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Contamination of cereal grain by *Fusarium* micromycetes and their mycotoxins under Lithuanian climatic conditions

Rimutė Mačkinaitė,

Audrius Kačergius,

Albinas Lugauskas,

Jūratė Repečkienė

Institute of Botany,
Žaliųjų Ežerų Str. 49, LT-08406 Vilnius, Lithuania.
E-mail: rimute@botanika.lt

In 2003–2005, cereal grains grown in Lithuanian climatic conditions were strongly contaminated by mould fungi. *Fusarium* was among the most common fungal genera identified in wheat and barley grains. Fungi of the genus *Fusarium* contaminated 92.1% of the grain samples tested. The spread and subsequent development of fusaria in cereal grains depend on climatic conditions, particularly rainfall and temperature. The vegetation period in 2005 was sufficiently warm and wet and thus most convenient for the distribution of *Fusarium*, especially of *F. graminearum.*

Twenty four taxa of *Fusarium* were identified in wheat and barley grains. *F. graminearum*, *F. poae*, *F. avenaceum*, *F. equiseti*, *F. sporotrichioides* and *F. sambucinum* were most frequently isolated from grain surface, while *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. graminearum* and *F. culmorum* dominated in the internal tissues with a decreasing prevalence. The complex of *Fusarium* species ascertained in wheat and barley grains in various years differed in composition as well as in isolation frequency and the relative density of separate species. These indexes depended on climatic conditions of a separate year and on the species of cereal as well. Different *Fusarium* complexes which developed on the surface and in the internal tissues of grains were established.

F. sporotrichioides and *F. graminearum* were ascertained as the main producers of T-2 toxin and its derivates in wheat and barley grains. *F. culmorum* can be the main source of nivalenol, *F. avenaceum* produces enniatins and beauvericin. Producers of trichotecenes A dominated in the grain samples studied.

Key words: *Fusarium,* species diversity, frequency of occurrence, cereal grain, mycotoxins, climatic conditions

INTRODUCTION

The genus *Fusarium* comprises a diverse array of fungi which are distributed world-wide as important plant pathogens, as well as opportunistic colonizers of plant and agricultural commodities or as saprophytes on debris and cellulosic plant material (Logrieco et al., 2003). Pathogenic species cause a range of plant diseases such as vascular wilt, root and stem rot, seedling blight, cereal ear rot. *Fusarium* pathogens on cereals are responsible for two forms of disease. These are 'foot rot', which affects roots and crowns and may cause seedling blight at early stages, and 'head blight' (FHB) which affects individual kernels, single ear spikelets or entire heads and results in kernel 'scab' (Logrieco et al., 2003). In the last ten years, the increased level of colonization and infection by *Fusarium*, particularly of ripening ears of cereals, has attracted much attention: firstly, because of the significant effects on yield and the quality of harvested grains, and secondly, because of the ability of *Fusarium* species to produce a wide range of mycotoxins which can enter the human and animal food chains (Magan et al., 2002). The mycotoxins in wheat and barley, which constitute almost two-thirds of the world's and almost 80% of European small-grain cereal production, is of particular concern because of the extent of infection and contamination of food products (Bottalico, Perrone, 2002).

Cereal grains can be colonized by up to 17 different *Fusarium* species, however, only few of them predominate in a particular host-agroclimatic system. *F. graminearum*, *F. culmorum*, *F. avenaceum* are the species predominantly associated with cereal grain. *F. poae*, *F. cerealis*, *F. equiseti*, *F. sporotrichioides* and *F. tricinctum* are among the most common *Fusariam* as well. *F. acuminatum*, *F. subglutinans*, *F. solani*, *F. oxysporum*, *F. incarnatum*, *F. verticillioides* and *F. proliferatum* are isolated more rarely (Parry et al., 1995; Bottalico, Perrone, 2002; Logrieco et al., 2003).

Different *Fusarium* species thrive in different climates (Dardis, Walsh, 2002). They differ in their climatic distribution and in the optimum climatic conditions required for their persistence (Doohan et al., 2003). *F. graminearum* is most common in moist-warm regions, while *F. culmorum* and *F. avenaceum* prefer cooler climate areas. Temperature and humidity/wetness are the main climatic factors influencing the development of *Fusarium* on cereals, although the influence of these climatic factors is not independent of other environmental and host factors (Doohan et al., 2003; Xu, 2003). Environmental factors may exert some selective pressure influencing the community structure and dominance of individual species (Martin et al., 1998; Magan et al., 2003).

The conditions favourable for *Fusarium* development are also favourable for mycotoxin production on cereal grain (Doohan et al., 2003). *Fusarium* fungi produce more than 40 known mycotoxins and many of *Fusarium* species can produce mycotoxins in grains (Dardis, Walsh, 2002). Mycotoxins can be formed in pre-harvest infected plants still standing in the fields, or in stored grains (Bottalico, 1998). In turn, the optimum climatic conditions for mycotoxin production in infected grains depend on the substrate, *Fusarium* species and isolate. High moisture favours the production of mycotoxins, but the optimum temperatures for mycotoxin production in *Fusarium*-infected grain appears to be specific to the substrate, species and individual metabolites (Doohan et al., 2003). The most common *Fusarium* mycotoxins are trichothecenes, zearalenones, and fumonisins. In addition, moniliformin, beauvericin, and fusaproliferin may be occasionally present (Logrieco et al., 2003).

Trichothecenes include T-2 toxin and its derivates, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), neosolanol (NEO), as well as deoxynivalenol (DON), nivalenol (NIV) and fusarenone X (FUS) and their derivates. These toxins are produced by strains of *F. sporotrichioides*, *F. acuminatum*, *F. poae*, *F. sambucinum*, *F. equiseti*, *F. graminearum*, *F. culmorum*, *F. cerealis*. Zearalenones: zearalenone (ZEA) and zearalenols (ZOH) are produced by *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti* and *F. incarnatum* and are among the most widely distributed *Fusarium* mycotoxins in agricultural commodities. Amongst the characterized compounds of fumonisins, fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) present the greatest mycotoxycological concern. They were isolated from *F. verticillioides* and *F. proliferatum.* Moniliformin (MON) has been found in the cultures of *F. proliferatum*, *F. subglutinans*, *F. avenaceum*, *F. tricinctum*, while beauvericin (BEA) and enniatins have been reported to be produced by *F. incarnatum*, *F. subglutinans* and *F. proliferatum.* Fusaproliferin (FUP) is a novel sesquiterpene produced by strains of *F. proliferatum* and *F. subglutinans* (Logrieco et al., 2003; Bottalico, Perrone, 2002).

Fusarium population consists of different individuals having different pathogenic characters including toxin-producing ability (Masretházy, 2002). Interactions between *Fusarium* species markedly influence mycotoxin production, some species stimulating and others inhibiting it. The interactions between these fungi are complex and strongly affected by the prevailing and changing environmental factors. Water activity, temperature and nutrient substrate are essential in the overlap and fluctuation of dominate mycotoxigenic species (Winclow et al., 1980; Martin at al., 1998; Magan at al., 2003).

The objectives of this study were to evaluate *Fusarium* contamination of wheat and barley grains grown under Lithuanian climatic conditions; to determine the prevailing *Fusarium* species on the surface and in the internal tissues of grains and their ability to produce toxic compounds.

MATERIALS AND METHODS

The investigation was carried out in 2003–2005 at the Institute of Botany. Seventy one grain samples of commercial wheat (*Triticum aestivum* L.) and barley (*Hordeum distichon* L.) grown under Lithuanian climatic conditions in different localities were sampled for testing after harvest.

For the evaluation of grain surface contamination by mould fungi, the dilution plating technique was applied (Samson et al., 1992). 10 g of grains was placed in 100 ml of sterile water, shaken for 10 min., dilutions (1:100, 1:1000, 1:10000) were made, and 1 ml of the suspension was drawn into a Petri dish and poured over with malt extract agar (MEA) with addition of chloramphenicol (50 mg/l). The analysis of each sample was performed in three replications. Fungi were cultivated for 7–10 days at 28 °C. The isolation frequency (IF) of a dominant species was calculated as a relation of the number of samples in which a species was found to the total number of the samples tested, expressed in percentage (Мирчинк, 1988; González et al., 1995).

To determine the internal contamination by mould fungi of grains, the agar plate method (Наумова, 1971; Mathur et Kongsdal, 2003) was applied. One hundred grains were tested for each sample. The grains were sterilized with 3% sodium hypochlorite for 2 min and rinsed three times with sterile water, then drained with sterile filter paper and placed on Petri dishes containing MEA with streptomycin addition (250 mg/l). The plates were incubated for seven days at 26 °C. After incubation, the number of micromycete species detected in each grain was calculated. The species frequency of occurrence (FO) as a relation of the number of grains in which the species was detected to the total number of grains tested, expressed in percentage, and the species relative density (RD) as a relation of the number of isolates of a particular species to the total number of isolates, expressed in percentage, in the grains were calculated (Booth, 1971; Мирчинк, 1988; González et al., 1995).

The species were identified on the basis of their morphological and cultural characteristics according to Ellis (1971, 1976), Билай (1977), Arx (1981), Gerlach, Nirenberg (1982), Nelson et al. (1983). The morphological identification of *Fusarium* isolates was confirmed by PCR analysis with species-specific primers (Kačergius, Mačkinaitė, 2005).

Single spore cultures of selected isolates were transferred on MEA and SNA for preservation and for studies of their ability to produce secondary metabolites. The method used for detecting the production of secondary metabolites by fungal strains using thin layer chromatography (TLC) was described previously (Kačergius & Mačkinaitė, 2005; Kačergius et al., 2005). For testing the production of secondary metabolites, the strains were grown on MEA, including 4% yeast extract and 4% glucose. The cultures were incubated at 27 °C for 3 weeks. The analysis of secondary metabolites was carried out by thin layer chromatography (TLC). An agar plug with a mycelium 8 mm in diameter was cut out of the colony and extracted with 1 ml of a chloroform / methanol mixture (2:1) overnight. The production of secondary metabolites is usually highest in the centre (oldest) of a colony. After extraction, 20 µl of extract was placed onto TLC plates. Toxin standards T-2, deoxynivalenol, nivalenol, neosolanol (SIGMA) were used at a concentration of 1 mg/ml. For analysis, precoated plates of silica gel (20 by 20 cm, 0.20 mm thick; Macherey–Nagel, Germany) containing a fluorescence indicator (F254) or no indicator were used. The solvent system of toluene / ethyl acetate / formic acid (90%) (TEF) 5:4:1 was chosen. After elution and drying, the TLC plates were examined in visible light and in UV-light (UV 254/366 nm). The 5% spray reagent $AlCl_3$ was used for visualising of secondary metabolites.

The pure cultures of *Fusarium* strains with a high mycotoxin production level have been deposited in the collection of the Institute of Botany, Vilnius, Lithuania.

Climatic conditions. The contamination of cereal grains by fungi is, to a large extent, determined by climatic parameters, particularly temperature and humidity. The distribution and predominance of fungi on cereal grains are influenced by the weather conditions, especially in the second half of June, in July and early in August, i.e. at the time of grain ripening and harvesting.

According to the data of the Lithuanian Hydrometeorology Service, the weather of summer in 2003 was various. The beginning of June was warm and dry, while its second part was cool, rainy, with late frosts. The mean temperature of June was 14.3–15.8 °C. In some west and south regions it was near the average temperature of several years' standing, and elsewhere was 0.4–1.1 °C lower. The amount of precipitation for the whole June in the biggest part of Lithuania reached 40–70 mm and made up 60% of the norm. In July, hot weather prevailed. The average temperature of the month was 19.3–20.6 °C (2.7–3.7 °C higher than the average of several years' standing) and in the last third 21.5–23.3 °C (even 5–6 °C higher than the average). The precipitation for July in many regions of west and middle Lithuania made up 40–70% of the norm, while in the northeast and some south regions it reached 1.3–2 norms. The hot weather of July was favourable for grain ripening. The hot and dry first part of August was convenient for harvesting. In many regions the average temperature of the month was by 0.5–1.1 °C higher than the norm. In some regions the draught began. The draughty weather came to the end only with a strong rainfall in the end of the second ten-day period.

The June 2004 was cool and rainy. Its average temperature was by 0.6-2.1 °C lower than the average of several years. The amount of precipitation in many regions made up 130–180% of the average. July was cool and damp as well. The average temperature of the whole month was close to the mean of several years' standing (15.2–17.1 °C). In many regions, the amount of precipitation was close to the average (66–107 mm), however, the rains were scattered unevenly. The cool and damp weather conditions were favourable for plant vegetation. The cereal crops grew up vigorously, but their ripening was prolonged and harvesting was late. Although August was rather warm (17.2–19.6 °C), the rain delayed the harvesting.

Early in June 2005 cool weather prevailed. The second ten-day period was warm, however, it got colder to the end of month. The average temperature was by 0.1–0.9 °C lower than the mean of several years' standing. The precipitation was of rainfall nature frequently. Its amount in the north and southeast regions exceeded 1.5–2 times the average, elsewhere being close to the norm. The weather of the first half of July was warm and dry, the second half being warm and damp. The mean temperature of the month was 1.1–3.8 °C higher than the average of several years' standing, and the amount of precipitation made up 30–65% of the average. Early in August cool and rainy and later warm and dry weather prevailed. The mean temperature of the whole month was close to the average (15.5–17.9 °C). The amount of precipitation exceeded 1.5–3.5 times the average in many regions during the month. The soil became wet, and the fields in lower localities were flooded by a strong rainfall. The rain and wind lodged cereal crops.

RESULTS AND DISCUSSION

Our research has shown that cereal grains grown under Lithuanian climatic conditions are strongly contaminated by mould fungi. The contamination of all grains sampled in 2003–2005 was close to 100%. Fungi of 26 genera were obtained. *Alternaria* Nees, *Ulocladium* Preuss, *Fusarium* Link: Fr., *Penicillium* Link: Fr., *Aspergillus* P. Michael ex Link: Fr. and *Bipolaris* Shoemaker (in barley grains) were most common. Fungi of

the genus *Fusarium* were identified in 92.1% of the grain samples tested. Their frequency of occurrence (FO) made up 16.1%.

Twenty four taxa of *Fusarium* were identified in the present study (Table 1). *F. avenaceum*, *F. graminearum*, *F. poae* and *F. sporotrichioides* dominated on the grain surface as well as in the internal tissues. *F. graminearum*, *F. poae*, *F. avenaceum*, *F. equiseti*, *F. sporotrichioides* and *F. sambucinum* were most frequently isolated (with a decreasing prevalence) from the grain surface, while *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. graminearum* and *F. culmorum* dominated in the internal tissues. *F. equiseti* was common only on the grain surface and while *F. culmorum* only in the internal tissues.

Contamination of barley by *Fusarium* was higher than of wheat and reached 97.1% on the average. *F. graminearum* (IF 65.7%), *F. avenaceum* (IF 51.4%), *F. equiseti* (IF 42.8%) prevailed on the surface of barley grain. *F. sporotrichioides* (IF 40.0%) and *F. sambucinum* (IF 37.1%) were common as well (Fig.1). Fungi of the genus *Fusarium* made up 15.8% of the total amount of isolates in the internal tissues of barley grains, and their frequency of occurrence reached 22.5%. *F. sporotrichioides* (FO 6.9%) and *F. poae* (FO 6.4%) dominated among them. *F. avenaceum* (FO 2.7%), *F. culmorum* (FO 1.8%) and *F. graminearum* (FO 1.2%) were among the most common species as well (Fig. 2).

The contamination of wheat grains by fusaria made up 87.8%. *F. poae* (IF 41.5%) prevailed on the surface. *F. graminearum*, *F. moniliforme* and *F. oxysporum* with IF 34.1% were among the most common *Fusarium* species. The isolation frequency of *F. avenaceum* made up 29.3% and of *F. equiseti* and *F. sporotrichioides* 26.8% (Fig.1). Fungi of genus the *Fusarium* made up 8.6% of

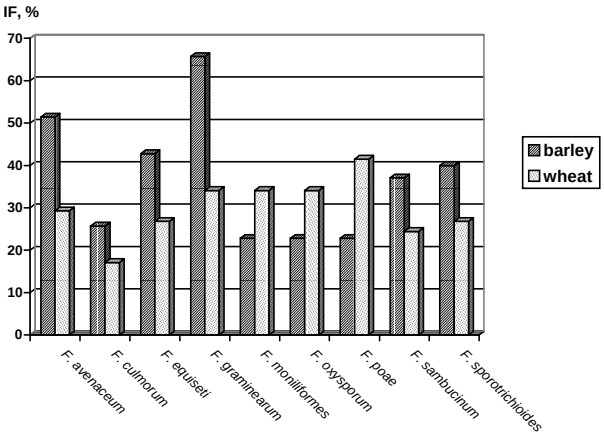

Fig. 1. The isolation frequency (IF) of dominant *Fusarium* species from wheat and barley grain surface

Table 1. ***Fusarium*** **species identified in cereal grains**

Fusarium species	IF (%) from grain surface	FO (%) in the internal tissues of grains
F. acuminatum Ellis et Everh. (*Gibberella acuminata* C. Booth)	0	0.11
F. anthophylum (A. Braun) Wollenw.	1.3	0.06
F. aqueductuum (Radlk. et Rabenh.) Lagerh.	0	0.01
F. avenaceum (Fr.) Sacc.(*Gibberella avenacea* R. J. Cook)	39.5	1.99
F. chlamidosporum Wollenw. et Reinking	0	0.03
F. culmorum (W. G. Sm.) Sacc.	21.1	0.94
F. equiseti (Corda) Sacc. (*Gibberella intricans* Wollenw.)	34.2	0.14
F. graminearum Schwabe (*Gibberella zeae* (Schwein.) Petch)	48.7	1.30
F. graminum Corda	0	0.35
F. heterosporum Nees et T. Nees (*Gibberella gordoni* C. Booth)	1.3	0.08
F. incarnarum (Desm.) Sacc.	10.5	0.30
F. merismoides Corda	3.9	0
F. moniliforme J. Sheld. (*Gibberella moniliformis* Wineland)	28.9	0
F. nivale (Fr.) Ces. (*Microdochium nivale* (Fr.) Samuels et J.C. Hallett = *Monographella nivalis* (Schaffnit) E. Müll.)	6.6	0
F. oxysporum Schltdl.	28.9	0.03
F. oxysporum f. sp. *conglutinans* W. S. Snyder et H. N. Hansen	0	0.06
F. poae (Peck) Wollenw.	46.0	4.61
F. proliferatum (Matsush.) Nirenberg ex Nirenberg et Gerlach	7.9	0
F. sambucinum Fuckel (*Gibberella pulicaris* (Fr.) Sacc.)	30.3	0.34
F. sambucinum var. *sambucinum* Fuckel	0	0.23
F. solani (Mart.) Sacc. (*Nectria haematococca* Berk. Et Broome)	17.1	0.10
F. sporotrichioides Scherb.	32.9	4.41
F. subglutinans (Wollenw. et Reinking) P. E. Nelson, Toussoun et Marasas	0	0.10
F. tricinctum (Corda) Sacc. (*Gibberella pulicaris* (Fr.) Sacc.)	17.1	0.11
Fusarium spp.	5.3	0.76

Note: IF – isolation frequency; FO – occurence frequency.

the total amount of isolates, and their frequency of occurrence reached 10.7% in the internal tissues of wheat grains. *F. poae* (FO 3.2%) and *F. sporotrichioides* (FO 2.4%) were predominant, *F. graminearum* and *F. avenaceum* with FO 1.4% being common as well (Fig. 2).

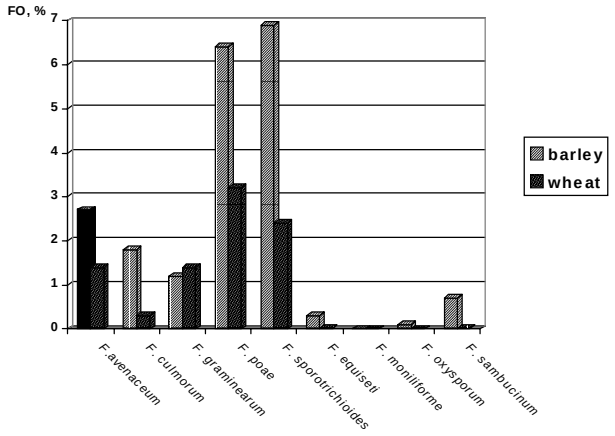

Fig. 2. The occurence frequency (FO) of dominant *Fusarium* species in the internal tissue of wheat and barley grain

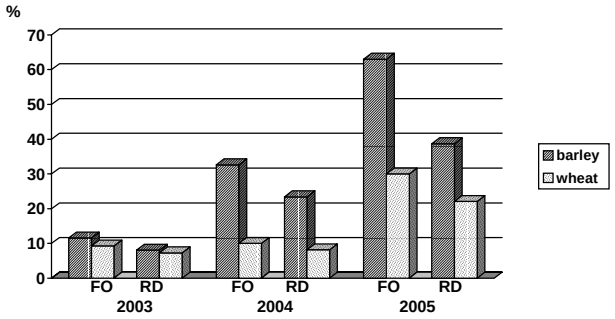

Fig. 3. The frequency of occurence (FO, %) and relative density (RD, %) of *Fusarium* in cereal grains in different years

The spread and subsequent development of micromycetes in cereal grains depend on several agronomic factors such as soil cultivation, nitrogen fertilization, fungicides, crop rotation, host genotype, however, primarily on climatic conditions, particularly rain and temperature (Botalico, Perrone, 2002). The most favourable factor for the spread of *Fusarium* fungi is rainy and warm weather during cereal anthesis, although the infection may be prolonged from the period of ears formation to early wax ripeness (Parry et al., 1995; McMullen et al., 1997).

In our investigation, the rather warm and wet weather prevailing in the vegetation period of 2005 was especially convenient for the spread of *Fusarium.* Although the diversity of *Fusarium* species was not very high, their frequency of occurrence in the internal tissues of barley and wheat grains reached even 63.0% and 30.0%, respectively (Fig. 3). The isolates of *Fusarium* spp. made up a considerable part among the other micromycetes obtained in grains of the 2005 harvest. The relative density of the isolates reached 38.7% and 22.1% in wheat and barley grains, respectively. This index in barley grain in 2003 and 2004 was 8.1% and 23.4% and in wheat grain 7.3 and 8.2%, respectively (Fig. 3).

The complex of *Fusarium* ascertained in wheat and barley grain in various years differed in their species composition as well as in isolation frequency and the relative density of separate species. These indices depended not only on the climatic conditions of a year, but also on grain species. Different *Fusarium* complexes have been formed on grain surface and in the internal tissues.

Twenty-one taxa of *Fusarium* were identified in cereal grains harvested in 2003. *F. avenaceum* and

Table 2. ***Fusarium*** **species on the surface of wheat grains of 2003–2005 harvest**

Fusarium species	Isolation frequency, %		
	2003	2004	2005
F. avenaceum	31.2	23.8	50.0
F. culmorum	0	28.6	25.0
F. equiseti	12.5	33.3	50.0
F. graminearum	31.2	23.8	100.0
F. heterosporum	0	0	25.0
F. incarnatum	6.2	9.5	0
F. merismoides	0	4.8	0
F. moniliforme	56.2	9.5	75.0
F. nivale	6.2	4.8	0
F. oxysporum	31.2	28.6	75.0
F. poae	25.0	52.4	50.0
F. proliferatum	6.2	4.8	25.0
F. sambucinum	18.8	28.6	25.0
F. solani	6.2	19.0	25.0
F. sporotrichioides	6.2	42.8	25.0
F. tricinctum	18.8	23.8	0
Fusarium spp.	12.5	0	0

Table 3. ***Fusarium*** **species on the surface of barley grains of 2003–2005 harvest**

Fusarium species	Isolation frequency, %		
	2003	2004	2005
F. anthophilum	0	7.1	0
F. avenaceum	62.5	42.8	0
F. culmorum	12.5	42.8	33.3
F. equiseti	43.8	42.8	66.7
F. graminearum	62.5	64.3	100.0
F. incarnatum	12.5	21.4	0
F. merismoides	0	14.3	0
F. moniliforme	43.8	0	0
F. nivale	0	21.4	0
F. oxysporum	6.2	35.7	66.7
F. poae	12.5	21.4	100.0
F. proliferatum	0	14.3	33.3
F. sambucinum	37.5	42.8	33.3
F. solani	18.8	28.6	0
F. sporotrichioides	25.0	71.4	0
F. tricinctum	0	35.7	0
Fusarium spp.	12.5	0	0

Table 4. ***Fusarium*** **species in the internal tissues of wheat grains of 2003–2005 harvest**

Fusarium species	2003		2004		2005	
	FO, %	RD, %	FO, %	RD, %	FO, %	RD, %
F. acuminatum	0.1	07	0	0	1.5	5.0
F. anthophylum	0.1	0.7	0	0	0	0
F. aqueductuum	0	0	0.1	0.5	0	0
F. avenaceum	0.3	3.4	1.2	11.9	12.0	40.0
F. chlamidosporum	0.1	0.7	0	0	0.5	1.7
F. culmorum	0.3	3.4	0.3	2.9	0	0
F. equiseti	0	0	0.1	0,5	0	0
F. graminearum	0.5	5.4	1.8	18.1	4.5	15.0
F. graminum	0.1	0.7	0.2	2.4	0	0
F. heterosporum	0.2	2.0	0.1	0.5	0	0
F. incarnarum	0.2	2.0	0.2	2,4	3.5	11.7
F. oxysporum var. *orthoceras*	0.3	2.7	0	0	0	0
F. poae	2.4	26.2	3.7	36.7	3.5	11.7
F. sambucinum	0.1	0.7	0	0	0	0
F. sambucinum var. *sambucinum*	0.3	2.7	1.0	0.1	0	0
F. solani	0.1	0.7	0.1	1.0	0	0
F. sporotrichioides	3.2	34.2	1.6	16.2	3.5	11.7
F. subglutinans	0	0	0.2	1.9	0	0
F. tricinctum	0.1	0.7	0	0	0	0
Fusarium spp.	1.3	13.4	0.5	4.8	1.0	3.3

F. graminearum (IF 62.5%) were dominant, *F. equiseti* and *F. moniliforme* (IF 43.8%) as well as *F. sambucinum* (IF 37.5%) were among the most common on the surface of barley grains (Table 3). *F. sporotrichioides* (FO 3.5%) was most frequently isolated from the internal tissues of barley grains harvested in 2003. *F. avenaceum* (FO 1.6%), *F. sambucinum* (FO 1.4%) and *F. poae* (FO 1.2%) were rather common as well (Table 5). *F. equiseti* and *F. moniliforme* were not ascertained in the internal tissues of barley grains, although they were among the most common fungi on the grain surface. *F. sporotrichioides* and *F. poae*, predominant in the internal tissues of barley grains, were rather rare on their surface (IF 25.0% and 12.5%, respectively).

F. moniliforme (IF 56.2%) prevailed and *F. avenaceum*, *F. graminearum*, *F. oxysporum* (IF 31.2%) were common on the surface, while *F. sporotrichioides* (FO 3.2%) and *F. poae* (FO 2.4%) dominated in the internal tissues of wheat grains of the 2003 harvest. The latter were rare on wheat and barley grain surface (IF 6.2% and 25.0%, respectively), while *F. moniliforme* and *F. oxysporum,* prevailing on the grain surface, were not found in the internal tissues of wheat grains (Tables 2, 4).

Twenty taxa of *Fusarium* were identified in cereal grains of the 2004 harvest. *F. sporotrichioides* (IF 71.4%) and *F. graminearum* (IF 64.3%) prevailed on barley grain surface. *F. avenaceum*, *F. culmorum*, *F. equiseti* and *F. sambucinum* (IF 42.8%) were also rather common (Table 3). *F. poae* (FO 11.7%) and *F. sporotrichioides* (FO 10.9%) as well as *F. avenaceum* (FO 2.9%), *F. culmorum* (2.6%), *F. graminearum* (FO 1.9%) were most frequently isolated from the internal tissues of barley grains (Table 5). *F. sambucinum* prevailed on the grain surface, and *F. equiseti* (FO 0.6%) was rare in internal tissues.

Table 5. ***Fusarium*** **species in the internal tissues of barley grains of 2003–2005 harvest**

Fusarium species	2003		2004		2005	
	FO, %	RD, %	FO, %	RD, %	FO, %	RD, %
F. acuminatum	0.2	2.0	0	0	0	0
F. anthophyllum	0.2	1.5	0	0	0	0
F. avenaceum	1.6	13.6	2.9	9.0	19.0	30.2
F. culmorum	0.5	4.5	2.6	8.1	10.0	15.9
F. equiseti	0	0	0.6	2.0	0	0
F. graminearum	0.4	3.5	1.9	5,9	3.0	4.8
F. graminum	0.5	4.5	0.7	2.2	0	0
F. heterosporum	0.1	0.5	0	0	1.0	1.6
F. incarnatum	0.2	2.0	0.1	0.4	0	0
F. oxysporum	0	0	0.1	0.4	0	0
F. poae	1.2	10.6	11.7	35.9	20.0	31.7
F. sambucinum	1.4	11.6	0	0	0	0
F. sambucinum var. *sambucinum*	0.2	2.0	0.5	1.5	0	0
F. solani	0	0	0.3	0.9	0	0
F. sporotrichioides	3.5	30.3	10.9	33.3	9.0	14.3
F. subglutinans	0	0	0.2	0.7	0	0
F. tricinctum	0.4	3.0	0	0	1.0	1.6
Fusarium spp.	1.2	10.1	0.1	0.4	0	0

F. poae (IF 52.4%), *F. sporotrichioides* (IF 42.8%) and *F. equiseti* (IF 33.3%) were most frequently isolated from the surface of wheat grains harvested in 2004 (Table 2). *F. poae* (FO 3.7%) was frequent in grain internal tissues as well. *F. graminearum* (FO 1.8%), *F. sporotrichioides* (FO 1.6%) and *F. avenaceum* (FO 1.2%) were among the most common fusaria identified in the internal tissues of wheat grains (Table 4). *F. equiseti*, common on the wheat grain surface, was rare in internal tissues (FO 0.1% only).

Only 14 taxa of *Fusarium* were identified in cereal grains of the 2005 harvest. *F. graminearum* and *F. poae* (IF 100%) prevailed on the surface on barley grains. *F. equiseti* and *F. oxysporum* (IF 66.7%) were also common (Table 3). *F. poae* (FO 20.0%), *F. avenaceum* (FO 19.0%) as well as *F. culmorum* (FO 10.0%) and *F. sporotrichioides* (FO 9.0%) were most frequently identified in the internal tissues of grains (Table 5). *F. equiseti* and *F. oxysporum* prevailing on barley grain surface, were not detected in internal tissues. *F. avenaceum* and *F. sporotrichioides*, though prevailing in the internal tissues of grains, were not isolated from their surface.

Table 6. **Ability of *Fusarium* strains to produce toxic secondary metabolites *in vitro***

Fusarium species	Amount of strains studied	Amount of strains producing toxic compounds	Mycotoxins
F. avenaceum	12	10	Unknown
F. culmorum	18	16	T-2 toxin (7*), diacetoxyscirpenol (1), deoxynivalenol (2), nivalenol (13)
F. equiseti	4	2	Unknown
F. graminearum	12	6	T-2 toxin (4), deoxynivalenol (1) unknown (1)
F. incarnatum	5	4	Unknown
F. poae	25	2	T-2 toxin (1), diacetoxyscirpenol (1), neosolanol (1)
F. sporotrichioides	20	10	T-2 (8), diacetoxyscirpenol (1), neosolanol (2), unknown (2)

* In brackets the amount of strains producing respectively compound is indicated.

Table 7. **Characteristics of chromatography (constant of TLC in TEF) and the preliminary identification of unknown compounds produced by micromycetes *in vitro***

Fusarium species	Amount of strains	Constant of TLC R_f	Toxic compounds
F. avenaceum	4	0.74	Enniatin
F. avenaceum	4	0.50	Unknown
F. avenaceum	2	0.56	Beauvericin
F. equiseti	2	0.90	Unknown
F. graminearum	1	0.76	Deoxynivalenol derivate
F. incarnatum	4	0.61	Beauvericin
F. sporotrichioides	2	0.12	Unknown
F. sporotrichioides	1	0.46	HT-2 toxin

F. graminearum (IF 100%), *F. moniliforme* and *F. oxysporum* (IF 75.0%) were among the *Fusarium* species most frequently isolated from wheat grain surface (Table 2). *F. avenaceum* (FO 12.0%) prevailed in the internal tissues of grains. *F. graminearum* (FO 4.5%), *F. incarnatum*, *F. poae* and *F. sporotrichioides* with FO 3.5% were common as well (Table 4). *F. moniliforme* and *F. oxysporum*, which prevailed on grain surface, were not detected in internal tissues. *F. equiseti*, rather common in the internal tissues of wheat grains of the 2005 harvest, was not isolated from their surface, although it was among the dominant *Fusarium* species on grains harvested in 2003 and 2004.

The data of our investigation indicated that although the rather warm and wet vegetation period of 2005 was most suitable for *Fusarium* distribution in general, not all *Fusarium* species preferred it equally. This year was most favourable for the distribution of *F. graminearum*. Its isolation frequency from grain surface reached even 100%. The year 2005 was also favourable for *F. avenaceum*, *F. equiseti*, *F. moniliforme* and *F. oxysporum* development, however, *F. avenaceum* was most frequently identified in the internal tissues of grains, while others were found only on grain surface. The year 2005 was favourable for *F. poae* also, although this fungus was also rather common in wheat grain of 2004 harvest. The climatic conditions in 2004 were more convenient for the development *F. culmorum* and *F. sporotrichioides*, while the meteorological conditions of the year seem to have had no strong influence on the distribution of *F. sambucinum.* The year 2005 was least favourable for the development of *F. tricinctum*, *F. solani* and *F. nivale*.

It is very important to detect the ability of particular fungal strains isolated from grains to produce toxic compounds contaminating the raw material. During this study, we tested in total 96 strains of fusaria isolated from different grain samples. We noted that not all fungal strains were able to produce toxic secondary metabolites. Only 50 strains of the 96 tested showed this ability (Table 6). For some metabolites, only preliminary identification was done. The TLC constant in the TEF solvent system was detected for those metabolites (Table 7). The fungal species *F. sporotrichioides* and *F. graminearum* were ascertained as the main producers of T-2 toxin and its derivates in grains. The main source of nivalenol in the the grain samples could be *F. culmorum.* Another active producer of toxic compounds was *F. avenaceum.* According to literature data (Logrieco et al., 2003) and our preliminary results, these species are a source of enniatins and beauvericin in raw material. It should be noted that low the number of deoxynivalenol-producing strains was low (only three, including an unknown DON derivate) (Tables 6, 7). In general, producers of group A trichotecenes prevailed in the grain samples studied.

CONCLUSIONS

The cereal grains grown under Lithuanian climatic conditions in 2003–2005 were strongly contaminated

by mould fungi. *Fusarium* species were most common among the fungi isolated from wheat and barley grains. They were obtained in 92.1% of the grain samples tested.

Twenty four taxa of *Fusarium* were identified in wheat and barley grains. *F. graminearum*, *F. poae*, *F. avenaceum*, *F. equiseti*, *F. sporotrichioides* and *F. sambucinum* were most frequently isolated from the grain surface, while *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. graminearum* and *F. culmorum* dominated in the internal tissues with a decreasing prevalence.

The complex of *Fusarium*, ascertained in wheat and barley grain in various years, differed in their species composition as well as in the isolation frequency and relative density of separate species. These indices depended not only on the climatic conditions of a year, but also on grain species. Different *Fusarium* complexes were formed on the surface and in the internal tissues of grains.

The rather warm and wet vegetation period of the year 2005 was most suitable for the development of *Fusaria* in general. That year was most favourable for the distribution of *F. graminearum* and least convenient for *F. tricinctum*, *F. solani* and *F. nivale*.

F. sporotrichioides and *F. graminearum* were ascertained as the main producers of T-2 toxin and its derivates in wheat and barley grains. *F. culmorum* can be the main source of nivalenol, *F. avenaceum* being the main producer of enniatins and beauvericin. Producers of group A trichotecenes dominated in the grain samples.

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Rimutė Mačkinaitė, Audrius Kačergius, Albinas Lugauskas, Jūratė Repečkienė

LIETUVOS GAMTINĖMIS SĄLYGOMIS IŠAUGINTŲ JAVŲ GRŪDŲ UŽTERŠTUMAS *FUSARIUM* GENTIES MIKROMICETAIS IR JŲ MIKOTOKSINAIS

S a n t r a u k a

Javų grūdai, išauginti 2003–2005 m. Lietuvos gamtinėmis sąlygomis, buvo labai užteršti mikroskopiniais grybais. *Fusarium* genties grybai buvo vieni dažniausiai identifikuojamų rūšių kviečių bei miežių grūduose. Jie užteršė 92% tirtų grūdų mėginių. *Fusarium* išplitimas ir jų vystymasis javų grūduose priklauso nuo aplinkos sąlygų, ypač kritulių ir temperatūros javų žydėjimo, brendimo bei derliaus nuėmimo metu. Tyrimai parodė, kad 2005 m. pakankamai drėgnas ir šiltas vegetacinis periodas buvo tinkamiausias *Fusarium* genties grybams plisti, tačiau ne visoms *Fusarium* rūšims jis buvo vienodai palankus. Palankiausias jis buvo *F. graminearum* plisti.

Tirtuose kviečių ir miežių grūduose buvo identifikuoti *Fusarium* genties grybų 24 taksonai. *F. graminearum*, *F. poae*, *F. avenaceum*, *F. equiseti*, *F. sporotrichioides* ir *F. sambucinum* buvo dažniausiai aptinkami, mažėjimo tvarka, grūdų paviršiuje, tuo tarpu *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. graminearum* ir *F. culmorum* vyravo vidiniuose grūdų audiniuose. Skirtingais metais kviečių bei miežių grūduose nustatyti *Fusarium* spp. kompleksai skyrėsi rūšių įvairove ir tam tikrų rūšių išskyrimo dažniu bei santykiniu tankiu, kurie priklausė ne tik nuo metų meteorologinių sąlygų, bet ir nuo javų rūšies. Skirtingi *Fusarium* kompleksai susiformavo grūdų paviršiuje bei viduje.

Tyrimai parodė, kad svarbiausi T-2 toksino ir jo darinių gamintojai kviečių ir miežių grūduose yra *F. sporotrichioides* ir *F. graminearum*. *F. culmorum* gali būti pagrindinis nivalenolo, o *F. avenaceum* – eniatinų ir beauvericino šaltinis. Tirtų javų grūduose vyravo A grupės trichotecenų gamintojai.

Raktažodžiai: *Fusarium*, rūšių įvairovė, aptikimo dažnis, javų grūdai, mikotoksinai, gamtinės sąlygos