection rate) differed significantly from that of all other varieties, while Radegast had the highest infection rate (20–22 %). Infection of Aksamit was almost identical at the two locations (9–11 %).



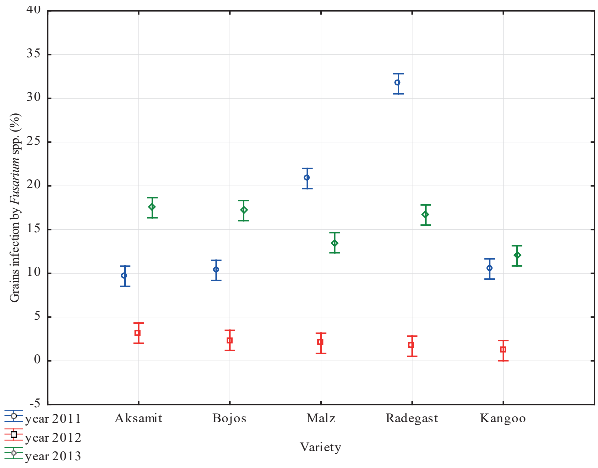
1: *Effect of year on barley grains' rate of infection by Fusarium spp.*
F (2, 60) = 979.51, p = 0.0000, α = 0.05



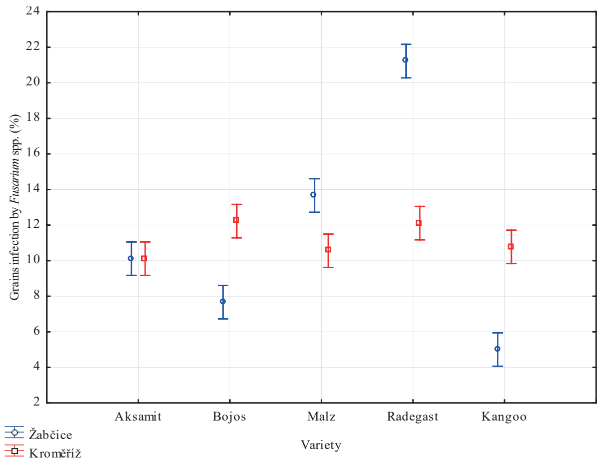
2: *Effect of location on barley grains' rate of infection by Fusarium spp.*
F (1, 60) = 1.6145, p = 0.20876, α = 0.05



3: *Effect of variety on barley grains' rate of infection by Fusarium spp.*
F (4, 60) = 100.31, p = 0.0000, α = 0.05



4: *Effect of interaction between variety and year on barley grains' rate of infection by Fusarium spp.*
F (8, 60) = 99.186, p = 0.0000, α = 0.05



5: *Effect of interaction between variety and location on barley grains' rate of infection by Fusarium spp.*
F (4, 60) = 82.648, p = 0.0000, α = 0.05

Determination of *Fusarium* Fungi on Barley Grains

During 2011–2013, the occurrence of *Fusarium* pathogens on naturally infected grains of malting barley varieties was determined at both locations. A total of five *Fusarium* species were determined: *F. poae, F. culmorum, F. graminearum, F. avenaceum*, and *F. tricinctum* (Tab. IV). During the monitored period, the occurrence and frequency of these species changed both among locations and within individual barley varieties. The most frequently detected species at both locations was *F. poae*, which was recorded in all cases except 2012 at Kroměříž.

In 2011 and 2013, mean infection rate at Žabčice and Kroměříž was 14.4–16.2 % and the *Fusarium* species spectrum included all stated species. In 2012, the naturally infected varieties' rate of infection by *Fusarium* pathogens did not exceed 2.4 % at either location.

Comparing individual species' proportions at the locations revealed the dominant species at Žabčice to be *F. poae* and *F. graminearum* and those at Kroměříž as *F. poae* and *F. culmorum*.

IV: *Frequency of occurrence of Fusarium spp., at Žabčice and Kroměříž, 2011–2013*

Location	Year	Infected (%)	FC (%)	FP (%)	FG (%)	FA (%)	FTRIC (%)
	2011	15.8	0.2	7.6	4.8	2.6	0.6
Žabčice	2012	2.4	0.2	1.8	0	0.4	0
	2013	14.4	2.2	11.0	0.8	0.2	0.2
	2011	15.4	2.0	11.6	1.2	0.4	0.2
Kroměříž	2012	1.4	0.8	0	0	0.6	0
		16.2	4.8	11.4	0	0	0

FC – *F. culmorum*, FP – *F. poae*, FG – *F. graminearum*, FA – *F. avenaceum*, FTRIC – *F. tricinctum*

Determination of *Fusarium* Fungi Spectrum on Barley Grains of Individual Varieties

Evaluating the *Fusarium* species spectrum revealed considerable differences among barley varieties. Only in 2011 all five *Fusarium* species were detected on Malz at Kroměříž, while in all other years only one or two species were detected. *F. poae* was most frequently isolated from Aksamit, Bojos, and Malz grains, while *F. avenaceum*, *F. culmorum*, and *F. graminearum* were recorded only sporadically.

F. tricinctum occurred only exceptionally (always one isolate) on Bojos, Malz, and Radegast, and it is currently not considered a pathogen threatening cultivated barley varieties.

Effect of Fungicides on *Fusarium* Infection

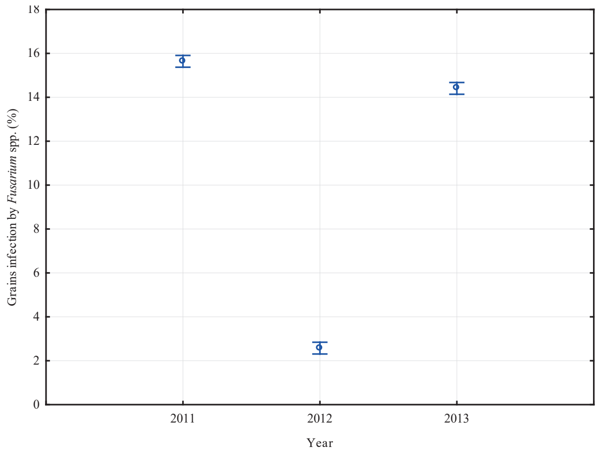
The effects of individual factors on suppression of *Fusarium* spp. are shown in Figs. 6–9. The frequency of occurrence of *Fusarium* pathogens significantly differed among individual years. The year 2012 was generally very dry and grains infection by *Fusarium* fungi was very low, with mean frequency below 4 %. In 2011 and 2013, this reached 14–16 % (Fig. 6). Examining the effect of treatment variant on grains infection by *Fusarium* fungi revealed no significant differences among individual locations during 2011–2013 (Fig. 7). As with the naturally infected (control) barley stands, the highest rate of grains infection by *Fusarium* fungi was recorded for Radegast, while Bojos and Kangoo had the lowest frequencies of occurrence, although this difference was not significant (Fig. 8).

In terms of the effectiveness of individual treatment variants, two variants significantly differed from the control (Fig. 9). The second variant (Hutton, BBCH 39 + Prosaro 250 EC, BBCH 65) was the most effective, followed by the first variant (Hutton, BBCH 39 + Zantara, BBCH 65). Following application of the third variant (Hutton, BBCH 25–30 + Prosaro 250 EC, BBCH 65), barley infection by *Fusarium* fungi slightly increased in comparison to the control.

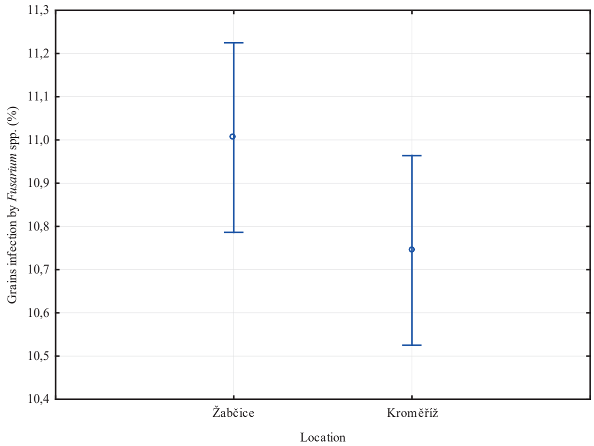
In 2012, climatic conditions unconducive to ear infection and very low grains infection rates prevented drawing conclusions from the results as to treatment variants' actual effectiveness. In 2011 and 2013, the first (Hutton + Zantara) and second (Hutton + Prosaro 250 EC) treatment variants applied at the first set of treatment dates (BBCH 39 and 69) had positive effects (Fig. 10).

The effectiveness of individual fungicide treatment variants differed between the two climatically different locations. At Žabčice, infection by *Fusarium* fungi significantly decreased after application of the first treatment variant, while the second variant was effective at Kroměříž (Fig. 11).

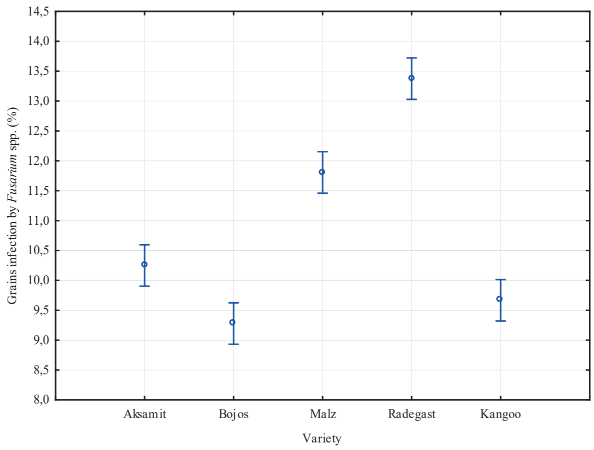
Treatment variants' effectiveness in suppressing *Fusarium* fungi on individual barley varieties varied widely (Fig. 12). For Aksamit, the effect of the first and second treatment variants suppressed *Fusarium* fungi. Following application of the third treatment variant (Hutton at BBCH 25–30 + Prosaro 250 EC at BBCH 65), infection increased from 10 % to ca 14.5 % in comparison with the control. Bojos responded positively in suppressing *Fusarium* fungi following application of the third treatment variant. For Malz, fungicide application had almost no effect on suppression or increase of *Fusarium* fungi in comparison with the control. Of the five barley varieties, rate of infection by *Fusarium* fungi significantly decreased (in comparison with the control) following application of all three treatment variants only for Radegast. For Kangoo, application of the second fungicide variant decreased infection by *Fusarium* spp., although this difference was not significant. In contrast, grains infection increased in comparison with the control for the two other treatment variants.



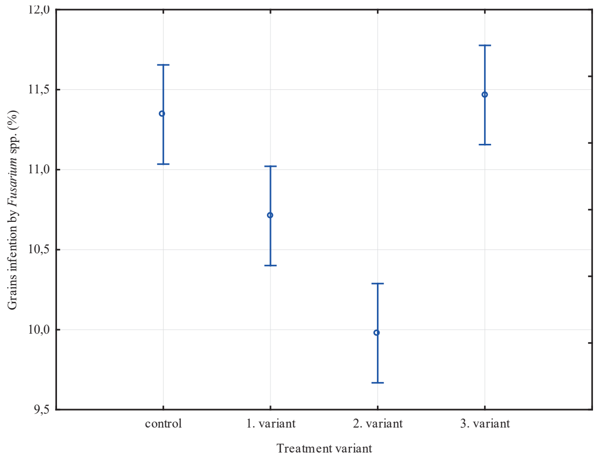
6: *Effect of year on barley grains' rate of infection by Fusarium spp. after fungicide treatment*
$F (2, 240) = 2800.1, p = 0.0000, \alpha = 0.05$



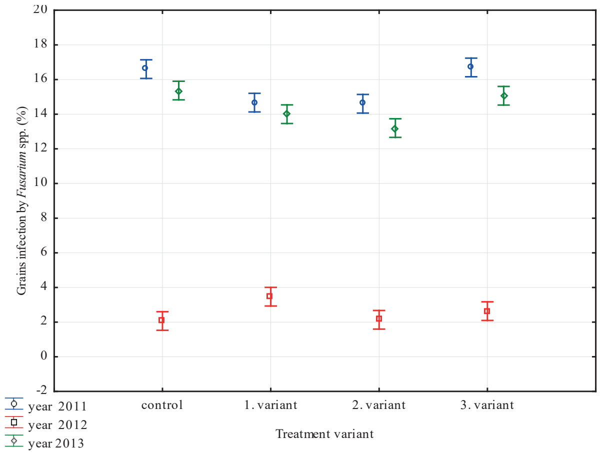
7: *Effect of location on barley grains' rate of infection by Fusarium spp. after fungicide treatment*
$F (1, 240) = 2.7509, p = 0.09850, \alpha = 0.05$



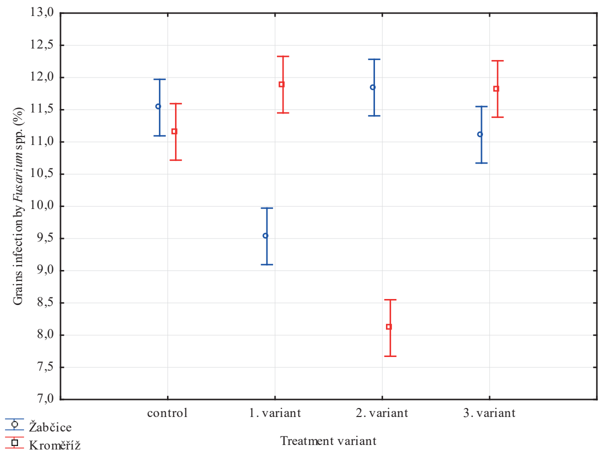
8: *Effect of variety on barley grains' rate of infection by Fusarium spp. after fungicide treatment*
$F (4, 240) = 92.945, p = 0.0000, \alpha = 0.05$



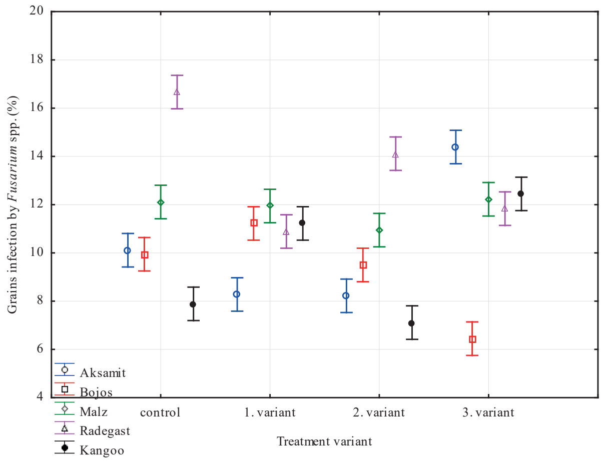
9: *Effect of treatment on barley grains' rate of infection by Fusarium spp.*
F (3, 240) = 18.861, p = 0.00000, α = 0.05
1. variant – Hutton, BBCH 39 + Zantara, BBCH 65
2. variant – Hutton, BBCH 39 + Prosaro 250 EC, BBCH 65
3. variant – Hutton, BBCH 25–30 + Prosaro 250 EC, BBCH 65



10: *Effect of interaction between treatment and year on barley grains' rate of infection by Fusarium spp.*
F (6, 240) = 9.1856, p = 0.00000, α = 0.05



11: *Effect of interaction between treatment and location on barley grains' rate of infection by Fusarium spp.*
F (3, 240) = 66.784, p = 0.0000, α = 0.05



12: *Effect of interaction between treatment and variety on barley grains' rate of infection by Fusarium spp.*
F (12, 240) = 47.902, p = 0.0000, α = 0.05

DISCUSSION

Monitoring the occurrence of individual *Fusarium* species on barley ears in the Czech Republic indicated that the number of *Fusarium* species remained (more or less) unchanged, although the proportions of individual species did change. Čača (1986) and Šrobárová and Vašková (1987) had studied the occurrence of *Fusarium* spp. on wheat at the turn of the 1970s to the 1980s but not *Fusarium* pathogens on barley. The most frequently recorded species had been *F. culmorum*, followed by *F. graminearum*, *F. oxysporum*, *F. avenaceum*, *F. nivale*, *F. moniliforme*, and *F. poae*.

During 1997–1998, Hýsek *et al.* (1999) found seven *Fusarium* species (*F. culmorum*, *F. poae*, *F. avenaceum*, *F. stilboides*, *F. merismoides*, *F. aquaeductum*, and *F. gigas*) on 360 spring barley samples at Kroměříž and Kojetín. As in previous years, the species most frequently present was *F. culmorum* (in more than 70 % of samples). Of the other species, *F. poae* had a high proportion (<20 % of samples) while the proportions of other species were only minimal (*F. avenaceum* > 2 %).

Polišenská *et al.* (2009) studied the occurrence of *Fusarium* spp. on wheat and barley in 2008. Wheat grains most frequently contained *F. graminearum* and *F. culmorum* with sporadic *F. poae*, although this latter species was dominant on barley grains. *F. poae* was confirmed as the dominant species on barley grains in the Czech Republic by Polišenská *et al.* (2010), Kmoch *et al.* (2012), Matušinský *et al.* (2013), and Běláková *et al.* (2014).

Our results correspond with these studies and confirm *F. poae* to be the dominant species in place of the former *F. culmorum* on barley grains during 2011–2013 at Žabčice and Kroměříž. In 2012, most barley stands in the Czech Republic were damaged as a result of drought, high temperatures, and only weak local precipitation during the flowering period in the southern parts of the country (Kopřivová, 2012). This damage was accompanied by very low frequency of occurrence and a narrow spectrum of *Fusarium* spp. (*F. culmorum*, *F. poae*, and *F. avenaceum*) on ears at both locations. *F. poae* presents a threat not only in infecting and damaging cereals but also in producing the mycotoxins T-2 toxin and nivalenol, which are more toxic to humans and animals than is deoxynivalenol (Desjardins, 2006).

Similarly in other parts of Europe, *Fusarium* species most frequently isolated from cereals were *F. graminearum* and *F. culmorum* (Bottalico and Perrone, 2002; Mesterházy, 2003). Stenglein (2009) stated that in Europe (England, Ireland, Germany, Hungary, Poland, Austria, Slovakia, Switzerland, Wales) and the Americas (Canada, Argentina) *F. poae* was among the pathogens most frequently isolated from cereals. The dominance of *F. poae* also has been confirmed by Gilbert and Tekauz (2011), who detected *F. poae* (56.6 %), *F. avenaceum* (17 %), *F. sporotrichioides* (15 %), *F. equiseti* (4 %), *F. graminearum* (2 %), and other *Fusarium* pathogens (3.2 %) in barley stands in Canada (Saskatchewan) during 2003–2007.

Of all tested treatment variants, the second (Hutton, BBCH 39 and Prosaro 250 EC, BBCH 65) was the most effective. The fungicides used in this variant contained active ingredients from the triazoles (tebuconazole) and triazolinthiones (prothioconazole), which recently have been confirmed as among the most effective against *Fusarium* pathogens on cereal ears (Jones, 2000; Wise, 2015). The present study confirms that other factors important for fungicides to have a positive effect against *Fusarium* spp. include suitable application date, temperature, and humidity (Simpson *et al.*, 2001; Hudec and Muchová, 2010). Within the climatic conditions of the Czech Republic, fungicide application during stem elongation and full flowering appeared to be effective.

CONCLUSION

During 2011–2013 in the Czech Republic, five *Fusarium* species were detected and the most frequently isolated cause of Fusarium head blight was *F. poae*. The variety Kangoo had the greatest resistance to *Fusarium* spp. and Radegast the lowest. Suppression of *Fusarium* pathogens from barley grains was the most effective following application of the second fungicide variant (Hutton and Prosaro 250 EC). The key factors for suppressing *Fusarium* pathogens are determining the proper application date and selecting the fungicides' active ingredients.

Acknowledgements

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