Fusarium fungi as potential toxicants on cereals and grain feed grown in Estonia during 1973–2001

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Abstract. This study was carried out to investigate the occurrence of the genus *Fusarium* Link ex Fr. on cereal grain grown in Estonia and grain feeds made of the named cereal grain. Within the period of 1973–1981, occurrence of genus *Fusarium* was identified in 1,065 grain seed samples, and, within 1997–2001, in 29 samples of grain feed. *Fusarium sp.* was identified in 67–100% of the studied wheat samples, and, depending on the year, infection was detected in 13–67% of the seeds. In the case of rye, *Fusarium* species were identified in 38–86% of the studied samples and infection was found in 8–23% of the seeds, with barley the figures were 45–97% and 14–46%; and oats 55–100% and 15–65%, respectively.

The study indicated that the infection spread more intensively when corn was lodged, as a result of rainy autumn and late harvest. 16 species and 4 varieties of *Fusarium* were found on seeds. According to the survey (in 707 samples), the most common species were the following: *F. avenaceum* (Fr.) Sacc., *F. poae* (Pk.) Wr., *F. oxysporum* (Schlecht) Snyd. et Hans., *F. ventricosum* App. et Wr., *F. sporotrichioides* Sherb. *var. minus* Wr., *F. verticillioides (Sacc.) Nirenberg* and *F. culmorum* (W.G.Sm) Sacc. Mycotoxin producing species *F. avenaceum, F. poae, F. sporotrichioides, F. oxysporum, F. verticilliodes, F. sambucinum* Fuck., *F. equiseti* (Corda) Sacc. and *F. culmorum* were detected in 50–60% of the studied samples. As a result of the studies on domestic grain feed (29 samples) carried out within 1997–2001, *Fusarium spp.* was found in 51.7% of the samples, whereas *Fusarium* toxins were detected in seven samples out of nine. 31.3% of *Fusarium* isolates were highly toxic, and 37.5% were toxic on *Bacillus stearothermophilus.* One isolate of *F. verticillioides,* one of *F. culmorum, F. tricinctum* (Corda) Sacc. and two of *F. sp*. proved to be highly toxic.

Key words: cereal grain, grain feed, *Fusarium* fungi, seed infection, mycotoxins, *Bacillus stearothermophilus*

INTRODUCTION

The representatives of the genus *Fusarium* Link ex Fr. can occur on grain as saprophytes and cause several diseases, of which the most well-known are pink snow mould, crown rot and head blight (Lõiveke, 1995).

Fusarium species also occur in the complex of root rot cereals (Lõiveke, 1993) and cause pink discolouration on rye seeds (Lõiveke, 1978). P. Soobik (1995) identified 13 *Fusarium* species on cereal grains grown in Estonia. However, the intensity of seed contamination was not studied.

Toxins produced by species of *Fusarium* can be present in grains and grain products and cause health disorders in humans and animals. Publications (Kadis et al., 1971; Bilai, 1977; Bilai & Pidoplitchko, 1970) define *Fusarium graminearum* Schwabe, *F. sporotrichiella* Bilai and *F. nivale* (Fr.) Ces. as toxin producing species, however, information about the existence of toxic strains of *F. culmorum* (W.G.Sm.) Sacc*., F. sambucinum* Fuckel*.., F. avenaceum* (Fr.) Sacc., *F. gibbosum* App. et Wr. Bilai and *F. oxysporum* (Schlecht) Snyd. et Hans. has also been provided. *F. culmorum* (W. G. Sm.) Sacc. and *F. tricinctum* (Corda) Sacc. produce five toxins – zearalenone, trichothecene T1, T2, HT2 and neosolanol (Ylimäki et al., 1979). According to Tutelyan and Kravtchenko (1985), toxins can also be produced by the isolates *F. solani* (Mart.) App. et Wr. and *F. monifilorme* Sheld. The most well-known mycotoxin producer *F. graminearum* Schwabe develops on wheat, rye, oats, barley, maize, rice, causing *Fusarium* head blight. Consumption of contaminated cereal grain for food results in development of toxicosis with symptoms similar to intoxication. Feeding cattle with contaminated feed causes vulvovaginitis, infertility, or spontaneous abortion. Continuous use of toxic grain could result in death. Consumption of cereal grain contaminated by *F. sporotrichiella* Bilai causes high temperature, gastric problems, sharp decrease in leucocytes and thrombocytes in blood and damage in bone marrow, etc. (alimentary toxic aleukia), which can be deadly. Similarly, studies in Japan revealed that consumption of grain feed contaminated by *F. nivale* caused lack of appetite and decrease in weight of animals as well as bleeding in lungs, stomach, brain and uterus, sometimes resulting in death. People experienced headache, vomiting, nausea, spasms (Bilai, 1977).

Feeding corn contaminated with *F. moniliforme* is often associated with pulmonary oedema syndrome in pigs (Prelusky et al., 1994). Consumption of grain feed contaminated by *Fusarium* metabolites causes infertility of pigs and cattle (Korpinen, 1972).

However, all strains and isolates of *Fusarium* species are not toxic. According to the studies of A. Z. Joffe (Kadis et al., 1971), carried out on cereal grain in the Orenburg region in Russia within the period of 1943–1949, the most toxic strains were found in the following Fusarium species: *F. sporotrichioides* Sherb., *F. poae* (Pk.) Wr.– in 93–97% of cases and *F. graminearum* Schw.– in 33% of cases. However, 19–20% of the strains of *F. avenaceum* (Fr.) Sacc., *F. culmorum,* (W.G.Sm.) Sacc*., F. equiseti* (Cda.) Sacc. and 15–17% of the strains of *F. solani,* (Mart.) App. et Wr., *F. redolens* Wr. Wr., *F. nivale* (Fr.) Ces., *F. moniliforme* Sheld. *and F. oxysporum* Schl. appeared to be toxic. Development of toxic strains depended directly on the time of crop harvesting. When grain was harvested in time, extensive development of microfungi (including *Fusarium* species) was detected neither on vegetative nor generative organs of cereal grain. In western Canadia Abramson et al. (2001) isolated 42 isolates of *F. graminearum*, 42 of *F. culmorum* and 42 of *F. avenaceum* from wheat. Toxin deoxynivalenol was found in 28 isolates of *F. graminearum* and in 42 isolates of *F. culmorum*. Toxin moniliformin was present in 40 isolates of *F. avenaceum*.

In Finland animal diseases and deaths caused by consumption of imported maize and domestic grain contaminated by *Fusarium* toxins *(F. graminearum* Schw*., F. poae* (Pk.) Wr.) were observed in 1982 and 1984. The above-mentioned two *Fusarium* species and six (diacetoxyscirpenol, deoxynivalenol, nivalenol, fusarenon –X, T-2, HT-

2) toxins were found in grain harvested in wet conditions and at feed industry production lines wet with condense water (Karppanen et al., 1985). According to the studies of and literature cited by Eskola et al. (2001), generation of toxin DON can be associated with the occurrence of *F. avenaceum* in cereal grain, and the studies also indicate that NIV toxin is generated by *F. tricinctum* and *F. sporotrichioide,* and HT –2 toxin by *F. tricinctum*.

A number of *Fusarium* species produce several toxins: for example *F. moniliforme* produces at least three – fumonisins, moniliformin and fusarin C. Different species may also cause complex contamination. Synergetic effect caused by co–occurrence is especially dangerous, moreover, *Fusarium* toxins can be carried over to milk, eggs and meat (Placinta et al., 1999).

The most well-known toxins produced by *Fusarium* species are zearalenone (F–2), deoxynivalenol (vomitoxin), T–2–toxin (Katchanova, 1983). The latter two belong to trichothecenes, in which case more than 40 different toxins can be specified. Zearalenone, which is estrogenically active, has earlier been found very often in Finland in grains and feeds that have caused diseases in farm animals (Korpinen, 1972).

According to D'Mello et al. (1997), trichothecenes, zearalenone, moniliformin and the fumonisins are the most important from the point of view of animal health and productivity. Trichothecenes are known to be immunosuppressive compounds and to inhibit protein synthesis and may cause tissue necrosis, vomiting and reduced feed intake (Prelusky et al., 1994). For example, trichothecenes are produced by *F. sporotrichioides*, *F. poae*, *F. culmorum* and *F. graminearum* (Placinta et al., 1999). Moniliformin is synthesised by *F. moniliforme* and *F. oxysporum*, zearalenone and fusarochromonones are the products of *F. equiseti* (D'Mello et al., 1997).

The purpose of this study is to define *Fusarium* species, the frequency and intensity of the occurrence of these species on cereal grain grown in Estonia, and on feed produced from it, and determine whether any of the species or isolates of *Fusarium* fungi produce mycotoxins.

MATERIALS AND METHODS

The occurrence of *Fusarium* fungi was studied on the basis of an analysis of cereal grain samples (1,065 samples – Table 1) collected on the experimental farms of the Estonian Research Institute of Agriculture within the period of 1973–1981 and grain feeds grown in 1997–2001 by Estonian farmers. Grain samples from the period of 1973–1981 were analysed for 4–5 weeks after collection. Samples from the period of 1997–2001 were taken by farmers from feed which had caused health disorders in domestic animals (catharsis, decrease in milk production, etc.).

The collection sites of the analysed samples have been presented in Fig. 1. The samples were taken and stored under conditions which excluded spread of contamination from one sample to another.

Moist chamber method (filter paper at the bottom of Petri dishes was saturated with sterilised water) was used to study the microflora of grain samples collected from experimental farms. 100 seeds of each sample were analysed. Seeds soaked for 24 hours were halved by means of a disinfected scalpel to ensure better exposure of inner infection. Samples were divided between 10 petri dishes, 20 half seeds per each dish.

Five dishes were incubated at a temperature of 20–30° and five dishes at 18–22° to enable *Fusarium* species a higher as well as lower temperature optimum to grow better. The results were assessed in two and four weeks – the number of seeds infected by *Fusarium* was counted and the infection rate (intensity -%) was determined. Preparations for assessing species (in 707 samples) included use of identifiers of V.J. Bilai (1955, 1977), W. Gerlach and H. Nirenberg (1982) and a microscope MBI–6. The number of microfungi in feed samples was assessed by pour plate method (Harrigan & McCance, 1976). The total number of fungi was estimated by a sowing from the first, second and third dilution on maltagar. The species and number of *Fusarium* were defined on the basis of the first and second dilution on Nash and Snyder selective medium.

Toxicity of corn feed, studied within the period of 1997–2001, was defined by means of a test organism *Bacillus stearothermophilus* (Watson & Lindsay, 1982). 16 Fusarium isolates were separated from nine samoles of domestic grain feed and their toxicity was tested in mm on the basis of the size of the growth inhibition zone of *Bacillus stearothermophilus*: toxicity was absent: 0–1 mm, toxic: 2–5 mm, very toxic: 6–10 mm.

The nomenclature of *Fusarium* used in this work is based on that of Gerlach and Nirenberg (1982).

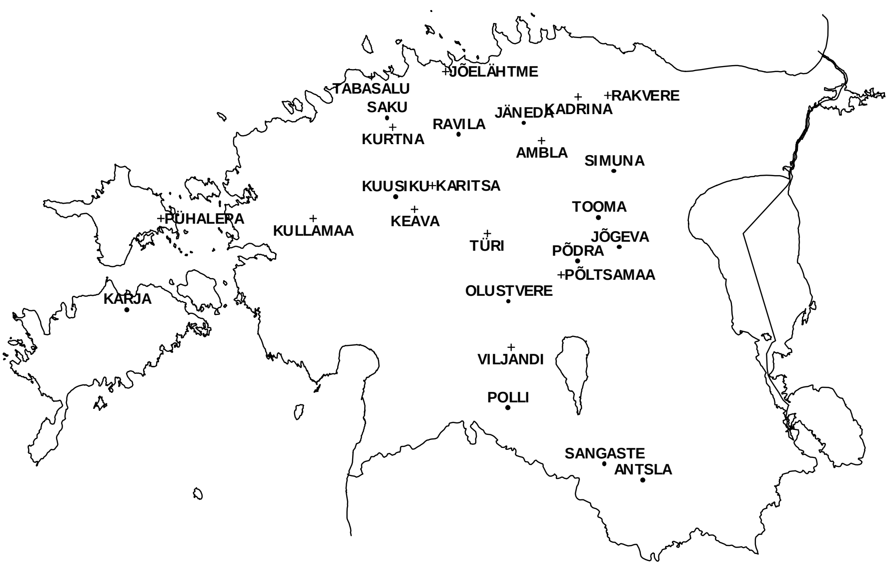

Fig. 1. Origin of analysed samples (location).

The places of origin of grain samples analysed in 1973–1981 are marked with dots, the places of collecting feed samples in the years 1997–2001 with crosses. The territory of the Estonian Republic is located in the area between the north latitude 57°30′ – 59°40′ and east longitude 21°45′ – 28°15′ (Agroclimatic resources of the Estonian SSR, 1976).

Table 1. The number of grain seed samples examined in 1973–1981.

Year	Number of samples				
	Wheat	Rye	Barley	Oat	Total
1973	4	13	65	11	93
1974	6	7	84	37	134
1975	3	21	63	22	109
1976	3	36	99	25	163
1977	6	-	95	29	130
1978	8	3	96	21	128
1979	6	-	88	22	116
1980	9	5	55	17	86
1981	12	-	75	19	106
Total	**57**	**85**	**720**	**203**	**1065**

RESULTS AND DISCUSSION

1. Occurrence and intensity of infection by year

The study of 57 samples of wheat produced in Estonia within 1973–1981 indicates that 67–100% of the samples were infected by Fusarium at an average rate of 13–67%. 38–86% of 85 rye samples were infected at an average rate of 8–23%. In the case of barley (720 samples), the figures were 45–97% and 14–46%, oats (203 samples) 55–100% and 15–65%, respectively (Table 2).

The average figures per year demonstrate that oats was most vulnerable to infection – 87% of the studied samples were infected at an average rate of 33%, with wheat the respective figures were 86% and 29%, in the case of barley – 79% and 29%, whereas rye appeared to be the most resistant – 62% of samples were infected at an average rate of 14%.

According to the data provided by Ylimäki (1981), the rate of infection of the samples of grain seed in Finland within the period of 1966–1973 was 67–76% in the case of rye, 56–67% in the case of winter wheat, 61–98% in the case of spring wheat, 82–97% in the case of barley and 100% in the case of oats, whereas the infection rate of grain kernels was 3.3–15.7% within 1966–1971, consequently, significantly lower than in Estonia during the period of 1973–1981. At the same time, infection rates of grain samples in Estonia fluctuated a great deal in the case of all grain species (Table 2). The highest average rate of infection (48%) of grain samples of the study period was detected in 1978 characterised by the highest amount of precipitation (480 mm) during the growing season (June–September). The August and September of 1978 were extremely wet – the amount of precipitation in different regions of Estonia was 1.5–2.4 times higher than the standard amount. Also, the growing season of the year 1981 was characterised by significantly high precipitation (1.5 times higher compared to 1980). In 1974 grain crop was lodged as a result of rain and strong winds during the growing season. Consequently, in 1974, 1978 and 1981, the average rate of the samples infected by *Fusarium* was considerably higher due to the above-named weather conditions (Table 2). At the same time, daily average temperatures seem to be less important factors.

Table 2. Percentage of grain samples infected by *Fusarium* and the average contamination rate (intensity) of contaminated samples in 1973–1981. The amounts of precipitation and average temperatures during June–September in 1973–1981.

Crop Indicator	1973	1974	1975	1976	1977	1978	1979	1980	1981	Ave-rage
WHEAT										
Contaminated samples, %	75	83	100	100	67	88	100	78	92	86.0
Amplitude of contamination of samples	60–81	5–55	30–62	6–24	14–36	18–70	12–32	10–22	4–56	-
Average contamination of samples, %	67	19	47	13	24	41	22	17	32	29.1
RYE										
Contaminated samples, %	85	86	38	61	-	67	-	80	-	62.3
Amplitude of contamination of samples	6–24	6–76	2–18	6–17	-	22–24	-	4–23	-	-
Average contamination of samples, %	20	23	8	2	-	23	-	14	-	14.1
BARLEY										
Contaminated samples, %	45	96	97	61	71	85	81	96	85	78.9
Amplitude of contamination of samples	13–75	6–95	4–63	4–42	6–50	8–90	6–58	3–36	4–78	-
Average contamination of samples, %	29	46	28	14	20	45	23	14	32	29.2
OAT										
Contaminated samples, %	55	92	100	60	90	95	91	100	84	86.7
Amplitude of contamination of samples	7–25	5–95	14–98	4–32	8–42	28–92	6–56	4–37	8–36	-
Average contamination of samples, %	21	48	33	15	20	65	30	16	22	32.5
Samples, total	93	134	109	163	130	128	116	86	106	1065
Contaminated samples, total	49	126	94	100	97	111	97	81	81	846
Contaminated samples, total. %	52.7	94.0	86.2	61.3	74.6	86.7	83.6	94.2	85.8	79.4
Average contamination of samples, %	26.5	43.9	28.2	13.4	20.1	48.1	24.6	14.9	30.3	29.2
Precipitation VI–IX, mm	295	297	205	228	283	480	286	270	405	-
Average temperature VI–IX, °C	14.6	14.2	13.0	12.9	13.2	13.1	14.1	14.6	14.1	-

Finnish scientists (Ylimäki, 1981; Avikainen & Hannukkala, 2001) also refer to the favourable effect of warm and wet late summer on the infection of grain ears and kernels with *Fusarium* fungi. The climate of the Northern and Central Estonia, where the majority of the samples of 1973–1981 originate from, can be compared to that of southwestern Finland. Avikainen and Hannukala have conducted studies of samples taken from 18 rye fields of the named region in Finland in 2000. All the studied fields were contaminated with *Fusarium* species (mostly *F. avenaceum*) with an average infection of 30% of the kernels, characterised by an amplitude from 9% to 64% (Avikainen & Hannukkala, 2001).

Table 3. Frequence of the main *Fusarium* species in grain samples in 1973–1981.

Fusarium sp. by Gerlach and Nirenberg (1982)	1973	1974	1975	1976	1977	1978	1979	1981	Average
	Contaminated samples, %								
F. avenaceum (Fr.) Sacc.	7.5	9.9	-	33.3	3.2	61.7	52.6	39.6	30.3
F. sporotrichioides Sherb.*var.minus* Wr.	1.0	7.0	-	2.0	1.0	7.0	3.0	8.5	5.0
F. poae (Pk.) Wr.	3.3	15.0	-	6.3	3.0	14.9	8.2	17.0	9.5
F. solani (Mart.) Sacc.	10.0	8.0	-	1.0	0.2	0.2	-	6.0	3.0
F. ventricosum App. et Wr.	16.9	17.3	4.0	1.8	0.6	0.6	-	11.0	6.9
F. verticillioides (Sacc.) Nirenberg	4.8	6.0	2.4	8.3	2.8	1.5	5.1	7.6	4.5
F. oxysporum (Schlecht) Snyd. et Hans.	11.8	9.9	9.5	8.3	-	7.0	6.0	12.3	7.4
F. culmorum (W.G.Sm.) Sacc.	2.2	4.4	4.8	13.9	-	1.6	6.0	7.5	4.1
F. sambucinum Fuck.	2.2	3.3	9.5	-	0.8	2.3	6.0	5.7	4.0
F. semitectum Berk. et Rav.	-	-	-	-	1.6	7.0	-	13.2	3.5
F. equiseti (Corda) Sacc.	2.2	2.2	-	2.8	-	2.3	1.7	3.8	2.1

The high infection rate of Estonian grain (38–100% of the samples) shows that our climatic conditions are favourable for the development of *Fusarium* on kernels. Reasons for the high fluctuation of infection intensity (8–67% of kernels) can be the following: different growing and weather conditions, microclimate of fields, characteristic features of varieties and agrotechnical characteristics. Nutrition of plants and previous crop, also, cultivation of land exert significant effects on the infection of cereal grain with *Fusarium* species (Oldenburg et al., 2001; Oldenburg & Brunotte, 2001; Dill-Macky & Jones, 1999).

Samples were taken from seed production holdings where the main agrotechnical requirements had been fulfilled, and grain was dried and cleaned as soon as possible after harvesting. Storage conditions in agricultural holdings generally fulfilled the requirements set for storing of seed grain.

It is a well-known fact from publications (Westermarck-Rosendahl & Ylimäki, 1978) and experience that harvested moist grain is a reason for the development of several microorganisms, including *Fusarium spp,* and self-heating. Consequently, the result of a sample analysis depends on growing conditions as well as the period after harvesting, i.e. how fast the harvested grain can be dried and transferred to conservation conditions with a moisture content of 13–14%.

Table 4. Occurrence of *Fusarium* fungi in grain samples in 1973–1981.

Fusarium sp. and var. by Gerlach and Nirenberg (1982)	BARLEY		OAT		WHEAT		RYE		Cereals Total	
	contaminated samples									
	No	%	No	%	No	%	No	%	No	%
F. avenaceum (Fr.) Sacc.	142	30	48	40.3	10	30.3	14	17.5	214	30.3
F. sporotrichioides Sherb.*var.minus* Wr.	26	5.5	7	5.9	1	3	1	1.3	35	5
F. poae (Pk.) Wr.]	48	10.1	14	11.8	2	6.1	3	3.8	67	9.5
*F. solani (*Mart.) Sacc.	15	3.2	1	0.8	1	3.3	4	5	21	3
F.ventricosum App. et Wr.	34	7.2	3	2.5	2	6.1	10	12.5	49	6.9
F. verticillioides (Sacc.) Nirenberg	25	5.3	3	2.5	1	3	3	3.8	32	4.5
F. oxysporum (Schlecht) Snyd. et Hans.	39	8.2	5	4.2	3	9.1	5	6.3	52	7.4
F. culmorum (W.G.Sm.) Sacc.	18	3.8	3	2.5	1	3	7	8.8	29	4.1
F. sambucinum Fuck.	15	3.2	11	9.2	0	0	2	2.5	28	4
F. semitectum Berk. et Rav.	17	3.6	8	6.7	0	0	0	0	25	3.5
F. equiseti (Corda) Sacc.	11	2.3	2	1.7	1	3.0	1	1.3	15	2.1
Fusarium spp.	33	6.9	4	3.4	1	3	4	5	42	5.9
Total number of samples	475	-	119	-	33	-	80	-	707	-

2. Composition of *Fusarium* species in grain samples

The study indicated that the frequency of the occurrence of a *Fusarium* species in samples depended on the grain species and the year (Tables 3 and 4). In the two consecutive years 1973 and 1974, the prevailing species was *F. ventricosum,* whereas 1976, 1978, 1979 and 1981 were predominated by *F. avenaceum*. In 1975, the driest year, the composition of *Fusarium* species was the smallest; *F. avenaceum* and *F. sporotrichioides* did not occur at all (Table 3). In 1978, the year of the highest amount of precipitation, *F. avenaceum* occurred more frequently than ever – in 62% of the studied samples. In about 40% of the samples, the occurrence of two or more *Fusarium* species was detected simultaneously (co-occurrence). Studies by Eskola et al. (2001), in Finland in 1998, also demonstrate that as a result of the cold and wet growing season, grain was most frequently infected by *F. avenaceum*.

16 *Fusarium* species and 4 varieties were identified on the studied seeds (Table 4). The most common species were *F. avenaceum, F. poae, F. oxysporum, F. ventricosum, F. sporotrichioides, F verticillioides and F. culmorum*. *F. avenaceum* and *F. poae* were the dominating *Fusarium* species on barley and oat. *F. avenaceum* with *F. ventricosum* dominated on rye, with *F. oxysporum* on wheat. *F. avenaceum, F. poae, F. sporotrichioides, F oxysporum, F. sambucinum*, *F. verticillioides, F. culmorum* and *F. equiseti* – known as toxin-forming *Fusarium* species occurred on 50–60% of all studied grain samples. No occurrence of the most dangerous toxin-forming *Fusarium* species *F. graminearum* was observed. P. Soobik (1995) does not provide either any information about occurrence of *F. graminearum* on grain in Estonia.

Table 5. Number of micofungi and toxin-forming *Fusarium* in grain feeds in 1997–2001.

Grain and grain products	Microfungi* on maltagar 10^3	% of *Fusarium* of total fungi	Toxicity Growth inhibition on *Bacillus stearothermophilus*		
			0–1 mm	2–5 mm	6–10 mm
Barley	913	+	-	*F. sp.*	-
Barley	474	+	*F. sp.*	-	-
Oat	118	5.3	-	*2 F. sp.*	-
Wheat	1.4	>2.6	-	*F. sp.*	-
Triticale	21.6	+	*2 F. sp.*	*F. sp.*	*F. verticillioides* (Sacc.) Nirenberg
Malt	47.7	<0.6	-	-	*F. culmorum* Sacc.
Meal	825	8.2	*F. sporotrichioides* Sherb. *var. minus* Wr. *F. sp.*	-	-
Pot-barley	9.3	6.8	-	-	*F. sp.*
Grain (dry original)	726	1.7	-	*F. sporo–trichioides* Sherb. *var. minus* Wr.	*F. sp.* *F. tricinctum* (Corda) Sacc.

* Main species: *Penicillium sp. – P. cyclopium, P. expansum*
Aspergillus sp. – A. oryzae, A. ochraceus,
Rhizopus nigricans, Cladosporium sp., Mucor sp., Alternaria sp.

Results of a survey of grain in Finland within 1966–1971 (Ylimäki, 1981) demonstrated that the most widely spread *Fusarium* species were *F. culmorum*, *F. poae*, *F. tricinctum*, *F. avenaceum*, as well as *F. arthrosporioides, F. graminearum* and *F. oxysporum*. Similarly to our studies, the occurrence of *F. nivale* in Finland was very rare. The frequency of the occurrence of *F. solani* in Finland was almost the same, only a little higher on barley (16.0% of samples) than in Estonia. *F. moniliforme* was detected very rarely in Finland – only on rye and barley. At the same time, *F. moniliforme* Sheld.=*F. verticillioides* Sacc. Nirenberg is the sixth frequently occurring species of *Fusarium* in Estonia – detected in 4.5% of all samples. Consequently, the composition of *Fusarium* species on grain seeds is relatively similar in Estonia and Finland. The lack of *F. graminearum* on Estonian grain, its moderate occurrence in samples of rye, wheat and barley (3.3–12.5%) and its extremely frequent occurrence in samples of oats in Finland (100%) could be considered significant exceptions here.

According to the studies of Ylimäki et al. (1979) conducted in Finland during 1976 and 1977, infection of grain seeds was most frequently caused by *three Fusarium species: F. avenaceum, F. tricinctum, F. culmorum,* whereas *F. arthrosporioides, F. poae, F. graminearum* and *F. sambucinum* occurred less frequently. Seeds of grain grown in the year of high precipitation (1977) and stored without drying was characterised by a high rate of infection.

The most common species detected on cereal grains by Ylimäki and Jamalainen (1986) have been *F. culmorum, F. avenaceum, F. arthrosporioides* and *F. graminearum*, however, contamination with *F. poe, F. tricinctum* and *F. oxysporum* has also been very heavy in all cereals.

The studies of Estonian as well as Finnish scientists demonstrate that the *Fusarium* species are not adapted to a certain grain species (they are not specific) and the composition of a *Fusarium* species depends, first and foremost, on the conditions of the growing season and post-harvest handling of cereal grain. The composition of *Fusarium* in grain seems to be typical of a geographical region.

3. Microflora of domestic grain feed

The most frequently occurring microfungi were (Table 5) *Penicillium spp*. – *P. cyclopium, P. expansum* and *Aspergillus spp. – A. oryzae*, *A. ochraceus*, as well as *Rhizopus nigricans*, *Cladosporium sp*., *Mucor sp*., *Alternaria sp*. The named genera of fungi were also frequent in Finnish grain feed (Ylimäki, 1981).

38% of domestic grain feed produced within 1997–2001 was characterised by signs of damage – in samples the total number of microfungi exceeded the limit value, i.e. 200,000 1/g by Schmidt-Lorenz (1980). *Fusarium sp.* was found in 52% of the samples.

4. Toxicity of Fusarium isolates

The toxicity of 16 Fusarium isolates was tested by the growth inhibition of *Bacillus stearothermophilus.* There were only 31% (5 isolates) of non-toxic isolates and 69% of toxic and very toxic isolates. Table 5 illustrates that toxins of *Fusarium* species were found in 7 samples out of the studied 9, which shows that the occurrence of *Fusarium* toxins in feed with signs of damage is very frequent. Very often it has been caused by a rate of moisture content of grain feed, exceeding the permitted limit. Ylimäki et al. (1979) stress the importance of drying grain immediately after harvesting to achieve a moisture content of less than 14%, which is one of the most efficient methods for preventing generation of *Fusarium* toxins.

The last sample of the yield of the year 2000, was obtained from Selja farm of Kullamaa rural municipality, where the harvested grain was so dry that it was expected to store it without drying. The results presented in Table 5 demonstrate that the experiment was a failure – the grain was damaged and became toxic.

One isolate of *F. tricinctum*, one of *F. culmorum*, one of *F. verticillioides* and two of *F. sp*. revealed high toxicicity. One isolate of *F. sporotrichioides var. minus* was toxic, while the other did not produce any toxins.

CONCLUSIONS

The composition of *Fusarium* species in grain seems to be quite similar in Estonia and Finland. These *Fusarium* species on grain in Estonia are not specialized on certain cereal species. Infection with *Fusarium* fungi, including potential toxicants and phytopathogenic species, often occurs on grain grown in Estonia, however, the rate of infection greatly depends on weather, the conditions of the growing season, crucial in the period of ear-forming and grain ripening. Post-harvest grain treatment (the speed of drying and cleaning of the harvested grain) and storage conditions are decisive as well.

Unsuitable storage conditions (a dripping roof of a storehouse, forming of condense water in seed mass, bad thermo- and hydroisolation, storehouse pests) will result in development of microfungi, including *Fusarium* fungi producing mycotoxins, causing health problems in animals and humans. Our studies showed that *Fusarium* strains (isolates) occurring on spoiled grain feed are sometimes highly toxic to *Bacillus stearothermophilus*, causing serious health problems or even death of young stock. Co-occurrence of several toxin-forming *Fusarium* species (strains) in studied grain feed samples refers to an increased risk to animal and human health.

Consequently, occurrence of toxicants from *Fusarium* species in grain and grain feed currently used in Estonia is an essential factor, and great attention should be paid to preventing infection of cereal grain during the growing season, post-harvest treatment, feed production and storage.

Solution of the problem in Estonia should be based on scientific research results and practical experience of other countries in grain growing and storing.

Seed dressing, use of fungicides during the growing season (McMullen et al., 1999; Grybauskas, 1999) and use of growth regulators to prevent cereal grain from lodging are essential for decreasing contamination of grain seeds with *Fusarium.*

As a conclusion, it could be stated that one of the most efficient possibilities for decreasing infection of grain by *Fusarium* toxins is to breed varieties resistant to the pathogenic *Fusarium* species (D'Mello & Macdonald, 1998; Hansen et al., 1999).

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