

Fungal contamination and *Fusarium* mycotoxins in cereals grown in different tillage systems

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ABSTRACT

The impact of two tillage systems (conventional and no-tillage) on cereal contamination with fungi and *Fusarium* mycotoxins was investigated. Samples of soil, cereal seeds, seedlings and freshly harvested grain were taken from plots of an agricultural cooperative in Lithuania. All samples were subjected to mycological analyses. Grain samples were also used for analysis of mycotoxins. In the soil under no-tillage, contamination with fungal spores was 92.9% higher compared to the soil under conventional tillage. In the soil of the no-tillage system we found 20 fungal genera, while in the soil under conventional tillage the number of genera was 15% lower. On cereal seedlings in the no-tillage system fungal spore content was 24.6% higher ($P > 0.05$) and detection frequency of *Fusarium* fungi was 22.9% higher ($P > 0.05$) than on seedlings in the conventional system. During harvest in feeding grain grown in the conventional tillage system, 2.2–8.0 \log_{10} CFU g^{-1} fungal spores were detected, compared to 2.5–12.0 \log_{10} CFU g^{-1} in grain in the no-tillage system. A significantly higher grain contamination with *Fusarium* spp. was detected in the no-tillage system. However, the deoxynivalenol, zearalenone and T-2 toxin content of grain was not considerably influenced by the different tillage systems, although it varied between species.

KEYWORDS: cereals, deoxynivalenol, fungi, grain, mycotoxins, tillage, zearalenone

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INTRODUCTION

In the last few years, particular interest was directed towards an increased risk of *Fusarium* infection and the production of the associated mycotoxins in small-grain cereals (Champeil et al., 2004; Vogelgsang et al., 2006) and maize (Maiorano et al., 2008). Different *Fusarium* spp. thrive in different climates. *F. graminearum* is most common in moist-warm regions, while *F. culmorum* and *F. avenaceum* prefer cooler zones (Doohan et al., 2003). In Lithuania, weather conditions are also favourable for *Fusarium* spp. In 2004, the detection frequency of *Fusarium* fungi was 38.5-50.0%, and in 2005, 45.0-70.8%. The most frequently isolated *Fusarium* spp. are *F. culmorum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides* (Mačkinaitė et al., 2006). Infections can result in yield losses but more important is the contamination of grain by mycotoxins produced by the pathogens. Mycotoxins can enter the food chain in the field, during storage, or later.

The prevailing mycotoxins - deoxynivalenol, nivalenol, zearalenone, fumonisins and T2/HT2 toxins - are produced by various *Fusarium* spp. (Lugauskas et al., 2002). Levels of the mycotoxins produced by *Fusarium* cannot be reduced after harvest by means of classical transformation procedures (Eriksen and Alexander, 1998). These toxins are chemically and thermally stable. They may, therefore, be found both in raw material (grain) and in transformed products (flour, cakes, feed) or in products from animals fed with contaminated grain (meat, milk, eggs) (CAST, 2003). Mycotoxins may also cause poisoning incidents, the principal symptoms of which are nausea, lethargy, fatty and cancerous infiltrations and possibly even death (Eriksen and Alexander, 1998).

Different tillage systems are used to protect the soil from erosion and to maintain soil fertility and productivity (Hobbs, 2007). Conventional agriculture, based on intensive tillage with heavy machinery, is a real threat to soil fertility. Soil structure becomes increasingly unstable, the soil loses its infiltration capacity and is eventually degraded (Ekeberg and Riley, 1997). The no-tillage system (also known as no-till) is a plant production system without any tillage from previous harvest to direct seeding. This system offers the best conditions to conserve and improve soil structure. The litter layer protects the soil from rain (battering and erosion), and increases surface humidity (by limiting evaporation), soil temperature and inertia (Auskalnis and Feiza, 2006).

The main plant cultivation measures, however, influence also the intensity of *Fusarium* infection. Narrow cereal rotation systems, in combination with conservation tillage, are potentially more likely to promote *Fusarium* infection than wide cereal rotations including legumes or catch crops, and/or ploughing. It is therefore recommended to optimize tillage systems to control *Fusarium* infection in cereal production (Oldenburg et al., 2007).

The aim of the present study was to estimate the effect of different tillage systems on quality cereal infection with fungi and contamination with *Fusarium* mycotoxins.

MATERIAL AND METHODS

Tillage

Field trials were established in plots of an agricultural cooperative, Lithuania in the Kaunas region. Soil type was identified as Endocalcari-Endohypogleyic Cambisol. Two tillage systems were applied for growing feeding cereals: 1. conventional tillage - deep ploughing (23-25 cm); 2. no-tillage - direct drilling. The other growing conditions of different cereals were similar.

Samples

Samples of soil, cereal seeds before sowing, seedlings and freshly harvested grain of spring and winter wheat (*Triticum aestivum* L.; cultivar - Širvinta 1), spring and winter barley (*Hordeum vulgare* f. *distichon* L.; spring barley cultivar - Auksiniai 3 and winter barley - Tilia), winter triticale (\times *Triticosecale* Wittm.; cultivar - Tornado) and maize (*Zea mays* L.; cultivar - Kakon) were taken from single experimental plots (6 rotation crops in total, approximate size of each plot about 1.0 ha each).

The samples of soil, cereal seeds, seedlings and part of each grain sample were subjected to analysis of mycological contamination, and about 50 g of each grain sample were air-dried and kept at -20°C until analysis of mycotoxin content.

Analysis of sample contamination by fungi

For determination of fungal colony-forming units per sample (CFU g⁻¹), we used the dilution plating method (Lugauskas et al., 2004).

Internal grain contamination with *Fusarium* fungi was determined by plating the surface-sterilized grains (200 for each sample) on Petri dishes with potato dextrose agar, incubated for 7-8 days at 26±2°C (Mathur and Kongsdal, 2003). The infection level of grain was evaluated as detection frequency (%). The purified single-spore cultures of *Fusarium* spp. were identified on the basis of their cultural and morphological characteristics according to Nelson et al. (1983), Samson and van Reenen-Hoekstra (1988) and Lugauskas et al. (2002).

Mycotoxin analysis

The approximately 50 g grain samples were analysed by the ELISA (enzyme-linked immunosorbent assay) method (Trucksess et al., 1995). Contamination with deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin was tested. The method is based on the antibody-antigen interaction. The Veratox test kits (Neogen Corporation, Scotland), approved by the AOAC (1999) (Certificate No. 950702) were used for the analysis. Mycotoxin extraction and tests were performed according to the manufacturer's instructions. The results were estimated taking into account the lowest calibration curve's mycotoxin concentration value (LOD-limit of detection, in $\mu\text{g kg}^{-1}$), which is 100.0 for DON, 10.0 for ZEN and 7.5 for the T-2 toxin.

Statistical analysis

Results of microbiological analysis of total fungal count (colony-forming units) in samples give into \log_{10} CFU g^{-1} and processed using the SPSS for Windows 12.0 programme package (SPSS Inc. IL., USA, 2003). Analysis of variance (ANOVA) was applied for the statistical processing of data, and we calculated mean values and standard deviation. P-values of less than 0.05 were considered significant.

RESULTS

Monthly mean values of temperature and rainfall in the study area in the flowering-ripening period of cereals in 2007 are given in Table 1. In the spring of 2007, warm and dry weather prevailed, but excessive moisture was observed from May throughout the growing season. The weather of the first half of July was warm and dry, the second half being warm and damp. During the cereal flowering stage (mid and late June and early July), the conditions were conducive to the development and spread of fungi, especially *Fusarium* spp.

Table 1. Weather conditions in the study area in 2007

Parameter	April	May	June	July	August	September
Average temperature, °C	6.9	13.5	17.6	17.2	18.7	14.4
± deviation from long-term mean, °C	+0.4	+1.3	+2.0	-0.4	+2.1	+2.4
Average rainfall, mm	15.8	98.2	61.5	118.1	50.8	26.6
% of long-term mean	42.5	187.1	100.3	160.5	69.0	51.3
Days with rainfall ≥ 1 mm	4	12	9	16	11	2

Soil is the main source of grain contamination by fungi. Under conventional tillage, the soil samples were contaminated with $7.0 \pm 0.5 \log_{10}$ CFU g^{-1} fungal

spores on average. In the soil under conventional tillage, 17 genera of fungi were found. The most common genera were *Alternaria* (detection frequency 37%), *Fusarium* (70%), *Mucor* (10%), *Penicillium* (10%) and *Trichoderma* (62%). The genus *Fusarium* was identified in 80% soil samples of conventional tillage. The dominant species were *Fusarium poae*, *F. solani* and *F. oxysporium*.

The soil under no-tillage was contaminated with $13.5 \pm 12.5 \log_{10}$ CFU g⁻¹ fungal spores ($P < 0.05$), i.e. 92.9% higher than under conventional tillage. In the soil of the no-tillage system, 20 genera of fungi were found. The species diversity of fungi was also higher. The most common genera were: *Fusarium* (84%), *Trichoderma* (70%), *Pythium* and *Verticillium* (50%), *Alternaria*, *Mortierella*, *Mucor*, *Rhizopus*, *Septoria* (38%) and *Drechslera* (25%). The following fungi were most frequent in the soil of the no-tillage system: *Alternaria alternaria*, *Drechslera sorokiniana*, *Epicoccum nigrum*, *Fusarium poae*, *F. oxysporium*, *F. solani*, *F. semitectum*, *Mortierella alpina*, *Rhizopus stolonifer* var. *stolonifer*, *Pythium sylvaticum*, *Septoria linicola*, *Sclerotinia sclerotiorum*, *Trichoderma viride* and *Verticillium albo-atrum*.

The analysis of fungal contamination of chemically treated cereal seeds before sowing revealed that spring and winter wheat were contaminated by 3.0 ± 0.2 and $2.5 \pm 0.1 \log_{10}$ CFU g⁻¹, respectively; spring and winter barely seeds by 1.3 ± 0.1 and $2.0 \pm 0.3 \log_{10}$ CFU g⁻¹, respectively; winter triticale by $1.0 \pm 0.4 \log_{10}$ CFU g⁻¹; and maize by $0.3 \pm 0.1 \log_{10}$ CFU g⁻¹. Species of *Alternaria*, *Cladosporium*, *Fusarium* and *Penicillium* were most frequent in seeds, and *Alternaria alternaria* dominated among them. Fungi of the genus *Fusarium* were detected in 2.0 and 5.0% of seeds of spring and winter wheat, respectively; 6.7 and 3.7% of spring and winter barely, respectively; 3.5% of winter triticale, and 0% of maize seeds.

Cereal seedling contamination by fungal spores is shown in Figure 1. When the conventional tillage system was used, seedling contamination with fungal

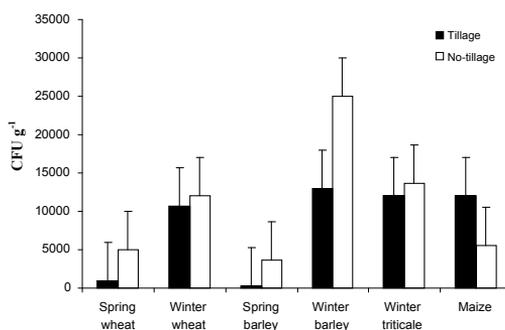


Figure 1. Contamination of various cereal seedlings by fungal spores (CFU g⁻¹). Error bar denotes standard deviation

spores reached 0.3-1.0 \log_{10} CFU g^{-1} . When no-tillage system was used, 3.7-25.0 \log_{10} CFU g^{-1} fungal spores were detected. On cereal seedlings in the no-tillage system, fungal spore content was on average 24.6% higher ($P>0.05$) than on cereal seedlings in the conventional system. Among fungi isolated from winter and spring cereal seedlings, the most common genera were *Alternaria* (16.0-75.0%), *Cladosporium* (1.3-46.6%) and *Fusarium* (7.0-47.0%). The highest fungal contamination was detected on winter barley seedlings, where in the no-tillage system, fungal contamination was 92.9% higher ($P<0.05$) than in the conventional system.

Our research has shown that in cereal seedlings under no-tillage the detection frequency of *Fusarium* spp. was 22.9% higher ($P>0.05$), compared to cereal seedlings in the conventional system (Figure 2). Winter triticale seedlings in the conventional system were most contaminated with *Fusarium* spp. (36.7%), while spring wheat was the least contaminated (7.0%). Where we used no-tillage, the contamination by *Fusarium* spp. ranged from 26.7% of maize seedlings to 47.7% of winter wheat seedlings.

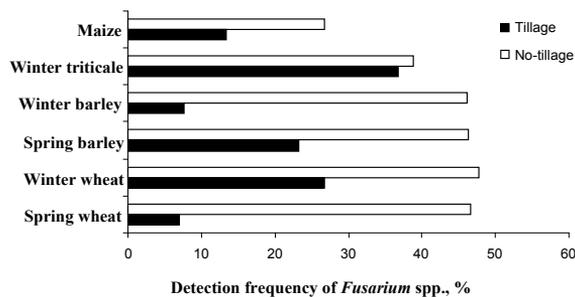


Figure 2. Detection frequency of *Fusarium* spp. on cereal seedlings

Fourteen species of *Fusarium* were identified in this study, but *F. avenaceum*, *F. graminearum*, *F. poae* and *F. sporotrichioides* dominated on cereal seedlings.

During harvest, 2.2-8.0 \log_{10} CFU g^{-1} fungal spores were detected in grain in the conventional system, compared to 2.5-12.0 \log_{10} CFU g^{-1} fungal spores in grain in the no-tillage system (Figure 3). The most severe fungal contamination was recorded for winter wheat in both systems, but under no-tillage it was contaminated 50% higher than under conventional tillage. Also generally, grain contamination with fungal spores was higher under conventional tillage but differences between the two tillage systems were not significant. An exception was maize, where an inverse trend was observed and the difference was significant.

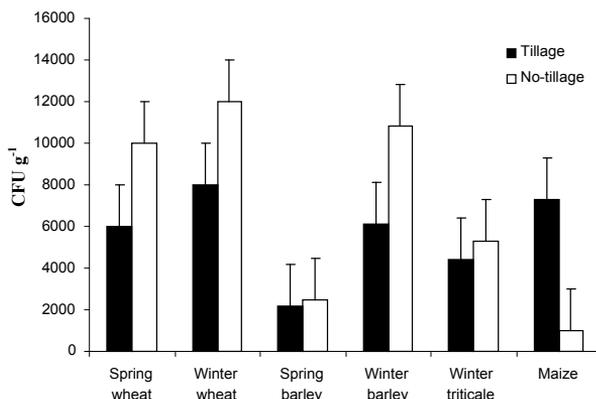


Figure 3. Grain surface contamination by fungal spores (CFU g⁻¹) during harvesting. Error bar denotes standard deviation

The predominant fungi present in freshly harvested grain samples were species of the genera *Alternaria* (30.0-50.0%), *Cladosporium* (26.7-47.0%), *Fusarium* (36.0-77.0%) and *Bipolaris* (7.0%) (in barley grain).

The average detection frequency of *Fusarium* in grain samples (Figure 4) was significantly higher in the no-tillage system. The highest *Fusarium* frequency was observed in spring barley and maize from the no-tillage system.

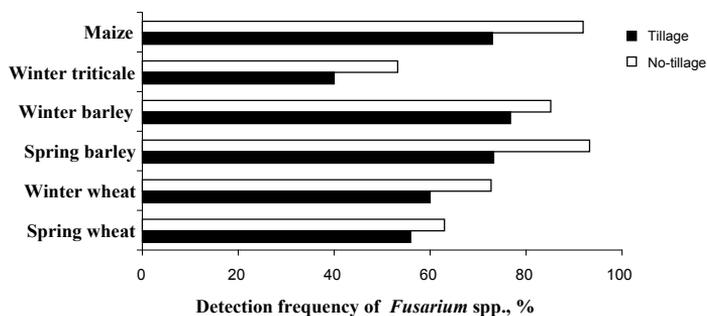


Figure 4. Detection frequency of *Fusarium* spp. in freshly harvested grain

Fusarium mycotoxin content of grain samples (Table 2) is presented separately for deoxynivalenol, zearalenone and T-2 toxin. Their content varied but it was not considerably influenced by the different tillage systems applied in the present study.

Table 2. Mycotoxin content of freshly harvested grain, $\mu\text{g kg}^{-1}$

Grain	Tillage system					
	conventional tillage			no-tillage		
	DON ¹	ZEN ²	T-2 toxin	DON ¹	ZEN ²	T-2 toxin
Spring wheat	tr	21.0	10.0	tr	21.0	15.0
Winter wheat	tr	Tr	13.0	tr	20.0	tr
Spring barley	180.0	20.0	tr	110.0	30.0	10.0
Winter barley	100.0	10.0	tr	100.0	25.0	8.0
Winter triticale	50.0	10.0	tr	170.0	30.0	tr
Maize	100.0	103.0	tr	120.0	20.0	tr

¹ DON - deoxynivalenol; ² ZEN - zearalenone; tr - traces

DISCUSSION

Our experimental evidence indicates that the no-tillage system increases the frequency of fungal spores, whereas deep tillage decreases it. Similarly, limited tillage systems increase the number of *Fusarium* spores in the soil (Parry et al., 1995; Oldenburg et al., 2007).

Fungi can also affect seed quality and several pathogens may infect seedlings from contaminated seeds. This applies to *Fusarium*, *Drechslera*, *Alternaria*, *Cladosporium*, *Penicillium* and other fungal genera (Forsberg et al., 2005). The seed-borne fungi may not only lower seed germination but also reduce seed vigour, resulting in a low yield. Some pathogenic fungi destroy the young seedlings before they appear on the surface of the soil. Other fungi can cause a range of plant diseases (Steinkellner et al., 2002). Chemically treated cereal seeds were also contaminated by fungal spores: from 0.3 \log_{10} CFU g^{-1} on maize to 3.0 \log_{10} CFU g^{-1} on winter wheat.

The climate plays a role at all stages of development of the fungus. In spring, the climate determines the type and quality of spores produced, together with the date of their dispersal and thus the intensity of the disease. Indeed, the coincidence of the dates of spore dispersal and crop flowering, marks the start of the critical phase of infection for plants. In summer, together with the virulence characteristics of the fungal strain and the intrinsic resistance of the plant, climate controls the competition between pathogen species, thereby controlling the rate of mycelium growth in the plant (Champeil et al., 2004). The climate may also affect the production of fungal toxins in the field (CAST, 2003).

The most prevalent fungi in cereal grain in the temperate zone are *Fusarium* spp. 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 the yield and quality of freshly harvested grain, and secondly, because of the ability of *Fusarium* spp. to produce

a wide range of mycotoxins, which can enter the human and animal food chains (Maiorano et al., 2008). In Lithuania, weather conditions are also favourable for the occurrence of *Fusarium* spp., where the frequency of detection of these fungi is sometimes as high as 93.5% (Mačkinitė et al., 2006).

Our research has shown that 26.7-47.7% of cereal seedlings were contaminated by *Fusarium* spp. in the no-tillage system, compared to 7.0-36.7% of seedlings in the conventional system.

As a result of fungal growth, cereal grain is often contaminated by mycotoxins. Food safety issues, due to mycotoxins of *Fusarium*, can be very important for the food chain.

Deoxynivalenol (DON) and zearalenone (ZEN) were prevalent mycotoxins in the analysed grain samples. The highest DON concentration was observed in barley and maize, but differences between soil cultivation methods were generally low. The only exception was triticale, where DON and ZEN content was at least 3-fold higher in the no-tillage system. This corresponds with Schaafsma et al. (2005), who reported that environmental effects accounted for 48% of variation in DON content in wheat grain, followed by variety (27%). Those authors also found that the effect of soil cultivation was very small in most experimental years. Koch et al. (2006) concluded from their on-farm large-scale experiments that annual weather conditions affect *Fusarium* infection and DON concentration to the same extent as cultivar susceptibility, and in comparison with these factors, the applied tillage system is less important. Dill-Macky and Jones (2000) found only small differences in DON content between mouldboard plough, chisel plough and no-till plots.

By contrast, Champeil et al. (2004) suggested that the level of grain contamination with mycotoxins depends on both climate and cropping system. Mycotoxin levels were highest in the year 2000, when disease severity was the highest. Contamination levels were highest in the direct drilling (i.e. no-tillage) system.

CONCLUSIONS

Our data indicate a significant effect of tillage system on the detection frequency of *Fusarium* spp. in grain, but no significant effect on grain contamination with deoxynivalenol, zearalenone and T-2 toxin. The no-tillage system promotes the development of fungi in grain and thus may lower the quality of grain, but fungal species diversity is higher in this system.

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