doi: 10.17221/708/2015-PSE

Changes in the content of various *Fusarium* mycotoxins forms in germinating winter wheat and spring barley kernels

K. Pazderů[1], Z. Vepříková[2], I. Capouchová[1], P. Konvalina[3], E. Prokinová[1], D. Janovská[4], A. Škeříková[1], H. Honsová[1]

[1]*Department of Crop Production, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Institute of Chemical Technology, Prague, Czech Republic*
[3]*University of South Bohemia, České Budějovice, Czech Republic*
[4]*Crop Research Institute, Prague, Czech Republic*

ABSTRACT

Fusarium mycotoxins are frequent contaminants in cereals at temperate zone. Next to deoxynivalenol (DON), there are other masked forms of DON, deoxynivalenol-3-glucoside (D3G) or 3(15)-acetyl-deoxynivalenol (3(15)-ADON), but changes among these forms are still insufficiently known. All these forms were evaluated in germinating kernels of wheat and malting barley from deliberated inoculated field plots. Results showed possible occurrence of high initial content of all evaluated DON forms. Total contents of all forms were detected as very steady from start to end of the germination process. It suggests high importance of evaluating not only DON content, but all DON forms simultaneously.

Keywords: *Triticum aestivum*; *Hordeum vulgare*; food chain; contamination; toxic metabolite

Cereals produced in climatic conditions of temperate zone can be frequently contaminated with mycotoxins, secondary toxic metabolites produced by the microscopic fungi of *Fusarium* genus (Zachariášová et al. 2014). The contamination of cereals kernels is greatly influenced by weather conditions, especially by the sum of precipitation and course of temperatures, risk factors for the formation and development of infection (Champeil et al. 2004).

Mycotoxins in cereals are a very serious problem in food chain; despite high temperature, physical and chemical stability, and even conditions at processing technology it is not possible to fully reduce their content (Kostelanská et al. 2011). The most frequent *Fusarium* mycotoxins are trichotecenes, especially deoxynivalenol (DON), considered as marker mycotoxin, indicating the level of *F. culmorum* and *F. graminearum* contamination (Tutelyan 2004).

Maximum limit for DON contamination is 200 µg/kg in processed cereal products for baby food, 1250 µg/kg in non-processed wheat and barley grain, and even 1750 µg/kg for non-processed durum wheat, oat and maize (EU regulation No. 1126/2007).

Next to DON, there are other conjugated masked forms of DON present in cereals, for example deoxynivalenol-3-glucoside (D3G) or 3(15)-acetyl-deoxynivalenol (3(15)-ADON) and others, detected in cereal products (Lancová et al. 2008, Juan et al. 2012). Vestraete (2009) specifies this problem as priority for monitoring. Conjugated forms can be potentially toxic for animals and humans (Berthiller et al. 2011), because they are split in digestive tract from conjugated forms to original DON.

Conjugated forms arise from DON as detoxification products decreasing toxicity of DON in plants (Berthiller et al. 2005) or they are intermediate products on DON biosynthetic pathway in *Fusarium* (McCormick et al. 2011), as in case of ADONs. It is evident that testing of primary produced DON in

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QI111B154.

doi: 10.17221/708/2015-PSE

plants is insufficient for evaluation of total content of *Fusarium* mycotoxins, both in non-processed raw material and processed products. It is very important to collect more data from this area.

MATERIAL AND METHODS

In this experiment 3 winter wheat (cv. Bohemia) and 2 spring malt barley (cv. Malz) lots were used, from deliberated *Fusarium* inoculation at early flowering stage (61 BBCH). The isolates of *F. culmorum* and *F. graminearum* were obtained from the mycological collection of the Crop Research Institute in Prague. All materials were harvested at maturation stage from small-scale field plots at Experimental and Research Station of Department of Crop Production, Czech University of Life Sciences, Prague. Kernels were treated before germination with fungicide to suppress subsequent *Fusarium* growth on seeds.

Seeds were germinated 1–7 days in plastic boxes, 3 replications per 50 g of cereals, moistened with 50 g of water on the start of germination. Whole samples (replications) were homogenized and used for extraction of mycotoxins. Samples were extracted to 200 mL of deionized water 60 min on shaker at 240 rpm. DON, D3G and 3(15)-ADONs were detected via the U-HPLC-HRMS (Aquity UPLC, Waters; Exactive Orbitrap MS, Thermo Fisher Scientific) from 1 mL of extracted micro-filtered samples (Pazderů et al. 2013).

The obtained results were statistically evaluated by the general linear model (GLM ANOVA) method in the SAS package (version 9.3., Carry, USA) at a significance level $\alpha = 0.05$. Differences between means were evaluated by the Tukey's *HSD* (honestly significant difference) test.

RESULTS AND DISCUSSION

The contents of DON, D3G and 3(15)-ADONs in germinating wheat and barley kernels were evaluated. The observed data confirmed high ability of

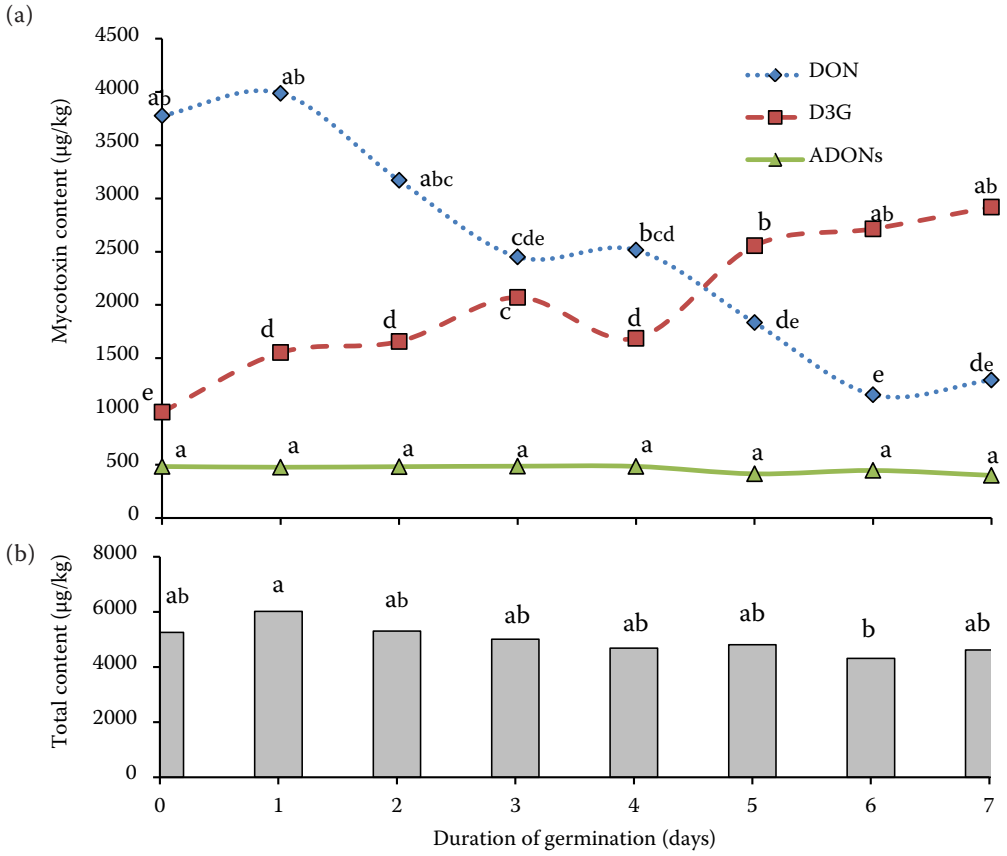

Figure 1. Changes in the content of deoxynivalenol (DON) forms in winter wheat seeds (a). Total mycotoxin content is sum of all three mycotoxins in each germination day (b). Letters of statistical significance correspond to $\alpha = 0.05$. D3G – deoxynivalenol-3-glucoside; 3(15)-ADON – 3(15)-acetyl-deoxynivalenol

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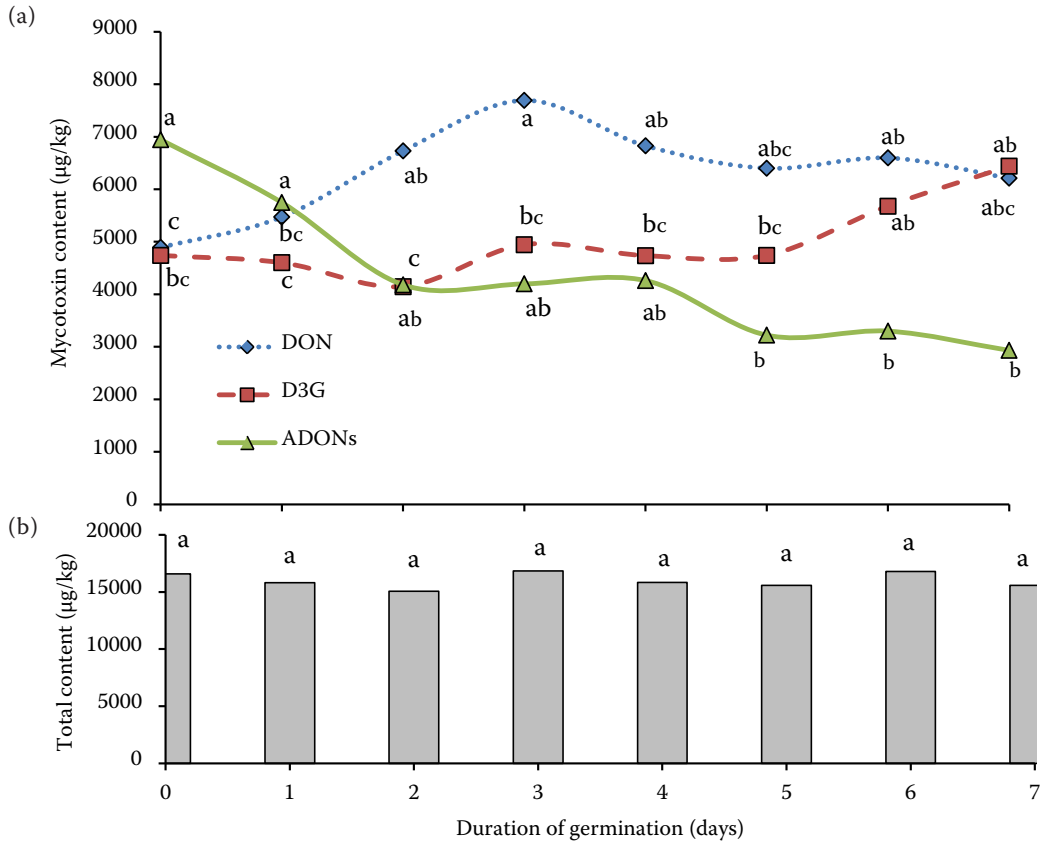

Figure 2. Changes in the content of deoxynivalenol (DON) forms in spring barley seeds (a). Total mycotoxin content is sum of all three mycotoxins in each germination day (b). Letters of statistical significance correspond to α = 0.05. D3G – deoxynivalenol-3-glucoside; 3(15)-ADON – 3(15)-acetyl-deoxynivalenol

seeds to solve their intoxication by DON thanks to inner regulation mechanism. Similarly, Maul et al. (2012) confirmed the ability of living seeds to transform DON to D3G. Lancová et al. (2008) detected similar transformations of *Fusarium* mycotoxins in barley from field to malt and beer.

Next to DON, both other tested mycotoxins D3G and 3(15)-ADONs were identified in all tested samples. Changes of all three evaluated DON forms during germination are demonstrated in Figures 1a and 2a. Initial levels were different in case of both cereal species, higher in malting barley (Figure 2b, more than 16 000 µg/kg of all three forms together in sum) than in wheat kernels (Figure 1b, about 5 000 µg/kg of all three forms). The content of ADONs in barley was even higher than DON content (Figure 2a) on the start of the germination test. In figures there is a visible increase of D3G in germinating kernels of both crops from start to end of the germination, statistically significant in case of wheat (293% increase of initial level, Table 1). D3G increase in barley kernels was only 136% in comparison with initial level of D3G, Table 2). DON content during germination decreased in wheat kernels (to 34% of initial level) and increased in case of barley kernels (127% of initial level), simultaneously with a decrease of ADONs in barley (42% of initial level).

Maul et al. (2012) determined 30% reduction of DON in steeping process. Berthiller et al. (2005) detected 1:3 ratio of D3G to DON in wheat kernels. From our results, it was concluded that the ratio could be higher in harvested kernels, even 1:1 (Figure 2a, barley). In germination process, when seeds try to survive, transformed D3G can increase more, even to 3:1 ratio (Figure 1a, wheat). D3G levels depend on ability of cereal genotype transform DON to D3G, as confirmed Faltusová et al. (2015) from comparison of susceptible and more resistant cultivars. Plant breeders consider this ability as a key trait for resistance of genotypes to *Fusarium* in breeding (Shin et al. 2012). However,

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Table 1. Shares of tested mycotoxins from start to end of wheat kernels germination

Mycotoxin	Day of germination							
	0*	1	2	3	4	5	6	7
DON (µg/kg)	3778 ± 281	3988 ± 85	3170 ± 301	2452 ± 729	2515 ± 253	1835 ± 245	1158 ± 149	1295 ± 404
(%)	100	106	84	65	67	49	31	34
D3G (µg/kg)	995 ± 28	1553 ± 48	1657 ± 55	2071 ± 21	1686 ± 40	2557 ± 112	2714 ± 31	2920 ± 223
(%)	100	156	166	208	169	257	273	293
ADONs (µg/kg)	483 ± 8	477 ± 21	48 2± 26	486 ± 19	484 ± 56	414 ± 11	446 ± 25	400 ± 23
(%)	100	99	100	101	100	86	92	83

*shares are expressed as percentage to original content of detected mycotoxins forms. DON – deoxynivalenol; D3G – deoxynivalenol-3-glucoside; 3(15)-ADON – 3(15)-acetyl-deoxynivalenol

breeders measure only DON content and prefer such genotypes that have low amount of DON. The contents of masked forms are still hidden.

The study confirms that total amounts of all evaluated forms of DON were the same at the start of the germination in comparison with levels at the end of the germination process. Kernels were able to slightly reduce total mycotoxins content, but possibly all tested mycotoxins were only transformed to other, non-evaluated forms of DON.

The increase of DON in case of barley probably resulted from transformation of ADONs. We can agree with McCormick et al. (2011), that ADONs present in kernels are probably intermediate products in DON biosynthesis pathway. They can be transformed into non-toxic D3G together with DON molecules in germination process (Figure 2a, barley), respectively in living plants. In our research, high level of ADONs in harvested kernels (Figure 2a) documents inactivation of DON biosynthesis pathway in time of maturation drying of seeds on mother plant. Similarly, all other metabolites in maturated seeds are only inactivated thanks to their low amount of water.

The detected total amount of all evaluated DON forms before and after germination test suggests the idea of very high stability of these mycotoxins in time. As Maresca (2013) noted, all masked forms can be risky for humans and for animals. The transformation of toxic DON in germinating seeds enables plants to survive. Yet, the increase of D3G can be potentially dangerous for animals and humans consuming products from germinated cereals.

Table 2. Shares of tested mycotoxins from start to end of barley kernels germination

Mycotoxin	Day of germination							
	0*	1	2	3	4	5	6	7
DON (µg/kg)	4892 ± 296	5471 ± 471	6730 ± 578	7692 ± 242	6828 ± 594	6400 ± 472	6597 ± 22	6211 ± 309
(%)	100	112	138	157	140	131	135	127
D3G (µg/kg)	4740 ± 139	4602 ± 203	4147 ± 390	4946 ± 220	4736 ± 342	4741 ± 50	5679 ± 243	6441 ± 268
(%)	100	97	87	104	100	100	120	136
ADONs (µg/kg)	6943 ± 274	5747 ± 523	4181 ± 674	4198 ± 674	4261 ± 159	3222 ± 176	3299 ± 112	2932 ± 272
(%)	100	83	60	60	61	46	48	42

*shares are expressed as percentage to original content of detected mycotoxins forms. DON – deoxynivalenol; D3G – deoxynivalenol-3-glucoside; 3(15)-ADON – 3(15)-acetyl-deoxynivalenol

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Received on November 16, 2015
Accepted on January 3, 2016

Corresponding author:

Ing. Kateřina Pazderů, Ph.D., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Katedra rostlinné výroby, Kamýcká 129, 165 21 Praha 6-Suchdol, Česká republika
e-mail: pazderu@af.czu.cz