FUSARIUM SPP. AS AN IMPORTANT PROBLEM IN CEREAL PRODUCTION IN ESTONIA

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Abstract. An overview of investigations on occurrence and hazardousness of *Fusarium* spp. on Estonian cereal grain and in spoiled grain feeds, and the toxicity of *Fusarium* isolates, carried out from the year 1973 up to the present. The problems of pure production without *Fusarium* spp. and fusariotoxins will be discussed.
Cereal grain samples from seed cultivation farms and from disease control trials were analysed by the moist chamber method. Grain feed samples were investigated by the pour plate method (Harrigan & McCance, 1976). The species and number of *Fusarium* 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 Bilai (1955, 1977) and Gerlach & Nirenberg (1982). The toxicity of *Fusarium* isolates was tested on the basis of the growth inhibition zone of *Bacillus stearothermophilus* (Watson & Lindsay, 1982).
On grain produced within the period of 1973–1981, *Fusarium* spp. were identified in 38–100% of samples with infection level 8–67% of seeds. In 67–100% of the studied wheat samples the infection was detected on 13–67% of seeds. In the case of rye, *Fusarium* spp. were identified in 38–86% of the studied samples and infection was found in 8–23% of seeds, with barley the figures were 45–97% and 14–46%; and with oats 55–100% and 15–65%, respectively. *F. avenaceum* (Fr.) Sacc., *F. poae* (Pk.) Wr., *F*: *sporotrichioides* Sherb. *var. minus* Wr., *F. oxysporum* (Schlecht) Snyd. et Hans., *F. verticillioides* (Sacc.) Nirenberg, *F. sambucinum* Fuck., *F. equiseti* (Corda) Sacc. and *F. culmorum* (W.G.Sm.) Sacc. – known as the toxin forming *Fusarium* species (toxicants) – occurred on 50–60% of the studied samples. 90% of samples from wheat grain of the year 1992 were infected with an infection level of 2–21% on spring wheat and 36–59% on winter wheat. The most frequent species were *F. oxysporum, F. semitectum* and *F. sporotrichioides*, and during the years 2002–2003 the species F. *semitectum, F. poae, F.culmorum,* and *F. avenaceum.*
In domestic grain feeds *Fusarium* spp. were found in 52% of the samples, whereas most of them (78% of the cases) were toxic to *B. stearothermophilus*. 31.3% of the studied *Fusarium* isolates were highly toxic and 37.5% medium toxic. Very toxic isolates belonged to the species *F. verticillioides, F. culmorum,* and *F. tricinctum*.
None of the tried fungicide variants (20) saved the yield from *Fusarium* spp. completely. The best effect on decreasing the number of *Fusarium* spp. was exerted by Tilt (propiconazole) and Corbel (fenpropimorph).

Key words: cereal grain, grain feed, toxicant, *Fusarium* spp., mycotoxin, *Bacillus stearothermophilus*, fungicide.

Introduction. *Fusarium* species have been a serious problem in production of plant and cattle breeding products already centuries. Reducing the realisation value of cereals as food, feed and grain cereal, toxins produced by the fungi cause chronic and acute poisoning and allergic signs both to animals and humans. Therefore, throughout the world great attention is paid to investigating *Fusarium* species and elaborating means for controlling them. Contamination of cereals with *Fusarium* toxins is a global problem, occurring in Europe, the Americas, Asia, and Australia (Placinta et al., 1999). A programme initiated by the European Commission "Agriculturally important toxigenic fungi. COST Action 835" focused on the problem of the existence of *Fusarium* species in cereals: disease forms, pathogenicity, species composition, resistance breeding, toxicology, inactivation of toxins. Most European countries participated in the programme (Annual Report 1998. Directorate-General for Research. 2000. EUR 19694).

The Scientific Committee on Plants of the European Commission in its document "Opinion on the relationship between the use of plant protection products on food plants and the occurrence of mycotoxins in food", issued on 30 November 1999, recommends to conduct research to elucidate effects of pesticides for preventing of diseases and production of mycotoxins as well as carry out more monitoring of mycotoxins in foodstuffs used in preparation of food for children and the underaged. Most of the attention should be directed towards toxins produced by *Fusarium, Aspergillus* and *Penicillium* species.

At a session of the cereals workgroup of the European Commission held on 4–5 December 2003 it was admitted that the contamination of cereals with *Fusarium* toxins is a problem in whole Europe. Tens of millions of cereals lay unused until elucidation of their level of contamination, according to which their usage could be determined. It is possible that part of the lots are not fit either as food or feed. At the same time, there was a 10% shortage of necessary grain due to the reduction of the cultivation areas.

Fusarium spp. produce strong toxins, exceeding the toxicity of pesticides used in cereal cultivation hundreds and thousands of times. For example, in peroral administration to mice of LD_{50} mg/kg per liveweight there are toxins: nivalenol (NIV)=4.1; toxin T-2=4.8–5.2; moniliformin=4.0; fusarenon X=3.4. Pesticides: seed dressing preparation Baytan Universal 19.5 WS=3338; the fungicide Tilt 250 EC=2000; the insecticide Actellic 50 EC=1522; the herbicide Basagran M=3200.

Fusarium toxins do not decompose in the animal organism but are transferred into products – eggs, meat and milk, jeopardising thus also human health (Schachermayr & Fried, 2000). It has been found by many scientists that most of the toxins are rather thermostable and do not decompose during thermal processing like boiling, cooking, steaming, and reach our table in brown and white bread (Obenauf, 2002).

This research was carried out to establish the level of contamination of Estonian cereal grain and grain feeds with *Fusarium* species, the potential toxicity of isolates and possibilities to avoid (or reduce) the occurrence of *Fusarium* species in yields and their production of toxic isolates.

For compiling the present overview the results from author`s long-term studies were used (Lõiveke, 1987; Lõiveke, Laitamm, Sarand, 2003; Lõiveke, Ilumäe, Toome, 2004) as well as data published by other researches from different regions. Other authors have studied neither distribution nor harmfulness of *Fusarium* spp. on grain in Estonia.

Methods. Mycological survey. Grain (wheat, rye, barley, oats) samples of yields from 1973–1981 and winter and summer wheat samples from 1992 were collected for mycological testing according to the requirements of average sample composition and analysed after 4–5 weeks. The grain samples were taken from the field trials and seed growing farms of the Estonian Research Institute of Agriculture, where general and special agrotechnical requirements, necessary for production of high reproduction seeds, were followed. In mycological analysis the wet chamber method was applied: microfungi were reared up from grains on filter paper in Petri dishes at a temperature of 20–30°C and 18–22°C (in the years 1974–1982) or at one regime of 20°C (in 1992). After two and four weeks the percentage of seed contamination with *Fusarium* species was determined. To identify *Fusarium* species under microscope, preparations were made.

Microbiological survey. Microbiological samples from grains of field trials in 1993–1994 and samples of grain feeds with spoiling signs from 1997–2002 delivered by animal breeders were analysed by the pour plate method (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 Bilai (1955, 1977) and Gerlach & Nirenberg (1982).

The toxicity of *Fusarium* isolates was tested on the basis of the growth inhibition zone of *Bacillus stearothermophilus* (Watson & Lindsay, 1982): 0-1 mm – non-toxic, 2-5 mm – medium toxic, 6-10 mm – highly toxic.

Field trials with fungicides to control grain diseases were carried out in Üksnurme experimental fields of the Estonian Research Institute of Agriculture by EPPO guidelines PP 1/152(2) in the years 1993 and 1994. In 1993 the trials included 9 variants of the barley \`Ida\` and the summer wheat \`Satu\` whereas in 1994 the number of variants was 11. Following 14 fungicides were tested: Alto 400 SC (active substance agent – cyproconazole), Alto Elite (cyproconazole, carbendazim), Alto Combi (cyproconazole, chlorothalonil), Archer 425 EC (propiconazole, fenpropimorph), Calixin (tridemorph), Corbel (fenpropimorph), Folicur BT 225 EC (tebuconazole, triadimefon), Folicur 250 EW (tebuconazole), Rider 400 EC (propiconazole, fenpropidin); Sportak 45 EC (prochloraze); Tango (tridemorph, epoxiconazole); Tilt 250 EC (propiconazole), Tilt Premium (propiconazole), and Tiptor (cyproconazole, prochloraze).

Barley was sprayed for controlling diseases caused by *Cochliobolus sativus, Pyrenophora teres*, *Rhynchosporium secalis* at the stage GS 37-39 (27.06.1993 and 01.07.1994) and wheat was sprayed for controlling diseases caused by *Mycosphaerella graminicola, Leptosphaeria nodorum* at the stage GS 39-40 (01.07.1993 and 04.07.1994). The trials were harvested at the full-ripening stage on 26.08. and 13.09. in 1993; on 22.08. and 01.09. in 1994, thus 52-73 days following the pesticide application..

Results and discussion. Occurrence and intensity of *Fusarium* infection in 1973–1981. On grain produced within the period of 1973–1981 *Fusarium* spp. were identified in 38–100% (average 79%) of samples (1065), the infection level being 8–67% (average 29%) of seeds. In 67–100% of the studied wheat samples (57) the infection was detected on 13–67% of seeds. In the case of rye, *Fusarium* spp. were identified in 38–86% of the studied samples (85) and infection was found in 8–23% of seeds, with barley (720 samples) the figures were 45–97% and 14–46%; and oats (203 samples) 55–100% and 15–65%, respectively. 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 the samples were infected at an average rate of 14%. The highest average rate of infection (48%) of grain samples was detected in 1978 characterised by the highest amount of precipitation (480 mm) during the growing season (June–September). Harvest time – 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 (Lõiveke et al., 2003). 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.

Composition of *Fusarium* species in 1973–1981. The frequency of the occurrence of *Fusarium* species (by Gerlach & Nirenbeg, 1982) depended on the grain species and the year. In 1973 and 1974 the prevailing species was *F. ventricosum* App. et Wr., whereas in 1976, 1978, 1979 and 1981 *F. avenaceum* (Fr.) Sacc. predominated. In 1975, the driest year, the composition of *Fusarium* species was the smallest: *F. avenaceum* and *F. sporotrichioides* Sherb. *var. minus* Wr. did not occur at all. In 1978, the year of the highest amount of precipitation, *F. avenaceum* occurred more frequently than ever – in 62% of the studied samples. The most common species were *F. avenaceum, F. poae* (Pk.) Wr., *F. oxysporum* (Schlecht) Snyd. et Hans, *F. ventricosum, F. sporotrichioides, F. verticillioides* (Sacc.) Nirenberg, and *F. culmorum* (W.G.Sm.) Sacc. In about 40% of the samples, the occurrence of two or more *Fusarium* species was detected simultaneously. *F. avenaceum*, *F. poae*, *F. sporotrichioides*, *F. oxysporum*, *F. verticillioides*, *F. sambucinum* Fuck., *F. equiseti* (Corda) Sacc. and *F. culmorum* – known as the toxin forming *Fusarium* species (toxicants) were detected on 50–60% of the studied samples (Lõiveke et al., 2003). The mentioned species are capable of producing many toxins – DON (deoxynivalenol), 3-ADON, 15-ADON, NIV (nivalenol), ZEN (zearalenone), HT-2, fumonisins, moniliformin, fusarin C, wortmannin, sambutoxin, fusarenon X, T-2, DAS (diacetoxyscirpenol), etc. (Bilai, 1977; Kadis et al., 1971; Miller & Trenholm, 1997).

Compared with *Fusarium* flora detected in cereals in Finland (Ylimäki, 1981; Ylimäki & Jamalainen, 1986), Estonian cereals are mostly infected by the same species. A difference is greater occurrence of *F. graminearum* and more modest occurrence of *F. verticillioides* in Finland than in Estonia.

Fusarium species in wheat in 1992, 2002, and 2003. 90% of samples from wheat grain of the year 1992 were infected with an infection level of 2–21% (average 12%) on spring wheat and 36–59% (average 47%) on winter wheat. The most frequent species were (by Gerlach & Nirenbeg, 1982) *F. oxysporum, F. semitectum* and *F. sporotrichioides*, and during the years 2002–2003 the species *F. semitectum, F. poae, F. culmorum,* and *F. avenaceum.* As revealed by a comparison of results from 1992 and 1973–1981, in the case of wheat, contamination with *Fusarium* species has not diminished at all, allowing us to conclude that the contamination of other cereals has not changed either.

Consequently, in Estonia there exist factors favouring *Fusarium* infection in grains, although head blight is very rare. The favouring factors are primarily rainy growing seasons and excessively wet pre-harvest periods. Development of *Fusarium* species in harvested grains can be inhibited only by quick quality drying to 13–14% moisture and storing in conditions avoiding excessive moisture (Ylimäki et al., 1979). In case grain has been infected with *F. graminearum*, it is considered necessary to dry the grain to moisture 10–13%, to avoid generation of mycotoxins in the future as well (Jevseyeva, 1992). One cannot always ensure operative drying in production conditions, which has caused grain and feeds to be spoiled because of *Fusarium* species and other microorganisms.

Fusarium species in domestic grain feeds in 1997–2002. In feeds with spoiling signs *Fusarium* spp. sometimes made up 6.8–8.2% of the total number of fungi, whereas also the known toxic species *F. culmorum, F. tricinctum,* F. *verticillioides* and *F.*

sporotrichioides were present. Fusariums were found in 52% of the 32 studied samples of feed that had caused health problems to animals: decrease in production or, in some cases, even death of animals. Could this be caused by *Fusarium* species in addition to other toxic moulds (*Acremonium, Cladosporium, Aspergillus, Penicillium, Rhizopus*, *Trichothecium, Paecilomyces*)? To elucidate it, *Fusarium* species were isolated into pure culture and their toxicity was studied.

<u>Toxicity of *Fusarium* isolates in domestic grain feeds.</u> The toxicity of *Fusarium* isolates separated from feeds was controlled by biotest by the size of the growth inhibition zone of *Bacillus stearothermophilus.* 78% of samples contaminated with *Fusarium* spp. contained *Fusarium* isolates toxic to *B. stearothermophilus*. Thus approximately 40% of the samples contained toxic isolates, showing that *Fusarium* species often are contaminators of feeds with mycotoxins. Of the 16 isolates, 31.3% were highly toxic (growth inhibition 6–10 mm) and 37.5% medium toxic (2–5 mm). The highly toxic isolates belonged to the species *F. verticillioides, F. culmorum,* and *F. tricinctum*.

A monitoring conducted by the Agricultural Research Centre in 1998–2002 revealed that there are *Fusarium* toxins in grain feeds and feed mixtures used in Estonia. In 1998 ZEN was found in 20%, in 1999 in 24%, in 2000 in 50%, in 2001 in 19%, and in 2002 in 14% of samples. DON was present in 1999 in 1.6% and in 2000 in 4.5% of samples.

<u>Toxicity of *Fusarium* isolates in grain.</u> 68 *Fusarium* isolates were separated from grain of 2002 and 2003 and the toxicity of the isolates was determined. In the case of 26 isolates obtained from wheat, the growth inhibition zone was 2–7 mm, with 24 barley isolates 2–5 mm, with 15 oats isolates 2–7 mm, and with 3 rye isolates 3–4 mm. Of wheat isolates from 2002, the most toxic were *Fusarium* sp., *F. culmorum,* and *F. verticillioides* (4–7 mm). Although the named year had a relatively dry growth period (precipitation in Saku 52 % of the norm), the toxicity of separated isolates was 3–7 (mm). Consequently, the variable *Fusarium* flora on grains contains *Fusarium* strains (forms) of very different toxicity.

<u>The effect of fungicides on the occurrence of *Fusarium* species in yields.</u> To suppress both typical pathogens and saphrophytic mycoflora, fungicides are usually applied in grain cultivation when 1–5% of the surface of the third leave from the top has been damaged. As it may occur 60–70 days prior harvest, it is important that the effect of fungicides is possibly long-lasting to protect grain from becoming infected in rainy autumn with delayed harvest. By analysing microbiologically barley and summer wheat yields of 1993 and 1994 it was tried to elucidate whether it was possible to reduce occurrence of *Fusarium* spp. in yield by using fungicides. Corbel (fenpropimorph) reduced the number of *Fusarium* species in yield up to 10 times, Sportak 45 EC (prochloraze) – up to 7 times, Tilt 250 EC (propiconazole) - up to 6 times, Folicur BT 225 EC (tebuconazole, triadimefon) – up to 5 times, Rider (fenpropimorph) – up to 3 times, and Calixin (tridemorph) – up to 2 times. The best of the named can considered Tilt and Corbel that were effective in 75–100% of the tests. The effect of other fungicides was unstable, fluctuating from reducing the number of *Fusarium* species to increasing the number.

According to data in literature, contradictory results have been obtained in experiments with fungicides for controlling fusarioses. D`Mello et al. (1998) suggest on the basis of the *in vitro* experiments that fungicides are often ineffective in controlling the production of mycotoxins by *Fusarium* and *Aspergillus* species. Consequently, fungicides are not effective in restricting the number of the species either. The results obtained cannot, however, be transferred to field conditions, where the *Fusarium* flora consists of many species and strains. *In vitro* experiments have been carried out with one specific *Fusarium* strain. Also in field conditions, fungicides have either reduced or increased infection with *Fusarium* fungi and the production of toxins. In Japan Topsin M (thiophanate-methyl) reduced damage by *F. graminearum* on wheat and barley as well as the content of trichotecenes, DON, NIV in yield (Ueda, Yoshizawa, 1988). Several authors (Suty et al., 1996; etc.) report the good effect of Folicur (tebuconazole) in controlling head blight and reducing toxin content in yield. Gareis and Ceynova (1994), on the other hand, found that the fungicide Matador (tebuconazole, triadimenol) considerably reduced the incidence of head blight in wheat infected with *F. culmorum* but increased the content of NIV in yield 16 times. An increase of toxin content in yield has been noticed particularly in using strobilurines. Oldenburg et al. (2000) tested comparatively azoles and strobilurines in controlling *F. graminearum* and *F. culmorum* on winter wheat. If fungicides containing tebuconazole and metconazole reduced the DON content compared with control, strobilurines, on the other hand, increased it. Tischner and Doleschel (2003) also noticed similar increase of the DON content in using strobilurines. In the same experiments, azoles reduced both spreading of the disease and the DON content in yield by 40–70%. Therefore, the use of highly effective strobilurines is possible only in combination with azoles. Such a fungicide combination system must, during the growth period, suppress development of all plant diseases, restrict effectively reproduction of saprophytic fungi and also ensure unhindered production of *Fusarium* spp. and fusariotoxins.

Conclusions. *Fusarium* spp. often occur in Estonian grain – 38–100% of samples, whereas the level of seed infection (8–67%) primarily depends on the weather of the growth period. The most contaminated cereal in 1973–1981 was oats (87% of samples, 33% of seeds), followed by wheat (86% and 29%, respectively) and barley (79% and 29%), the least contaminated being rye (62% and 14%). At present the infection of grain with *Fusarium* species has not diminished. Infection of grain increases with rainy growing seasons, lodging, and delayed harvest. The most widely spread species are *F. avenaceum, F. poae, F. semitectum, F. oxysporum, F. ventricosum, F. sporotrichioides, F. verticillioides,* and *F. culmorum.* The composition of *Fusarium* flora depends on weather as well. Species known as toxicant often occur in grain (in 50–60% samples). Fusariums are a genus of microorganisms contributing to the spoiling process of grain feeds (cereal grain). *Fusarium* species in grain feeds with spoiling signs sometimes made up 6.8–8.2% of the total number of fungi and they were found in 52% of samples, including also the toxicants: *F. culmorum, F. tricinctum,* F. *verticillioides,* and *F. sporotrichioides.* 40% of feed samples contained *Fusarium* isolates toxic to *Bacillus stearothermophilus*. Of the isolates studied, most (69%) were medium or highly toxic. The most toxic ones were *F. verticillioides, F. culmorum* and *F. tricinctum* isolates. When separated from dry grains of quality, the toxicity of *Fusarium* isolates fluctuated from non-toxicity to high toxicity. Of the 14 fungicides tested, Tilt 250 EC (propiconazole) and Corbel (fenpropimorph) reduced most (6–10 times) the number of Fusariums in yield.

To obtain production free of *Fusarium* spp. and fusariotoxins, it is necessary to apply a system of measures, where both (chemical dressing of seeds sown, spraying of fields with fungicides, use of retardants to avoid lodging) and agrotechnical (optimum sowing norm and time, balanced fertilisation, timely harvest, etc.) methods are used. It is also necessary to elaborate a system of combining strobilurines and azoles (and other fungicides) that would be effective for controlling the majority of diseases and saphrophytic fungi appearing at the end of growth period. Quick drying and cleaning of harvested grain and its storing in suitable conditions are the most important factors for avoiding subsequent production of toxins and ensuring quality storing of grain.

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