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Fusarium head blight in Oat

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Abstract

Ten oat varieties (six hulled: Atego, Flämingsprofi, Neklan, Pogon, Salo, Veli and four naked: Abel, Avenuda, Izak and Saul) were grown on small 2.5 m² plots in the allotment of University of South Bohemia and Agricultural Research Institute Kroměříž in the Czech Republic. The experiment was carried out during the years 2009-2012. Each variety was tested by quantitative and qualitative assessment, during vegetation and after harvesting. Harvested samples were tested for occurrence of micro fungi and then analysed for the presence of mycotoxins, especially trichothecenes (T-2 and HT-2 toxin, deoxynivalenol, nivalenol and DON-3-glucoside) using ELISA method and HPLC (LC/MS-MS). The purpose of this experiment was to gain information about *Fusarium* sp. contamination in oat in local growing conditions and to compare the susceptibility of each variety.

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Introduction

The occurrence of dangerous diseases in agricultural plants has been the principal problem for farmers since 19th century, when the first disease was described in England by W. G. Smith (Wegulo *et al.*, 2015). In fact, the problem of micro fungi diseases has been largely solved, though for wheat and barley only. Recent years have shown that *Fusarium* head blight is a serious problem for oat, which was previously known as a very healthy crop. Cultivation of oat is currently very popular in ecological agriculture. Repeated cultivation of oat year after year is not an ideal choice due to higher frequency of possible pest occurrence - *Oscinela frit* or *Heterodera avenae*. Oat is also susceptible to occurrence of *Puccinia coronata*, *Puccinia graminis* or powdery smut *Ustilago avenae*, where the spots occur mainly on the leaves and also *Fusarium* ssp. shown to affect the panicle.

Fusarium spp. belong to the most important genera of pathogenic fungi causing plant diseases and decreasing grain quality through higher levels of mycotoxin contamination and high yield loss (Madgwick *et al.*, 2011). In the inoculation studies, yield reductions up to 74% were established in small grain cereals (McMullen *et al.*, 2012). Scab or Fusarium head blight is caused by a complex of 17 species of which *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *Microdochium nivale* are predominant (Brennan *et al.*, 2007). This genus includes about 1000 species; it belongs to phylum *Ascomycota*, order *Hypocreales*.

It hibernates in fields on plant remnants and is spread by soil and infected seeds in favourable conditions (high moisture and temperatures around 20°C during flowering growth stage). It causes diseases that damage different parts of the targeted plant. The primary reservoir of inoculum is plant remnants from the preceding crop (Xu, 2003). A warm, moist environment characterized by frequent precipitation or heavy dew is highly favourable for fungal growth, infection and development of disease in head tissues (Lawrence and Stein, 2007). The symptoms of infection are varied and can be exhibited by necrotic changes or bleached spikes with pink, yellow, brownish, reddish, violet or lilac-tinted sporodochias. Most frequently the bleaching begins in the centre of the spike (Samson *et al.*, 1996 and Fernando *et al.*, 1997).

Another negative influence of micro fungi for crops is the production of secondary metabolites, mycotoxins. Trichothecens of type A (HT-2 a T-2-toxin, diacetoxyscirpenol, neosolaniol, fusarenon X) and type B (Deoxynivalenol, nivalenol), zearalenon and fumonisins belong to the most important toxic substances (Desjardins, 2006). Increased presence of mycotoxins in food and feed can cause acute and chronic health complications. This influence on live organisms is manifested in various ways, e.g. HT-2 and T-2 toxin have immunosuppressive cytotoxic affect and damages blood, deoxynivalenol has immunosuppressive effect and it causes vomiting, nivalenol has carcinogenic effect and it causes regurgitation and zearalenon has carcinogenic or estrogenic effect. Production of mycotoxins is influenced by several factors such as composition of soil, genotype, seeds, position, agro technology, environmental conditions (frequency and quantity of precipitation, day temperature, air humidity, sunshine hours and oxygen level) and stocking.

Reduction in grain quality is due to the presence of shrivelled or discoloured kernels known as *Fusarium* damaged kernels or “tombstones” which are unsuitable for milling, baking or malting (McMullen *et al.*, 1997). When using oat in food and feed processing, it is necessary to keep to strict grain quality and primarily eliminate the occurrence of dangerous mycotoxins. The determination of contaminants content is the subject of Commission regulation EU No.1881/2006, which also gives strict maximum levels for any *Fusarium* mycotoxins in food and feed within the European Union (Czembor *et al.*, 2015). Maximum levels were determinated for deoxynivalenol, zearalenone, fumonisins, aflatoxins and ochratoxin A. The aim of this study was to obtain information about occurrence of key mycotoxins during growing of oats in the conditions of the Czech Republic.

Materials and methods

Grain samples

Ten oat varieties were compared in quantitative and qualitative assessment as a part of project NAZV QH81060. The purpose of this experiment was to detect the level of contamination by *Fusarium* mycotoxins in the selected oats. Six hulled (Atego, Flämingsprofi, Neklan, Pogon, Salo and Veli) and four naked varieties (Abel, Avenuda, Izak and Saul) were compared. For subsequent tests of mycotoxicology quality of oats, small 2.5 m² plots were made (3 rows with pitch 12.5 cm). Stripes of oats were sown as protection around these plots. Each variety was sown in two repetitions, after three preceding crops (rape, oat, corn). The experiment was carried out using the small plots strictly sowed by Hege machine in the earliest possible period. The seeding rate was 5 million per ha for hulled grain varieties and 5.5 million per ha for naked grain varieties.

During the vegetation period, germination, number of panicles per square metre, lodging rate and visual symptoms of the *Fusarium* head blight occurrence were determined. After harvest, 1000 grain weight, number of grains in one panicle, weight of grain in one panicle, volume weight, percentage of hulls and the grain size grading were established.

Isolation of Fusarium species

After harvest, grains from each two replicates were milled and mixed. Representative grain samples of half a kilogram of each variety were collected and used for analyses. Determination of each sample was carried out by the Crop Research Institute in Ruzyně and the following chemical analysis was carried out by University of Chemistry and Technology in Prague, where the samples were analysed for the presence of trichothecenes.

The grain samples were soaked in distilled water with the addition of Savo® and TWEEN 20 for surface disinfection and rinsed 3 times in distilled water, then dried on sterile filter paper, placed into Petri dishes and incubated at 22°C in darkness. After 14 days' incubation of each culture was placed on nutrient mediums (PDA, PSA or OA) and purified. Pure cultures were grown at 22°C in Petri dishes under black light (310-360 nm). After 7-14 days' main characteristics of most colonies were visible.

The principle of PCR reaction is the amplification of part of DNA with a couple of species-specific primers (forward and revers). The first step is denaturation of the double helix by high temperatures then the respective primers are attached to DNA sequence according to the principle of complementarity. The reaction result is multiple multiplication of the DNA section. The product of PCR reaction was detected on agarose gel under UV light, after being colorized by ethidium bromide. Species specific primers for more species were added to the reaction mixture when detecting multiple species simultaneously in case of multiplex PCR.

Meteorological data

The experiment was carried out in the allotment of University of South Bohemia and Agricultural Research Institute Kroměříž. The allotment is situated in South Bohemian basin, 381 meters above sea level, in potato productive area (48°58'29 "latitude and 14°28'29" longitude). The soil in the area is clayey and fine-grained with brown gley soil prevailing. Long-term annual average temperature is 8.1°C, soil pH is weakly acidic (pH 5.9) and the average annual precipitation is 623 mm. Table 1. Shows temperature and precipitation during the experiment.

Table 1. Temperature and precipitation.

2008	March	April	May	June	July	August	Σ precipitation/vegetation
Average temperature (°C)	2.6	7.3	13.2	16.9	17.4	17.0	12.4
Average precicpitation (mm)	62	55	56	67	85	70	395
2009	March	April	May	June	July	August	Σ precipitation/vegetation
Average temperature (°C)	2.8	11.4	12.9	14.5	17.5	17.9	12.8
Average precicpitation (mm)	71	30	101	166	117	89	574

2010	March	April	May	June	July	August	Σ precipitation/vegetation
Average temperature (°C)	2.1	7.6	11.3	16.0	19.3	16.4	12.1
Average precicpitation (mm)	31	53	107	95	128	131	545
2011	March	April	May	June	July	August	Σ precipitation/vegetation
Average temperature (°C)	3.2	9.7	12.6	16.2	15.7	17.3	12.5
Average precicpitation (mm)	35	34	81	72	145	61	428
2012	March	April	May	June	July	August	Σ precipitation/vegetation
Average temperature (°C)	4.9	7.5	13.4	16.4	17.2	17.3	12.8
Average precicpitation (mm)	12	54	55	103	133	120	477

Statistical analyses

The average values for individual samples were compared using analysis of variance. Comparison of each assessment with preceding crops, seasons and varieties were conducted using the Kruskal-Wallis and Mann-Whitney test. Stat Soft was used for all statistical analyses.

Mycotoxin analyses

The toxin analyses were carried out using HPLC (LC/MS-MS) method and ELISA method at University of Chemistry and Technology in Prague (the ELISA method was used only for samples from Kroměříž). High-performance liquid chromatography is an improved form of column chromatography while the ELISA method is an enzyme-linked immunosorbent assay. Both methods are used for identification and quantification of present mycotoxins. Results from the ELISA method were somewhat higher, with possible reason of a cross-reaction with related mycotoxins, while ELISA remains the faster and cheaper method for mycotoxin detection. In this project, high-performance liquid chromatography with tandem mass spectrometer QTRAP 5500, AB Sciex (U-PLC-MS/MS) was used for the analyses. For every sample the limit of quantitation (LOQ) was initially estimated. LOQ is the lowest calibrating value for sample determination with still acceptable precision. For the ELISA method, test kits RIDASCREEN FAST®T-2 and RIDASCREEN FAST®HT-2 made by R-Biopharm (Germany) were used.

List of varieties

This experiment involved ten oat varieties (four naked and six hulled). Naked varieties were represented by Abel, Izak, Avenuda and Saul, hulled by Atego, Flämingsprofi, Neklan, Pogon, Salo and Veli.

Results

Lodging rate

Lodging rate varieties were evaluated on a scale 1-9 (9 = the lowest lodging rate, 1 = the highest lodging rate). The varieties with the highest lodging rate were Neklan, Atego and Salo. Reversely, in our experiments the varieties with the lowest lodging rate were Flämingsprofi, Abel and Avenuda. The best preceding crop was corn, followed by oat and rape.

Average number of panicles per m²

The highest average number of panicles per m² was achieved with the Salo variety when grown after oat with 416 panicles, 394 panicles after rape and 388 after corn, followed by Abel variety with 380 panicles per m² after rape and Atego with 373 panicles per m² after rape. Best results were achieved with Veli and rape as the preceding crop-with 672 panicles per m², Abel with 656 panicles per m² and Flämingsprofi with 640 panicles per m².

Number of grains per panicle

Highest number of grains in a panicle was found in Flämingsprofi (76 after oat), Pogon (76 after oat) and Veli (72 after corn) varieties, reversely, the lowest grain number was reached by Salo (42 after rape), Avenuda (43 after rape) and Izak (46 after rape) varieties. When comparing preceding crops, the best results were established after oat with Flämingsprofi (123 grains), after rape with Atego (119 grains) and after corn with Neklan (119 grains).

Grain weight per panicle

When assessing grain weight per panicle, highest values were established for Neklan (4.24g after corn), Pogon (4.03g after corn) and Flämingsprofi (3.99g after oat); reversely the lowest values were found for Avenuda (0.07g after corn), Abel (0.11g after corn) and Saul (0.16g after corn). On average the most suitable preceding crop was oat, followed by rape and corn.

1000-seed weight analysis

When comparing results from the 1000-seed weight analysis, the best variety was Flämingsprofi (46.3g after oat and 44.9g after corn) followed by Atego (42.4g after rape). Lowest values were found for Fläminsprofi (16.79g after oat), Izak (17.24g after rape) and Avenuda (17.5g after corn). The difference for the various preceding crops was negligible and in tenth of grams only.

Grain size

When assessing grain size, highest values were established for hulled varieties with Neklan (96.84 after corn), Pogon (96.67 after corn) and Izak (96.66 after rape). The least uniform varieties were Veli (12.63 after rape), Avenuda (17.76 after rape) and Flämingsprofi (22.48 after rape).

Presence of hulled grains

When testing the grains, Izak (1.4%) and Saul (1.97%) were established as varieties with the lowest presence of hulled grains. The best preceding crop in this case was rape (2.11%), followed by oat (2.13%) while corn (2.26%) was the preceding crop with the highest number of hulled grains. The highest percentage of hulls in the hulled varieties was achieved with Pogon (26.29) and Flämingsprofi (25.59) with insignificant influence from the preceding crops – corn (24.42), rape (24.45) and oat (25.8).

Occurrence of fungi species

The occurrence of individual fungi species was very specifically dependent on the preceding crop and season. Most frequent fungi occurrence for all varieties grown after all preceding crops occurred in 2010. The following fungi species were detected: *Alternaria, Acremonium, Beauveria, Cladosporium, Fusarium, Gliocladium, Paecilomyces, Penicilium, Trichothecium, Trichoderma* and sterile mycelium (Table 2). Reversely the lowest occurrence was detected in the year 2011. The results from the four-year experiment have shown that the best preceding crop on average with the lowest fungi occurrence was rape, followed by oat while the worst preceding crop was corn. The highest *Fusarium sp*. occurrence was in the year 2012 after oat as the preceding crop and in the years 2010 and 2012 after corn. The lowest *Fusarium* occurrence was found in varieties grown after rape, followed by corn and the highest after oat. In our experiment the following *Fusarium* species were detected: *F. avenaceum, F. equiseti, F. graminearum, F. poae, F. sporotrichoides* and *F. tricinctum*. The species frequency is shown in the Table 3.

Table 2. Microfungi occurrence after every single preceding crops.

Year	2009			2010			2011			2012		
Previous crop	Rape	Oat	Corn	Rape	Oat	Corn	Rape	Oat	Corn	Rape	Oat	Corn
Alternaria sp.	4	1	6	5	5	7	2	3	2	1	3	3
Fusarium sp.	3	2	0	2	4	5	3	3	3	3	5	5
Gliocladium sp.	0	0	0	1	0	0	0	0	0	0	1	0
Penicilium sp.	0	0	1	1	1	2	1	1	2	1	1	2
Paecilomyces sp.	0	0	0	1	0	0	0	0	1	1	1	3
Acremonium sp.	0	0	0	2	1	1	0	1	0	0	0	0
Beauveria sp.	0	0	0	1	0	0	0	0	0	0	0	0
Trichothecium sp.	0	2	0	0	1	0	0	0	0	0	0	0
Trichoderma sp.	2	2	0	0	0	0	1	0	0	0	0	0
Cladosporium sp.	0	0	1	0	0	0	0	1	0	0	0	0
Sterile mycelium	1	2	0	4	0	1	0	0	0	0	1	0

Note: Total number of species microfungi.

Table 3. *Fusarium* occurrence.

Year/	2009			2010		
Previous crop	Rape	Oat	Corn	Rape	Oat	Corn
F. poae	Fläm,Salo	Fläm,Salo		Izak	Atego, Izak	Salo
F. tricinctum	Salo					
F. avenaceum				Salo	Abel, Neklan	Pogon
F. equiseti						Abel, Atego, Fläm
F. graminearum						
F. sporotrichoides						
Year/	2011			2012		
Previous crop	Rape	Oat	Corn	Rape	Oat	Corn
F. poae	Fläm	Izak, veli				Neklan, Pogon
F. tricinctum						
F. avenaceum	Veli	Salo	Abel	Saul	Izak, Salo	Salo, Veli
F. equiseti	Atego		Salo		Veli	Fläm
F. graminearum			Avenuda	Veli	Avenuda	
F. sporotrichoides				Neklan		

Fläm = Flämingsprofi.

Mycotoxins

In the year 2009 T-2 toxin was detected in 40% of the tested samples while HT-2 toxin was found in 47% of tested samples. The average values were 27 µg/kg for T-2 and 145 µg/kg for HT-2 toxin, respectively. DON was detected in 63% of tested samples with average value of 47 µg/kg. NIV was detected in 23% of tested samples with an average content of 72 µg/kg. In the year 2010 the highest amount of samples positive with T-2 toxin 65% and HT-2 toxin 75% were found with average values of 30 µg/kg for T-2 toxin and 47 µg/kg for HT-2 toxin. With the regard to DON content, 25% of the samples were contaminated. Almost 95% of samples were contaminated with conjugated form of D3G with average value of 24 µg/kg. Quantity of samples contaminated with NIV was almost 95% and the average value was 135µg/kg. In the year 2011 the overall levels of mycotoxins were very low, only NIV had the highest values this year compared to all the tested years. The DON content was not higher than 5µg/kg in any sample. The lower quantity of positive samples was detected for T-2 and HT-2 toxins with values 10% for T-2 and 47% for HT-2. The conjugated form D3G was not detected in any sample. All the samples were contaminated with NIV with average values 640µg/kg. The mycotoxin occurrence is shown in Table 4.

Table 4. Mycotoxins occurrence.

Previous crop	Variety	HT-2 (µg/kg)		T-2 (µg/kg)		DON (µg/kg)		NIV (µg/kg)		D3G (µg/kg)	
		2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Oat	Abel	9,9	30,3	<3	16,7	<3	<3	92,5	177	<5	31
	Atego	14,2	89,8	5,5	53,2	5,9	<3	<10	295	<5	23,9
	Avenuda	10,6	34,5	<3	42,2	<3	<3	<10	210	<5	25,1
	Flämingsprofi	18,1	44,4	<3	29,1	58	6,4	<10	144	<5	21,6
	Izak	14	<3	3,1	<3	<3	<3	107	286	<5	25,4
	Neklan	45,2	62,4	5,3	39,5	66,2	<3	<10	81,2	<5	21,5
	Pogon	<3	116,5	<3	69,4	26,7	42,1	<10	67,8	<5	19,1
	Salo	19,6	69,2	4,1	29,6	24,3	<3	<10	163	<5	27,2
	Saul	14	37	2,6	17,5	<3	<3	<10	109	<5	29
	Veli	21,8	39,1	5,1	38,2	24,9	14,5	<10	104	<5	21,7
average oat		18,6	58,1	4,3	37,3	34,3	21,0	99,6	163,6		24,6
Rape	Abel	14,2	<3	3,4	14,6	<3	<3	<10	24,6	<5	29
	Atego	<3	49,5	4,1	33,7	22,9	<3	50,2	20,4	<5	20,4
	Avenuda	22,4	<3	<3	<3	<3	<3	89,5	19,4	<5	25,6
	Flämingsprofi	27,1	<3	14,1	12,5	98,9	<3	<10	32,9	<5	17,9
	Izak	15,4	-	<3	-	<3	-	<10	-	<5	-
	Neklan	<3	12,4	<3	17,5	41,3	<3	<10	60,9	<5	20,2
	Pogon	<3	<3	<3	<3	49,7	3,9	73	52,5	<5	18,8
	Salo	<3	13,3	<3	19,9	41	<3	<10	426	<5	24,7
	Saul	<3	7,9	<3	<3	<3	<3	<10	13,1	<5	26,5
	Veli	<3	<3	4,7	<3	88	<3	<10	<10	9,4	<5

Previous crop	Variety	HT-2 (µg/kg)		T-2 (µg/kg)		DON (µg/kg)		NIV (µg/kg)		D3G (µg/kg)	
		2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
average rape		19,8	20,8	6,6	19,6	57	3,9	70,9	81,2	9,4	22,9
Corn	Abel	<3	-	5,3	-	<3	-	<10	-	<5	-
	Atego	<3	-	<3	-	43,2	-	41,3	-	<5	-
	Avenuda	<3	-	<3	-	<3	-	<10	-	<5	-
	Flämingsprofi	<3	-	<3	-	182	-	49,3	-	<5	-
	Izak	<3	-	<3	-	<3	-	<10	-	<5	-
	Neklan	<3	-	<3	-	3,1	-	<10	-	<5	-
	Pogon	27,8	-	10,5	-	74,1	-	<10	-	<5	-
	Salo	<3	<3	<3	12,5	21	22,7	<10	277	<5	22
	Saul	<3	-	<3	-	3,8	-	<10	-	<5	-
	Veli	<3	-	<3	-	19,4	-	<10	-	<5	-
average corn		27,8		7,9	12,5	49,6	22,7	45,3	277		22
LOQ		3	3	3	3	3	3	10	10	5	5

The DON and D3G values were very low and they oscillated at the detection limit. The NIV average contents were 73µg/kg after rape, 72 µg/kg after oat and after corn only one sample with NIV was detected with 106 µg/kg. In the year 2011 comparison among varieties was almost not possible due to low mycotoxins content. The found exception was NIV, where its content was lower with naked varieties as opposed to hulled varieties. By comparing previous crops, it was proved a statistically significant difference in the presence of mycotoxins. According to Table 5. and 6. the oat contained significant more HT- 2 toxin than the other previous crop. In case of the content of T-2 toxin, DON, NIV and D3G is not significant difference among previous crops (Table 7, 8, 9 and 10).

Table 5. Kruskal-Wallis test - HT-2 and pre-crop.

	Kruskal-Wallis ANOVA based on order; HT-2 Independent variable: Pre-crop Kruskal-Wallis test: H (2, N=50) = 20,56084 p = ,0000		
Dependent:	Number of	Sum of	Mean
Ht-2	valid	order	order
Rape	19	396,5000	20,8684
Oat	20	719,5000	35,9750
Corn	11	159,0000	14,4545

Table 6. Kruskal-Wallis test - HT-2 and pre-crop 2.

	Multiple comparisons p values (two-tailed); HT-2 Independent variable: Pre-crop Kruskal-Wallis test: H (2, N=50) = 20,56084 p = ,0000		
Dependent:	rape	cereal	corn
Ht-2	R:20,868	R:35,975	R:14,455
Rape		0,003652	0,736530
Oat	0,003652		0,000252
Corn	0,736530	0,000252	

Table 7. Kruskal-Wallis test - T-2 and pre-crop.

	Kruskal-Wallis ANOVA based on order; T-2 Independent variable: Pre-crop Kruskal-Wallis test: H (2, N=50) = 6,144534 p = ,0513		
Dependent:	Number of	Sum of	Mean
T-2	valid	order	order
Rape	19	450,5000	23,71053
Oat	20	619,5000	30,97500
Corn	11	205,0000	18,63636

Table 8. Kruskal-Wallis test - DON and pre-crop.

	Kruskal-Wallis ANOVA based on order; DON Independent variable: Pre-crop Kruskal-Wallis test: H (2, N=50) = 2,106754 p = ,3488		
Dependent:	Number of	Sum of	Mean
DON	valid	order	order
Rape	19	449,0000	23,63158
Oat	20	488,5000	24,42500
Corn	11	337,5000	30,68182

Table 9. Kruskal-Wallis test - NIV and pre-crop.

	Kruskal-Wallis ANOVA based on order; NIV Independent variable: Pre-crop Kruskal-Wallis test: H (2, N=50) = 5,315813 p = ,0701		
Dependent:	Number of	Sum of	Mean
NIV	valid	order	order
Rape	19	458,0000	24,10526
Oat	20	609,0000	30,45000
Corn	11	208,0000	18,90909

Table 10. Kruskal-Wallis test – D3G and pre-crop.

	Kruskal-Wallis ANOVA based on order; D3G Independent variable: Pre-crop Kruskal-Wallis test: H (2, N=50) = 5,429525 p = ,0662		
Dependent:	Number of	Sum of	Mean
D3G	valid	order	order
rape	19	503,5000	26,50000
oat	20	576,5000	28,82500
corn	11	195,0000	17,72727

The content of individual mycotoxins during the year 2009 and 2010 were significantly statistically different. Higher content of mycotoxin HT-2, T-2, NIV and D3G was in year 2010. On the other hand, higher content of mycotoxin DON was in the year 2009. There is not a significant statistically difference between varieties in case of HT-2, T-2, NIV and D3G. According to Table 16 content of DON is more significant different between varieties Pogon and Abel, Pogon and Avenuda in case of occurrence DON.

Table 11. Mann-Whitney U test – HT-2 and year.

	Mann-Whitney U test Acc. variable: Year Marked tests are significant at a significance level p <0,05000					
	Sum of the order	Sum of the order	Z	p	N valid Group 1	N valid Group 2
Variable	Group 1	Group 2				
HT-2	662	613	-2,030	0,0420	30	20

Table 12. Mann-Whitney U test – T-2 and year.

	Mann-Whitney U test Acc. variable: Year Marked tests are significant at a significance level p <0,05000					
	Sum of the order	Sum of the order	Z	p	N valid Group 1	N valid Group 2
Variable	Group 1	Group 2				
T-2	567	708	-3,911	0,0001	30	20

Table 13. Mann-Whitney U test – DON and year.

	Mann-Whitney U test Acc. variable: Year Marked tests are significant at a significance level p <0,05000					
	Sum of the order	Sum of the order	Z	p	N valid Group 1	N valid Group 2
Variable	Group 1	Group 2				
DON	903,5	371,5	2,733	0,006	30	20

Table 14. Mann-Whitney U test – NIV and year.

	Mann-Whitney U test Acc. variable: Year Marked tests are significant at a significance level p <0,05000					
	Sum of the order	Sum of the order	Z	p	N valid Group 1	N valid Group 2
Variable	Group 1	Group 2				
NIV	534,5	740,5	-4,555	0,000005	30	20

Table 15. Mann-Whitney U test – HT-2 and year.

	Mann-Whitney U test Acc. variable: Year Marked tests are significant at a significance level p <0,05000					
	Sum of the order	Sum of the order	Z	p	N valid Group 1	N valid Group 2
Variable	Group 1	Group 2				
D3G	480,5	794,5	-5,624	0,000	30	20

Table 16. Mann-Whitney U test – DON and year (variety).

	Multiple comparisons p values (two-tailed); DON Independent variable: Variety Kruskal-Wallis test: H (9, N=50) = 25,43657 p =,0025									
	Abel	Atego	Avenuda	Flämings	Izak	Neklan	Pogon	Salo	Saul	Veli
Dependent:	R:13,500	R:27,200	R:13,500	profi	R:13,500	R:28,200	R:40,200	R:28,833	R:16,400	R:32,900
DON				R:37,700						
Abel		1,000	1,000	0,390	1,000	1,000	0,047	1,000	1,000	1,000
Atego	1,000		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Avenuda	1,000	1,000		0,390	1,000	1,000	0,047	1,000	1,000	1,000
Flämingsprofi	0,390	1,000	0,390		0,600	1,000	1,000	1,000	0,939	1,000
Izak	1,000	1,000	1,000	0,600		1,000	0,285	1,000	1,000	1,000
Neklan	1,000	1,000	1,000	1,000	1,000		1,000	1,000	1,000	1,000
Pogon	0,047	1,000	0,047	1,000	0,285	1,000		1,000	0,443	1,000
Salo	1,000	1,000	1,000	1,000	1,000	1,000	1,000		1,000	1,000
Saul	1,000	1,000	1,000	0,939	1,000	1,000	0,443	1,000		1,000
Veli	1,000	1,000	1,000	0,600	1,000	1,000	1,000	1,000	1,000	

Discussion

The collected data show that *Fusarium* occurrence and mycotoxin content depend on a combination of several agricultural practices. Especially difference in fungal growth and toxin production may be caused by due to meteorological factors. In our research individual varieties of oats were compared grown after three different preceding crops for several years and thereby in different weather conditions.

According to results obtained from statistical analysis the naked varieties clearly showed better results regarding most of the studied parameters than the hulled oats. Correlation analyses of Doehlert *et al.* (2001) suggested that warm, bright (high solar radiation) spring weather, and cooler summer without excessive rains during grain filling generated the best oat yields with high quality grain.

The growth of microscopic fungi was supported by higher temperature and higher precipitation during the flowering in the year 2009 and 2012. Higher average temperature during June and July 2010 had the similar effect for occurrence microscopic fungi with lower values of precipitation. According to our results were these conditions suitable for occurrence another species of microfungi (*Gliocladium* sp. *Acremonium* sp. *Beauveria* sp.) and higher presence of *Alternaria* sp. Generally, in this year there was a quite high occurrence of microfungi, especially *Fusarium* sp. In our experiment *Fusarium* poae, *Tricinctum, Equiseti* and *Avenaceum* were most frequently detected. In oat samples harvested in 2010 and 2011 in South, East and West Sweden Fredlund *et al.* (2013) detected species *Fusarium langsethiae, Avena ceum* and *Poae.*

The preceding crop had significant influence on mycotoxin content in the year 2009 where the highest trichothecens type A (T-2 and HT-2 toxin) content was detected with varieties grown after oat, reversely the lowest trichothecens type B (DON, NIV) content was detected after oat. The lower mycotoxin contents were detected with naked varieties compared to hulled varieties due to presence of glumes, which is built better conditions for *Fusarium* growth. Equally to preceding year, in 2010 the preceding crop also showed large impact on mycotoxin content as similar results for trichothecens occurrence were found. Again the naked varieties showed lower mycotoxin contents of mycotoxin, especially DON.

In the experiment of Scudamore *et al.* (2007) concerning the occurrence of HT-2 and T-2 toxins in oats grown in the UK in 2002-2005, higher level of HT-2 was always detected as compared to T-2. No correlation could be confirmed between HT-2 and DON as these mycotoxins were formed by different *Fusarium* species. However, DON and NIV were produced by different chemotypes of the same species. Reversely in our experiment the difference between the occurrence of HT-2 and T-2 toxins depended on the season and the preceding crop and we could not confirm that HT-2 toxin always had higher levels than T-2.

Generally, the most stable preceding crop was rape as the varieties grown after rape had the lowest mycotoxin values.

In the experiment by Obst *et al.* (2000) it was detected that corn as a preceding crop presented high risk due to DON occurrence. The same DON results have also been confirmed in our experiments.

The temperature and precipitation are important factors affecting *Fusarium* occurrence in cereals. The average temperature and precipitation was for the four experimental years very similar. For *Fusarium* occurrence the precipitation was essential especially during the heading and flowering growth stages. High DON contamination was determined after dry springs and wet flowering growth stage and NIV, HT-2 and T-2 toxins were determined after dry and warm flowering growth stage. In the year 2009, the average precipitation was higher as compared to the annual average but despite this fact the mycotoxin content was lower.

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