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Incidence of *Fusarium* spp. on several field crops in Estonia and their toxicity towards *Bacillus stearothermophilus*

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Abstract. The article provides an overview of the occurrence of *Fusarium* spp. on grain produced in Estonia from 1973–2004, the occurrence of *Fusarium* spp. in the common root rot complex of cereals in 1977–1985 and potato tubers with dry rot symptoms in the yield of 1996–2000. The dominating species on grain were (contaminated samples, %) *F. avenaceum* (Fr.) Sacc. - 28.0–30.3; *F. poae* (Pk.) Wr. - 7.4–9.5 *F. semitectum* Berk. et Rav. - 7.0–9.0; *F. oxysporum* (Schlecht) Snyd. et Hans. - 7.4-8.0; accompanied by *F. ventricosum* App. et Wr., *F. sporotrichioides* Sherb. *var. minus* Wr., *F. verticillioides* (Sacc.) Nirenberg, *F. culmorum* (W. G. Sm.) Sacc. and *F. sambucinum* Fuck. In the common root rot complex of barley, *F. culmorum* - 16.8*; F. sambucinum* - 2.5*; F. avenaceum* - 2.2*; F. oxysporum* - 1.7*;* and *F. poae* - 1.3 (contaminated samples, %) dominated. In potato with dry rot, *F. culmorum* - 26.7*; F. solani* (Mart.) Sacc. - 20.0; *F. poae* - 15.0*; F. oxysporum* - 13.3 and *F. sulphureum* Schlecht - 8.3 (contaminated tubers, %) dominated. The occurrence of toxic isolates both on grain and potato was established. Of *Fusarium* isolates recovered from grain (total 287) 5.6% were highly toxic, and 88.1% mildly or medium toxic to *Bacillus stearothermophilus*. Of 15 isolates found on potato, 1 was highly toxic and 9 were mildly to medium toxic. *Fusarium* spp. has been presented according to Gerlach & Nirenberg (1982).

Key words: *Fusarium* spp., cereal crops, potato, toxicity of isolates, *Bacillus stearothermophilus*

INTRODUCTION

Fusarium Link: Fr. genus is widely spread in nature, mostly at the conidial stage, more rarely at the reproductive stage. Having labile properties, *Fusarium* spp. may, depending on the environmental conditions and the health state of plants, be parasites, saprophytes or reveal even symbiotrophic properties. *Fusarium* spp. may cause the dangerous *Fusarium* head blight, but may also occur on grain as epiphytes, feeding on plant secretions and dead organic matter on the grain surface without causing any loss of yield. *Fusarium* spp. also occur in most soils, particularly in cultured soils (Bilaj, 1977). On roots of annual plants, Fusariums cause mycorhiza, which is important and necessary for feeding of the plant. With weakening of the host plant, the Fusariums forming mycorhiza pass from symbiotic relations to parasitism, accompanied by the adaptation necessary for conquering the substrate-excreting toxins and poisoning the host plant. In the case of cultures grown in the same crop rotation, such as cereals and potato, several diseases caused by *Fusarium* fungi must be considered: common root rot, damping-off, crown root rot in cereals and Fusarium tuber dry rot in potato. As

Fusariums are polyphags, it is necessary to know, when growing cereals and potato in the same crop rotation, which species damage one or another culture, to be able to plan further control methods. From the position of food hygiene, it is important to have information on the potential toxicity of representatives of *Fusarium* spp. on cereal seeds and potato tubers.

The present overview aims to explain which *Fusarium* species occur on cereal seeds grown in Estonia and the common root rot complex of cereals, as well as the species composition of Fusariums in the disease ratio of Estonian potato with dry rot during the storage period. The results of testing the toxicity of isolates separated from grain and potato to *Bacillus stearothermophilus* are also presented. The results of earlier studies by the author (grain – 1973–1981; 1992, 2002, 2003; potato – 1996–2000) were also used, supplemented by data from recent years (2004, 2005).

MATERIALS AND METHODS

Grain samples (total 1265) for mycological survey were collected according to the aggregate sample (weight 1–3 kg) requirements and analysed after 4–5 weeks. In mycological analysis, the wet chamber method was applied; after 2 and 4 weeks, 100 seeds of each sample were analysed. Seeds soaked for 24 hours were halved 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 to emerge at higher as well as optimum lower temperature. The results were assessed in two and four weeks: the number of seeds contaminated by *Fusarium* spp. was counted and the infection rate (number of contaminated grain, %) was determined. In the case of the microbiological analysis, the pour plate method on malt extract agar was used (Harrigan & McCance, 1976). The species and number of *Fusarium* spp. were defined on the basis of the first and second dilution on Nash & Snyder selective medium (Booth, 1971). The identification of *Fusarium* spp. has been made according to Bilaj (1955, 1977) and Gerlach & Nirenberg (1982). In this article, the author´s research results are presented only according to the taxonomy of Gerlach & Nirenberg.

The occurrence of *Fusarium* spp. in the common root rot complex of gramineous plants was investigated on the basis of barley root samples (total 6825) collected from long-term crop rotations. For cultivation of the pathogens, in 1977–1981 malt extract agar (Baumgart, 1993) and in 1982–1985, the wet chamber method was used. Ten (10) root pieces per one petri dish, washed and dried on filter paper, were used. Samples in three replicates were studied at 20–30° during 2–3 weeks.

Potato samples (total 1100 tubers) with dry rot symptoms from yields of 1996–2000 were collected from storage at the end of the storing period, in March-April, and analysed. The pathogens were cultivated on malt extract agar in petri dishes at 20–30° C for 2–3 weeks, or determined by the fungus mycel on the tuber surface or spore clusters, and transferred into pure cultures.

The toxicity of *Fusarium* isolates separated both from grain and potato tubers was determined by the growth inhibition zone of *Bacillus stearothermophilus* No 3011 (Watson & Lindsay, 1982): 0–1 mm - non-toxic, 2–5 mm - mildly to medium toxic, 6–10 mm - highly toxic.

The data were processed statistically by calculating the arithmetic mean and standard deviation.

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).

RESULTS AND DISCUSSION

On average, o 79% of the samples of Estonian grain produced in 1973–1981, were contaminated with *Fusarium* spp., the level being 29% of grain. The most contaminated cereal was oats – on average 87±8% of samples - and 33±6% of grain and wheat with 86±3% and 29±1%, respectively. Barley was slightly less contaminated, with 79±6% and 29±4%; the least contaminated was rye with 62±7% and 14±1%, respectively (Lõiveke et al., 2003). The contamination was increased by the greater amount of precipitation both in the growth period (June-September) and the harvest period (August-September) and by late ripening for harvest. Oats ripen late due to a long growing time and, therefore, their harvest period often has high precipitation; contamination of the grain with *Fusarium* spp. is greater. On average, 85±5% of summer and winter wheat in 1992 and 2004, and 35±3% of grain were contaminated, from which it may be concluded that, compared with 1973–1981, contamination of wheat, and probably other cereals with Fusariums has not decreased in Estonia.

The most often occurring *Fusarium* species (contaminated samples, %) in 1973–1981 were *F. avenaceum* (Fr.) Sacc. - 30.3; *F. poae* (Pk.) Wr. - 9.5; *F. oxysporum* (Schlecht) Snyd. et Hans. - 7.4; *F. ventricosum* App. et Wr. - 6.9; *F. sporotrichioides* Sherb. var. *minus* Wr. - 5.0; *F. verticillioides* (Sacc.) Nirenberg - 4.5; *F. culmorum* (W. G. Sm.) Sacc. - 4.1 and *F. sambucinum* Fuck. - 4.0. While in drier years *F. oxysporum* and *F. sambucinum* dominated, in wetter years *F. avenaceum* or *F. ventricosum* were dominant.. The species composition of the *Fusarium* flora also depended, to some extent, on the grain species. *F. avenaceum* and *F. ventricosum* dominated on rye, *F. avenaceum* and *F. poae* on barley and oats, *F. avenaceum*, *F. ventricosum* and *F. oxysporum* on wheat. Later wheat research, in 1992, and 2002-2004, also revealed most often contaminated samples (%) with *F. avenaceum* – 28.0; *F. semitectum* Berk. et Rav.- 9.0; *F. oxysporum* - 8.0; *F. poae* - 7.4, accompanied by *F. sporotrichioides* and *F. culmorum.* The dominating species in 1992 were *F. oxysporum* and *F. semitectum,* and *F. semitectum* and *F. poae* in 2002–2004.

Of the *Fusarium* spp. identified, many are considered toxin-producing: *F. avenaceum, F. poae*, *F. sporotrichioides, F. oxysporum*, *F. verticillioides, F. sambucinum* and *F. culmorum* (Bilaj, 1977; Miller & Trenholm, 1997; Galvano & Dominy, 2005). These occurred in 50–60% of samples studied; not all strains and isolates within a species are toxic.

The absence of *F. graminearum* Schwabe, the most dangerous species, was observed. Similarly to our studies, Soobik in his research work “Microfungi on grain in Estonia”, published in 1995, does not provide any information about the occurrence of *F. graminearum* in Estonia. But in Finland *F. graminearum* (Ylimäki, 1981) belongs to well known species in grain.

Table 1. Growth inhibition zone of *Bacillus stearothermophilus* (mm) caused by *Fusarium* isolates found on grain and storage potatoes.

Crop	2002	2003	2004	2005	Average of years	Total number of isolates
Wheat	5-7	2-5	2-7	2-6	4.0	164
Barley	-	2-5	-	2-6	3.5	75
Oats	-	2-7	-	-	3.5	14
Rye	3-5	3-4	-	-	3.5	6
Winter triticale	-	-	-	3-6	4.0	28
Potato	-	-	-	3-6	3,5	15

Table 2. Occurrence of *Fusarium* species in barley root samples (%) as determined on malt extract agar during 1977–1981 in Estonia.

Fusarium spp. *	1977	1978	1979	1980	1981	Average of years
F. culmorum	19.8	23.8	19.7	21.3	2.5	16.8
F.sambucinum	1.8	1.0	0	4.9	2.5	2.5
F. avenaceum	0.0	0.3	20.5	0,3	0	2.2
F. oxysporum	0.9	1.1	8.7	1.0	0.7	1.7
F. poae	3.6	1.8	0.4	1.0	1.0	1.3
F.verticillioides	0.9	0.2	0.9	0.1	1.3	0.6
F. equiseti	1.8	0.6	0	0.8	2.8	0.8
F. solani	2.7	1.1	0	0	0.7	0.6
Fusarium spp.	7.2	25.8	31.0	44.9	8.8	20.6
Samples, no	111	625	229	719	603	2287
Infected samples, %	39.6	32.6	81.2	71.8	20.2	46.9

*identified according to Gerlach & Nirenberg (1982)

Table 3. Composition of *Fusarium* spp. (%) isolated from storage potatoes with dry rot during 1996–2000 in Estonia.

Fusarium spp. *	1996	1997	1998	1999	2000	Average of years
F. culmorum	20.0	6.3	29.4	50.0	16.7	26.7
F. solani	20.0	25.0	17.6	6.3	50.0	20.0
F.poae	0	6.3	17.6	31.3	0	15.0
F. oxysporum	40.0	12.5	17.6	6.3	0	13.3
F. sulphureum	0	12.5	17.6	0	0	8.3
F.verticillioides	20.0	6.3	0	6.3	0	5.0
F. nivale	0	6.3	0	0	16.6	3.3
F. coeruleum	0	0	0	0	16.7	1.7
Fusarium spp.	0	25.0	0	0	0	6.7
Isolates, no	50	160	170	160	60	120

* identified according to Gerlach & Nirenberg (1982)

The toxicity of *Fusarium* isolates separated from the grain yield in 2002–2005 was tested by means *B. stearothermophilus* (Table 1). The growth inhibition zone in the isolates studied (287) was mostly 2–5 mm, i.e. the isolates were mildly to medium toxic; only the growth inhibition zone of 5.6% of the isolates exceeded 6 mm,; these can be considered highly toxic. The growth inhibition zone of 6.3% isolates was below 2 mm, i.e. they had no toxic properties. As the average of the years, the indicator of the isolates' toxicity was the highest (4.0 mm) in wheat and winter triticale, whereas that of other cereals was 3.5–3.7 mm. Isolates with higher toxicity belonged to the species *F. verticillioides, F. sambucinum, F. poae* and *F. culmorum.*

To determine which seeds might be affected by *Fusarium spp.* occurring in the root rot complex of barley, when grown in a crop rotation, barley root samples collected in the growing period in 1977–1985 were examined (Lõiveke & Pari, 2005). On average, on malt extract agar, *Fusarium* spp. emerged in 47±12% of barley root samples, whereas the most frequently occurring species were *F. culmorum* in 16.8%, *F. sambucinum* in 2.5%, F. avenaceum in 2.2%, *F. oxysporum* in 1.7% and *F. poae* in 1.3% of samples (Table 2). However, using the wet chamber method in 1982–1985, *Fusarium* spp. were detected in only 8±2% of samples. Infection of roots with *Fusarium spp.* depended more on the level of fertiliser background than on the pre-crop, and was greater on a more heavily fertilized background. The infection of barley roots grew constantly in the growth period. When, at the stalking stage, *Fusarium* spp. occurred in 0–60% of roots, the indicator at the milk wax stage was 13–88%.

Although, depending on the environment, the species composition of *Fusarium* spp. on barley seeds and roots is dominated by different species, the incidence of the same species is a connecting link. Thus, in cases of storing of cereals, their contact with soil may be one reason for contamination of the grain with *Fusarium* spp. In soil and the root rot complex, the prevailing species are probably better adapted (*F. culmorum*) to compete with the antagonistic microflora of the soil (Bilaj, 1977). Also in the risosphere of gramineous plants, *F: sambucinum, F. culmorum, F. oxysporum* and *F. solani* dominate (Repšene, 1975). Non-decomposed grain root residue in soil is certainly a source of the *Fusarium* spp. infection.

In their growing period, damage to potatoes by dry rot develops primarily through pests or mechanical injury, along with (?) the existence of the infection in soil. The infection is transferred with soil and tubers into storage preserves, and other tubers become infected through injuries caused by sorting the potatoes. Dry rot caused by *Fusarium* spp. on tubers during their storage period is one of the most damaging potato diseases.

In 1996–2000, *Fusarium* spp. was isolated in 62% of potato tubers with dry rot. The most often occurring isolates were 26.7% and 20.20%, respectively, *F. culmorum* and *F. solani* (Mart.) Sacc., 15.0% - *F poae*, 13.3% - *F. oxysporum*, 8.3% - *F. sulphureum* Schlecht, 5.0% - *F. verticillioides*, 3.3% - *F. nivale* (Fr.) Ces. ex Sacc., 1.7% - *F. coeruleum* (Libert) ex Sacc., 6.7% - *Fusarium* spp. (Table 3). Of the named species, only *F. sulphureum* and *F. coeruleum* were not found on grain and the root rot complex of cereals.

According to Dorozhkin & Belskaya (1979), the following Fusarium species are considered to cause potato dry rot: *F. coeruleum, F. solani, F. sambucinum, F. oxysporum,* and *F. culmorum*. According to Mihaltshik (1977, quoted by Dorozhkin &

Belskaya, 1979) in Byelorussia, species most often separated from tubers with dry rot were *F. sambucinum var. minus* (from 57.9% of tubers) and *F. sambucinum* (from 23.1% of tubers). F. *avenaceum* occurred on 7.4%, *F oxysporum* on 5.4% and *F. culmorum* on 2.7% of tubers.In Finland, Seppänen (1981) found (on tubers with dry rot) mostly *F. solani var. coeruleum* (Sacc.) Booth – on 30% of tubers and *F. avenaceum* (Corda ex Fr.) Sacc. - on 30% of tubers, followed by *F. culmorum* (W. G. Sm.) Sacc. and *F. sulphureum* Schlecht. Schwartz & Gent (2005) in the USA consider *F. solani* the most important, and *F. sambucinum, F.avenaceum, F. culmorum* and *F. oxysporum* less important species. Therefore, the composition of the causes of dry rot may vary in areas, depending on the *Fusarium* flora of the soils.

Several researchers have established the role of *Fusarium* species occurring on potato tubers as toxicants. In Korea, Kim & Lee (1994) found toxic isolates in the species *F. sambucinum, F. oxysporum* and *F. solani*, which caused several health disorders and ultimately the death of tested rats. Rotkiewiecz et al. (1993) revealed that both fungi *F. solani var. coeruleum* and *F. sulphureum* and effected tubers had hepatotoxic and nephrotoxic effects on the rats. El-Banna et al. (1984) established formation of the toxins deoxynivalenol, acetyldeoxynivalenol and HT-2 toxin in tubers damaged by *F. solani var. coeruleum* (Sacc.) Booth or *F. sambucinum* Fuckel..

In 2005, 15 *Fusarium* isolates were separated from potatoes with dry rot in Estonia; their toxicity to *B. stearothermophilus* was determined. Five (5) of the isolates tested were not toxic, 9 were mildly to medium toxic and 1 highly toxic. However, other investigators have found that all isolates are not toxic. For example, in research by Kim & Lee (1994).), only 16 of the 80 isolates were toxic, causing death of the tested rats.

Considering the occurrence of toxic isolates on potato crops in Estonia, potatoes damaged by *Fusarium* spp. must be considered potentially dangerous, and their use as food and fodder should be abandoned.

CONCLUSIONS

Fusarium species often occur on Estonian grain, mostly as epiphytes. Sixteen (16) *Fusarium* species and 4 varieties are represented, the dominating species of which are *F. avenaceum, F. poae, F. semitectum, F. oxysporum, F. ventricosum, F. sporotrichioides, F. verticillioides,* and *F. culmorum*. The contamination is greater on oats and wheat, less on barley and rye, depending primarily on weather conditions during the growing and harvest periods. Also in recent years, contamination with Fusarium species has not decreased, indicating the importance of local weather conditions in contamination of grain with *Fusarium* spp. Although potential toxicant species were established in 50–60% of samples, only 5.6% of the isolates were highly toxic; the majority (88.1%) were mildly to medium toxic. On average, toxicity of the isolates to *B. stearothermophilus* was higher on wheat and winter triticale, and are therefore, more in danger than other cereals.

Fusariums on grain also often occurred in the root rot complex of gramineous plants (13 species), although, in this case, other species were dominant, primarily *F. culmorum, F. sambucinum, F. avenaceum, F. oxysporum,* and *F. poae*, found in 24.5 % of barley root samples. Some *Fusarium* species which were carried to soil with untreated seeds are pathogenic, being able to cause root rot in grain; some are not.

Fusarium species inhabiting soil and non-decomposed plant residue also cause the disease. Occurrence of the same species on cereal seeds and roots refers to a potential common source of infection – soil. When growing potato and cereals in the same crop rotation, the transfer of polyphagous *Fusarium* spp. as soil infection from one culture to another is possible.

On potato tubers with dry rot symptoms, 9 *Fusarium* species were identified, the dominant of which were *F. culmorum, F. solani, F. poae, F. oxysporum* and *F. sulphureum.* Part of the *Fusarium* isolates which were separated were highly toxic to *B.stearothermophilus,* indicating potential danger when potatoes with dry rot symptoms are consumed.

Representatives of *Fusarium* Link. ex Fr. genus often occur in grain grown in Estonia, in the root rot complex of cereals and potato tubers with dry rot. Some of the species have toxic strains (isolates) that may damage the quality and safety of food and fodders. More attention must be paid to reducing and avoiding the harmful effects of *Fusarium* spp.

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