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Manuskript, zu finden in www.fal.de

Published in: Landbauforschung Völkenrode 52(2002)2, pp. 91-96

Braunschweig
Bundesforschungsanstalt für Landwirtschaft (FAL)
2002

Fusarium mycotoxins in conventionally and organically grown grain from Thuringia/Germany

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Abstract

The *fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZON) were determined in conventionally and organically grown grain harvested in 1998 in Thuringia/Germany. A total of 196 wheat samples and 69 rye samples was analysed.

DON concentrations of conventionally grown wheat were found to be significantly higher than in organically grown wheat. Of conventionally grown wheat, 69 % tested positive, containing a mean concentration of 1540 µg/kg dry matter (DM). In 54 % of the organically grown wheat samples DON was detected with a mean value of 760 µg/kg DM. DON concentration in rye and ZON concentration in wheat showed similar tendencies.

The different cultivars of conventionally grown wheat showed large differences in DON contamination.

In the year of investigation, high concentrations of *fusarium* mycotoxins were typical in grain grown in Germany, such as the DON concentrations found here. ZON contamination of the investigated samples was rather low, probably due to the early harvest before the heavy rainfalls in September.

If the analysed wheat were to be included in a feed ration for pigs at 50 %, the DON concentrations of 12 % (respectively 4 %) in the rations mixed with conventionally grown wheat (respectively organically grown wheat) would exceed the orientation value for critical concentrations in feedstuffs given by the German Federal Ministry of Nutrition, Agriculture and Forestry (2000).

From the standpoint of human nutrition, 43 % of the conventionally grown wheat and 37 % of the organically grown wheat contain toxin concentrations which exceed the tolerable daily intake (TDI) of DON established by the European Scientific Committee on Food, assuming a mean body weight of 60 kg at a mean wheat consumption

Keywords: Deoxynivalenol, zearalenone, organic farming, cultivars, wheat, rye

Zusammenfassung

***Fusarium* Mykotoxine in konventionell und ökologisch angebautem Getreide aus Thüringen/Deutschland**

Die Fusarientoxine Deoxynivalenol (DON) und Zearalenon (ZON) wurden in konventionell und ökologisch angebautem Getreide der Ernte 1998 aus Thüringen/Deutschland untersucht. Insgesamt wurden 196 Weizen- und 69 Roggenproben analysiert.

Der Weizen aus konventionellem Anbau war signifikant höher mit DON belastet als der aus ökologischem Anbau. 69 % des konventionell angebauten Weizens waren positiv, mit einem mittleren Gehalt von 1540 µg/kg Trockensubstanz (T). In 54 % des ökologisch angebauten Weizens wurde DON mit einer mittleren Konzentration von 760 µg/kg T nachgewiesen. Die DON Konzentrationen im Roggen und die ZON Konzentrationen im Weizen zeigten ähnliche Tendenzen.

Die verschiedenen Sorten des konventionell angebauten Weizens zeigten deutliche Unterschiede in den ermittelten DON Konzentrationen.

Im Erntejahr 1998 waren hohe Fusarientoxinbelastungen, wie die hier gefundenen DON- Konzentrationen, in deutschem Getreide üblich. Die ZON Belastung des untersuchten Getreides war gering. Ein möglicher Grund hierfür könnte die zeitige Ernte des Getreides vor den starken Niederschlägen im September sein.

Wenn der untersuchte Weizen in Schweinefutter mit einem Anteil von 50 % der Ration eingesetzt würde, überschritten 12 % der mit konventionellem Weizen hergestellten Futtermischungen und 4% der Futtermischungen mit ökologisch angebautem Weizen den Orientierungswert für DON Konzentrationen im Futtermittel des Bundesministerium für Ernährung, Landwirtschaft und Forsten (2000).

Aus Sicht der Humanernährung enthalten 43 % des konventionell angebauten Weizens und 37 % des ökologisch angebauten Weizens Konzentrationen, die zu einem Überschreiten des von dem European Scientific Committee on Food erarbeiteten „tolerable daily intake“ (TDI) für DON, bei einen angenommenen mittleren Körpergewicht von 60 kg und einem mittleren Weizenkonsum, führen.

Schlüsselworte: Deoxynivalenol, Zearalenone, Ökologischer Landbau, Sorten, Weizen, Roggen

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1 Introduction

Fusarium and *fusarium* toxins represent a worldwide problem for agriculture, food industries and consumers (Tanaka et al., 1988).

The infection of grain with *fusarium* results in reduced crop yields, inferior quality and possibly additional costs for special treatments of infected grain (Charmley et al., 1994).

As summarized by Dänicke et al. (2000) feeding of *fusarium* toxin contaminated grain to farm animals could result in adverse effects on performance and animal health, especially the immune system and the reproductive tract are concerned. The effects of *fusarium* toxins on human health were mainly deduced from animal studies.

To minimize the problems caused by *fusarium*, it is important to investigate the occurrence of *fusarium* and *fusarium* mycotoxins and factors influencing the infection and the formation of toxins. Reviewing the previous literature Oldenburg et al. (2000) were able to post a ranking of factors influencing infection of grain, especially wheat. The authors found the climate to have the greatest impact, followed by infection pressure/tillage, corn as preceding crop, plant protection, cultivars and plant nutrition. In consideration of these influencing factors, the crop production system of organic farming seems to hold some advantages concerning crop rotation and tillage as compared to conventional farming. There are controversial reports on varying mycotoxin contamination levels of conventionally and organically grown grain (Berleth et al., 1998; Marx et al., 1995; Meier et al., 1999; Stähle et al., 1998).

In the present study, DON and ZON were determined in 265 samples of conventionally and organically grown grain from Thuringia/Germany harvested in 1998, a year with high incidence of *fusarium* mycotoxins.

2 Materials and Methods

A total of 265 grain samples, grown in 1998 in Thuringia/Germany was provided by the Thuringian Institute of Agriculture (TLL)[2], of which 200 samples, 150 wheat and 50 rye, were grown conventionally and 65 samples, 46 wheat and 19 rye, were grown organically. The samples were taken after harvest and frozen until analysis. For the samples out of conventional farming further information (origin, cultivars, date of harvest) were provided.

Weather data, monthly precipitation and mean temperatures from 1998, and the respective long-term mean were provided by the Deutscher Wetterdienst of Thuringia (Weimar, Germany).

DON was determined in all samples with the competitive enzyme immunoassay RIDASCREEN®FAST DON (R-Biopharm, Darmstadt, Germany). The detection limit was 111 µg/kg.

ZON was determined in 126 wheat samples using HPLC with fluorescence detection following a modified method described by Valenta and Oldenburg (1995). The modification was to use ethylacetate for the extraction and to work without clean up by solid-phase-extraction. The detection limit was 2 µg/kg. The positive tested samples were confirmed by the competitive enzyme immunoassay RIDASCREEN®Zearalenon (R-Biopharm, Darmstadt, Germany). Another 55 wheat samples were analysed by the TLL applying an HPLC method with a detection limit of 20 µg/kg and the results were incorporated in the present study (TLL, 2000).

All the ZON data were evaluated using a detection limit of 20 µg/kg.

For statistical analyses Microsoft SPSS was used, applying the t-test.

3 Results and discussion

DON was detected in 147 out of the 265 analysed samples. The mean DON concentration of the positive samples was 1380 µg/kg in dry matter (DM) in wheat and 450µg/kg DM in rye. It can be seen that in analogy with the results of Lepschy et al. (1989) and Tanaka et al. (1988), wheat was more frequently and more highly contaminated than rye (Table 1).

Table 1
DON content in wheat and rye

	n	Positive [%]	Mean[1)] [µg/kg DM]	Range [µg/kg DM]
Wheat	196	65	1 380	120 - 11 660
Rye	69	28	450	120 - 3 090

[1)] of positive samples

ZON was detected in 11 of 181 analysed wheat samples with a mean concentration of 69 µg/kg DM.

Conventional and organic farming

DON was investigated in 200 samples, 150 wheat and 50 rye, of conventionally grown grain and in 65 samples , 46 wheat and 19 rye, of organically grown grain (Table 2).

The conventionally grown wheat contained significantly more DON than the organically grown wheat ($p<0.05$). DON was detected in 69 % of the conventionally grown wheat containing a mean concentration of 1540 µg/kg DM and in 54 % of the organically grown wheat with a mean concentration of 760 µg/kg DM.

Of the 50 analysed rye samples produced in conventional farming systems 34 % were tested to be positive with a mean DON concentration of 490 µg/kg DM. In 2 (11 %)

2 We thank Dr. U. Kirchheim of the Thuringian Institute of Agricultur for providing the samples and the sample information.

Table 2
DON content of conventionally and organically grown wheat and rye

	n	Positive [%]	Mean[1)] [µg/kg DM]	Median[2)] [µg/kg DM]	Max. [µg/kg DM]
C.w.	150	69	1540	270	11660
O.w.	46	54	760	230	4220
C.r.	50	34	490	<d.l.	3090
O.r.	19	11	130	<d.l.	130

[1)] of positive samples; [2)] of all samples; c.-conventionally grown; o.-organically grown; w.-wheat; r.-rye; d.l.-detection limit; max.-maximum

of the 19 samples of organically grown rye DON could be determined with a concentration of 120 and 130 µg/kg DM respectively. For these samples statistical analyses could not be performed because of the limited number of replications.

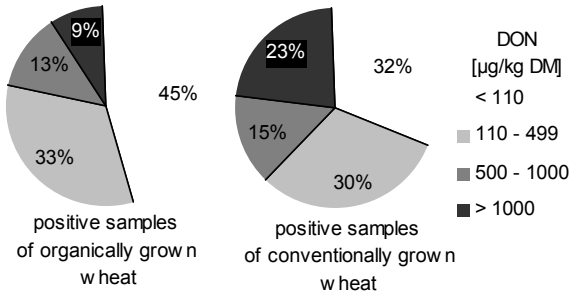

Fig. 1
Ranges of DON concentrations of the contaminated organically and conventionally grown wheat

135 samples of conventionally and in 46 samples of organically grown wheat were analysed for ZON. This mycotoxin was detected in 7 % of the conventionally grown wheat with a mean concentration of 74 µg/kg DM and in 4 % of the organically grown wheat with a mean of 47 µg/kg DM respectively (Table 3).

Table 3
ZON content of conventionally and organically grown wheat

	n	Positive [%]	Mean[1)] [µg/kg DM]	Median[2)] [µg/kg DM]	Max. [µg/kg DM]
C.w.	135	7	74	<d.l.	250
O.w.	46	4	47	<d.l.	55

[1)] of positive samples; [2)] of all samples; c.-conventionally grown; o.-organically grown; w.-wheat; d.l.-detection limit; max.-maximum

The significant differences in the DON contamination of the investigated wheat as well as the tendencies found for the DON content of rye and the ZON content of wheat for the different farming systems could result from the differences in practices of crop rotation, tillage, fertilisation and/or other husbandry treatments.

Meier et al. (1999) investigated wheat of the 1997 and 1998 harvest in the Rhineland (Germany) and found higher DON concentrations in conventionally grown wheat than in organically grown wheat as well. Also, Stähle et al. (1998) ascribed the low DON concentrations found in wheat harvested in 1997 to the organic farming since investigations of conventionally grown wheat of the same year showed higher contents. In contrast to the mentioned authors and the presented results, Marx et al. (1995) detected more DON as well as ZON in organically grown wheat and rye than in conventionally grown grain. The authors investigated grain harvested in Bavaria (Germany) in 1991.

Cultivars

The different cultivars of conventionally grown wheat samples showed distinct differences in DON contamination.

Cultivars with five or more samples are included in the discussion.

The cultivars Ritmo, Kontrast, Bandit and Zentos with median DON contents of 1060, 1010, 960 and 840 µg/kg DM showed the highest contamination. With median concentrations of 530 and 450 µg/kg DM, samples of the cultivars Tambor and Aron were also high contaminated. Tarso, Monopol and Batis contained median amounts of 150, 140, and 120 µg DON/kg DM. The medians for the DON contents of the cultivars Pegasos, Flair and Bussard were below detection limit.

The total number of ZON-positive samples was too small to show clear differences between the cultivars.

The varying DON contamination of the different cultivars confirm the results of Langseth and Stabbetorp (1996), Obst et al. (1997), Klingenhagen and Frahm (1999) and Lauren et al. (1996) who reported a varying

Table 4
DON content of wheat of different cultivars

Cultivar	n	Positive [%]	Mean[1)] [µg/kg DM]	Median[2)] [µg/kg DM]	Max. [µg/kg DM]
Ritmo	18	88	2310	1060	11660
Kontrast	5	100	2390	1010	9400
Bandit	5	100	1570	960	4320
Zentos	11	91	990	840	4080
Tambor	7	71	2190	530	7390
Aron	16	81	1790	450	11440
Tarso	7	57	440	150	790
Monopol	5	60	630	140	1560
Batis	20	55	480	120	1210
Bussard	11	36	160	<d.l.	180
Flair	6	17	250	<d.l.	250
Pegasos	9	22	1820	<d.l.	3250

[1)] of positive samples; [2)] of all samples; d.l.-detection limit; max.-maximum

susceptibility of the different cultivars to *fusarium* infection and contamination with mycotoxins. Klingenhagen and Frahm (1999) also investigated the cultivars Batis, Bussard, Flair, Pegasos, Ritmo and Zentos for *fusarium* ear infections. The authors found Ritmo, a cultivar which was highly contaminated with DON in the present investigation, to be highly infected. For the cultivars Batis, Bussard, Flair and Pegasos Klinghagen and Frahm (1999) found low infection rates, these cultuvars showed low DON contamination in the present study. In contrast to the present results where a high DON contamination of the wheat Zentos was found the authors reported low infection of Zentos.

Climate

Comparing the present results with literature data it is conspicuous that the detected DON concentrations are higher than in the previous years (Table 5). In the years 1989, 1990, 1991, 1992 and 1993, the wheat investigated by Müller et al. (1997) contained mean DON concentrations of 150, 600, 360, 340 and 390µg/kg original substance. In the year 1987 a mean concentration, which is close to the present results, of 1690 µg/kg was detected. This year was called a "mycotoxin-year", like 1996, where Kuhlmann et al. (1999) detected a mean of 760 µg/kg in wheat. The summer months of the years 1987 and 1996 were characterised by heavy rain falls (Müller et al., 1997; Kuhlmann et al., 1999).

Table 5
DON content of wheat harvested within different years in Germany

Year	Orig.	n	Positive [%]	Mean[1)] [µg/kg]	Range [µg/kg]	Ref.
1987	BW	84	96	1690	4-20540	a
1989	BW	78	69	150	3-1190	a
1990	BW	80	96	600	10-8970	a
1991	BW	80	96	360	4-4630	a
1992	BW	78	95	340	20-5410	a
1993	BW	45	96	390	20-6170	a
1996	TH	21	90	760[2)]	110-2840[2)]	b
1998	TH	150	69	1540[2)]	120-11660[2)]	c

[1)] of positive samples; [2)] DM; BW-Baden-Württemberg; TH-Thuringia; orig.-origin; ref.-reference; a: Müller et al., 1997; b: Kuhlmann et al., 1999; c: present results

Comparing to the high DON contamination the ZON contamination, with 7 % positive samples containing mean concentrations of 74 µg/kg, was rather low compared to the other so called "mycotoxin-years" (Table 6). Lew (1995) investigated why *fusarium* infected wheat contained high amounts of DON and small amounts of ZON while the same *fusarium* isolates produced equal amounts of DON and ZON when cultured in vitro. The author noticed that high amounts of DON were present at an early state of fungal growth while ZON was produced at a later state of growth.

Table 6
ZON content of wheat harvested within different years in Germany

Year	Orig.	n	Positive [%]	Mean[1)] [µg/kg]	Range [µg/kg]	Ref.
1987	BW	84	80	180	1-8040	a
1989	BW	78	14	3	1-10	a
1990	BW	80	11	10	1-20	a
1991	BW	80	13	20	1-110	a
1992	BW	78	19	4	1-20	a
1993	BW	45	62	10	2-50	a
1996	TH	21	62	40[2)]	3-220[2)]	b
1998	TH	135	7	74[2)]	20 - 250[2)]	c

[1)] of positive samples; [2)] DM; BW-Baden-Württemberg; TH-Thuringia; orig.-origin; ref.-reference; a: Müller et al., 1997; b: Kuhlmann et al., 1999; c: present results

The infection of grain with *fusarium* and the production of mycotoxins is influenced by the weather conditions, especially at anthesis since the ears are most susceptible to *fusarium* infection at that time (Obst et al., 1997; Mills, 1990; Lacey, 1990; Langseth et al., 1995). A drop in temperature below 18°C and heavy rainfalls during anthesis increases the risk of mycotoxin formation in the following period (Obst et al., 1997).

Furthermore, the weather conditions at the time of harvest have a high impact on mycotoxin production. In the years 1987 and 1996 the harvest was delayed because of heavy rainfalls during summer and high concentrations of ZON were found in wheat samples (Müller et al 1997, Kuhlmann et al. 1999). In the year of grain sampling for the present study, heavy rainfalls occurred in September (Figure 2). With the exception of one, all samples investigated were harvested between the 6th and the 22nd of August 1998, so the rainfalls in September could have hardly affected the grain contamination.

Reutter (1999) investigated wheat of the harvest 1998 grown in Schleswig-Holstein/Germany and harvested late because of heavy rainfall. The author reported DON contaminations (Table 7) similar to the ones found in the presented investigation but ZON contamination (Table 8) was much higher. This leads to the conclusion that the *fusarium* infection caused the DON contamination but the ZON contamination was probably prevented by early harvest.

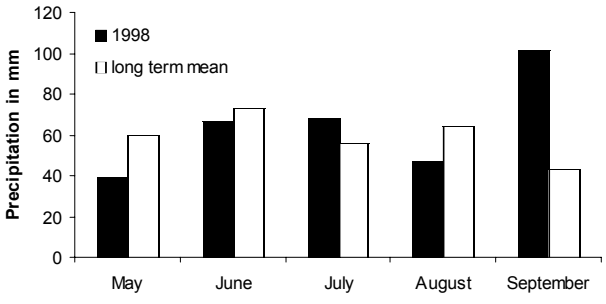

Fig. 2
Monthly precipitation in Thuringia in 1998 and the long term mean

Table 7
DON content of wheat harvested in different region of Germany in 1998

Year	Orig.	n	Positive [%]	Mean[1)] [µg/kg]	Range [µg/kg]	Ref.
1998	SH	116	86	2700	300-10800	d
1998	TH	150	69	1540[2)]	120-11660[2)]	c

[1)] of positive samples; [2)] DM; SH-Schleswig-Holstein; TH-Thuringia; orig.-origin; ref.-reference; c: recent results; d: Reutter, 1999

Table 8
ZON content of wheat harvested in different region of Germany in 1998

Year	Orig.	n	Positive [%]	Mean[1)] [µg/kg]	Range [µg/kg]	Ref.
1998	SH	125	74	69	10-346	d
1998	TH	135	7	74[2)]	20-250[2)]	c

[1)] of positive samples; [2)] DM; SH-Schleswig-Holstein; TH-Thuringia; orig.-origin; ref.-reference; c: recent results; d: Reutter, 1999

Toxicological relevance

Assuming the examined grain was mixed into diets for pigs, which are most sensitive to *fusarium* toxins, some of the toxin concentrations reached would be considered to be critical according to the orientation values of the German Federal Ministry of Nutrition, Agriculture and Forestry (BML, 2000a). If 50 % of the analysed grain would be included into a feed ration the DON concentrations of 12 % (respectively 4 %) of the rations mixed with conventionally grown wheat (respectively organically grown wheat) would exceed the orientation value of 1.0 mg DON/kg complete diet (88 % DM). Only 2 % (1 sample) of the conventionally grown rye and none of the organically grown rye could cause a critical DON concentration in a feed ration for pigs if it would be included at 50 %. For ZON there are different critical concentrations for pre-pubertal gilts and fattening pigs and sows of 0.05 mg/kg and 0.25 mg/kg diet (88 % DM) respectively. Two of the conventionally grown wheats and one of the organically grown wheats would exceed the critical ZON concentration in a diet containing 50 % wheat for prepubertal gilts.

For human nutrition the European Scientific Committee on Food (SCF) has established a tolerable daily intake (TDI) for DON of 1µg/kg body weight (bw)/d (2002) and a temporary TDI of 0.2µg/kg bw/d for ZON (2000). In the year 1998/99 the per capita consumption of wheat flour in Germany was 58.6 kg (BML, 2000b). Assuming a mean body weight of 60 kg which results in a mean wheat intake of 2.7g/kg bw, the DON concentration in wheat which should not be exceeded according to the TDI, is 370 µg/kg and 74 µg/kg for ZON. For DON, 43 % of the conventionally grown wheat and 37 % of the organically grown wheat, and for ZON 2 % of the conventionally grown wheat do not meet these criteria. A survey of the National Institute of Public Health and the Environment (Pieters, 1999), Netherlands, showed that wheat consumption is not evenly distributed among the population. Children aged 1 to 4 had the highest wheat intake, i.e., 4.5 - 8.5 g/kg bw/d. To meet the TDI of a young child with a high wheat intake the DON content should be less than 118 µg/kg and the ZON content less than 24 µg/kg. The detection limits for the presented results were 111µg DON/kg and 20 µg ZON/kg. This means that all of the positive results found in wheat near or exceed the TDI of a young child with a high wheat intake.

Conclusions

The organic farming system with the practice of a well-balanced crop rotation, tillage and fertilization showed benefits concerning the contamination with *fusarium* mycotoxins, especially DON, in this investigation of a large number of grain samples from Thuringia/Germany in the year 1998. The beneficial factors causing the differences between the farming systems need to be scrutinized by investigations under defined conditions, but in conventional farming more attention should be paid to conspicuous differences such as crop rotation. The cultivation of less susceptible cultivars can decrease the contamination with mycotoxins as well.

Considering that reached mycotoxin concentrations exceeded critical concentrations for animal as well as human nutrition, harvested grain should be monitored for *fusarium* toxins, especially in years with a high risk of contamination.

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