CONFIRMING THE RELATIONSHIP BETWEEN INFESTATION OF WINTER WHEAT WITH *TILLETIA CARIES* AND THE QUANTITY OF *FUSARIUM* MYCOTOXINS IN HARVESTED GRAIN*

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The objective of the study was to confirm or to disprove the connection between infestation of winter wheat with *Tilletia caries* and the quantity of *Fusarium* toxins in the harvested grain. From 2007 to 2009, small-plot experiments were conducted in Kroměříž. A range of winter wheat varieties were sown, with 26 varieties tested in 2007, 35 varieties tested in the year 2008. In the year 2009 46 winter wheat varieties *T. caries*-inoculated plus 30 varieties without the *T. caries* inoculation were tested. The set of varieties included those with varied levels of susceptibility to infestation with *Tilletia caries* and Fusarium head blight (FHB). The percentage of spikes infested with blight was evaluated symptomatically. The percentage of grains infected with *Fusarium* spp. was found out using the wet chamber method. The Ridascreen Fast DON immunochemical kit was used in determining the deoxynivalenol (DON) content, the Ridascreen Fast Zearalenon was used for determining zearalenone (ZEA) and the T-2 toxin level was determined using the Ridascreen Fast T-2 toxin (R-Biopharm, Germany). Three-year results showed that there was no correlation between infestation of winter wheat with *T. caries* and *Fusarium* spp. (the coefficient of 0.11648). There was no evidence of correlation between blight infection and the DON content (the coefficient of 0.227845), ZEA content (the coefficient of 0.32315) or T-2 (the coefficient of 0.342288). As expected, a close direct relationship was confirmed between the frequency of grains infected with *Fusarium* spp. and the DON content (the coefficient of 0.71648) and the ZEA content (the coefficient of 0.674679). Any connection between the infestation with *Fusarium* spp. and the content of T-2 toxin (the coefficient of –0.15401) was not confirmed. However, there was a statistically significant difference in the content of DON and ZEA at different rates of blight infection, between the groups with *T. caries* infestation rate 0–5% and over 25%.

winter wheat; *Tilletia caries*; Fusarium head blight; mycotoxins

INTRODUCTION

The wheat smut disease caused by the fungi *Tilletia caries* (syn. *T. tritici*), *T. controversa* and *T. laevis* (syn. *T. foetida*) is widespread in many countries; nonetheless, the disease occurs in most years with less frequency and low impact on the yield rate, with qualitative losses being rather higher, since the fungi produce a substance of strong smell, trimethylamine. Common bunt is still an important disease of winter wheat. The control of this disease has not been yet fully resolved despite considerable knowledge, with potential calamitous occurrence in some years. The infestation of the plant occurs during germination, with chiefly temperature and snow cover in the period following sprouting being responsible for any further development of infection, as suggested by J. A. Faris as early as in 1924 and recently confirmed in 2009 (Liatukas, Ruzgas, 2009). In the infected spike, bunt balls filled with spores of the pathogen are formed instead of grain. Production of any toxin is not known. Pathogens of the *Tilletia* genus have been studied from many aspects. Any details about a possible relationship to other fungi are very rare (e.g. Willingale, Mantle, 1987).

Fusarium head blight is one of global importance and frequently occurring disease in wheat or cereals in general. Infestations with this disease lead to both quantitative and qualitative losses. Toxins produced by *Fusarium* fungi – Fusarium head blight agents – contaminate cereal grains in varying degrees (Štočková et al., 2008). Such contamination is undesirable, both in food grade and feed grade wheat (Čonková et al., 2003; Creppy, 2002). Infestation of grains by *Fusarium* fungi also significantly worsens the quality of seeds (Hershmann, 2009). A number of countries including the CR therefore currently apply the content of deoxynivalenol (DON) as one of the criteria when purchasing food grade wheat, as the

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former is regarded as a marker of *Fusarium* toxins. As a result, the growers' aim is to achieve a minimum infestation of spike with *Fusarium* fungi.

It is well-known fact that *Fusarium* spp. is among facultative pathogens, which are usually more likely to infect stressed plants. The preconditions of the emergence and spread of infection and factors that influence the process are dealt with a number of studies. Virtually all those available address the rate of impact of abiotic factors, especially the importance of temperature and humidity (Brennan et al., 2005; Saharan et al., 2004), in addition to the importance of preceding crops (Schaafsma et al., 2005) and the effect of various fungicidal compounds (Menniti et al., 2003). Some works mention the importance of primary infestation of ears by pests for the subsequent infection caused by *Fusarium* fungi (Bagga, Kaur, 2004). The number of papers studying the resistance of wheat to spike infection is also high (Šíp et al., 2010). Less attention has been paid to interactions among microfungi – spike disease agents. Those studying the importance of representation and relations amongst the individual species – Fusarium head blight agents – from the mycotoxin production aspect included Xu et al. (2007).

When addressing smut disease in winter wheat and FHB, the occurrence of *Fusarium* spp. spores was found out both on and inside the *Tilletia* spp. balls. (Similarly, abundant colonisation of corn smut galls by *Fusarium* fungi is also frequent. Indicative comparison of the content of *Fusarium* toxins in corn ears contaminated by *Ustilago maydis* and ears without smuts showed a significantly higher content of mycotoxins in ears infested; see Table 1 – Prokinová, Štočková, 2006, unpublished.) This led to the hypothesis about the possibility of the importance of primary infection of wheat by obligate biotrophic pathogen *T. caries* as a biotic stressor, which makes the plant prone to infestations by facultative pathogens *Fusarium* spp.

The objective of this paper is to confirm or disprove the connection between infestation of winter wheat with *Tilletia caries* and the quantity of *Fusarium* toxins in harvested grain.

Table 1. DON content in smut-infected and healthy corn ears

Description	DON (mg/kg)
Healthy ears, Sample 1	< LOQ
Infested ears, Sample 1	2.1
Healthy ears, Sample 2	< LOQ
Infested ears, Sample 2	2.6

MATERIAL AND METHODS

Small-plot experiments were underway on the land of Agrotest fyto Ltd. in Kroměříž from 2007 to 2009. A range of winter wheat varieties were sown, with 26 varieties tested in 2007, 35 varieties tested in the year one and 46 winter wheat varieties, *T. caries* inoculation option, plus 30 varieties, without the *T. caries* inoculation option, tested in 2009. The set of varieties included those with varied levels of susceptibility to infestation by with *Tilletia caries* and Fusarium head blight. In 2007 and 2008, only seed artificially inoculated with spores of *T. caries* was used, dose of 2 g of spores per 1 kg of seed. In 2009, there were two general options: #1 – sowing healthy seed, #2 – sowing seeds artificially inoculated with spores of *T. caries*, with 4 repetitions per option. In 2007 and 2008, the level of *Fusarium* mycotoxins was rated only on the basis of natural infection. Because natural conditions do not warrant spike infection each year, artificial inoculation was conducted in 2009 on all plots using a mixture of *F. graminearum* and *F. culmorum* spores in a 1:1 ratio. The isolates used originated from the collection of microscopic fungi kept by the Mycology Department of the Research Institute of Crop Production in Prague-Ruzyně, the density used was 10^6 of spores per 1 ml of suspension. The inoculum was applied by spraying in three different periods according to the flowering time of specific winter wheat varieties, each time at the beginning of the flowering phase. The percentage of spikes infested with *T. caries* was evaluated symptomatically, with 4 x 200 spikes rated per option, immediately before harvesting. The percentage of grains infected with *Fusarium* spp. was found using the wet chamber method, with 4 x 100 grains rated per option. Mycotoxin content in grain was determined by the following procedure: each sample was ground by the IKA A 11 laboratory mill. The Ridascreen Fast DON immunochemical kit was used in determining the deoxynivalenol (DON) content, the Ridascreen Fast Zearalenon was used for determining zearalenone (ZEA) and the T-2 toxin level was determined using the Ridascreen Fast T-2 toxin (R-Biopharm, Germany). The determination procedure was conducted under the protocol prescribed by the specific kit producer. To determine DON, the sample is extracted by twenty fold volume of distilled water and the filtrate directly used for determination. For the determination of ZEA and T-2 toxin, the sample is extracted by quintuple volume of 70% methanol, with samples diluted with 1:1 distilled water before applying onto the titration plate, making the final concentration of methanol in the sample prepared for determination to be 35%. The rate above must be upheld in any further dilution of the sample, unless the content of ZEA and/or T-2 toxin exceeds 400 ppb, i.e. dilution is done using 35% methanol., instead of distilled water. Toxin yield in each method of determination indicated above was calculated through analysis of reference materials for DON and zearalenone, and using wheat meal spike for T-2 toxin. LOQ values: 0.2 $mg.kg^{-1}$ for DON and 50 $\mu g.kg^{-1}$ for ZEA as well as T-2.

RESULTS

In 2007, the seed was only inoculated using *T. caries* spores naturally infected with *Fusarium* spp., with the corresponding low content of mycotoxins determined (Table 2). Of the tested varieties, the lowest proportion of blight infection was found in Karolinum (0%), while the

Table 2. 2007 year results

Variety	% of spikes with *Tilletia* infection	% of grain colonized with *Fusarium* spp.	DON ($mg.kg^{-1}$)	ZEA ($\mu g.kg^{-1}$)	T-2 ($\mu g.kg^{-1}$)
AKTEUR	22.61	6	0.490	< LOQ (1.69)	190.05
BANQUET	24.25	4.5	< LOQ (0.188)	<LOQ (8.06)	110.75
BARROKO	5.35	2	< LOQ (0.020)	< LOQ (5.67)	< LOQ (44.75)
BATIS	26.68	5.25	< LOQ (0.108)	< LOQ (4.42)	150.70
BILL	14.2	0	0.299	< LOQ (11.18)	129.85
BISCAY	33.1	7.25	0.580	< LOQ (3.81)	156.62
CAPHORN	21.4	2	< LOQ (0.046)	< LOQ (2.25)	60.75
CLARUS	22.51	3.5	< LOQ (0.117)	< LOQ (7.17)	89.57
CLEVER	23.31	6.75	< LOQ (0.095)	< LOQ (17.22)	92.84
COMPLETE	9.46	2.5	< LOQ (0.099)	< LOQ (14.88)	< LOQ (39.45)
CUBUS	33.39	8.25	< LOQ (0.076)	< LOQ (4.32)	< LOQ (41.10)
DARWIN	12.6	5.5	< LOQ (0.047)	< LOQ (36.50)	66.91
DRIFTER	45.82	10	0.893	< LOQ (12.02)	334.46
EBI	31.23	9.25	0.693	< LOQ (23.62)	87.39
HEDVIKA	27.83	8.5	< LOQ (0.027)	< LOQ (6.31)	< LOQ (48.54)
HERALDO	17.15	5.25	0.585	< LOQ (16.43)	70.43
ILIAS	31.77	8	0.295	257.29	109.99
KAROLINUM	0	1	< LOQ (0.035)	< LOQ (28.76)	< LOQ (41.49)
LUDWIG	34.3	7.5	< LOQ (0.113)	< LOQ (9.44)	89.71
MERITTO	24.86	9	< LOQ (0.150)	< LOQ (28.82)	113.64
NIAGARA	26.47	8.25	0.363	< LOQ (27.18)	195.78
RAPSODIA	30.05	6.5	0.541	< LOQ (17.36)	82.55
RHEIA	31.33	7.25	< LOQ (0.064)	< LOQ (17.36)	45.78
SIMILA	39.8	4.75	0.237	< LOQ (9.44)	64.45
SULAMIT	31.56	5	0.312	< LOQ (19.26)	121.91
TREND	25.98	5.75	< LOQ (0.142)	< LOQ (15.30)	114.96

highest level was determined in Drifter (45.82%), with the values of remaining 24 varieties found within the range indicated above. In addition, Drifter was the variety with the highest DON content (0.893 $mg.kg^{-1}$) found, while the minimum content was found in Barroko (0.02 $mg.kg^{-1}$). In the latter case, this is a statistically insignificant value, with calculation refined using ELISA analysis evaluating software. ZEA content values for all varieties occurred under the LOQ value, with the calculation completed using software as well to allow for a certain level of comparison of the varieties, with the exception of Ilias, the ZEA content of which was 257.29 $\mu g.kg^{-1}$. For T-2 toxin, the maximum value (334.46 $\mu g.kg^{-1}$) was detected in Drifter, with the content below the LOQ found in five varieties. The other 20 varieties ranged from 60.75 to 334.46 $\mu g.kg^{-1}$ as indicated above.

In 2008, *T. caries* did not infest Alibaba and Globus at all, while Ilias was the most infected variety. In addition, the *Fusarium* toxin content in the grain was relatively low in this year as a result of low natural infection with *Fusarium* spp. Nonetheless, zero DON content was found even in the most infected variety, Ilias, compared to 2007. On the other hand, of all the varieties, Ilias was one with the highest content of zearalenone. DON content in all samples analysed was below the LOQ level, ZEA content in 4 samples was above the LOQ value and the content of T-2 toxin was exceeding the LOQ level in only two samples analysed (see Table 3). Calculation of content values below the LOQ limit was completed just by software alike in 2007 to allow a certain level of comparison of varieties. Throughout the set of samples, no significant correlation between the level of mycotoxins and smut-infested spikes (the correlation coefficient of 0.249987) were found out. A significant positive correlation between the percentage of bunt-infested spikes and content of T-2 toxin was reported in the set of varieties with bunt infestation rate above 15% (the correlation coefficient 0.78283).

In 2009, *Tilletia* did not infect Bill and Brilliant at all, while Eurofit and Kerubino were the most affected varieties. Thanks to artificial inoculation of ears using a suspension of *Fusarium* spp. spores, the mycotoxin contents were higher and the difference among the varieties was shown to a greater extent. Even in this year, the most bunt-infested varieties were not those with the highest DON content, while showing rather higher, but not the highest content of ZEA. Calculation of content values below the LOQ level was again completed just by software to allow for evaluation (Tables 4 and 5). Generally, a higher DON content was found in the options without infection with *T. caries*, but the difference was not significant. In con-

Table 3. 2008 year results

Variety	% of spikes with *Tilletia* infection	% of grain colonized with *Fusarium* spp.	DON (mg.kg^{-1})	ZEA (μg.kg^{-1})	T-2 (μg.kg^{-1})
Akteur	5	19.25	< LOQ (0)	0	< LOQ (22.98)
Alana	1.2	16	< LOQ (0	< LOQ (10.25)	< LOQ (18.55)
Alibaba	0	18.5	< LOQ (0.009	< LOQ (12.75)	< LOQ (34.89)
Anduril	14.4	28.5	< LOQ (0.005	< LOQ (14.63)	< LOQ (23.31)
Banquet	2.2	12	< LOQ (0.021	< LOQ (7.25)	< LOQ (23.55)
Barryton	24.4	26	0	< LOQ (22.21)	< LOQ (37.71)
Batis	17.3	14.25	0	< LOQ (7)	< LOQ (46.55)
Bill	1.9	1	< LOQ (0.006	< LOQ (9.45)	< LOQ (28.42)
Bohemia	3.6	18	0	< LOQ (2.93)	< LOQ (22.32)
Buteo	21.1	22	0	< LOQ (7.25)	< LOQ (29.27)
Caphorn	13.8	24.5	0	< LOQ (4.83)	50.1
Clever	14.8	16.25	0	< LOQ (8.66)	< LOQ (39.58)
Cubus	3.9	18.5	0	< LOQ (15.08)	< LOQ (24.3)
Darwin	2.4	32	< LOQ (0.049	0	< LOQ (31.52)
Dromos	3.7	4.5	< LOQ (0.009	< LOQ (22.55)	< LOQ (30.13)
Drifter	13.4	14.5	< LOQ (0.003	< LOQ (29.66)	< LOQ (25.38)
Ebi	16.4	22	< LOQ (0.002	< LOQ (24.08)	< LOQ (39.4)
Etela	7.5	44	< LOQ (0.033	0	< LOQ (18.88)
Eurofit	20.1	21.5	< LOQ (0.019	< LOQ (11.07)	< LOQ (11.52)
Florett	10.5	20	< LOQ (0.001	< LOQ (45.35)	< LOQ (39.32)
Globus	0	16.75	0	< LOQ (34.69)	< LOQ (29.33)
Hedvika	16.8	28	< LOQ (0.02	< LOQ (40.39)	< LOQ (37.54)
Ilias	31.2	22	0	99.71	< LOQ (37.84)
Kerubino	17.1	27.5	< LOQ (0.016	< LOQ (2.42)	< LOQ (15.81)
Ludwig	7.3	46	0	< LOQ (40.39)	< LOQ (11.02)
Meritto	14.5	29.5	0	62.97	< LOQ (43.23)
Mulan	6.4	24	0	72.84	< LOQ (32.68)
Nela	1.2	17.5	0	< LOQ (18.87)	< LOQ (10.8)
Raduza	13.2	16.5	0.016	< LOQ (22.94)	< LOQ (46.41)
Rheia	23.1	5.75	0	< LOQ (28.47)	< LOQ (32.11)
Sakura	15.9	24	0	< LOQ (22.33)	< LOQ (23.97)
Samanta	10.2	10.25	0	0	< LOQ (6.54))
Simila	6.9	14.25	< LOQ (0.017	< LOQ (31.77)	< LOQ (16.12)
Sulamit	4.7	28.75	< LOQ (0.006	123.67	80.85
Vlasta	9.7	18	< LOQ (0.012	36.58	< LOQ (30)

trast, a higher content of ZEA was detected in varieties infected with blight, but even this difference was not significant. Assuming the values calculated using software for T-2 toxin, the content of this toxin was significantly higher in the varieties infected with *T. caries* (Fig. 1). The difference was highly significant at the level of α 0.01.

Three-year results showed that there was no correlation between infestation of winter wheat with *T. caries* and *Fusarium* spp. (the coefficient of 0.11648). There was no evidence of a correlation between blight infection and the content of DON (the coefficient of 0.227845), ZEA (the coefficient of 0.32315) or T-2 (the coefficient of 0.342288). As expected, a close direct relationship was confirmed between the frequency of grains infected with *Fusarium* spp. and the DON content (the coefficient of 0.71648) and the content of ZEA (the coefficient of 0.674679). Any connection between the infestation with *Fusarium* spp. and the content of T-2 toxin (the coefficient of –0.15401) was not confirmed. However, there was a statistically significant difference in the content of DON and ZEA at different rates of bunt infection, between the groups with *T. caries* infestation rate 0–5% and over 25%. Among all bunt infestation rate groups assessed, there was a significant difference in the content of T-2 toxin (Fig. 2). Unlike the overall negative result of assessment of the relationship between the bunt infestation and content of DON and ZEA, the average three-year results show that the higher infestation with *T. caries*, the higher content of T-2 toxin,

Table 4. 2009 year results; seed inoculated with *Tilletia caries*

Variety	% of spikes with *Tilletia* infection	% of grain colonized with *Fusarium* spp.	DON (mg.kg^{-1})	ZEA (μg.kg^{-1})	T-2 (μg.kg^{-1})
Akteur	39.8	29	7.8	123.5	< LOQ (45.91)
Anduril	56	37.75	9.43	237.58	68.52
Bagou (SUR.98504/20)	28.3	27.5	9.66	522.44	56.1
Bakfis	4.4	3.75	< LOQ (0.093)	57.15	66.1
Baletka	6.6	5.75	0.71	88.66	65.2
Bardotka	14.6	8.75	2.65	75.5	50.17
Barroko	15.6	42	14.72	130.85	46.37
Barryton	48.1	31.25	9.91	142.37	64.85
Bill	0	1	< LOQ (0.168)	< LOQ (25.52)	< LOQ (37.63)
Biscay	32	30	12.19	251.77	86.61
Bohemia	23.6	22.5	8	146.96	51.44
Boncap	32.2	60	36.8	387.4	< LOQ (47.60)
Brilliant	0	47	13.72	227.72	< LOD (36.36)
Citrus	55.2	37.75	12.63	333.66	104.59
Darwin	14.1	49	29.17	188.4	72.47
Drifter	30.3	1	< LOD (0.030)	< LOQ (43.10)	< LOQ (38.26)
Dromos	22.8	18	5.22	132.32	63.43
Ebi	51.1	44.5	5.9	166.12	65.56
Etela	8.5	51.5	20.03	323.01	61.19
Eurofit	58.4	28	7.55	166.38	50.1
Federer (BR 04-080)	47.9	14.5	4.28	151.18	69.53
Hedvika	34.4	26.5	5.19	121.63	59.51
Herman	54.1	25.25	10.58	109.17	< LOQ (44.69)
Iridium (MH 04-12)	28.8	36	16.54	308.95	46.94
Karolinum	35.1	40.5	11.66	248.67	< LOQ (40.13)
Kerubino	57.7	33.5	9.54	198.16	< LOQ (39.3)
Kodex	34.3	45.5	1.14	339.34	96.07
Ludwig	22	49	7.96	139.91	< LOQ (43.7)
Manager	15.3	26.25	5.75	188.25	90.92
Megas	47.8	21.25	3.62	173.47	47.05
Meritto	22.3	32.75	12.14	254.44	69.81
Mulan	31.6	24.75	4.48	124.63	101.08
Nikol	13.3	8	2.57	90.56	85.3
Orlando	21.8	9	3.42	127.29	83.41
Pitbull	57.1	17.25	9.18	141.96	115.8
Raduza	59	25.75	4.54	113.76	55.48
Rapsodia	17.5	43.25	20.02	379.9	87.55
Rheia	31.5	9	3.52	79.3	< LOQ (31.60)
Sakura	40.3	30.5	3.86	129.98	60.68
Samanta	12.3	13.25	2.68	56.64	< LOQ (21.71)
Secese (SG-S 1038-04)	8.2	30.25	9.04	169.85	57.1
Seladon (SG-S 1337-04)	32.1	41.5	17.67	333.66	< LOQ (47.39)
Sogood	11	62	30.56	391.87	153.62
Sulamit	8.6	41.5	< LOD (0.034)	< LOQ (38.31)	< LOQ (40.92)
Sultan	14.1	27	6.08	153.76	64.33
Trend	14.3	46	13.56	265.43	< LOQ (46.2)

Table 5. 2009 year results; seed without *Tilletia carie* inoculation

Variety	% of spikes with *Tilletia* infection	% of grain colonized with *Fusarium* spp.	DON (mg.kg^{-1})	ZEA (µg.kg^{-1})	T-2 (µg.kg^{-1})
Ludwig	0	63	12.855	96.46	< LOQ (41.65)
Merrito	0	67	11.645	192.41	< LOQ (46.90)
Etela	0	60	22.065	217.32	< LOQ (40.92)
Rapsodia	0	64	16.97	141.94	< LOQ (38.41)
Trend	0	73	14.59	575.73	< LOQ (41.50)
Hedvika	0	70	13.73	248.97	< LOQ (43.87)
Akteur	0	69	14.13	153.42	< LOQ (36.85)
Biscay	0	68	22.315	211.94	< LOQ (36.29)
Sakura	0	54	5.925	205.42	< LOQ (45.30)
Dromos	0	61	4.67	158.26	< LOQ (47.68)
Eurofit	0	58	14.08	118.64	< LOQ (38.76)
Kerubino	0	56	8.065	149.19	< LOQ (39.19)
Barryton	0	21	6.895	73.51	< LOQ (24.20)
Manager	0	30	5.43	68.58	< LOQ (20.89)
Pitbull	0	31	10.135	85.29	< LOQ (19.81)
Bohemia	0	19	2.53	< LOQ (20.02)	< LOQ (26.35)
Mulan	0	11	5.245	69.71	< LOQ (27.83)
Baletka	0	5	0.516	< LOD (14.19)	< LOQ (38.49)
Bakfis	0	3	0.312	< LOD (6.83)	< LOQ (25.54)
Orlando	0	32	14.45	102.72	< LOQ (36.11)
Sultan	0	26	10.2	58.75	< LOQ (28.79)
Kodex	0	25	9.68	161.79	< LOQ (45.74)
Megas	0	35	10.89	189.53	< LOQ (35.88)
Federer (BR 04-080)	0	26	6.69	78.42	< LOQ (34.45)
Brilliant	0	51	14.97	139.86	< LOQ (36.95)
Seladon (SG-S 1337-04)	0	50	14.51	235.88	< LOQ (47.92)
Iridium (MH 04-12)	0	60	21.99	244.64	< LOQ (32.50)
Nikol	0	12	2.15	51.81	< LOQ (35.51)
Secese (SG-S 1038-04)	0	47.25	13.69	183.23	< LOQ (38.72)
Bagou (SUR.98504/20)	0	47.25	10.67	116.06	< LOQ (41.94)

although even here the variety played some role (Figs 3 and 4).

Of the total range, ten varieties were evaluated in each of the three years. In terms of resistance, only the Bill variety showed a higher resistance to infection caused by both *T. caries* and *Fusarium* spp., and to the accumulation of toxins. Higher levels of toxins were corresponding to the high rate of infestation with *Fusarium* spp. only in Darwin and Meritto varieties. For the other varieties, any correspondence was not clear. For instance, the content of DON and ZEA in Ludwig was rather low within the set under survey despite this being the variety most affected by *Fusarium* spp. At the same time, the content of T-2 was the lowest in Ludwig (see Fig. 5).

DISCUSSION AND CONCLUSIONS

The hypothesis about the possibility of the importance of primary infection of wheat by obligate biotrophic pathogen *T. caries* as a biotic stressor, which makes the plant prone to infestation with optional pathogens *Fusarium* spp. was not confirmed; no direct connection between the infection by *T. caries* and that with *Fusarium* spp. was shown. In terms of infection, the primary infestation of plants with *T. caries* has therefore no effect on any subsequent infection of spike by *Fusarium* fungi. Especially for Fusarium head blight, genetically based resistance plays a dominant role. Resistance of different varieties to *Tilletia* is of importance as well (Dumalasová, Bartoš, 2007). The difference in the degree of resistance to infection by *T. caries* was evident in our experiments as well. The results show a direct link between the frequency of infection by *Fusarium* spp. and DON content, which confirms the results published so far (Chrpová et al., 2009). The frequency of *Fusarium* spp. corresponded to the higher content of ZEA. This connection however does not apply to T-2 toxin; the results obtained do not confirm the ‘the higher attack, the higher the content of the toxin’ presumption in this case.

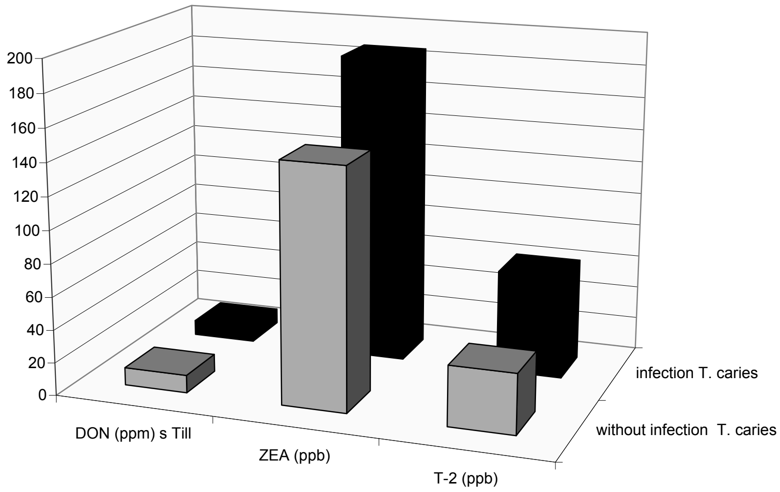

Fig. 1. Content of toxins in grain, 2009

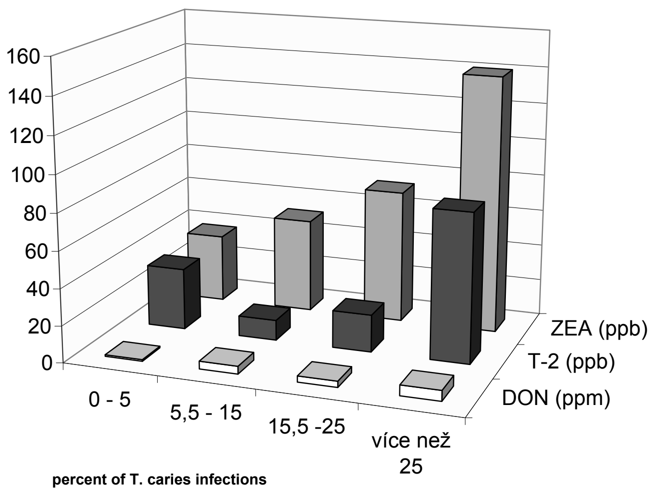

Fig. 2. Content of toxins on various level of *T. caries* infection

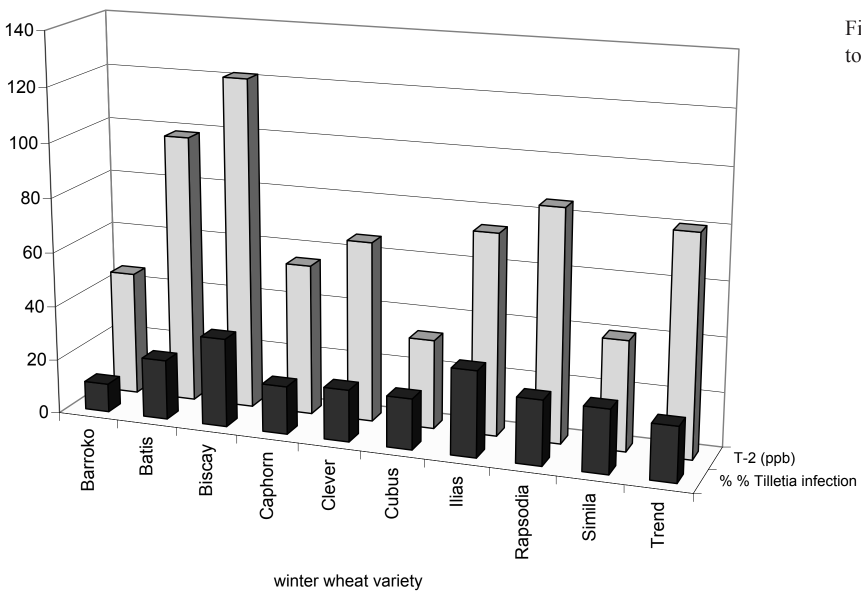

Fig. 3. Percent of *T. caries* infection and T-2 toxin content; two-years results

The objective of this study was to confirm or disprove the connection between infestation of winter wheat with *Tilletia caries* and the quantity of *Fusarium* toxins in harvested grain. There was no demonstrable link between infestation with *T. caries* and DON content. However, the results show that the infestation with *T. caries* can affect the production of ZEA and influences the production and accumulation of T-2 toxin. For T-2 toxin, possible explanations include the association with metabolism of glucose that is necessary for the formation of the toxin and is currently one of the building components of the cell walls of *Tilletia* spores (P r i l l i n g e r et al., 1991).

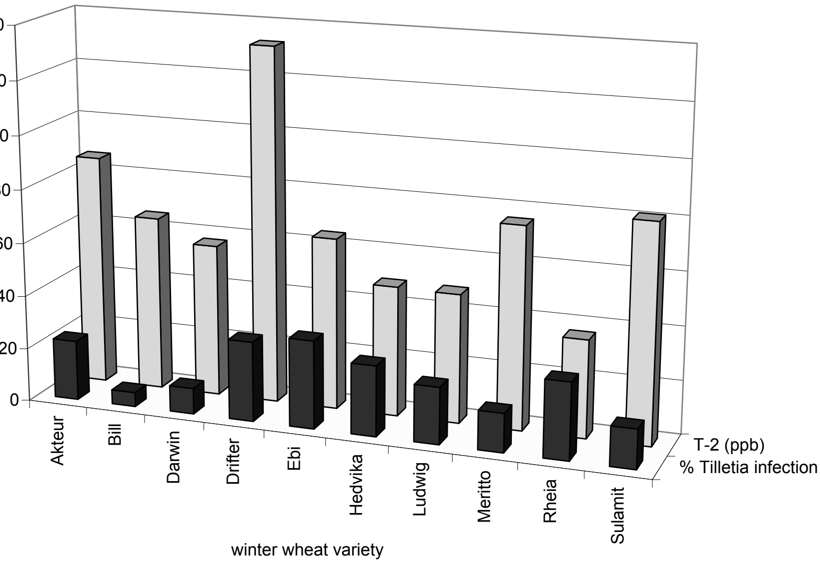

Fig. 4. Percent of *T. caries* infection and T-2 toxin content; three-years results

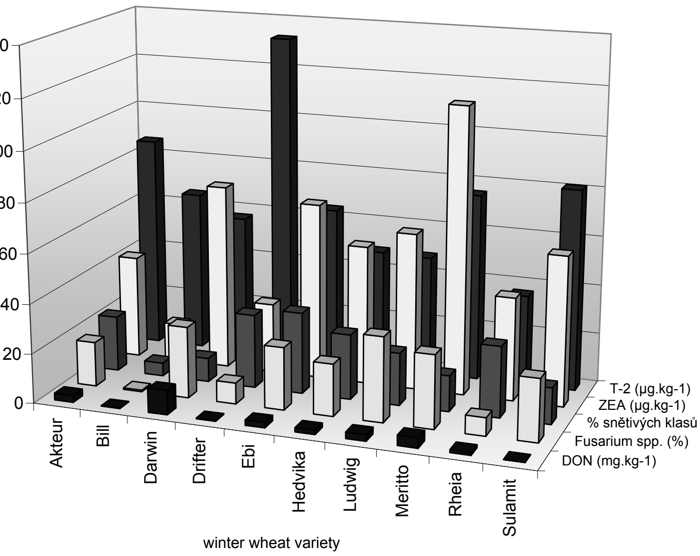

Fig. 5. Content of DON, ZEA, T-2 toxin and percent of *Fusarium* infection of grains and *Tilletia* infection spikes; three-years results

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Ověření vztahu mezi napadením ozimé pšenice *Tilletia caries* a množstvím fuzariových mykotoxinů ve sklizeném zrnu.

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Snětivost pšenice způsobovaná houbami *Tilletia caries* (syn. *T. tritici*), *T. controversa* a *T. laevis* (syn. *T. foetida*) je rozšířená v mnoha zemích světa, onemocnění se ale ve většině let vyskytuje s menší četností a malým dopadem na výši výnosu. Větší ztráty bývají kvalitativní, protože houby produkují intenzivně páchnoucí látku trimethylamin. Fuzarióza klasů patří k celosvětově rozšířeným, často se vyskytujícím chorobám pšenice, resp. obilnin. Napadení touto chorobou vede ke ztrátám kvantitativním i kvalitativním. Toxiny produkované houbami rodu *Fusarium* – původci klasové fuzariózy – kontaminují v různé míře zrna obilnin. Tato kontaminace je nežádoucí jak v potravinářské, tak v krmné pšenici, napadení zrn houbami rodu *Fusarium* také výrazně zhoršuje kvalitu osiva. Při řešení problematiky snětivosti ozimé pšenice a fuzarióz klasů jsme se setkávali s výskytem spór *Fusarium* spp. na a v hálkách *Tilletia* spp. To vedlo k vyslovení hypotézy o možnosti významu primárního napadení pšenice obligátním biotrofním patogenem *T. caries* jako biotického stresoru, který disponuje rostlinu k napadení fakultativními patogeny *Fusarium* spp.

Cílem práce je potvrdit nebo vyvrátit souvislost mezi napadením ozimé pšenice *Tilletia caries* a množstvím fuzariových toxinů ve sklizeném zrnu. V letech 2007–2009 probíhaly na pozemcích Agrotest fyto, s. r. o., v Kroměříži maloparcelkové pokusy. Bylo vyseto spektrum odrůd ozimé pšenice – v roce 2007 bylo testováno 26 odrůd, v roce 2008 35 odrůd a v roce 2009 46 odrůd ozimé pšenice ve variantě inokulované *T. caries*, 30 odrůd ve variantě bez inokulace *T. caries*. Soubor odrůd zahrnoval odrůdy různě citlivé k napadení *Tilletia caries* a klasovým fuzariózám. Symptomaticky bylo v době zralosti před sklizní vyhodnoceno procento klasů napadených snětí. Metodou vlhké komůrky bylo v průměrném vzorku zjištěno procento zrn napadených *Fusarium* spp. Pro stanovení obsahu deoxynivalenolu (DON) byly použity imunochemické kity Ridascreen Fast DON, pro stanovení zearalenonu (ZEA) kity Ridascreen Fast Zearalenon a pro stanovení T-2 toxinu Ridascreen Fast T-2 toxin (R-Biopharm, SRN).

Hypotéza o možnosti významu primárního napadení pšenice obligátním biotrofním patogenem *T. caries* jako biotického stresoru, který disponuje rostlinu k napadení fakultativními patogeny *Fusarium* spp., se nepotvrdila, nebyla prokázána přímá vazba mezi napadením *T. caries* a napadením *Fusarium* spp. Z výsledků je patrná přímá vazba mezi četností napadení *Fusarium* spp. a obsahem DON, což potvrzuje dosud publikované výsledky (Chrpová et al., 2009). Četnosti *Fusarium* spp. odpovídal i vyšší obsah ZEA. Tato vazba ale neplatí pro T-2 toxin, dosažené výsledky nepotvrzují v tomto případě předpoklad čím vyšší napadení, tím vyšší obsah toxinu. Nebyla zjištěna žádná prokazatelná vazba mezi napadením *T. caries* a obsahem DON. Výsledky ale ukazují, že napadení snětí může ovlivnit produkci ZEA a ovlivňuje produkci a kumulaci T-2 toxinu.

pšenice ozimá; *Tilletia caries*; fuzarióza klasů; mykotoxiny

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