Fusarium spp. on several field crops and toxicity of *Fusarium* isolates

H. Lõiveke

Estonian Research Institute of Agriculture, Teaduse 13, EE75501 Saku, Harjumaa, Estonia; e-mail: heino.loiveke@eria.ee

Abstract. The article provides an overview of the occurrence of *Fusarium* spp. on grain produced in Estonia from 1973 to 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 *F. avenaceum* (Fr.) Sacc., *F. poae* (Pk.) Wr., *F. semitectum* Berk. et Rav., *F. oxysporum* (Schlecht) Snyd. et Hans., *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*, *F. sambucinum*, *F. avenaceum*, *F. oxysporum,* and *F. poae* dominated. In potato with dry rot, *F. culmorum*, *F. solani* (Mart.) Sacc., *F. poae, F. oxysporum* and *F. sulphureum* Schlecht dominated. The occurrence of toxic isolates both on grain and potato was established. Of *Fusarium* isolates separated from grain, 5.6% were highly toxic, and 88.1% mildly or medium toxic, to *Bacillus stearothermophilus*. Of 15 isolates separated from potato, 2 were highly toxic and 3 were mildly to medium toxic. *Fusarium* spp. have been presented according to Gerlach & Nirenberg (1982).

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

Introduction

Fusarium Link. ex 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 they may also occur on grain as epiphytes, without causing any loss of yield, feeding on plant secretions and dead organic matter on the grain surface. *Fusarium* spp. also occur in most soils, particularly in cultured soils (Bilaj, 1977). On roots of annual plants, Fusariums cause mycorisa, which is important and necessary for feeding of the plant. With weakening of the host plant, the Fusariums forming mycorisa 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 diseasedness 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 the recent years (2004, 2005).

Materials and methods

Grain samples for mycological and microbiological survey were collected according to the requirements of average sample composition and analysed after 4-5 weeks. In mycological analysis, the wet chamber method was applied; after 2 and 4 weeks, the percentage of seeds contamination with *Fusarium* spp. was determined. In case of the microbiological analysis, the pour plate method was used (Harrigan & McCance, 1976). The species and number of Fusariums were defined on the basis of the first and second dilution on Nash & Snyder selective medium. 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 collected from long-term crop rotations. For rearing out the pathogens, in 1977-1981 maltagar and in 1982-1985 the wet chamber method was used.

Potato samples with dry rot symptoms from yields of 1996-2000 were collected from storages at the end of the storing period, in March-April, and analysed. The pathogens were reared out on maltagar in Petri dishes at 20-30^0 C during 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* (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.

Results and discussion

Of Estonian grain produced in 1973-1981, on average 79% of samples 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. Little less contaminated was barley 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 the late arrival of the ripeness for harvest. Oats with a long growth time ripen late and, therefore, their harvest period has often high precipitation, and contamination of the grain with Fusariums 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, in Estonia with Fusariums has not decreased.

The most often occurring *Fusarium* species (by Gerlach & Nirenberg, 1982) in 1973-1981 were *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, *F. culmorum* (W. G. Sm.) Sacc. and *F. sambucinum* Fuck. When in drier years *F. oxysporum* and *F. sambucinum* dominated, in more wet years *F. avenaceum* or *F. ventricosum* dominated. 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 oates, *F. avenaceum*, *F. ventricosum* and *F. oxysporum* on wheat. Later wheat research, in 1992, 2002-2004, also revealed most often *F. avenaceum*, *F. oxysporum, F. poae* and *F. semitectum* Berk. et Rav. , 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% samples studied. Whereas, not all strains and isolates within a species are toxic.

The toxicity of *Fusarium* isolates separated from grain yield in 2002-2005 was checked 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% isolates exceeded 6 mm, i.e. 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.*

Fusarium spp. occurring on which seeds may occur in the root rot complex of barley when grown in a crop rotation was studied by examining barley root samples collected in the growth period in 1977-1985 (Lõiveke & Pari, 2005). On maltagar *Fusarium spp.* grew out in 47±12% of barley root samples on average, whereas the most often 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, with using the wet chamber method in 1982-1985, *Fusarium* spp. were detected in 8±2% of samples only. Infection of roots with *Fusarium spp.* depended more on the level of fertiliser background than on the pre-crop, being greater on a higher fertiliser 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* flora on barley seeds and roots is dominated by different species, the incidence of the same species is a connecting link. With lodging of cereals, their contact with soil may thus be one reason for contamination of the grain with *Fusarium* spp. In soil and the root rot complex, the prevailing species are probably the species better adapted (*F. culmorum*) to competing with the antagonistic microflora of 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 Fusariums' infection.

On growing potatoes damage by dry rot develops primarily through pests or mechanical injury with the existence of the infection in soil. The infection transferred with soil and tubers into storage preserves and other tubers become infected through injuries caused by sorting the potatoes. Dry rot caused by Fusariums on tubers is one of the most important potato diseases during their storage period

From potatoes with dry rot in 1996-2000, *Fusarium* spp. was separated in 62% of tubers. The most often occurring isolates were – 26.7% and 20.20%, respectively, *F. culmorum* and *F. solani* (Mart.) Sacc., 13.3% - *F. poae,* 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 the causers of potato dry rot: *F. coeruleum, F. solani, F. sambucinum, F. oxysporum,* and *F. culmorum*. Mihaltshik (according to 1977, quoted Dorozhkin & Belskaya, 1979) in Byelorussia separated from tubers with dry rot most often *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. Seppänen (1981) in Finland 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 causers 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. Kim & Lee (1994).) found in Korea toxic isolates in the species *F. sambucinum, F. oxysporum* and *F. solani*, which caused several health disorders and finally 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 forming of the toxins deoxynivalenol, acetyldeoxynivalenol and HT-2 toxin in tubers damaged by *F. solani var. coeruleum* (Sacc.) Booth or *F. sambucinum* Fuckel..

From potatoes with dry rot in Estonia, 15 *Fusarium* isolates were separated, the toxicity of which to *B. stearothermophilus* was determined. 10 of the isolates tested were not toxic, 3 were mildly to medium toxic and 2 highly toxic. Other investigators have also 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 also on potato grown 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. 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, lesser on barley and rye, depending primarily on the weather conditions of the growth 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 biggest part (88.1%) were mildly to medium toxic. Toxicity of the isolates to *B. stearothermophilus* was averagely higher on wheat and winter triticale, which, therefore, are more in danger than other cereals.

Fusariums on grain often occurred also in the root rot complex of gramineous plants (13 species), although, in this case, other species were dominating: primarily *F. culmorum, F. sambucinum, F. avenaceum, F. oxysporum,* and *F. poae*, found in 24.5 % of barley root samples. Part of *Fusarium* species carried to soil with untreated seeds are pathogenic, being able to cause root rot in grain, part 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 dominating of which were *F. culmorum, F. solani, F. poae, F. oxysporum* and *F. sulphureum.* Part of the separated *Fusarium* isolates 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. Part 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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Table 1. Growth inhibition zone of *Bacillus stearothermophilus* (mm) with *Fusarium* isolates separated from grain

Cereal species	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

Table 2. Occurrence of *Fusarium* species in barley root samples (%) on maltagar in 1977-1981

Fusarium sp. by Gerlach & Nirenberg (1982)	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

Table 3. Composition of *Fusarium* spp. (%) separated from potatoes with dry rot

Fusarium sp. by Gerlach & Nirenberg (1982)	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	600