Effect of Fusarium Head Blight on Mycotoxin Content in Grain of Spring Barley Cultivars

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Abstract: The results are based on field infection experiments with six spring barley cultivars registered in the Czech Republic and resistance sources Chevron and CI 4196. One of the four *Fusarium* isolates used for inoculations was a predominant nivalenol producer, while the other isolates were deoxynivalenol (DON) producers. Out of the other mycotoxins 3-AcDON was found in grain at a relatively higher concentration. Significant cultivar differences in DON content, examined yield traits, percentage of *Fusarium* colonies and percentage of non-germinating seeds were detected after inoculum spraying on two dates and mist irrigation of infected plots. When inoculated with aggressive isolate of *F. culmorum* Chevron and CI 4196 cvs. showed high resistance and the cultivars Jersey, Olbram and Scarlett moderate resistance to DON accumulation in grain. Treatment with fungicide Horizon 250 EW (active ingredient tebuconazole) led on average to a 52.5% reduction of DON content, but the efficacy of fungicide treatment was highly influenced by year and cultivar. Fungicide treatment did not have a significant effect on grain weight per spike and, in general, the influence of infection on examined yield traits was low in these experiments. DON content was closely related only with the parameter C_T Fus (transformed) from quantitative real time PCR analysis. Using the developed PCR system it was possible to specify clearly cultivar responses to infection and effects of fungicide treatment on DON content.

Keywords: spring barley; *Fusarium culmorum*; *Fusarium graminearum;* head blight; mycotoxin content; yield traits; cultivar resistance; fungicide treatment; quantitative PCR analysis

Since the beginning of the twentieth century Fusarium head blight (FHB) of barley has been recognised as a devastating disease in periods of epidemics. Significant losses to barley producers were reported in several states of the USA (Jones & Mirocha 1999) and Canada (Tekauz *et al.* 2000; Campbell *et al.* 2000). This pathogen has reduced both the yield and the quality of barley and has raised food safety problems due to the contamination of grain by mycotoxins. FHB significantly affects the malting and brewing quality of barley grain. Beer gushing is perhaps the most insidious problem caused by *Fusarium* (Steffenson 1998).

In some regions the species *Fusarium graminearum* was found to be the principal pathogen of barley heads (Salas *et al*. 1999). Besides *Fusarium culmorum* this toxigenic pathogen now becomes more important also in conditions of Central and Northern Europe (Sýkorová *et al*. 2003; Waalwijk *et al*. 2003). Both species were reported to be prevalent producers of deoxynivalenol (DON), but also the ability to produce nivalenol (NIV) or acetyldeoxynivalenol (3-AcDON or 15-AcDON) has been recognised in some isolates of both *F. culmorum* and *F. graminearum* (Mirocha *et al*. 1994; Perkowski *et al*. 1997; Salas *et al.* 1999; Chelkowski *et al*. 2000).

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Four primary strategies for the control of FHB include biological, agricultural, chemical, and genetic methods (Steffenson 1999). Development of a biological agent to control FHB effectively (suppress the pathogen) is undoubtedly a difficult task. Agricultural practices that rely on a reduction of the amount of *Fusarium* inoculum residing in crop debris cannot guarantee sufficient protection either. Fungicides based on tebuconazole and metconazole were reported to suppress FHB and accumulation of mycotoxins (Magan *et al.* 2002), however, the protection by these products is not generally sufficient yet either in barley or in wheat (Jones 2000; Mesterházy *et al.* 2003; Šíp *et al.* 2004). The deployment of barley cultivars with genetic resistance is the most cost effective and environmentally sound way of controlling FHB. It is important that valuable sources of resistance to FHB and DON accumulation were detected when evaluating barley germplasm. Particularly Chevron cv., a six rowed, nonmalting barley originating from Switzerland, CI 4196 (a landrace from China) and Svanhals (a landrace from Sweden) exhibited low levels of FHB and DON under epidemic conditions of North Dakota (Steffenson 1999). Chelkowski *et al.* (2000) detected the line MP7 from a doubled haploid set of lines with the lowest mycotoxin accumulation and reduction in yield traits. A survey of different resistance sources and results of breeding barley for FHB resistance was given by Rudd *et al.* (2001). Accelerated FHB resistance breeding programs that use the detected resistance sources have been established in different parts of the world including Europe. The introduction of high resistance into modern barley cultivars has not been successful yet, but new moderately resistant materials have been developed within these programs. Greater problems than in wheat may arise in barley in field resistance tests because cultivar resistance is not so easy to identify properly with the use of simply determined parameters (Jones & Mirocha 1999). Particularly the assessment of disease amount according to the number of blighted kernels is complicated in barley due to frequent misidentifications of FHB lesions in grain samples (Prokinová 1999; Salas *et al.* 1999). However, molecular markers have already been developed and it can be expected that in near future marker assisted selection will facilitate the transfer of resistance into adapted germplasm. Furthermore, the analyses on a molecular level, able to determine the quantity of pathogen DNA, are expected to contribute significantly to better identification of resistance level and understanding of factors that influence FHB, which is important for eliminating the risk of mycotoxin contamination of grains and foodstuffs (Nicholson *et al.* 2003).

The objective of our present study was to examine spikes infected with different isolates of *F. culmorum* and *F. graminearum* for mycotoxin content and analyze variations in different traits in artificially inoculated spring barley genotypes.

MATERIAL AND METHODS

Plant material. Material for this study comprised eight spring barley cultivars with different levels of resistance. The line CI 4196 (two-rowed landrace from China) and Chevron cultivar (six-rowed landrace from Switzerland) were included as the most resistant materials to both FHB and DON accumulation in grain (Steffenson 1999). The other six cultivars Jersey, Scarlett, Olbram, Akcent, Tolar and Kompakt, registered in the Czech Republic (Bulletin of the Central Institute for Supervising and Testing in Agriculture, Brno, 2003) and widely used in practice, are two-rowed spring barleys that were found (unpublished data) highly variable in FHB resistance and DON content in previous experiments. All these cultivars are medium early (Olbram, Scarlett and Kompakt) or medium late (Jersey, Akcent and Tolar) malting barleys. Cultivar differences in heading date did not exceed five days.

Description of field experiments. This study is based on two types of experiments that included eight spring barley materials. In the first experimental series carried out at the locality Prague-Ruzyně for two years (2002–2003) 30 spikes were inoculated in each cultivar at the stage of full flowering by brushing 2 ml of conidial suspension (5×10^6 in l ml) of the fungus (Chelkowski *et al.* 2000). The following isolates were used for inoculation: (1) isolate A of *F. culmorum* (CZ); (2) isolate B (Stupice) of *F. culmorum* (CZ); (3) isolate 821 of *F. graminearum* (PL), (4) isolate 608 of *F. graminearum* (CZ). The isolates were selected on the basis of previous experiments with wheat (Šíp *et al.* 2003) in which data were obtained on their aggressiveness and chemotype. After inoculations spikes were covered with polythene bags for 48 hours.

In the second experimental series at two localities (Prague-Ruzyně and Kroměříž) and in two years

(2002–2003) cultivars were grown on 2.5m^2 plots in three replications of four variants: (1) Infection variant (I); (2) Infection variant treated with fungicide (IF); (3) Control uninfected variant (C); (4) uninfected variant treated with fungicide (CF). Infection and control variants were grown in two separate blocks isolated by five meters wide wheat stand that was kept free from diseases by protective chemicals. Highly pathogenic isolate (B) of *Fusarium culmorum* (Šíp *et al.* 2002a) was used for inoculation. The spore mixture (0.8×10^7 per ml) was applied with hand sprayer directly onto the whole plot on two dates: at full flowering (> 50% of flowering spikes) and one week later. Fungal infection was promoted by mist irrigation of plots (applied in all variants). In both years and in both localities the fungicide Horizon 250 EW (active ingredient Tebuconazole; supplier Bayer, Aktivengesellschaft, Leverkusen, Germany) was applied in IF and CF variants following the manufacturer's instructions. Inoculation with *Fusarium* conidia suspension followed in IF variant after 24 hours, when the positive occurrence of fungicide in plant tissue was assured.

Determination of FHB resistance traits. In the first experimental series both the inoculated spikes and the control uninfected spikes were analysed for number of grains per spike (GNS), thousand grain weight (TGW) and grain weight per spike (GWS). Determination of resistance traits in the second series of experiments was based on seed samples obtained in each plot from randomly selected 50 spikes. In all variants the traits grains per spike, thousand grain weight and grain weight per spike were determined and tolerance to the infection was expressed as percentage reduction (R) in these traits in relation to uninfected, control variant C. In both series of experiments spikes were threshed at a low wind not to lose light infected scabby kernels.

The analysis of the extent of infection penetration into grain was based on a procedure described by Amelung (1996), determining the percentage of *Fusarium* colonies and percentage of non-germinating seeds from samples that contained 100 randomly selected seeds.

Chemical analyses. In the first experimental series seed samples of each cultivar obtained after inoculations with four isolates were analysed for the content of trichothecene mycotoxins and zearalenone using either GC/ECD and clean-up on MycoSep 225 column (Institute of Chemical Technology, Department of Food Chemistry and Analysis, Prague) or ELISA (Research Institute of Crop Production, Prague-Ruzyně; Sýkorová *et al.* 2003). Determination of DON content by ELISA in the first and second experimental series was based on RIDASCREEN® FAST DON kits from R-Biopharm GmbH, Darmstadt, Germany. A representative sample was ground and thoroughly mixed. After that 5 g of ground sample was shaken with 100 ml of distilled water and filtered. 50 µl of the filtrate was used for the test. Samples and standards were applied according to the manufacturer's instructions. Absorption of final solution was measured at 450 nm, using SUNRISE spectrophotometer. RIDAWIN® software was used for data processing.

DNA isolation. DNAs were isolated using DNeasy Plant Mini Kit (QIAGEN) from mycelia and from infected/non infected plant tissues. DNA concentrations and qualities were checked electrophoretically and spectrophotometrically in Gene Quant Pro instrument.

Taq Man and primers for real time PCR. The PCR primers and TaqMan probe used for the real time PCR were designed using Primer Express software (Applied Biosystems). The input sequences were obtained by sequencing of amplified DNA regions flanked with species specific primers (Doohan *et al*. 1998). The TaqMan probe was labelled at the 5′-end with the FAM reporter dye and at the 3′-end with the MGB quencher. Newly designed primer set and TaqMan probe were used to amplify and detect a 92-bp long DNA fragment.

Real time quantitative PCR. Real time PCR assay consisted of 50µl PCR mixture containing 12.5µl of TaqMan universal PCR master mix (Applied Biosystems) composed of 5mM MgCl2, ROX as an internal reference, 200 µM each dATP, dCTP and dGTP, 400 µM dUTP, 1U AmpliTaq Gold DNA polymerase and 1U AmpErase uracil-N-glycosylase. The concentrations of primers were respectively 200nM and 300nM. Program consisting of 2 min at 50°C 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C was used. Amplifications and detections were performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems).

Post-PCR data analysis was performed using the Sequence Detector Software (Applied Biosystems). The threshold cycle (CT) indicates the fraction cycle number at which the amount of amplified target reaches a fixed threshold. CT value for *Fusarium*

target amplicons (CT Fus) was transformed in the following way:

$$CT\ Fus\ transf = 107 * 2\text{-}CT\ Fus$$

Statistical analyses. The UNISTAT 5.0 package (UNISTAT Ltd., London W9 3DY, UK) was used for statistical analyses of the data.

RESULTS AND DISCUSSION

Mycotoxin assays on inoculated spikes

The results of GC analyses of mycotoxin spectra in grains of spikes inoculated by the "brushing" method (Table 1) showed prevalent production of the mycotoxin DON in three out of four isolates (range 1.537 to 4.784 mg/kg), similarly like in experiments with winter wheat (Šíp *et al*. 2003). Isolate 608 of *F. graminearum* was found a predominant NIV producer (0.475 mg/kg). The isolates of *F. culmorum* (particularly isolate A) produced relatively higher quantities of DON and isolate 821 of *F. graminearum*, which showed the highest aggressiveness in experiments with wheat, caused below-average accumulation of DON in barley genotypes. From the other thrichothecene mycotoxins 3-AcDON had a relatively higher content (range 0.049 to 0.478 mg/kg), particularly after infection with *F. culmorum* isolates. The examined isolates also produced small quantities of 15-AcDON (range 0.007 to 0.043 mg/kg) and zearalenone (ZEA) (range 0.003 to 0.053 mg/kg). The content 0.053 mg/kg of the highly deleterious mycotoxin ZEA was detected after inoculation with isolate B of *F. culmorum*. No mycotoxins were detected in the control samples.

There is a sufficient evidence of DON presence in infected barley grains, but the production of other toxins was found highly dependent on the *Fusarium* species and isolate used (Perkowski *et al*. 1997; Salas *et al.* 1999; Chelkowski *et al*. 2000). Therefore the studies on isolate chemotypes are highly desirable.

The results of DON determination by the ELISA method were compared with the results of gas chromatography and highly significant and positive correlations were obtained for all four isolates (Table 1). Comparative testing by GC method showed a relatively higher average DON content with the use of ELISA method, which could be explained by the high specificity of used antibodies and consequently by high recovery (106%) of ELISA (Reference Material No. 379 – Deoxynivalenol in wheat – medium level; Commission of the European Communities, Community Bureau of Reference BCR No. 00462). Besides, by immunochemical methods it was not possible to completely exclude the cross reactivity with some other trichothecene substances (present at very low quantities). As shown in Table 1, coefficients of correlation between DON content determined by ELISA method and contents of other "more important" toxins (NIV and 3-AcDON) were, however, significant only sporadically, which indicates that specific studies

Table 1. Average values (mg/kg) of trichothecene mycotoxins and zearalenone in grain of 8 cultivars inoculated with four *Fusarium* isolates (F.c. – *F. culmorum*; F.g. – *F. graminearum*) and coefficients of correlation between DON-ELISA and toxins determined by GC

Isolate	F.c. – A	F.c. – B	F.g. – 821	F.g. – 608
DON-ELISA	6.337	3.775	3.165	0.478
DON-GC	6.157	3.348	1.978	0.086
NIV	0.037	0.026	0.037	0.475
3-AcDON	0.478	0.290	0.196	0.049
15-AcDON	0.043	0.028	0.013	0.009
ZEA	0.004	0.053	0.003	0.003
DON-ELISA/DON-GC	**0.94**	**0.88**	**0.99**	**0.77**
DON-ELISA/3-AcDON	**0.91**	0.57	0.95	0.05
DON-ELISA/NIV	0.01	0.35	0.68	0.54

Pearson's correlation coefficients significant at $P = 0.01$ are in bold letters

Table 2. ANOVA mean squares for different traits in two series of experiments

Inoculation	Source of variation	df	DON content (mg/kg)	Grain number/ spike reduction	Thousand grain wt. reduction	Grain wt./spike reduction	% of *Fusarium* colonies	% of non-germinating seeds
"Spraying" technique	cultivar	7	577***	327***	938***	2040***	670***	3030***
	year/locality (YL)	3	1253***	488***	6147***	10877***	14234***	6234***
	treatment (I/IF)	1	1997***	56*	127*	46	2327***	2262***
	cultivar × YL	17	158**	784***	770***	2755***	763***	1106***
	cultivar × treatment	7	381***	54***	88***	238***	172	317*
	YL × treatment	3	187*	299***	1143**	2699***	1598***	401*
	error	81	63	11	14	44	154	132
"Brushing" technique	cultivar	7	8	93*	104**	233*		
	year	1	78***	144	283**	722**		
	isolate	3	33**	85	95**	152		
	cultivar × year	7	6	125*	86**	238*		
	cultivar × isolate	21	4	42	21	83		
	year × isolate	3	35***	20	6	21		
	error	21	4	36	19	68		

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

on the accumulation of different mycotoxins are needed with respect to the isolate used.

Analyses of variance for DON content and examined disease severity traits

Analyses of variance with the aim to detect cultivar, year (locality), fungicide treatment and interaction effects on DON content and examined disease severity traits were performed in both experimental series. The results are given in Table 2. It is necessary to mention that "the brushing" series of experiments (series 1) with the use of isolate B of *F. culmorum* resulted in a relatively lower average DON content determined by ELISA (3.78 mg/kg) than experiments 2 with the spraying of inoculum onto the whole plot followed by mist irrigation of infected plants (14.14 mg/kg). In series 1, with the lower disease incidence, significant cultivar differences were not detected, but year, isolate and year × isolate interactions explained highly significant effects on DON accumulation. In the experiments with inoculum spraying (2) cultivars significantly differed in DON accumulation, however, the effects of years/localities and fungicide treatment were relatively higher. The interactions between these factors were also highly significant. Similar findings were obtained by ANOVAs for the traits percentage of *Fusarium* colonies and percentage of non-germinating seeds. The effect of environment (year/locality) was particularly high in the trait percentage of *Fusarium* colonies.

It is clear from Table 3 that the examined traits of grain yield showed on average very small differences between infected and control variants. It can be deduced from comparisons with the other examined parameters (particularly DON content) that the infection with *Fusarium culmorum* affected mainly grain quality in these experiments. ANOVAs for reductions of yield traits showed significant cultivar and environmental effects, but the effects of fungicide treatment, isolate used and specific interactions were not clear. There were not found any significant effects of fungus isolate and fungicide treatment on the reduction of grain weight per spike.

The detection of lower contents of DON with the use of "brushing techniques" in barley was

Table 3. Trait means in variants I (infection), IF (infection and fungicide), C (control, uninfected) and CF (control and fungicide) and comparison of I and IF for single traits

Variant	DON (mg/kg)	% of Fusarium colonies	% of non-sprouting grains	Grain number/ spike	Thousand grain wt. (g)	Grain wt./ spike (g)
I	14.14	43.68	39.46	23.24	46.33	1.07
IF	6.71	29.28	30.43	24.13	48.09	1.13
C	0.31	9.25	5.58	23.65	47.14	1.13
CF	0.21	6.58	1.25	24.33	48.50	1.18
% reduction (increase*)^	52.5	33.0	22.90	3.7*	3.7*	5.4
I-2002	14.61	48.32	22.87	23.09	45.65	1.05
IF-2002	9.75	29.77	9.05	23.28	46.80	1.07
% reduction (increase*)^ – 2002	33.2	38.4	60.4	0.8*	2.5*	1.9
I-2003	13.67	35.07	39.93	23.39	47.01	1.08
IF-2003	3.67	20.63	30.7	24.98	49.38	1.19
% reduction (increase*)^ – 2003	73.2	41.1	23.1	4.8*	6.3*	9.5*

^100 – IF/I (I/IF*) × 100

analogous to single floret inoculation experiments with wheat (Šíp *et al.* 2003). Experiments using the spraying (atomising) of inoculum onto the entire spike surface evidently created more favourable conditions for the disease development in mist-irrigated plots and enabled to detect cultivar differences in the examined traits. Spraying systems or grain spawn methods are widely used for FHB resistance screening in barley nurseries (Rudd *et al*. 2001).

Effects of genotype and fungicide treatment on different traits

Genotypic differences in DON content between cultivars in infection variants are shown in Figure 1. High resistance to DON accumulation was detected in Chevron and CI 4196, but the cultivars Jersey, Olbram and Scarlett could also be included in the first "resistant" group. Similar genotypic classification like for DON was obtained for the percentage of *Fusarium* colonies ($r = 0.74$; $P < 0.01$). In this trait Chevron and CI 4196, with 4.9% and 17.1%, were clearly separated from the other cultivars (which showed the range between 32% and 53%). Classification of genotypes in the other traits (percentage of non-germinating seeds and yield traits) was not related to classification according to DON content.

It follows from Tables 2 and 3 that fungicide treatment had a significant effect on the reduction of DON content in grain and also on the percentage of *Fusarium* colonies and percentage of non-germinating seeds. Mean fungicide efficacy for DON content was 52.5%, which indicates that prospects for the chemical control of FHB in barley are limited (Jones 2000) like in wheat (Mesterházy *et al*. 2003). Similarly conducted experiments with winter wheat (Šíp *et al.* 2004) showed on average 49% reduction of DON content for the years 2002 and 2003 as a result of fungicide treatment. However, both in wheat and barley there were large fluctuations in fungicide efficacy depending on the year. While in 2002 the efficacy reached 33.2% in barley, in 2003 it exceeded 70%. When compared with the other years (the period of 1992–2003 – Šíp *et al*. 2002b, 2004), the year 2003 was characterised by short disease development as a consequence of high temperatures following inoculation at water availability on mist-irrigated plots. The shortness of fungicide action could contribute to higher effectiveness in comparison with 2002. Fungicide treatment led to 33% decrease in the percentage of *Fusarium* colonies and 23% decrease in the per-

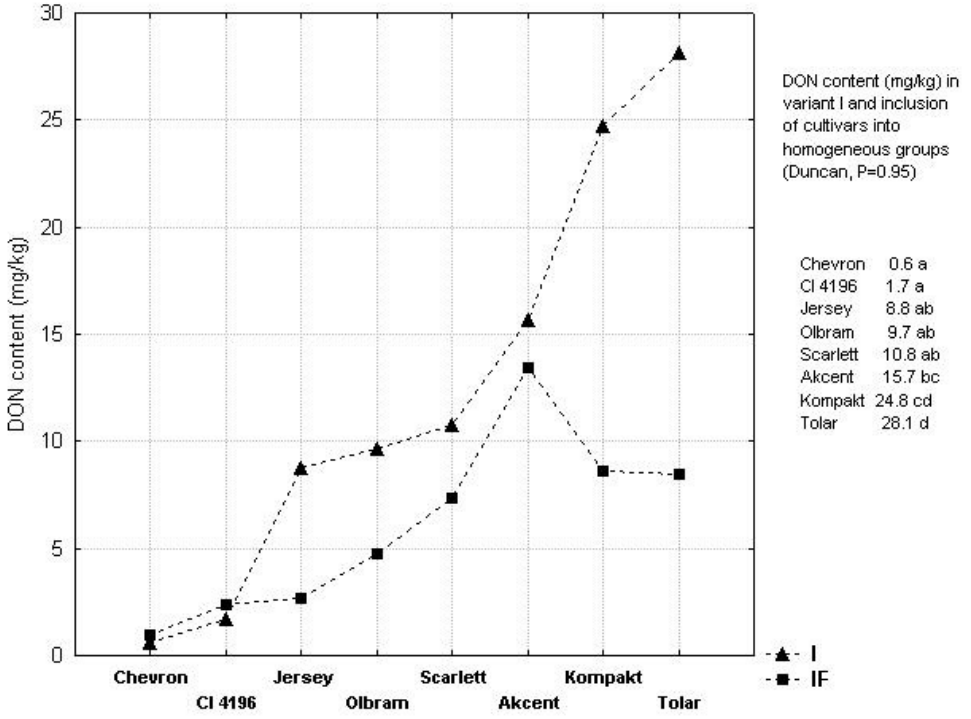

Figure 1. Average DON content (from two years and two localities) of 8 spring barley cultivars in variants I (infection) and IF (infection and fungicide) and results of multiple comparisons

centage of non-germinating seeds. Low (5%) was the effect of fungicide treatment on an increase in grain yield per spike, but it is necessary to take into consideration that in these experiments the infection with *Fusarium culmorum* had on average a low effect on yield traits (Table 3). The same grain yield per spike was detected in variants IF (infection and fungicide) and C (control variant). On the contrary, in 2002 and 2003 experiments "wheat" experiments showed 33% efficacy of fungicide treatment for grain weight per spike (Šíp *et al.* 2004).

As shown in Figure 1, the fungicide treatment was not identically effective in all cultivars. While in susceptible cultivars Kompakt and Tolar the

Table 4. Phenotypic coefficients of correlation between examined traits in 2002 (below diagonal) and 2003 (above diagonal)

	DON	FUC	NGS	GNS-R	TGW-R	GWS-R
C_T	0.92***	0.32	0.25	0.32	0.48**	0.42*
DON	–	0.38*	0.29	0.33	0.50**	0.43*
FUC	0.50**	–	0.68***	–0.34	–0.26	–0.33
NGS	0.04	0.82***	–	–0.27	–0.32	–0.16
GNS-R	–0.07	–0.30	–0.12	–-	0.81***	0.95***
TGW-R	–0.07	0.00	–0.02	0.33	–	0.95***
GWS-R	–0.15	–0.22	–0.14	0.84***	0.78***	–

C_T = C_T Fus transformed, DON = DON content, FUC = percentage of *Fusarium* colonies, NGS = percentage of non-germinating seeds, GNS-R = reduction of grain number per spike, TGW-R = reduction of thousand grain weight, GWS-R = reduction of grain weight per spike

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

application of fungicide led to 60–70% reduction of DON content, practically ineffective was this treatment in cultivar Akcent, which was also documented by the real time PCR analyses (Figure 2). In this cultivar the fungicide treatment evidently did not result in a substantial reduction of the disease (pathogen DNA). Fungicide treatment was ineffective in this cultivar also in 2001 (data not presented here). The explanation of this specific reaction was not found in these experiments because this cultivar, registered in the Czech Republic, was always treated similarly like the other cultivars. To explain different effects of fungicides on cultivars and *Fusarium* species, specific detailed studies of host × pathogen interactions on a molecular level are evidently needed (Doohan *et al.*, 1999; Nicholson *et al.* 2003). However, it follows from the obtained confounding results that in agricultural practice only "double protection" consisting in growing resistant cultivars and fungicide treatment may guarantee sufficient protection (Mielke & Weinert 1996), particularly in "endangered crops" (when maize is the preceding crop and with application of reduced tillage practices).

Significance of different traits for depiction of FHB infection harmfulness

In these experiments DON content in grain could be considered as decisive for the classification of disease severity and genotype and fungicide effects because the influence on yield traits was low. However, deleterious effects of *Fusarium* infection on barley yield cannot be neglected, as reported from the USA and Canada (Jones & Mirocha 1999; Tekauz *et al*. 2000).

In both years the low DON content was associated with low percentage of *Fusarium* colonies in examined grains and in 2003 also with lower reductions of thousand grain weight and grain weight per spike (Table 4). The value of the percentage of non-germinating seeds could not been justified from these aspects by these experiments because relations with this trait were insignificant (except for relations with % of *Fusarium* colonies).

These results are supported by the findings of Jones and Mirocha (1999), who found that the DON concentration in barley could not be effectively estimated by yield traits, visual index or by grain discolorations. We also tried to determine the grain infection score visually in these experiments, but due to grain discolorations observed also in control variants and in materials resistant to FHB, this trait could not be used as an indicator of FHB disease. Ample simultaneous discolorations due to the occurrence of *Bipolaris sorokiniana* and *Alternaria* spp. as well as *Cladosporium* or *Epicoccum* spp. were observed, similarly like in experiments of Prokinová (1999) and Salas *et al.* (1999). The visual scoring of symptoms (used without problems e.g. in wheat) could not be performed in barley because of the disease predominant "latency". Sig-

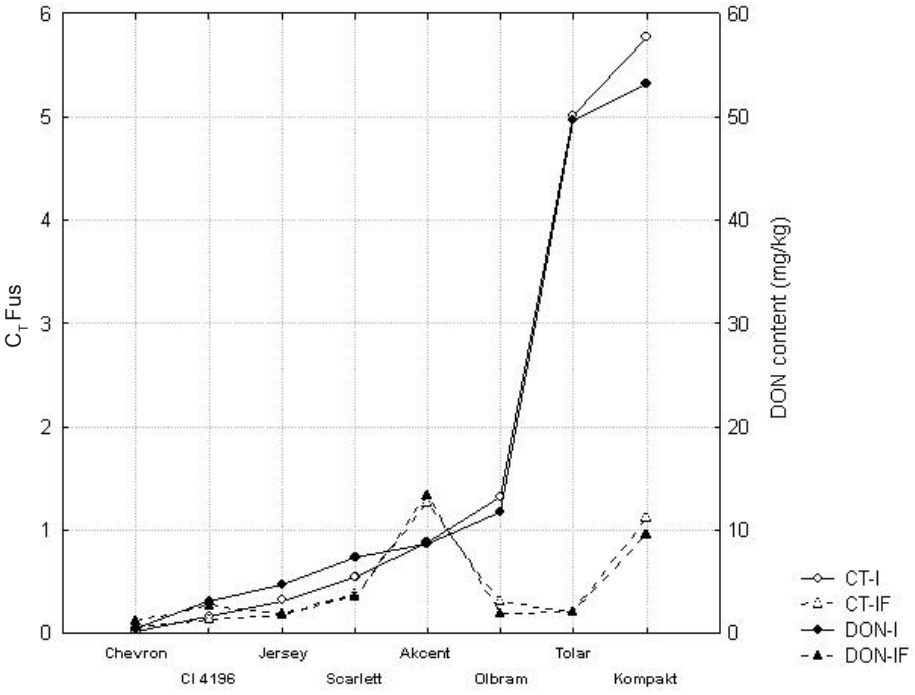

Figure 2. Correspondence between DON content in the grain of 8 barley cultivars and C_T Fus transformed (real time PCR) after infection with *Fusarium culmorum* (I) and fungicide treatment (IF) in 2003

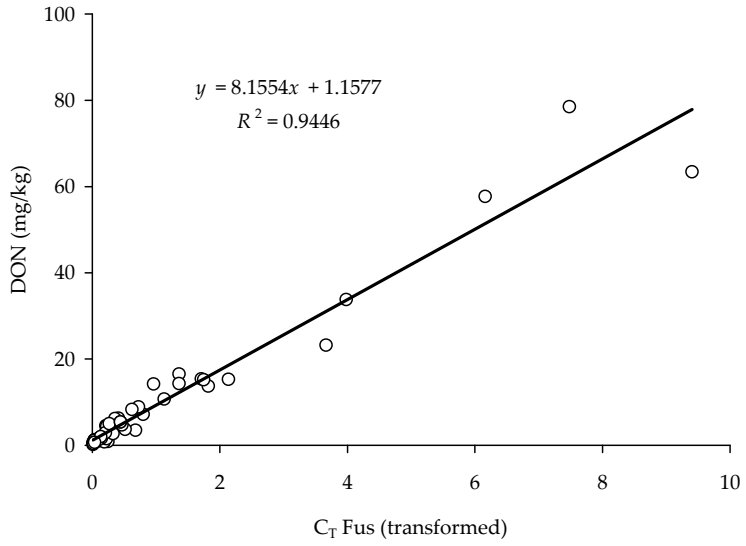

Figure 3. Relation between DON content and C_T Fus (transformed) obtained from real time PCR analysis across all tested samples

nificant relationships between mycotoxin content and different traits were observed (Adamski *et al.* 1999; Chelkowski *et al.* 2000), but it is evident that in general the observations of yield traits or visual assessments of the disease cannot replace direct determinations of mycotoxin content in barley.

Possible application of real time PCR assays for quantification of FHB causal agents in barley

Due to the fact that FHB causes very miscellaneous and often unclear symptoms of infection in barley, it was suggested that PCR analysis could be particularly helpful for quantifying FHB and for evaluating the efficacy of the fungicides. The method based on real time PCR was developed in RICP Prague-Ruzyně and the results concerning 2003 experiments with barley are presented here.

A close relationship was found between the detection of DON by ELISA and DNA content of the pathogen in analysed samples estimated by C_T Fus transformed ($r = 0.92$, $P < 0.001$) (Figure 3). Besides DON, C_T correlated significantly positively with reductions of thousand grain weight and grain weight per spike (Table 4). Cultivar differences in the effects of fungicide treatment on a reduction of DON content in grain were clearly described by C_T values as well (Figure 2).

The obtained results indicate that C_T can give reliable evidence of both the cultivar resistance to DON accumulation and FHB and the efficacy of fungicide treatment. It is very important that DON accumulation in grain was found closely related to the pathogen presence in infected grain. Therefore, from these aspects PCR analysis is expected to provide higher resolution than any other trait. Furthermore, it is possible to detect and quantify different metabolite (toxin) profiles by this technique. As shown, the analysis also allowed to discriminate fungicide efficacy that was not apparent from the examined yield traits. Doohan *et al.* (1999) reported that in wheat only PCR analysis enabled to determine fungicide effects on a reduction of colonisation of tissues by *F. culmorum* and *F. poae*. Great advantage of this technique lies in the fact that it enables to identify the causal organism and therefore it is superior to analyses measuring infection effects. It can be expected that molecular assays could replace the examinations of many characters that are due to the complicated character of the disease needed in practice and breeding for evaluating genotype resistance and efficacy of treatments. As described by Nicholson *et al.* (2003), the PCR offers a sensitive and potentially specific instrument to detect, identify and quantify different species present in plant tissues as well as to differentiate between chemotypes within a single species. A robust, multiplex PCR was developed in the Netherlands (Waalwijk *et al.* 2003), which enabled to screen a large series of isolates and detect major changes in *Fusarium* spp. on wheat. Different uses of competitive and real time PCR assays were lately reviewed by Nicholson *et al.* (2003). These authors gave evidence about the usefulness of these assays to increase our understanding of factors that influence FHB and

ability to control the disease. In barley, where the assessment of disease according to symptoms and other measurements is even more complicated than in wheat, these assays may significantly help to increase the effectiveness of disease control in this crop both by genetic instruments and by chemicals.

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Abstrakt

Šíp V., Tvarůžek L., Chrpová J., Sýkorová S. , Leišová L., Kučera L., Ovesná J. (2004): **Vliv stupně napadení fuzariózou klasu na obsah mykotoxinů v zrnu u ječmene**. Czech J. Genet. Plant Breed., **40**: 91–101.

Uskutečnili jsme polní infekční pokusy se 6 odrůdami ječmene jarního registrovanými v České republice a se zdroji rezistence Chevron a CI 4196. U jednoho ze čtyř izolátů používaných pro inokulace převládala produkce mykotoxinu nivalenol, zatímco ostatní izoláty byly producenty deoxynivalenolu (DON). Z dalších mykotoxinů se vyskytoval v relativně vyšší koncentraci 3-AcDON. Statisticky významné rozdíly mezi odrůdami v obsahu DON, ve sledovaných výnosových znacích, v procentu napadených zrn a v procentu neklíčivých zrn byly zjištěny v pokusech s plošnou infekcí postřikem ve dvou termínech na parcelách s mlhovou závlahou. Po aplikaci agresivního izolátu *F. culmorum* vykazovaly odrůdy Chevron a CI 4196 vysokou rezistenci k akumulaci DON v zrnu a odrůdy Jersey, Olbram a Scarlet mírnou rezistenci. Ošetření fungicidem Horizon 250 EW (účinná látka tebuconazole) vedlo v průměru k 52,5% redukci obsahu DON v zrnu, avšak efektivita fungicidního ošetření byla silně ovlivněna ročníkem a odrůdou. Fungicidní ošetření nemělo významný vliv na hmotnost zrna na klas. Redukce sledovaných výnosových znaků vlivem infekce byly v průměru nízké. Obsah DON byl v úzkém vztahu pouze s transformovanými hodnotami C_T Fus získanými pomocí kvantitativní PCR analýzy. Pomocí vytvořeného PCR systému bylo možné zřetelně specifikovat reakci odrůd na infekci a vliv fungicidního ošetření na obsah DON.

Klíčová slova: jarní ječmen; *Fusarium culmorum*; *Fusarium graminearum;* fuzarióza klasu; obsah mykotoxinů; výnosové znaky; rezistence odrůd; fungicidní ošetření; kvantitativní PCR analýza

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