Zeitschrift: Mitteilungen aus Lebensmitteluntersuchungen und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 95 (2004)

Heft: 3

Artikel: Comparison of trichothecene contaminations in wheat cultivated by three different farming systems in Switzerland : biodynamic, bioorganic and conventional

Autor: Griesshaber, Dorrit / Kuhn, Fabian / Berger, Urs

DOI: https://doi.org/10.5169/seals-981827

Comparison of trichothecene contaminations in wheat cultivated by three different farming systems in Switzerland: biodynamic, bioorganic and conventional

Dorrit Griesshaber[1], Fabian Kuhn[1], Urs Berger[2] and Michael Oehme[1]

[1]Organic Analytical Chemistry, University of Basel, Neuhausstrasse 31, CH-4057 Basel, Switzerland
[2]Norwegian Institute for Air Research (NILU), The Polar Environmental Centre, Hjalmar Johansens gate 14, NO-9296 Tromsø, Norway

Received 27 October 2003, accepted 29 April 2004

Introduction

Over the past years, contamination of cereal grains and animal feed with *Fusarium* mycotoxins has been reported more frequently (1). The fungi responsible for their production infect cereals world-wide both during growth and crop storage in humid environment, where production of mycotoxins can also take place. Trichothecenes are a group of more than 140 different mycotoxins, mainly produced by various species of *Fusarium* fungi (2). They are divided into four different groups according to their molecular structure. Type B-trichothecenes differ from type A by the presence of an additional carbonyl group and are mainly found in contaminated food. Type C-toxins are characterised by an additional epoxide function, and D-trichothecenes have macrocyclic structures. This diversity of trichothecenes causes a wide range of toxic effects in animals and humans such as food refusal, vomiting, anemia, hemorrhage and immunosuppression (3). However, only a limited number of the known trichothecenes have been identified in *Fusarium*-infected crops. Among these, deoxynivalenol (DON) is considered to be the most important *Fusarium* toxin in temperate zones (4).

The DOK trial has been carried out since 1978 in Therwil in the north-western part of Switzerland, to compare biodynamic (D), bioorganic (O) and conventional

Table 1

Average input (1978–1998) of nutrients, pesticides and fossil energy to the different DOK trials. Soluble nitrogen is the sum of NH_4-N and NO_3-N. Input of active pesticides is based on years 1985–1991. Energy for production of machinery and infrastructure, in fuel and for production of mineral fertilizer and pesticides was calculated for 1985–1991 (18)

*Farming system**)	*Total nitrogen [kg N ha^{-1} year^{-1}]*	*Soluble nitrogen [kg N ha^{-1} year^{-1}]*	*Phosphorus [kg P ha^{-1} year^{-1}]*	*Potassium [kg K ha^{-1} year^{-1}]*	*Pesticides [kg active ingredients ha^{-1} year^{-1}]*	*Energy [GJ ha^{-1} year^{-1}]*
D	99	34	24	158	0	12.8
O	93	31	28	131	0.21	13.3
K	149	96	43	268	6	20.9

*) D, biodynamic; O, bioorganic; K, conventional

(K) farming systems (see Table 1). It is a joint project of the Swiss Federal Research Station for Agroecology and Agriculture (FAL, Zürich-Reckenholz, Switzerland) and the Research Institute of Organic Agriculture (FiBL, Frick, Switzerland). The mentioned systems differ mainly in fertilization and plant protection. Furthermore, each farming system is subdivided in two treatments using different fertilizer intensities (trials 1 and 2). Crop and harvest are carried out in the same way for each treatment. The trial is designed as a randomised block of fields including four replicates of each farming system with a plot size of 5×20 m. The climate in Therwil is rather dry and mild with a mean precipitation of 785 mm per year and a mean temperature of 9.5 °C. The soil is a silty clay on loess (5).

The aim of this study was to test the robustness and applicability of a recently developed HPLC-MS method for trichothecenes (6) by studying the A- and B-trichothecene contamination of organic and conventionally grown winter wheat from two crop rotation periods, the third (1998) and fourth (2000) ones. Furthermore, it should be investigated, if trichothecene levels were significantly different between the farming systems and if some compounds could therefore serve as biomarkers. Based on their occurrence and levels in our region (4), the following mycotoxins were selected for this study: Nivalenol (NIV), deoxynivalenol (DON), neosolaniol (NEO), fusarenon-X (F-X), diacetoxyscirpenol (DAS), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), HT-2-toxin (HT-2) and T-2-toxin (T-2).

Experimental

Chemicals and reagents

Mycotoxin standards of certified purity were purchased from Sigma Chemie (Buchs, Switzerland): DON, 3-ADON, DAS (>99 %); NIV, NEO (≥99 %); F-X, 15-ADON (99 %); T-2, verrucarol (≥98 %) and HT-2 (>97.5 %). Hydrocortisone (purum, >97 %) was provided by Fluka Chemie (Buchs, Switzerland). Acetonitrile (190 far UV, >99.9 %) was obtained from Romil Ltd. (Cambridge, UK) and methanol (pestipur, >99.8 %) from SDS (Peypin, France). Water was obtained from an Elgastat Maxima HPLC water purification unit (Elga Ltd., Bucks, UK). Helium of 99.996 % and nitrogen of 99.995 % purities were used (Carbagas, Switzerland).

Wheat samples

A total of 48 winter wheat samples grown in 1998 and 2000 were selected from a long-term field trial in Therwil, Switzerland. 24 samples were collected per year (4 per farming system and fertilizer intensity). To minimize interferences, samples were taken from a 20 m^2 area inside of the 100 m^2 replicate. 300 g of each were randomly collected and 100 g were ground with a ultracentrifugal mill ZM 100 (Retsch GmbH & Co. KG, Haan, Germany) at 18000 rotations per minute and a 1 mm ring sieve. Samples were stored at 25 °C prior to analysis.

Extraction and sample cleanup

Various wheat samples were tested for the presence of verrucarol (VOL) and all of them were negative. To our knowledge there is no evidence in literature, that VOL is a naturally occuring trichothecene in wheat. Thus, 15 µg of the internal standard VOL in 150 µl of methanol were added to 10 g of ground corn. The mixture was shaken for 2 h with 40 ml of acetonitrile/H_2O (84+16 v/v) and filtrated through folded cellulose filters of medium porosity (no. 311845, Schleicher & Schuell, Feldbach, Switzerland). A 4 ml aliquot was cleaned up on a MycoSep 227 trichothecene cartridge and a final cleanup was performed on a cleanup column No. 216 (both Romer Labs Inc., USA).

Separation and detection

Prior to analysis 1.5 µg of the recovery standard hydrocortisone in 150 µl of methanol/water (1+3 v/v) was added to the sample solution. HPLC separation was carried out on a C18 modified stationary phase (Nucleosil, 120 Å pore size, 3 µm particles, normal density, 125 mm column length, 2 mm i.d., Macherey-Nagel, Oensingen, Switzerland). A linear binary gradient was applied (low-pressure binary gradient HPLC pump Rheos 4000; Flux Instruments, Basel, Switzerland) increasing from 25 % to 98 % methanol in water for 12 min, followed by 5 min rinsing with 98 % methanol. Then, the methanol content was lowered to 25 % within 1 min, and the column was re-equilibrated for 6 min. The flow rate of the mobile phase was 250 µl/min. An ion trap mass spectrometer (LCQ, Finnigan MAT, San Jose, USA) was used in the positive ion mode employing atmospheric pressure chemical ionisation (APCI(+)). Mass spectra were registered in the full-scan mode with a mass range of 150–500 u.

Quantification

Quantification was carried out using the mass chromatograms of the $[M+H]^+$ ions (internal and recovery standard, DON, HT-2) or the fragment ions *m/z* 294.9+312.7 for NIV. Detection limits were determined at a signal-to-noise ratio of 3:1 and limits of quantification (LOQ) at a ratio of 10:1. Since recovery rates of trichothecenes hardly varied over a large concentration range, quantitative results were corrected for recovery. Correction factors between the given toxin and the ISTD were not influenced by concentration over the whole calibration range (for NIV 1.2; DON 1.12 and HT-2 1.07 (7)).

Results and discussion

The applied methodology was quite robust. Recoveries ranged from 74 to 107 % with a mean recovery of 89 % in 1998 and 91 % in 2000 for DON. Typical coefficient of variation of the sample cleanup procedure was 4 % (n=4) and of the whole method 8 % (n=4). Except for a few outliers, differences between parallel determinations including extraction and cleanup did not exceed 20 %. This somewhat

Table 2

DON, NIV and HT-2 contents in wheat samples from the DOK trials in 1998 and 2000. The three different farming systems were biodynamic (D), bioorganic (O) and conventional (K) at two (1, 2) different fertilizer intensities. Values are given in µg/kg. Concentrations of DON between the limit of detection (LOD) and limit of quantification (LOQ) are marked in parenthesis

	DON	*Mean DON ± s_d*	*NIV*	*HT-2*
D1_1998	(31)	40±18	<10	<1
	(22)		<10	<1
	(45)		<10	<1
	63		<10	<1
D1_2000	(25)	27±24	[1]	<1
	(10)		[1]	<1
	61		[1]	<1
	(10)		111	<1
D2_1998	78	48±26	<10	<1
	(37)		<10	<1
	58		<10	[2]
	(18)		<10	<1
D2_2000	(41)	30±14	[1]	<1
	(30)		[1]	<1
	(40)		369	<1
	(10)		[1]	<1
O1_1998	142	75±51	[1]	<1
	(45)		[1]	<1
	84		[1]	<1
	(27)		<10	<1
O1_2000	(10)	48±44	[1]	<1
	84		[1]	<1
	(10)		[1]	<1
	89		[1]	<1
O2_1998	110	74±32	<10	<1
	(35)		<10	<1
	85		<10	[2]
	67		<10	[2]
O2_2000	(26)	46±34	[1]	13
	85		[1]	<1
	64		[1]	<1
	(10)		136	<1
K1_1998	129	105±60	[1]	<1
	113		[1]	[2]
	(20)		[1]	[2]
	159		[1]	13
K1_2000	(35)	52±23	[1]	<1
	(40)		[1]	<1
	(47)		[1]	<1
	85		[1]	<1
K2_1998	(21)	81±55	[1]	<1
	153		<10	<1
	62		<10	[2]
	87		<10	[2]
K2_2000	(29)	91±79	[1]	14
	206		170	<1
	62		[1]	<1
	66		177	[2]

[1] Value between LOD (10 µg/kg) and LOQ (100 µg/kg)
[2] Value between LOD (1 µg/kg) and LOQ (10 µg/kg)

higher value compared to method precision may be due to a slight to moderate heterogenity of the samples, which is difficult to overcome. Retention times of DON varied less than 8 % over a period of 12 h (n=10). Limits of detection were 10 µg/kg for NIV, 6 µg/kg for DON and 1 µg/kg for HT-2.

In 48 investigated samples only one type A-trichothecene (HT-2) and two type B-trichothecenes (NIV and DON) were present at detectable levels (see Table 2). DON was detected in all wheat samples from 1998 and 2000. However, concentrations were often (52 %) below the LOQ of 50 µg/kg and can therefore only be considered as semiquantitative with a typical coefficient of variation of ca. 30 %.

The range of DON concentrations was 10 to 206 µg/kg and the overall mean 60 µg/kg for both years and all farming systems. This is comparable to the results by Noser et al. (8) for wheat from the same region. DON contents below 300 µg/kg were reported for 1993 and 1994 and for 88 % of the samples in 1995. None of the observed DON levels in our study exceeded the Swiss tolerance value of 1 mg/kg on dry weight basis, the water content of 10–15 % having been taken into account. Moreover, the ubiquitous presence of DON in this region as documented by *Bucheli et al.* (4) was confirmed.

Berleth et al. (9) found a comparable range of 14–184 µg/kg DON for wheat and rye from Germany in 1996. However, *Schollenberger et al.* (10) reported DON contaminations of 15–1379 µg/kg in wheat samples from south Germany harvested in 1999. Since growth of *Fusarium* fungi is mainly triggered by humidity and relatively low temperatures (11), the lower concentrations of the investigation presented here may be explained by the climatic conditions of the summers 1998 and 2000. Weather was warmer and also dryer than the 10 year's mean (see Figure 1).

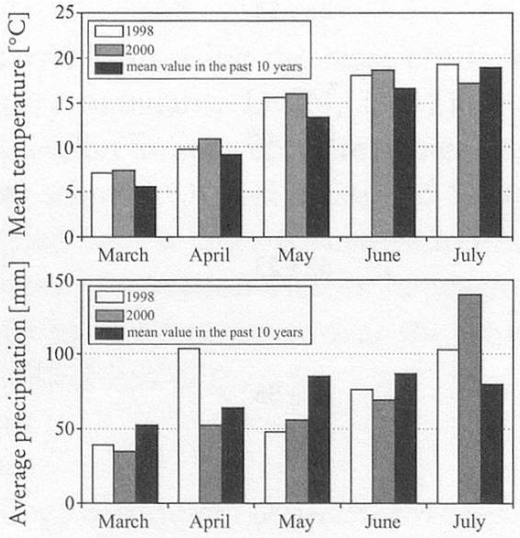

Figure 1 **Monthly average temperatures and precipitation for March to July 1998 and 2000 in Liestal, Basel-Landschaft, Switzerland. Long term mean values (10 years) are given for comparison (19)**

NIV and HT-2 were detected at concentrations around or below their LOQ (100 µg/kg for NIV and 10 µg/kg for HT-2). NIV was detected in 32 (67 %) and HT-2 in 12 samples (25 %) at levels similar to those observed by *Schollenberger et al.* (10) and *Müller et al.* (12). However, NIV and HT-2 were found at a higher percentage of samples than in other studies from the same region (12–14), where NIV was present in 11–64 % and HT-2 in none to 12.5 % of all samples. One sample from 2000 contained a relatively high NIV concentration of 369 µg/kg probably caused by an artefact (e.g. inhomogeneity of the wheat sample). In contrast to the studies mentioned above, this investigation did not detect further trichothecenes. Possible reasons could be climatic differences or the higher selectivity of the applied MS technique.

The mean of the DON concentrations was 30 % higher in 1998 (interquartile range 52 µg/kg in 1998 and 39 µg/kg in 2000). Though temperature was lower in 1998 (see Figure 1), the data do not allow to consider this difference as significant. NIV was detected in 33 % of the samples in 1998 compared to all in 2000. For HT-2 there was a decrease in the number of positive samples from 38 % in 1998 to 13 % in 2000.

A survey of the DON contamination of the three farming systems (biodynamic, bioorganic and conventional) is presented in Figure 2. Mean values and standard deviations are given for 1998 and 2000. A slightly higher content of DON could be observed for conventionally grown wheat, when concentrations below the LOQ (see Table 2) were included. However, the difference was not statistically significant ($P<0.99$ in 1998 and $P<0.95$ in 2000). A similar trend was also found in preceding studies (4, 9). *Schollenberger et al.* (10) reported higher concentrations in conventionally grown wheat (median 295 µg/kg) as compared to biodynamic farming (median

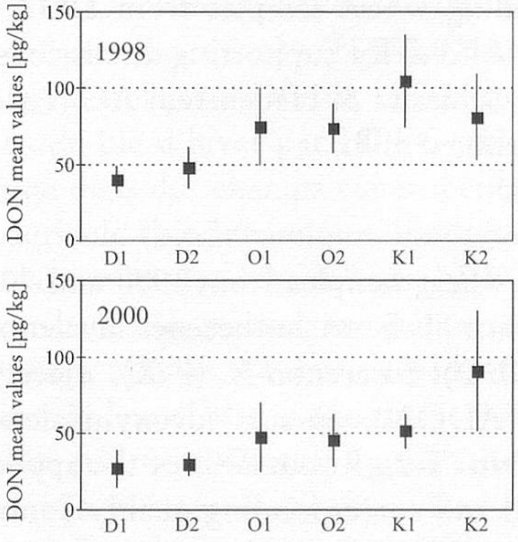

Figure 2 **Mean DON concentrations (n=4) and standard deviations (s_d) in wheat samples from the farming systems biodynamic (D), bioorganic (O) and conventional (K) at two different fertilizer intensities (1 and 2) for 1998 and 2000**

120 µg/kg). Moreover, *Döll et al.* (15) found in 23 % of the samples of conventionally grown wheat more than 1 mg/kg DON compared to 9 % in organically grown wheat. As in our study, the data from the conventional farming system were more scattered.

The literature comparing mycotoxin content in wheat from organic and conventional farming contains quite contradictory information. An investigation in South Germany (16) could not find any difference (occurrence in 88 % and 76 % of all samples and mean contents of 420 µg/kg and 486 µg/kg, respectively). *Malmauret et al.* (17) found significantly higher concentrations of DON in organically (median 106 µg/kg) than in conventionally grown wheat (median 55 µg/kg) from France. Additionally, they detected NIV, HT-2 and 3-ADON in organically grown wheat only (medians 10 µg/kg, 50 µg/kg and 10 µg/kg, respectively). Our study revealed more samples with NIV and HT-2 in conventionally farmed wheat in 1998. However, this was not the case for NIV in 2000.

In conclusion, for both years a tendency was observed of lower levels of DON in biodynamic wheat compared to bioorganic and conventional farming methods. However, the statistical significance could not be proven at a high enough confidence level. The low, rather scattered levels found in most samples would require at least one order of magnitude more samples to draw any conclusion, which is beyond any cost frame when applying HPLC-MS. Nevertheless, these results indicate that the presence and levels of (selected) trichothecenes may not be suited as marker to differentiate farming systems. Moreover, no difference could be observed between the two fertilizer intensities in both investigations.

Acknowledgement

The Research Institute of Organic Agriculture (FiBL, Frick, Switzerland) is acknowledged for providing wheat samples from DOK trial in 1998 and 2000. Many thanks to Thomas Alföldi for supporting discussions and explanations about the DOK trial as well as Schieritz & Hauenstein AG (Arlesheim, Switzerland) for the use of their ultracentrifugal mill.

Summary

A total of 48 winter wheat samples from 1998 and 2000 were investigated by HPLC-MS for the presence of the trichothecenes nivalenol (NIV), deoxynivalenol (DON), neosolaniol (NEO), fusarenon-X (F-X), diacetoxyscirpenol (DAS), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), HT-2-toxin (HT-2) and T-2-toxin (T-2). Robustness of the applied technique under routine conditions was good and corresponding quality control information is given. The wheat samples were cultivated on a long-term field trial in Switzerland with three different farming systems (biodynamic, bioorganic and conventional). DON was detected in 100 %, NIV in 67 % and HT-2 in 25 % of all wheat samples. Only 48 % of the detectable DON concentrations were above the limit of quantification (LOQ, 50 µg/kg). The range varied between 10–206 µg/kg. NIV and HT-2 were

detected at concentrations around or below their LOQ (NIV: 100 µg/kg; HT-2: 10 µg/kg). Statistically significant differences between the three farming systems could not be found. However, there were some indications that wheat from organic farming had lower DON contaminations than that from conventional one.

Zusammenfassung

Insgesamt 48 Proben des Winterweizens aus den Jahren 1998 und 2000 wurden mittels HPLC-MS auf die Anwesenheit der Trichothecene Nivalenol (NIV), Deoxynivalenol (DON), Neosolaniol (NEO), Fusarenon-X (F-X), Diacetoxyscirpenol (DAS), 3-Acetyldeoxynivalenol (3-ADON), 15-Acetyldeoxynivalenol (15-ADON), HT-2-toxin (HT-2) und T-2-toxin (T-2) untersucht. Die Robustheit der Methode unter Routinebedingungen war gut und entsprechende Qualitätskontrollinformation wird gegeben. Die Weizenproben wurden innerhalb eines Langzeit-Feldversuches in der Schweiz mittels drei verschiedenen Anbausystemen kultiviert. DON wurde in 100 %, NIV in 67 % und HT-2 in 25 % aller Weizenproben detektiert. Nur 48 % der detektierbaren DON-Konzentrationen waren oberhalb der Quantifizierungsgrenze (LOQ, 50 µg/kg). Die ermittelten Werte variierten zwischen 10–206 µg/kg. Die Konzentrationen von NIV und HT-2 lagen im Bereich ihrer LOQ (NIV: 100 µg/kg; HT-2: 10 µg/kg) oder darunter. Statistisch signifikante Unterschiede der drei Anbausysteme konnten nicht gefunden werden. Allerdings gab es Hinweise darauf, dass Weizen aus organischem Anbau niedrigere DON-Kontaminationen aufweist als aus konventionellem Anbau.

Résumé

Plusieurs échantillonnages réalisés entre 1998 et 2000 ont permis l'analyse des trichothécènes nivalénol (NIV), déoxynivalénol (DON), néosolaniol (NEO), fusarénone-X (F-X), diacétoxyscirpénol (DAS), 3-acétyldéoxynivalénol (3-ADON), 15-acétyldéoxynivalénol (15-ADON), HT-2-toxine (HT-2) et T-2-toxine (T-2) dans 48 échantillons de blé d'hiver par HPLC-MS. Les échantillons de blé furent cultivés à long terme dans des champs expérimentaux selon trois différents systèmes d'exploitation agricole (biodynamique, bioorganique et conventionnel). DON a été détecté dans la totalité des échantillons, NIV dans 67 % et HT-2 dans 25 %. Seules 48 % des concentrations détectées étaient au-dessus de la limite de quantification (LQ, 50 µg/kg). NIV et HT-2 ont été identifiés à des concentrations de l'ordre ou inférieures à leurs LQ respectives (NIV: 100 µg/kg; HT-2: 100 µg/kg). Statistiquement, aucune différence significative entre les systèmes d'exploitation cités ne fut observée. Néanmoins, il apparaît tout de même que les taux de contamination des blés issus de cultures organiques restent inférieurs à ceux du blé issus de cultures conventionnelles.

Key words

Trichothecenes, conventional farming, organic farming, wheat, deoxynivalenol

References

1 *Placinta C.M., D'Mello J.P.F. and Macdonald A.M.C.:* A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim. Feed Sci. Technol. **78**, 21–37 (1999)

2 *Grove J.F.:* Macrocyclic Trichothecenes. Nat. Prod. Rep. **10**, 429–448 (1993)

3 *Prelusky D.B., Rotter B.A. and Rotter R.G.:* Toxicology of mycotoxins, in Mycotoxins in grain: Compounds other than aflatoxins, 359–403, J.D. Miller and H.L. Trenholm, Editors, Eagan Press: St. Paul, MN, USA, (1994)

4 *Bucheli B., Diserens P., Rychener M., Tieche J.-D. und Trenkner N.:* Untersuchungen zum Fusarienbefall und zur Mykotoxinbelastung des schweizerischen Brotgetreides der Ernten 1992–1994. Mitt. Lebensm. Hyg. **87**, 84–102 (1996)

5 *Besson J.-M. und Niggli U.:* DOK-Versuch: Vergleichende Langzeit-Untersuchungen in drei Anbausystemen Biologisch-dynamisch, Organisch-biologisch und Konventionell. Schweiz. Landw. Fo. **31**(3), 79–109 (1991)

6 *Berger U., Oehme M. and Kuhn F.:* Quantitative determination and structure elucidation of type A- and B-trichothecenes by HPLC/ion trap multiple mass spectrometry. J. Agric. Food Chem. **47**(10), 4240–4245 (1999)

7 *Berger U.:* Structure elucidation and quantification of trichothecenes and bisphenol diglycidyl ether derivatives in food by HPLC combined with multiple mass spectrometry, PhD thesis, 29, University of Basel, Basel, Switzerland, 2000

8 *Noser J.R., Wenk P. und Sutter A.:* Deoxynivalenol, Zearalenon und Ochratoxin A in Weizen aus dem Kanton Basel-Landschaft. Mitt. Lebensm. Hyg. **87**, 574–586 (1996)

9 *Berleth B., Backes F. und Krämer J.:* Schimmelpilzspektrum und Mykotoxine (Deoxynivalenol und Ochratoxin A) in Getreideproben aus ökologischem und integriertem Anbau. Agribiol. Res. **51**(4), 369–376 (1999)

10 *Schollenberger M., Jara H.T., Suchy S., Drochner W. and Müller H.-M.: Fusarium* toxins in wheat flour collected in an area in southwest Germany. Internat. J. Food Microbiol. **72**(1–2), 85–89 (2002)

11 *Park J.J., Smalley E.B. and Chu F.S.:* Natural occurence of *Fusarium* mycotoxins in field samples from the 1992 Wisconsin corn crop. Appl. Environ. Microbiol. **62**(5), 1642–1648 (1996)

12 *Müller H.-M., Reimann J., Schumacher U. and Schwadorf K.:* Natural occurence of *Fusarium* toxins in barley harvested during five years in an area of southwest Germany. Mycopathologia **137**, 185–192 (1997)

13 *Müller H.-M., Reimann J., Schumacher U. and Schwadorf K.: Fusarium* toxins in wheat harvested during six years in an area of southwest Germany. Nat. Toxins **5**, 24–30 (1997)

14 *Schollenberger M., Suchy S., Jara H.T., Drochner W. and Müller H.-M.:* A survey of *Fusarium* toxins in cereal-based foods marketed in an area of southwest Germany. Mycopathologia **147**, 49–57 (1999)

15 *Döll S., Valenta H., Dänicke S. and Flachowsky G.: Fusarium* mycotoxins in conventionally and organically grown grain from Thuringia/Germany. Landbauforschung Völkenrode. **2**(52), 91–96 (2002)

16 *Marx H., Gedek B. und Kollarczik B.:* Vergleichende Untersuchungen zum mykotoxikologischen Status von ökologisch und konventionell angebautem Getreide. Z. Lebensm. Unters. Forsch. **201**, 83–86 (1995)

17 *Malmauret L., Parent-Massin D., Hardy J.-L. and Verger P.:* Contaminants in organic and conventional foodstuffs in France. Food Addit. Contam. **19**(6), 524–532 (2002)

18 *Mäder P., Fliessbach A., Dubois D., Gunst L., Fried P. and Niggli U.:* Soil fertility and biodiversity in organic farming. Science **296**, 1694–1697 (2002)

19 *Lufthygieneamt beider Basel,* Liestal, Schweiz. Jahresdaten, Luftqualität und Meteorologie, Jahresrückblick (1998+2000)

Corresponding author: Prof. Dr. Michael Oehme, University of Basel, Neuhausstrasse 31, 4057 Basel, Switzerland, E-mail: michael.oehme@unibas.ch