Vol. 13 (2004): 54–67.

Contents of trichothecenes in oats during official variety, organic cultivation and nitrogen fertilization trials in Finland

Veli Hietaniemi
MTT Agrifood Research Finland, Chemistry laboratory, FIN-31600 Jokioinen, Finland,
e-mail: veli.hietaniemi@mtt.fi

Markku Kontturi
MTT Agrifood Research Finland, Plant Production Research, FIN-31600 Jokioinen, Finland

Sari Rämö, Merja Eurola
MTT Agrifood Research Finland, Chemistry Laboratory, FIN-31600 Jokioinen, Finland

Arjo Kangas, Markku Niskanen
MTT Agrifood Research Finland, South Ostrobothnia Research Station, FIN-31600 Jokioinen, Finland

Marketta Saastamoinen
Satafood Development Association, Risto Rytin katu 70 C, FIN-32700 Huittinen, Finland

Natural toxins, such as mycotoxins, have emerged as a significant factor affecting the safety image of cereal grains as a raw material for the food and feed industry. The aim of the present study was to investigate the contents of trichothecenes in representative samples of oats during official variety, nitrogen fertilization and organic farming trials in Finland, 1997–1999. Further objectives were to promote industry and commerce by selection of high-quality oat varieties for various applications. The official variety trials conducted at 8–10 locations were managed following standard protocol. There were 2 types of agronomy trial, the first included comparison of oat cultivars grown in conventional and organic farming systems at 6 locations, and the second used 5 nitrogen rates (0, 40, 80, 120 and 160 kg N ha^{-1}) at 2 locations. Regardless of wet cold summer occurring in Finland during 1998, the concentrations of *Fusarium* toxins were lowest during this 3 year monitoring period. More mycotoxins were produced during the warm, dry summers of 1997 and 1999 than in 1998. In all, 55% of the oat samples in the official variety trials contained deoxynivalenol (DON) within the range of 50–896 µg kg^{-1}. The differences in DON concentrations between organic and conventional cultivation were small. The results showed also that the use of various nitrogen fertilization levels only slightly affected the trichothecene concentrations. The contents of trichothecenes in Finnish grains appeared to be similar to or lower than those reported earlier in the Northern Hemisphere.

Key words: oats, grains, mycotoxins, trichothecenes, *Fusarium* toxins, official variety trials, nitrogen, organic farming, cultivars

Manuscript received July 2003

Vol. 13 (2004): 54–67.

Introduction

The central goal of grain cultivation is the production of high-quality food or feed-related raw materials for the processing industry. Natural toxins, such as mycotoxins, have emerged as a significant factor affecting the safety image of cereal grains as a raw material for the food and feed industry. Many previous studies in Finland (Karppanen et al. 1985, Hietaniemi and Kumpulainen 1991, 1993, Rizzo 1993, Eskola et al. 2001, Rizzo et al. 2001) and other European countries (Tanaka et al. 1988, Langseth et al. 1989, 1999, Müller and Schwadorf 1993, Pettersson et al. 1995, Rizzo et al. 2001, Widestrand 2001, Döll et al. 2002, Schollenberger et al. 2002) as well as globally (Tanaka et al. 1988, Shephard et al. 1996, Groves et al. 1999, Janardhana et al. 1999, Salay and Mercadante 2002) have shown that there is reason to focus on *Fusarium* toxins and their appearance. A planned European Union directive will specify the maximum limits for trichothecenes such as 4-deoxynivalenol (DON), T-2 toxin and HT-2 toxin and for zearalenone (ZEN) and fumonisins. DON is the most frequently found contaminant of oats, barley, wheat and corn throughout the world (Scott 1989, WHO 1993, 2001). In addition to DON, T-2 toxin, HT-2 toxin and ZEN frequently occur in cereal crops cultivated in northern temperate regions (Hietaniemi and Kumpulainen 1991, Pettersson et al. 1995, Langseth et al. 1999, Rizzo et al. 2001, Thuvander et al. 2001, Eskola 2002, Lígia Martins and Martins 2002). On the other hand, fumonisins cause more extensive problems in the Southern Hemisphere than in the Northern (Shephard et al. 1996). The most frequently isolated *Fusarium* species are *F. graminearum*, *F. culmorum*, *F. moniliforme*, *F. poae*, *F. equiseti* and *F. proliferatum* (Ylimäki et al. 1979, Marasas et al. 1984, Hietaniemi and Kumpulainen 1991, Eriksen and Alexander 1998, Eskola et al. 2001, Creppy 2002).

Numerous studies on mould toxicoses have shown that type A trichothecenes such as T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS) and neosolaniol are more acutely toxic, whereas type B trichothecenes such as DON and nivalenol (NIV) are less toxic and are therefore implicated in more chronic toxicoses (Atroshi et al. 2002, Creppy 2002, Gutleb et al. 2002, Vilà et al. 2002, de Vries et al. 2003, Sudakin 2003). For example, the most frequently found of these substances (DON) in cereal crops is known to affect symptoms of intoxication in humans such as abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhoea, and blood in the stool (Eriksen and Alexander 1998). In the case of intoxication in animals, symptoms such as loss of appetite, reduced weight gain, vomiting, heavy diarrhoea and swelling have been reported (Joffe 1978, Ueno 1987). In addition, DON is a very stable compound occurring during both storage and processing of food and does not degrade at high temperatures (Scott 1991).

Increased attention has been focused on mycotoxins in Finland, especially the late 1980s, Hietaniemi and Kumpulainen (1991) showed that almost all grain and feed samples studied in Finland during 1987–1988 contained from 7 to 300 μg kg^{-1} of DON and smaller amounts (13–120 μg kg^{-1}) of 3-acetyldeoxynivalenol. The most toxic trichothecenes, T-2- and HT-2 toxin, as well as NIV and ZEN, were found at low concentrations in some samples. Based on the results of Rizzo (1993) trichothecene concentrations in Finnish grains (n = 292) have ranged 1–6300 μg kg^{-1} for DON, 1–1000 μg kg^{-1} for NIV and 5–238 μg kg^{-1} for T-2. In the study of Eskola et al. (2002) DON was detected in 54 of 68 Finnish cereal samples in the concentration range 5–111 μg kg^{-1}. NIV and HT-2 toxin were detected in 3 and 2 samples, respectively, in the concentration range 10–20 μg kg^{-1}. In addition, Eskola et al. reported the contents of *Fusarium* toxins in 47 cereal grain samples collected in different parts of the country. The mean concentrations of DON, 3-AcDON, NIV, HT-2 and ZEN in contaminated samples were 60, 30, 42, 43 and 5 μg kg^{-1}, respectively. T-2 was detected only once (23 μg kg^{-1}), while fusarenon X (FX) and diacetoxyscirpenol (DAS) were not detected.

There are differences in susceptibility to *Fusarium* contamination amongst the various types of grain. Oats are regarded as being more susceptible to *Fusarium* fungi in Nordic countries, although studies done in Norway (Langseth et al. 2001) suggest that contamination in barley and wheat occurs just as frequently. The species prevalent here and in the other Nordic countries is *F. avenaceum,* which does not produce DON and becomes especially abundant during rainy, cool summers, such as in 1998. According to Marasas et al. (1984) and Eriksen and Alexander (1998) high temperatures during the growing season favour significant toxin formulators; in particular, *F. culmorum, F. graminearum* and *F. poae* proliferate in warm temperatures, which was quite clearly found in Finland in the year 2001 and 2002. A belated harvest increases the growth of *Fusarium* fungi and toxin-related risk along with the rise in moisture. *Fusarium* fungi form toxins during the growing season, in warm-air drying, the fungi are preserved in grain, but trichothecene compounds no longer form under dry storage.

The aim of the present study during 1998–2000 was to investigate the contents of trichothecenes in representative samples of oats during official variety, nitrogen fertilization and organic farming trials in Finland, 1997–1999. Further objectives were to enhance producers procedures for growing and harvesting oats and promote industry and commerce by selection of high-quality oat varieties for various applications.

Material and methods

Samples

The oat samples were collected after harvest during official and agronomy trials conducted by MTT Agrifood Research Finland in 1997–1999. The official variety trials conducted at 8–10 locations were managed following standard protocol (Fig. 1). There were 2 types of agronomy trial, the first included comparison of oat cultivars grown in conventional and organic farming systems at 6 locations, and the second used 5 nitrogen rates (0, 40, 80, 120 and 160 kg N ha^{-1}) with 4 oat cultivars at 2 locations (Fig. 1). More detailed information on the trials was published previously (Järvi et al. 2000, Eurola et al. 2003). After harvest, the grains were immediately dried with warm air in a flat bed grain drier to a moisture content of below 14%. The oat grains were sorted with a 2.0-mm sieve and hulled with a laboratory hulling machine BT 459 using air pressure. Oat groats were milled with a falling number hammer mill using a 1.0-mm sieve. The total number of oat samples analysed were 147, 147 and 99 in 1997, 1998 and 1999, respectively.

The varieties studied were Leila, Kolbu, Salo, Belinda, Veli, Roope, Aarre, Katri, Puhti and Yty. Kolbu and Roope have yellow husks, and the other varieties have white husks. Leila and Kolbu are cultivars developed in Norway, Salo and

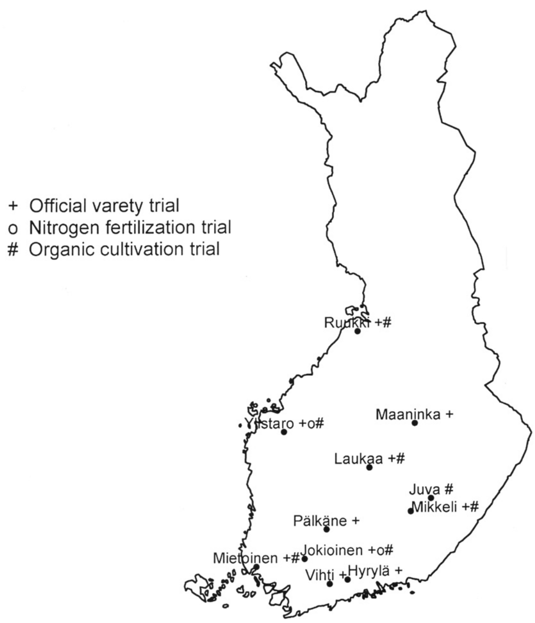


Fig. 1. Location of the trial sites in Finland.

Vol. 13 (2004): 54–67.

Belinda in Sweden, and Veli, Roope, Aarre, Katri, Puhti and Yty in Finland. The oat varieties selected for the project included the most popular cultivars as well as new varieties on the National list of cultivars in Finland.

For example, the variety Veli was the most widely cultivated variety of oat in Finland from 1997 to 1999. Veli gives high quantities, is early and possesses good quality. Cultivation-related reliability is favourable and the grain is white-hulled, mid-sized and rather thin-hulled. The protein content and the weight of the grain are high. Veli is a thriving variety, reliable under cultivation, that also produces good harvests during poor years. Veli is suitable as an oat for feed, food products and export. The year of National variety catalogue entry for this type, which was developed in Finland by Boreal Plant Breeding Ltd, was 1982.

On the other hand, Salo is a variety developed in Sweden by the Svalöf Weibull Company and emerged on the market in 1989. It has good lodging resistance and was the second most widely cultivated variety of oat in Finland from 1997 to 1999. Salo is a late variety with large grain size, low hull content and pure resistance against dryness in addition to being susceptible to oat leaf blotch. Due to its short, durable stem, Salo withstands 10–20 kg ha^{-1} of stronger nitrogen fertilization than normal. The main use of Salo has been as a food product.

Analytical methods

Sample clean-up, identification and quantification

To determine the presence of mycotoxins, the ground-up samples (25 g) were extracted through a blend of acetonitrile-water (84:16) and then suction-filtered. Part of the filtrate (7.5 ml) was extracted for activated carbon cleaning (MycoSep#227, Romer Labs), after which the sample was transferred to a silylated test tube and evaporated to dryness under a stream of nitrogen. 100 µl N-trimethylsilylimidazole (TMSI) reagent was added to the residue evaporated to dryness. DON, DAS, 3-acetyldeoxynivalenol (3-AcDON), FX, NIV, T-2 and HT-2 toxins and 19-nortestosterone (the internal standard) were identified and quantified as trimethylsilylether derivatives, employing GC-MS (Rizzo et al. 1986, Hietaniemi and Kumpulainen 1991, Saastamoinen and Saloniemi 1997).

Quality assurance

The reliability of the GC-MS method in trichothecene analysis was examined in repeatability, reproducibility, recovery and intercalibration studies by specifying the linearity of the method, the minimum detectable concentrations (quantification limit), utilizing certified reference materials and participating in FAPAS round tests. The recoveries for the compounds varied between 70–100% and the repeatability between 5% and 25%, while the minimum detectable concentrations were 50 µg kg^{-1} with all trichothecenes. The various linearity areas with respect to trichothecenes were as follows: DON (25–8000 µg kg^{-1}), DAS (25–500 µg kg^{-1}), 3-AcDON (25–800 µg kg^{-1}), FX (25–250 µg kg^{-1}), NIV (25–400 µg kg^{-1}), T-2 (25–600 µg kg^{-1}) and HT-2 (25–1300 µg kg^{-1}). BCR wheat flour, CRM 379 was employed as the reference material (DON concentration 670 µg kg^{-1}). The Chemistry Laboratory of Agrifood Research Finland follows a quality control system in accordance with ISO 17025. In the case of samples whose concentration level was below the limit of quantification, the value reported in the mean value, median and standard deviation calculations was estimated as 50% of the quantification limit.

For statistical analyses Data Desk 6.1.1 (Data exploration and visualization) was used (Velleman and Hoaglin 1981).

Results and discussion

Official variety trials

The results of the official variety trials showed that the mycotoxin DON was found most frequently in Finnish oats during 1997–1999. In 1997, the procent of positive toxin findings out of all oat samples studied (51) were as follows: DON 69%, DAS not detected, 3-AcDON 6%, FX not detected, NIV 14%, T-2 toxin 4% and HT-2 toxin 4%. The mean DON concentration of all samples was 222 μg kg^{-1} (median 79 μg kg^{-1}, standard deviation 206 μg kg^{-1}). A few individual, rather high contents of NIV were found within a range of < 50–575 μg kg^{-1}. The mean T-2- and HT-2 toxin concentrations were below the quantification limit of 50 μg kg^{-1}. The highest mean DON concentrations were determined in the areas of Hyrylä, Pälkäne, Mikkeli and Laukaa (Fig. 1). The mean contents of DON, amount of precipitation, effective temperature sum values, and pH and the type of soil as obtained for May – August are presented in Table 1. None of the above-mentioned background factors did not explain directly the higher toxin concentrations in these areas. The amounts of precipitation and effective temperature sums were similar to the average values observed in other areas, and not even the soil types could indicate a direct connection with elevated toxin concentrations (Table 1). The varieties, Leila, Salo, Veli, Belinda, Kolbu and Roope, were employed in the official trials. Figure 2 shows DON contents of six oat

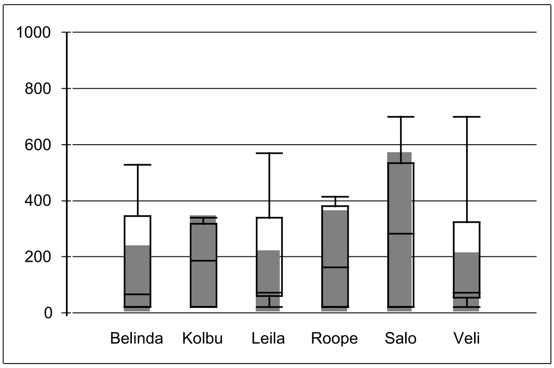
Table 1. Precipitation, effective temperature sum, soil pH, soil type and mean deoxynivalenol (DON) content of oats in official variety trials during 1997–1999 at different trial locations in Finland.

Location	Year	Precipitation May-Augmm	Effective temperature sum	Soil pH	Soil type	Mean DON content μgkg^{-1}
Jokioinen	1997	302	1217	6.3	sandy clay	64
	1998	318	1011	5.8	clay	< 50
	1999	146	1184	5.7	sandy clay	243
Mietoinen 1	1997	232	1285	5.3	clay	87
	1998	245	1036	5.9	sandy clay	97
	1999	92	1236	5.8	sandy clay	262
Hyrylä	1997	not available	not available	6.3	sandy clay loam	630
	1998	not available	not available	6.0	coarse silt	114
Pälkäne	1997	253	1250	5.7	fine silt	334
	1998	339	1043	6.0	fine silt	< 50
	1999	141	1231	5.9	fine silt	184
Mikkeli/Juva	1997	115	1150	6.9	fine sand	272
	1998	337	976	6.0	fine sand	< 50
	1999	243	1133	6.0	fine sand	< 50
Maaninka	1997	177	1153	5.8	coarse silt	233
	1998	340	938	6.1	coarse silt	165
	1999	183	1134	5.5	coarse silt	286
Laukaa	1997	182	1146	6.0	coarse silt	435
	1998	345	916	6.0	coarse silt	419
	1999	175	1112	6.0	coarse silt	88
Ylistaro 1	1997	155	1143	6.0	sandy clay loam	< 50
	1998	372	937	6.2	silty clay	< 50
	1999	120	1062	6.1	silty clay	< 50
Ylistaro 2	1997	155	1143	5.3	mould	< 50
	1998	372	937	5.7	mould	< 50
	1999	120	1062	5.5	mould	82
Ruukki 1	1999	182	952	5.4	coarse silt	102
Ruukki 2	1999	182	952	5.4	mould	< 50
Vihti	1998	389	997	5.9	coarse silt	70
	1999	132	1173	6.2	clay	260

Vol. 13 (2004): 54–67.

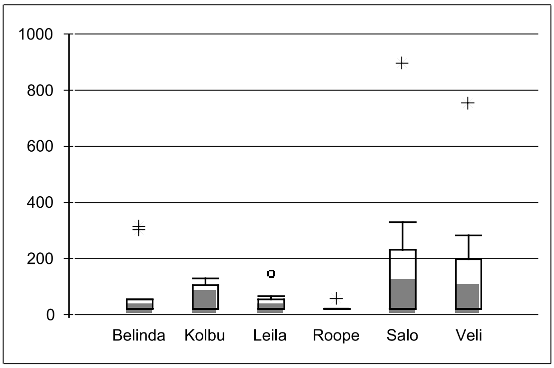
DON contents [μg/kg] of six oat cultivars in 1997

Oat cultivar	*Count*	*Mean*	*Median*	*StdDev*	*Min*	*Max*
Belinda	9	207	73	205	25	534
Kolbu	8	178	189	151	25	345
Leila	9	219	75	211	25	573
Roope	8	193	170	183	25	419
Salo	8	297	290	252	25	706
Veli	9	237	79	252	25	705



DON contents [μg/kg] of six oat cultivars in 1998

Oat cultivar	*Count*	*Mean*	*Median*	*StdDev*	*Min*	*Max*
Belinda	10	85	25	118	25	312
Kolbu	5	64	25	54	25	132
Leila	10	45	25	39	25	146
Roope	7	29	25	12	25	56
Salo	10	172	25	277	25	896
Veli	10	157	25	232	25	756



DON contents [μg/kg] of six oat cultivars in 1999

Oat cultivar	*Count*	*Mean*	*Median*	*StdDev*	*Min*	*Max*
Belinda	6	104	62	104	25	292
Kolbu	8	172	67	233	25	660
Leila	13	172	91	168	25	510
Roope	6	157	167	86	25	287
Salo	13	84	25	88	25	250
Veli	13	215	143	185	25	494

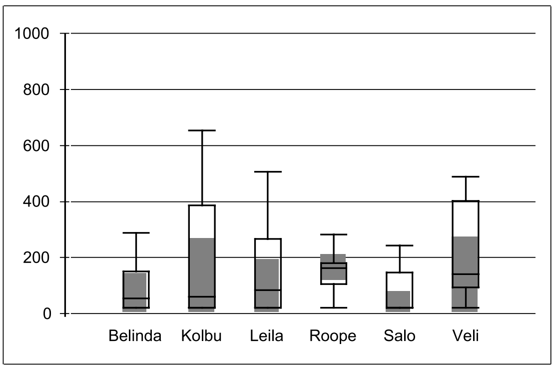


Fig. 2. Deoxynivalenol (DON) contents of six oats cultivars across all sites of official variety trials during 1997–1999 in Finland.

cultivars across all sites of official variety trials during 1997–1999. The highest individual DON concentrations were found in the varieties Veli and Salo. According to the statistical analyses no distinct differences were found in DON contents of various varieties. It is most likely that the climate had the greatest impact by fixing the time of heavy rainfalls in the right period of the growing season, for example during heading and harvesting time.

During these 3 research years, the lowest mycotoxin concentrations were determined from the oat harvest of 1998 (Table 2). The mean DON content from the 51 samples studied was 99 µg kg^{-1} (median < 50 µg kg^{-1}, standard deviation 171 µg kg^{-1}). Other toxins were found in only a few samples as small concentrations, e.g., no T-2 toxin content exceeding the quantification limit was found and only one HT-2 toxin concentration in excess of the quantification limit was determined. Summer 1998 was cool and rainy throughout the Finland. Despite this, conditions for the formation of toxins were not favourable. Nevertheless, fusarium head blight visibly appeared in Finnish grain, especially in rye. In the investigations of Eskola et al. (2000 and 2001) a lot of mouldiness of the Finnish grain in 1998 was found. Despite of the heavy contamination of the moulds, contents of trichothecenes were low. The findings in 1998 also indicated that Veli and Salo had the highest individual DON concentrations. The highest DON concentrations were detected in Laukaa, central Finland, where rainfall was also high and the effective temperature sum was low. However, similar amounts of rain were also observed in other areas, and there was no association with elevated toxin concentrations within the same area (Table 1).

As a research year 1999 was quite similar to 1997 (Table 2). In several areas, 1999 was clearly less subject to rain, but the effective temperature sums were similar to those in 1997. The average DON concentrations for the oat samples were, 154 µg kg^{-1}, and the number of positive findings out of the 59 samples studied was 37. The largest DON concentrations were detected in the variety Veli. As in 1998, no positive T-2 toxin results were measured, but 4 positive HT-2 toxin contents were found, the highest value being 240 µg kg^{-1}. NIV was found from 14 samples out of a total number of 59, with the concentrations ranging < 50–423 µg kg^{-1}. From the regional stand point, the most contaminated oat samples were found in southern, southwestern and eastern Finland (Fig. 1). The most precipitation occurred in eastern Finland within a range of 183–243 mm, and the least was measured in southwestern Finland at 92 mm. The year 1999 also indicated that a direct connection between the amounts of rain, effective temperature sums and contents of mycotoxins does not exist.

It still appears more probable that the 'right' period of rainfall during the growing season exerts greater significance. For example, if heavy rainfalls occurred during heading and harvesting time, the risk of growth of *Fusarium* fungi and production of toxins would exist. Oldenburg et al. (2000) studied the factors which influence most the 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. Langseth et al. (2001) reported from Norway that the DON level was especially high in 1988 and 1992, years with spring drought and much precipitation in July in the period during and after anthesis. According to McMullen et al. (1997) during the last two decades fusarium head blight disease occurs especially during rainy flowering seasons of the crop and besides of DON, an infection of the head by *Fusarium* can lead to severe yield loss and reduced kernel quality. On the other hand, rains occurring at harvest time may increase the risk of appearance of mycotoxins considerably. However, warm-air-drying as primarily employed in Finland is evidently a security-enhancing factor with respect to grain (Jayas and White 2003).

Nitrogen fertilization trial

Nitrogen fertilization trials at five nitrogen levels 0, 40, 80, 120 and 160 kg N ha^{-1} were imple-

Vol. 13 (2004): 54–67.

Table 2. Mean contents of trichothecenes in oats during official variety trials in Finland, 1997-1999.

		Number of samples	Range µgkg^{-1}	Mean µgkg^{-1}	Median µgkg^{-1}	StdDev µgkg^{-1}	Positive [%]
1997	DON	51	< 50–706	222	79	206	69
	DAS	51	Not detected				
	3-AcDON	51	< 50–219	< 50	< 50	41	6
	F-X	51	Not detected				
	NIV	51	< 50–575	74	< 50	129	14
	T-2	51	< 50–349	< 50	< 50	62	4
	HT-2	51	< 50–507	< 50	< 50	85	4
1998	DON	52	< 50–896	99	< 50	171	33
	DAS	52	Not detected				
	3-AcDON	52	< 50–310	< 50	< 50	50	10
	F-X	52	Not detected				
	NIV	52	< 50–530	53	< 50	95	10
	T-2	52	Not detected				
	HT-2	52	< 50–116				2
1999	DON	59	< 50–660	154	108	158	63
	DAS	59	Not detected				
	3-AcDON	59	Not detected				
	F-X	59	Not detected				
	NIV	59	< 50–423	< 50	< 50	63	24
	T-2	59	Not detected				
	HT-2	59	< 50–240	< 50	< 50	< 50	7

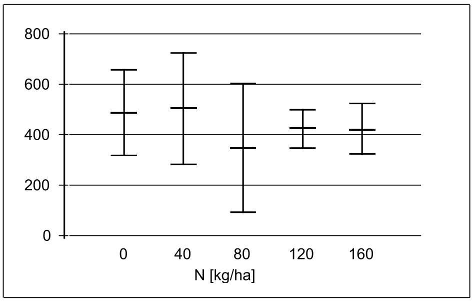
mented. Aarre, Katri, Kolbu and Salo were the varieties used in the trials, which were carried out in 2 locations: in Jokioinen, province of Häme and Ylistaro, South Ostrobothnia (Fig. 1). The results showed that the use of various nitrogen fertilization levels during 1997–1999 only slightly affected the mycotoxin concentrations (Fig. 3). The highest mean DON concentrations were observed in the data from 1999 within a range of 101–730 µg kg^{-1} (Fig. 3). The DON concentrations in Häme (Jokioinen) were, to some extent, higher than those in South Ostrobothnia (Ylistaro), although the amounts of rainfall were similar. No remarkable differences between the various varieties were found; the highest individual concentrations were determined from the variety Aarre (Fig. 3). Aarre is an early, high-yielding and high-quality oat variety and in addition has good lodging resistance. It is earlier than Veli by one day. Aarre is used for feed and food products and export and it is developed by Boreal Plant Breeding Ltd of Finland. It was introduced in the National variety catalogue in 1995.

Organic farming

The trials for conventional as well as organic cultivation were implemented during 1997–1998 at 6 locations: Jokioinen, Mietoinen, Laukaa, Partala, Ylistaro and Ruukki. Only at the Partala Research Station in eastern Finland was conventional cultivation not used as comparative data. More precise comparison of these cultivation methods could be carried out only with 2 varieties, Veli and Puhti, although 5 other varieties Aarre, Katri, Kolbu, Leila and Roope were also included in the organic cultivation trials. In contrast to the results obtained in the official variety trials and nitrogen fertilization trials, DON was also the most commonly appearing myco-

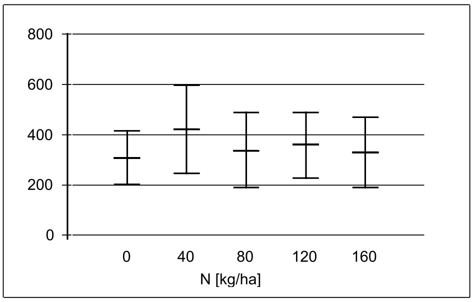
Mean DON contents [µg/kg] at five nitrogen levels in oats cultivar Aarre

N level [kg/ha]	*Count*	*Mean*	*Min*	*Max*
0	2	492	322	662
40	2	510	289	730
80	2	354	101	607
120	2	432	354	509
160	2	428	328	528



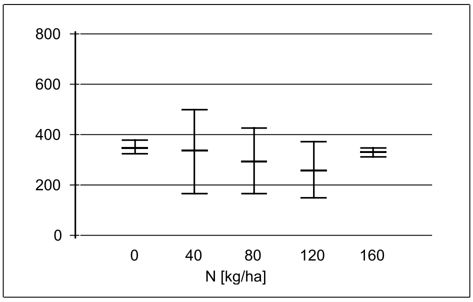
Mean DON contents [µg/kg] at five nitrogen levels in oats cultivar Katri

N level [kg/ha]	*Count*	*Mean*	*Min*	*Max*
0	2	314	207	421
40	2	429	252	606
80	2	344	194	493
120	2	364	232	496
160	2	335	194	476



Mean DON contents [µg/kg] at five nitrogen levels in oats cultivar Kolbu

N level [kg/ha]	*Count*	*Mean*	*Min*	*Max*
0	2	358	328	387
40	2	340	174	505
80	2	302	171	433
120	2	265	152	378
160	2	337	319	355



Mean DON contents [µg/kg] at five nitrogen levels in oats cultivar Salo

N level [kg/ha]	*Count*	*Mean*	*Min*	*Max*
0	2	292	210	373
40	2	447	431	463
80	2	293	183	402
120	2	245	181	308
160	2	192	161	222

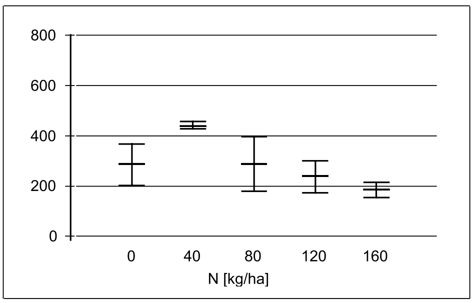
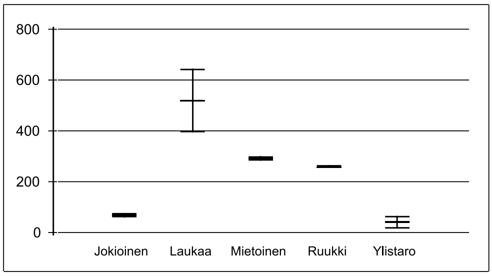


Fig. 3. Mean deoxynivalenol (DON) contents at five nitrogen levels in nitrogen fertilization trials in 1999 in Finland at two sites (Jokioinen and Ylistaro).

Vol. 13 (2004): 54–67.

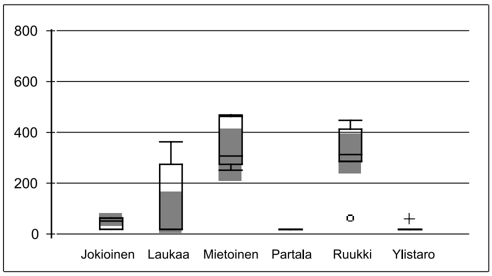
DON contents [µg/kg] in conventionally cultivated oats in 1997

Location	*Count*	*Mean*	*Median*	*StdDev*	*Min*	*Max*
Jokioinen	2	75			68	81
Laukaa	2	526			403	648
Mietoinen	2	295			289	300
Partala *	0	•			∞	-∞
Ruukki	2	266			264	268
Ylistaro	2	47			25	69



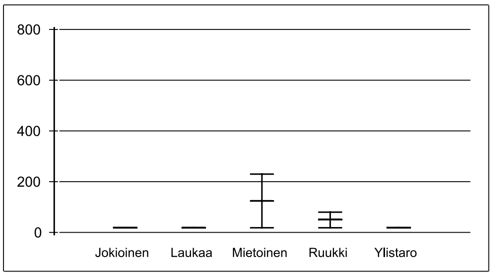
DON contents [µg/kg] in organically cultivated oats in 1997

Location	*Count*	*Mean*	*Median*	*StdDev*	*Min*	*Max*
Jokioinen	8	50	61	21	25	69
Laukaa	8	100	25	141	25	370
Mietoinen	8	345	316	81	258	474
Partala	7	25	25	0	25	25
Ruukki	7	315	318	127	66	455
Ylistaro	8	30	25	14	25	63



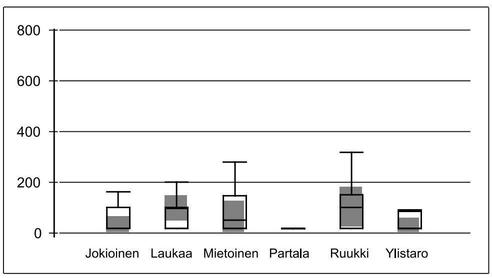
DON contents [µg/kg] in conventionally cultivated oats in 1998

Location	*Count*	*Mean*	*Median*	*StdDev*	*Min*	*Max*
Jokioinen	2	25			25	25
Laukaa	2	25			25	25
Mietoinen	2	131			25	237
Partala *	0	•			∞	-∞
Ruukki	2	57			25	88
Ylistaro	2	25			25	25



DON contents [µg/kg] in organically cultivated oats in 1998

Location	*Count*	*Mean*	*Median*	*StdDev*	*Min*	*Max*
Jokioinen	8	56	25	53	25	168
Laukaa	7	97	103	63	25	210
Mietoinen	8	97	59	94	25	286
Partala	7	25	25	0	25	25
Ruukki	7	124	108	101	25	324
Ylistaro	8	43	25	32	25	97



* The results of the conventional cultivation trials at Partala Research Station were not available.

Fig. 4. Mean deoxynivalenol (DON) contents in oats during organic and conventional cultivation trials in Finland in 1997 and 1998.

toxin in samples from the conventional and organic cultivation trials (Fig. 4). Correspondingly, the results corroborated previous indications that the toxin concentrations during the rainy, cold year of 1998 were clearly lower than in 1997. There were no large differences in DON concentrations in the cultivation methods studied. The mean levels appeared to be similar in organic and conventional cultivation (Fig. 4).

According to Leblanc et al. (2002) a particular group at risk for exposure to DON may be the consumers of organic products as they may regularly eat foods containing higher levels of DON than the general population. On the basis of the work of Schollenberger (2002) the DON content of flour samples originating from conventionally produced wheat was significantly higher than that of samples from organic production. Similar results were obtained by Döll et al. (2000) and Usleber et al. (2000), who found lower DON contents in wheat, rye and flour samples of organic origin than in those from conventional production. In agreement with this study, Marx et al. (1995) reported from southern Germany that the incidence of DON in conventionally and organically produced wheat was 88% and 76%, whereas the mean contents were 420 and 486 µg kg^{-1}, respectively. In addition, according to Döll et al. (2000) the organic farming system with the practice of a well-balanced crop rotation, tillage and fertilization showed benefits concerning the contamination with *Fusarium* toxins, especially DON.

Conclusions

The results of the official variety and nitrogen fertilization trials and comparison of conventional and organic cultivation, suggest that future research and follow-up on trichotecenes must be emphasized and continued. The results of the trials showed that the mycotoxin DON was found most frequently in Finnish oats during 1997–1999. According to the results on an average 55% of the oat samples respective to the official variety trials in 1997–1999 contained DON within the range of 50–896 µg kg^{-1}. Correspondingly, the range and frequencies of other toxin findings were as follows: 3-AcDON 50–310 µg kg^{-1} (5%), NIV 50–575 µg kg^{-1} (16%), T-2 toxin 50–349 µg kg^{-1} (1%) and HT-2 toxin 50–507 µg kg^{-1} (4%). A directive currently under preparation in the European Union will specify the tolerance limits for DON (1500 µg kg^{-1}). In comparison to previous studies, the contents of trichothecenes in grains appeared similar or lower to those reported earlier in the Northern or Southern Hemisphere (Karppanen et al. 1985, Tanaka et al. 1988, Hietaniemi and Kumpulainen 1991, Müller and Schwadorf 1993, Rizzo 1993, Pettersson et al. 1995, Groves et al. 1999, Janardhana et al. 1999, Döll et al. 2000, Langseth and Rundberget 2001, Rizzo et al. 2001, Eskola 2002, Schollenberger et al. 2002, Salay and Mercadante 2002). The results also showed that no distinct differences were found in DON contents of various varieties. The differences in DON concentrations between organic and conventional cultivation were small. In addition, the results showed that the use of various nitrogen fertilization levels only slightly affected the trichothecene concentrations.

Nevertheless, the importance of background factors with respect to samples is often forgotten in monitoring the quality of grains, not to mention a deeper familiarity with their impact and, through the same, better control over grain quality. Evidently, the incidence of rain during heading time represents a risk factor (McMullen et al. 1997, Döll et al. 2000, Oldenburg et al. 2000, Langseth et al. 2001), although it has not been indicated here. The present results also showed that more precise research into the effects of cultivation methods in relation to fungi and toxins is necessary (Norred 2000). Good familiarity with, the effects of the preceding crop, rotation, seed purity, various soil preparation methods, direct sowing and pesticides on the formation of mycotoxins is a minimal requirement. A question of its own right also concerns breeding for resistance against *Fusarium* fungi

Vol. 13 (2004): 54–67.

and their associated toxins (de Vries 2000, Hollins et al. 2003). Knowledge of these factors is a prerequisite for good cultivation-related directives that industry and farmers should follow to ensure high-quality production of oats both in Finland and internationally.

Acknowledgements. The authors wish to thank Ms. Kirsi Puisto, Ms. Leena Holkeri, Mr. Seppo Nummela and Mr. Tauno Koivisto for their skilful technical assistance and enthusiasm for this study. This study was financially supported by the Ministry of Agriculture and Forestry and the Finnish food and feed industry: Raisio Group, Suomen Viljava Ltd. and Kemira Agro Ltd.

References

Atroshi, F., Rizzo, A., Westermarck, T. & Ali-Vehmas, T. 2002. Antioxidant nutrients and mycotoxins. *Toxicology* 180: 151–167.

Creppy, E.E. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters* 127, 1–3: 19–28.

de Vries, G.E. 2000. New Canadian barley nursery. *Trends in Plant Science* 5, 11: 462.

de Vries, J.W., Trucksess, M.W. & Jackson, L.S. 2003. Mycotoxins and food safety. *Trends in Food Science & Technology* 14: 111–115.

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

Egmond, Van, H.P. 1989. Current situation on regulations of mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. *Food Additives and Contaminants* 6: 139–188.

Eriksen, G.S. & Alexander, J. (eds.). 1998. *Fusarium* toxins in cereals – a risk assessment. *TemaNord* 1998: 502. Nordic Council of Ministers. Copenhagen, Denmark. 146 p.

Eskola, M. 2002. Study on trichothecenes, zearalenone and ochratoxin A in Finnish cereals: occurrence and analytical techniques. *EELA publications* 3. National Veterinary and Food Research Institute (EELA) and University of Helsinki. Helsinki, Finland. 78 p. (Academic dissertation).

Eskola, M., Parikka, P. & Rizzo, A. 2001. Trichothecenes, ochratoxin A and zearalenone contamination and Fusarium infection in Finnish cereal samples in 1998. *Food Additives and Contaminants* 18: 707–718.

Eskola, M., Rizzo, A. & Soupas, L. 2000. Occurrence and amounts of some Fusarium toxins in Finnish cereal samples in 1998. *Acta Agriculturae Scandinavica., Sect. B, Soil and Plants Science* 50: 183–186.

Eurola, M., Hietaniemi, V., Kontturi, M., Tuuri, H., Pihlava, J.-M., Saastamoinen, M., Rantanen, O., Kangas, A. & Niskanen, M. 2003. Cadmium contents of oats (*Avena sativa* L.) in official variety, organic cultivation, and nitrogen fertilization trials during 1997–1999. *Journal of Agricultural and Food Chemistry* 51: 2608–2614.

Groves, F.D., Zhang, L., Chang, Y.-S., Ross, P.F., Casper, H., Norred, W.P., You, W.-C. & Fraumeni, J.F., Jr. 1999. Fusarium mycotoxins in corn and corn products in a high-risk area for gastric cancer in Shandong province, China. *Journal of AOAC International* 82, 3: 657–662.

Gutleb, A.C., Morrison, E. & Murk, A.J. 2002. Cytotoxicity assays for mycotoxins produced by *Fusarium* strains: a review. *Environmental Toxicology and Pharmacology* 11, 3–4: 309–320.

Hietaniemi, V. & Kumpulainen, J. 1991. Contents of *Fusarium* toxins in Finnish and imported grains and feeds. *Food Additives and Contaminants* 8, 2: 171–182.

Hietaniemi, V. & Kumpulainen, J. 1993. Mycotoxins in cereal grains and feeds in Finland. In: Tiina Aalto-Kaarlehto & Hannu Salovaara (eds.). Proceedings from the 25th Nordic Cereal Congress: The Nordic Cereal Industry in an Integrating Europe: June 6–9 1993, Helsinki. *EKT Series of the Food Science Programme, University of Helsinki* 930. p. 54–59.

Hollins, T.W., Ruckenbauer, P. & De Jong, H. Progress towards wheat varieties with resistance to fusarium head blight. *Food Control* 14, 4: 239–244.

Janardhana, G.R., Raveesha, K.A. & Shekar Shetty, H. 1999. Mycotoxin contamination of maize grains grown in Karnataka (India). *Food and Chemical Toxicology* 37, 8: 863–868.

Järvi, A., Kangas, A., Laine, A., Niskanen, M., Salo, Y., Vuorinen, M., Jauhiainen, L. & Mäkelä, L. 2000. Results of the official variety trials 1992–1999. Publications of Agricultural Research Centre of Finland. Serie A 70. 216 p.

Jayas, D.S. & White, N.D.G. 2003. Storage and drying of grain in Canada: low cost approaches. *Food Control* 14, 4: 255–261.

Joffe, A. 1978. *Fusarium poae* and *F. sporotrichioides* as principal causal agents of alimentary toxic Aleukia. In: Wyllie, T.D. & Morehouse, L.G. (eds.). *Mycotoxic fungi. Mycotoxins. Mycotoxicoses*. New York and Basel: Marcel Dekker. Vol. 3. p. 21–86.

Karppanen, E., Rizzo, A., Berg, S., Lindfors, E. & Aho, R. 1985. Fusarium mycotoxins as a problem in finnish feeds and cereals. *Journal of Agricultural Science in Finland* 57: 195–206.

Langseth, W., Elen, O. & Rundberget, T. 2001. Occurrence of mycotoxins in Norwegian cereals 1988–1999. In: Logrieco, A. (ed*.). Occurrence of toxigenic fungi and mycotoxins in plants, food and feed in Europe*. p. 37–43.

Langseth, W., Ellingen, Y., Nymoen, U. & Økland, E.M. 1989. High-performance liquid chromatographic determination of ZEN and ochratoxin A in cereals and feed. *Journal of chromatography* 478: 269–274.

Langseth, W. & Rundberget, T. 1999. The occurrence of HT-2 toxin and other trichothecenes in Norwegian cereals. *Mycopathologia* 147: 157–165.

Leblanc, J.-C., Malmauret, L., Delobel, D. & Verger, P. 2002. Simulation of the exposure to deoxynivalenol of French consumers of organic and conventional foodstuffs. *Regulatory Toxicology and Pharmacology* 36, 2: 149–154.

Le Guevel, R. & Pakdel, F. 2001. Assessment of oestrogenic potency of chemicals used as growth promoter by in vitro methods. *Human Reproduction* 16: 1030–1036.

Lígia Martins, M. & Martins, H.M. 2002. Influence of water activity, temperature and incubation time on the simultaneous production of deoxynivalenol and zearalenone in corn (*Zea mays*) by *Fusarium graminearum*. *Food Chemistry* 79, 3: 315–318.

McMullen, M., Jones, R. & Gallenberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devasting impact. *Plant disease* 81: 1340–1348.

Marasas, W.F.O., Nelson, P.E. & Tousson, T.A. 1984. *Toxigenic Fusarium species: identity and mycotoxicology.* University Park, Pennsylvania State University Press. 328 p.

Marx, H., Gedek, B. & Kollarczik, B. 1995. Vergleichende Untersuchungen zum mykotoxikologischen Status von ökologisch und konventionell angebautem Getreide. *Zeitschrift fur Lebensmittel-Untersuchung und Forschung* 201: 83–86.

Müller, H.-M. & Schwadorf, K. 1993. A Survey of the natural occurrence of *Fusarium* toxins in wheat grown in a southwestern area of Germany. *Mycopathologia* 120: 115–121.

Norred, W.P. 2000. Agriculturally important fungal toxins. *Chemical Health and Safety* 7, 4: 22–25.

Oldenburg, E., Valenta, H. & Sator, Ch. 2000. Risikoabschätzung und Vermeidungsstrategien bei der Futtermittelerzeugung. *Landbauforschung Völkenrode* SH 216: 5–34.

Pettersson, H., Hedman, R., Engström, B., Elwinger, K. & Fossum, O. 1995. Nivalenol in Swedish cereals – occurrence, production and toxicity towards chickens. *Food Additives and Contaminants* 12, 3: 373–376.

Rizzo, A.F., Saari, L. & Lindfors, E. 1986. Derivatization of trichothecenes and water treatment of their trimethylsilyl ethers in an anhydrous apolar solvent. *Journal of chromatography* 368: 381–386.

Rizzo, A. 1993. Mycotoxins in Finnish alimentary products. *Environmental Health* 24: 457–466.

Rizzo, A., Berg, S., Eskola, M., Perttilä, U. & Saari, L. 2001. Occurrence of mycotoxins in cereals and foodstuffs in Finland between years 1987–1999. In: Logrieco, A. (ed.). *Occurrence of toxigenic fungi and mycotoxins in plants, food and feed in Europe.* p. 37–43.

Rintala, R., Hirvi, T. & Hallikainen, A. 1995. A study on the ochratoxin A and zearalenone (F2) concentrations in bread corn and animal feed corn samples in 1992–1994, and the ochratoxin A concentrations in samples of animal origin in 1994. *National Food Administration Research Notes* 8/1995. Helsinki. 19 p.

Saastamoinen, I. & Saloniemi, H. 1997. Quantification and confirmation of trichothecenes by gas chromatography/mass spectrometry/selected ion monitoring. In: *Proceedings of the 9th International congress in animal hygiene, ISAH-97.* Poster abstract. p. 937–940.

Salay, E. & Mercadante, A.Z. 2002. Mycotoxins in Brazilian corn for animal feed: occurrence and incentives for the private sector to control the level of contamination. *Food Control* 13, 2: 87–92.

Schollenberger, M., Jara, H.T., Suchy, S., Drochner, W. & Müller, H.-M. 2002. Fusarium toxins in wheat flour collected in an area in southwest Germany. *International Journal of Food Microbiology* 72, 1–2: 85–89.

Scott, P.M. 1989. The natural occurrence of trichothecenes. In: Beasley, V.R. (ed.). *Trichothecene mycotoxicosis: pathophysiologic effects.* Boca Raton: CRC Press. Vol. 1. p. 1–26.

Scott, P.M. 1991. Possibilities of reduction or elimination of mycotoxins present in cereal grains. In: Chelkowski, J. (ed.). *Cereal grain. Mycotoxins, fungi and quality in drying and storage.* Elsevier, Amsterdam. p. 529–572.

Shephard, G.S., Thiei, P.G., Stockenström, S. & Sydenham, E.W. 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. *Journal of AOAC International* 79, 3: 671–687.

Smith, T.K. 2003. Mycotoxin management. In: *World grain.* p. 29–33.

Sudakin, D.L. 2003. Trichothecenes in the environment: relevance to human health. *Toxicology Letters* 143, 2: 97–107.

Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U.-S., Sugiura, Y. & Ueno, Y. 1988. Worldwide contamination of cereals by the mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1: survey of 19 countries. *Journal of Agricultural and Food Chemistry* 36: 979–983.

Thuvander, A., Möller, T., Barbieri, H.E., Jansson, A., Salomonsson, A.-C. & Olsen, M. 2001. Dietary intake of some important mycotoxins by the Swedish population. *Food Additives and Contaminants* 18, 8: 696–706.

Ueno, Y. 1987. Trichothecenes in food. In: Krogh, P. (ed.). *Mycotoxins in food.* London, Academic Press. p. 123–147.

Usleber, E., Lepschy, J. & Märtlbauer, E. 2000. Deoxynivalenol in Mehlproben des Jahres 1999 aus dem Einzelhandel. *Mycotoxin Research* 16A: 30–33.

Velleman & Hoaglin 1981. *Applications, basics, and computing of exploratory data analysis.* Boston, Duxbury Press, Chapter 3. 354 p.

Vilà, B., Jaradat, Z.W., Marquardt, R.R. & Frohlich, A.A. 2002. Effect of T-2 toxin on in vivo lipid peroxidation and vitamin E status in mice. *Food and Chemical Toxicology* 40, 4: 479–486.

WHO 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. *IARC monographs on the evaluation of carcinogenic risks to humans* 56: 397–444.

WHO 2001. Safety evaluation of certain mycotoxins in food: prepared by the fifty-sixth meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA). *WHO Food Additives Series:* 47. *FAO Food and Nutrition Paper:* 74. *IPCS-International Programme on Chemical Safety,* WHO Geneva, 2001. 701 p.

Vol. 13 (2004): 54–67.

Widestrand, J. 2001. *Assessment of trichothecenes contamination, chemical aspects and biological methodology*. Doctoral thesis, Swedish University of Agricultural Sciences. Uppsala, Sweden. 96 p.

Ylimäki, A., Koponen, H. Hintikka, E.-L., Nummi, M., Niku-Paavola, M.-L., Ilus, T. & Enari, T.-M. 1979. Mycoflora and occurrence of *Fusarium* toxins in Finnish grain. *Technical Research Centre of Finland, Materials and Processing Technology, Publication 21.* 28 p.

SELOSTUS

Kauran trikotekeenipitoisuus virallisissa lajikekokeissa sekä typpilannoitus- ja luomulajikekokeissa

Veli Hietaniemi, Markku Kontturi, Sari Rämö, Merja Eurola, Arjo Kangas, Markku Niskanen ja Marketta Saastamoinen

MTT (Maa- ja elintarviketalouden tutkimuskeskus) ja Satafood Kehittämisyhdistys ry

Viljojen luontaiset toksiinit, kuten hometoksiinit, ovat tärkeitä raaka-aineen laadun mittareita elintarvike- ja rehuteollisuuden kannalta. Tutkimuksessa määritettiin *Fusarium*-toksiinit ja trikotekeenit satokausien 1997–1999 virallisten lajike-, typpilannoitus- ja luomulajikekokeiden kauranäytteistä. Lisäksi tavoitteena oli lisätä tietoa eri kauralajikkeiden kemiallisesta koostumuksesta. Viralliset lajike-, typpilannoitus- ja luomulajikekokeet toteutettiin MTT:n tutkimusasemilla.

Virallisten lajikekokeiden tulosten perusteella homemyrkyistä deoksinivalenoli (DON) esiintyi useimmiten suomalaisessa kaurassa vuosina 1997–1999. Tutkituista näytteistä 55 % sisälsi DON:a < 50–896 µg kg^{-1}. Pienimmät trikotekeenipitoisuudet kolmen tutkimusvuoden aikana määritettiin vuoden 1998 kaurasadosta. Satokausi 1998 oli hyvin sateinen, kostea ja kylmä. Typpilannoituskokeissa käytettiin neljää eri typpitasoa 40, 80, 120 ja 160. Lisäksi vertailuaineistona oli nollataso, jossa lisätyppeä ei käytetty ollenkaan. Tulosten perusteella typpilannoitustaso ei vaikuttanut kauran mykotoksiinipitoisuuksiin vuosina 1997–1999. Suurimmat keskimääräiset DON-pitoisuudet havaittiin vuoden 1999 aineistosta vaihteluvälillä 192–510 µg kg^{-1}. Vastaavasti kuin edellä esitetyt tulokset virallisista lajikekokeista ja typpilannoituskokeista osoittivat, deoksinivalenoli oli yleisimmin esiintyvä *Fusarium*-toksiini myös tavanomaisen ja luomuviljelykokeiden näytteissä. Tutkittu viljelymenetelmä ei vaikuttanut DON-pitoisuuksiin.

Yhteenvetona voidaan todeta, että viljoissa esiintyviä trikotekeenejä on tärkeä tutkia lisää, ja järjestelmä pitoisuuksien seurantaan on luotava. EU:ssa valmisteilla oleva direktiivi tulee määrittämään suurimmat sallitut pitoisuudet deoksinivalenolille, T-2- ja HT-2-toksiinille, tsearalenonille ja fumonisiineille. Viljanäytteiden taustatietojen tunteminen ja niiden vaikutusten analysointi nousee yhä tärkeämmäksi laadun seurannassa. Tämä mahdollistaa nykyistä paremman viljan laadun hallinnan.