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The impact of tillage and fertilization on *Fusarium* infection and mycotoxin production in wheat grains

Skaidrė SUPRONIENĖ[1], Audronė MANKEVIČIENĖ[1], Gražina KADŽIENĖ[1], Audrius KAČERGIUS[2], Virginijus FEIZA[1], Dalia FEIZIENĖ[1], Roma SEMAŠKIENĖ[1], Zenonas DABKEVIČIUS[1], Kęstutis TAMOŠIŪNAS[1]

[1]Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: skaidre@lzi.lt

[2]Institute of Botany, Nature Research Centre
Žaliųjų Ežerų 49, Vilnius

Abstract

Fusarium head blight (FHB) is a worldwide disease of small grain cereals, which reduces grain yield and its quality. The aim of this study was to identify the influence of different tillage and fertilization practices on winter and spring wheat grain infection with *Fusarium* fungi and contamination by mycotoxins – deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin. A two-factor field experiment was carried out at the Lithuanian Institute of Agriculture during the period 2005–2008. The internal grain infection with *Fusarium* fungi were quantified using agar tests. Purified colonies were identified using different manuals. The mycotoxins were analyzed using ELISA method.
Meteorological conditions were not conducive to *Fusarium* head blight development during the 2005–2008 period, therefore *Fusarium* infection level was very low in harvested winter wheat grain (0–7.1%) and moderate in spring wheat grains (27.3–41.3%). The concentrations of DON (<100–166.3 μg kg^{-1}), ZEN (<10–10.8 μg kg^{-1}) and T-2 toxin (<7.5–13.2 μg kg^{-1}) in winter wheat grain samples most often were close to the limit of detection, while in spring wheat samples they were slightly higher. Tillage systems had no clearly evident influence on *Fusarium* infection level; however, some significant differences in the mycotoxin production were observed. The concentrations of DON (2008) and ZEN (2006 and 2008) in spring wheat and T-2 toxin (2006) in winter wheat significantly correlated with the number of productive tillers or number of plants m^{-2} and were significantly lower in no-tillage system. A significant influence of high fertilizer rates on spring wheat grain infection with *Fusarium* spp. was observed in 2007, and similar trends were found in 2006 and 2008. Higher concentrations of trichothecene producers were detected in the spring wheat grain from the conventionally tilled treatments applied with higher fertilizer rates.

Key words: tillage, fertilization, *Fusarium* spp., mycotoxin, *Triticum aestivum*, grain.

Introduction

Wheat (*Triticum aestivum* L.) is one of the major sources of food in many countries, including Lithuania. Due to its high yield potential, winter wheat prevails and occupies more farmland than any other cereal in Lithuania. The production area of spring wheat has also increased over the recent years (Statistiniai rodikliai, www.stat.gov.lt).

Fusarium fungi cause a devastating disease of small-grain cereals – *Fusarium* head blight (FHB) and are the major producers of mycotoxins in grains before, or immediately after harvest (Bottalico, Perrone, 2002; Thrane et al., 2004). The species predominantly found in association with FHB in small-grain cereals all over Europe are *Fusarium graminearum* Schw. (teleomorpha *Gibberella zeae* Schw., Pech), *F. avenaceum* (Coda: Fr.) Sacc. (*Gibberella avenacea* Cook), *F. culmorum* (W. G. Smith) Sacc., *F. poae* (Pech) Wollenw., *F. sporotrichioides* Sherb., *F. tricinctum* (Corda) Sacc. (*Gibberella tricincta* El-Gholl, McRithie, Scoulties), *F. equiseti* (Cda.) (*Gibberella intricans* Wollenw.), *F. langsethiae*, *F. moniliforme* Sheldon (= *F. verticillioides* (Sacc.) Nirenberg), etc. (Bottalico, Perrone, 2002; Kosiak et al., 2003).

The mycotoxins produced by *Fusarium* spp. include deoxynivalenol (DON), zearalenone (ZEN), nivalenol (NIV), T-2, HT-2, diacetoxyscirpenol (DAS), enniatins, moniliformin and others. *F. culmorum* and *F. graminearum* are the key producers of DON and ZEN. *F. avenaceum* does not produce trichothecenes, but does produce other mycotoxins such as enniatins and moniliformin. *F. sporotrichioides* and some isolates of *F. poae* produce T-2 and HT-2 toxins, but in general *F. poae* is the main producer of DAS and NIV (Thrane et al., 2004; Surai, Mezes, 2005).

Trichothecenes constitute the largest group of mycotoxins produced by *Fusarium* in cereal grain. Recently, highly conserved regions in the tri5 sequences of *Fusarium* have been utilised to set up polymerase chain reaction (PCR) based assays for the detection of trichothecene-producing *Fusarium* spp. (Niessen, Vogel, 1998; Edwards et al., 2001). The assays are being successfully applied to detect contamination in cereals and cereal products.

In Lithuania, FHB is not an epidemic disease in cereals; however, research conducted in recent years has suggested that cereal grain grown under the country's conditions is contaminated with mycotoxins DON, T-2 and ZEN (Baliukoniene et al., 2003; Mankevičienė et al., 2011). Moreover, the weather conditions in separate years are also favourable for the occurrence of *Fusarium* species, and the frequency of identification of these fungi is as high as 93.5% (Lugauskas et al., 2004). The most frequently occurring *Fusarium* species in Lithuanian cereal grain are *F. avenaceum*, *F. poae*, *F. culmorum*, *F. sporotrichioides* and some others (Lugauskas et al., 2004; Supronienе et al., 2010).

Development of *Fusarium* spp. infection in cereals has been associated primarily with the environmental conditions where climatic conditions are especially important (Doohan et al., 2003; Rohačik, Hudec, 2005). Warm and rainy weather (with relative air humidity of ≥80%) during cereal anthesis stage is the most conducive to the occurrence of *Fusarium* fungi infection in cereal grain (Rossi et al., 2001). The agronomic practice may also influence the occurrence of *Fusarium* fungi and the contamination of cereals with mycotoxins. There is some evidence that FHB can be affected by fertilizer regimes. Martin et al. (1991) and Lemmens et al. (2004) observed that increasing the amount of nitrogen applied to cereals resulted in increased incidence of FHB or *Fusarium*-infected grain. However Lori et al. (2009) reported that favourable weather conditions are a more important factor for FHB infection than tillage practice and fertilizer treatments.

Mouldboard ploughing is most common in Lithuania; however, direct drilling and other sustainable tillage practices are becoming increasingly popular. Reduced tillage has an advantage over the conventional tillage, because of reduced costs (Yalcin et al., 2005; Feizienė et al., 2006) and given environmental benefits: a reduction in fuel consumption, soil erosion, nitrate leaching, an increase in soil organic matter and activity of soil organisms, improvement in soil structure and soil moisture conservation (Subbulakshmi et al., 2009; Feiza et al., 2010). Nevertheless, contradictory data on the effect of tillage on *Fusarium* infection in cereals are available. No-tillage may result in increased FHB incidence and severity (Dill-Macky, Jones, 2000; Fernandez et al., 2005; Lori et al., 2009), especially in wheat following corn cultivation. However, some researchers have reported no increase in *Fusarium* infection and mycotoxin content in cereal grain in conservation tillage treatments (Miller et al., 1998; Steinkellner et al., 2002). In Lithuania, there was no research evidence on *Fusarium* infection and mycotoxin contents in grain under different soil tillage systems; the data on the impact of fertilizes are also scarce. As a result, the aim of our research work was to ascertain the influence of different soil tillage – fertilization systems on winter and spring wheat grain infection with *Fusarium* fungi and contamination by mycotoxins (DON, ZEN and T-2 toxin).

Materials and methods

Study site description. The study site is located at the Lithuanian Institute of Agriculture. Research was carried out on the basis of two long-term field trials, established in the autumn of 1999 (first trial) and 2000 (second trial). The soil is defined as an *Endocalcari-Epihypogleyic Cambisol* (*CMg-p-w-can*) according to the WRB (FAO) system. Experimental design was a split plot in four blocks (replications), with the tillage treatments: conventional tillage, reduced tillage and no-tillage as the main plots (Table 1). Fertilization rates of mineral NPK (none, moderate and high), designed as subplots, were calculated according to the soil properties and expected crop yield (Švedas, Tarakanovas, 2000). The gross area of each sub-plot was 3.3 × 20 m. Crop rotation was as follows: winter wheat → spring rape → spring wheat → spring barley → pea in both field experiments. For mycological study we used grain samples of commercial varieties of winter ('Zentos') and spring ('Triso') wheat collected during 2005–2008 (second crop rotation). Due to poor winter wheat overwinter survival in 2006, half of the field trial was re-sown with spring wheat (dividing trial plots in two equal parts across all treatments). Therefore in 2006 both winter and spring wheat grain samples were used for assessment. Plant residues of the pre-crops were collected and removed from the experimental field each year after harvest. Using a conventional crop rotation, unfavourable for wheat diseases, we expected to get results influenced only by tillage and fertilization. Each year, three weeks after harvesting of previous crop, non-selective herbicide (glyphosate at a dose of 1.44 kg ha^{-1} a.i.) was sprayed in no-tillage plots to control weeds and volunteer plants of pre-crop.

Table 1. Experimental design of different soil tillage and fertilization practices

Tillage systems (factor A)		
treatment	primary tillage	pre-sowing tillage
conventional tillage	deep ploughing (23–25 cm)	spring tine cultivation (4–5 cm)
reduced tillage	shallow ploughing (14–16 cm)	spring tine cultivation (4–5 cm)
direct drilling	no tillage	direct drilling combined with rotary cultivation (2–3 cm)
Fertilization (factor B)[a]		
1	no fertilization	
2	mineral NPK fertilizers according to soil nutrient status and expected yield (winter wheat – 6.5 t ha^{-1}, spring wheat – 4.5 t ha^{-1})	
3	mineral NPK fertilizers according to soil nutrient status and expected yield (winter wheat – 8.0 t ha^{-1}, spring wheat – 6.0 t ha^{-1})	

Note. [a] – fertilization rates were calculated according to the soil properties and expected crop yield, using the software *Trešimas* (Švedas, Tarakanovas, 2000).

Grain yield and yield components. Wheat plants and productive tillers m^{-2} were counted in each plot before harvesting. The crop was harvested and weighed from each plot at a complete maturity stage. The yield was adjusted to a moisture content of 15%. Grain samples were taken from each plot for further processing. Four sub-samples of 500 kernels per each plot were counted and weighed to determine a thousand kernel weight.

Grain infection with Fusarium fungi. For laboratory analyses, grain samples of 1.0 kg were taken from each plot at harvesting. Sub-samples were stored in plastic jars in a freezer at −20°C to prevent alterations in fun-

gi and mycotoxin contents until the conduct of analyses. Before analyzing, the grain was de-frosted up to room temperature. Agar plate method was used for internal grain infection estimation. Surface-sterilized (for 3 min in 1% NaOCl solution) grains were plated in Petri-dishes with potato dextrose agar (PDA: 250 g of potato, 10 g of glucose, 14 g of agar and 1 l of distilled water) and incubated for 7–8 days at 26 ± 2ºC in the dark (Mathur, Kongsdal, 2003). The fungal grain infection level per sample was expressed in percent. The overgrown *Fusarium* colonies were isolated, purified and identified according to the manuals of Nelson et al. (1983) and Leslie and Summerell (2006). Colonies which did not form conidia were attributed to *Fusarium* spp.

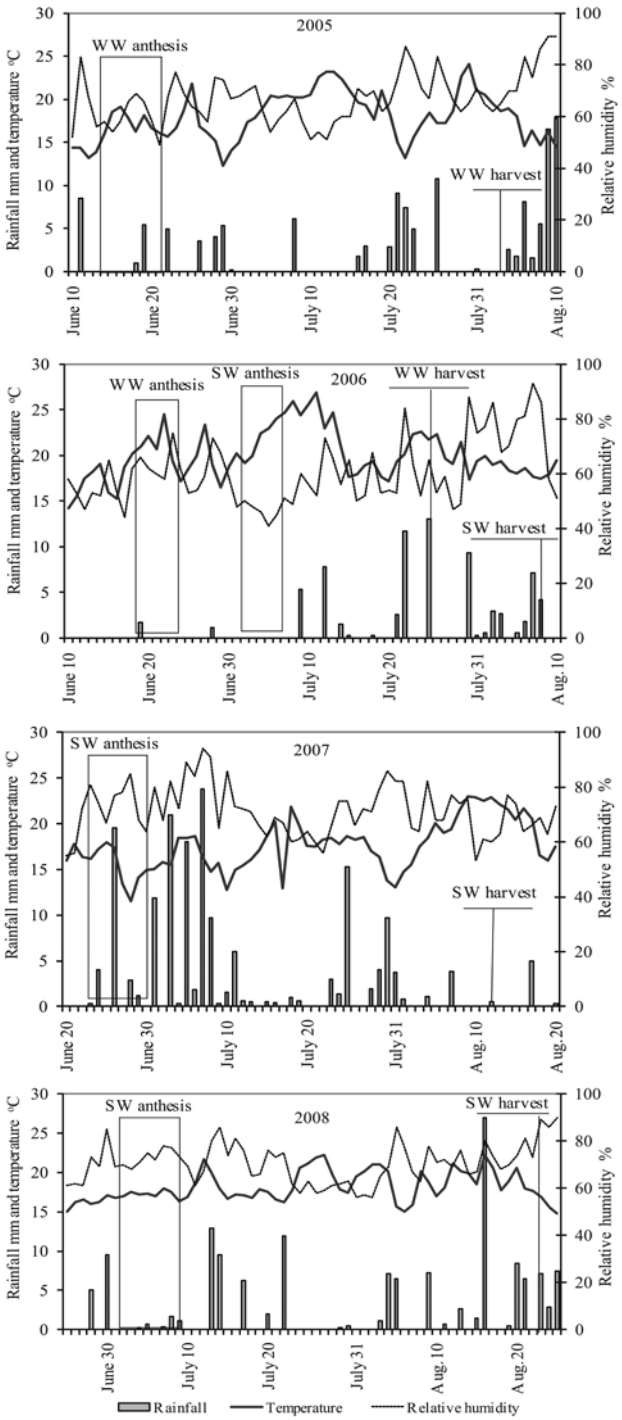
Analysis of mycotoxins. Grain contamination with deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin (T-2) was tested by the CD-ELISA (competitive direct enzyme-linked immunosorbent assay) method (Wilkinson et al., 1992). The Veratox® quantitative test kits ("Neogen", Scotland), approved by the AOAC Research Institute (Certificate No. 950702) were used for the analysis. The optical densities of samples and controls from standard curve were estimated by a photometer "Neogen Stat Fax®303 Plus" (USA), using a filter of 650 nm. Measured absorbances were automatically converted to the mycotoxin concentration units – μg kg^{-1}. The results were estimated taking into account the lowest calibration curve's mycotoxin concentration value (LOD-limit of detection), which is for: DON – 100.0 μg kg^{-1} (ppb), ZEN – 10.0 μg kg^{-1} (ppb), T-2 – 7.5 μg kg^{-1} (ppb). While assessing our data with regard to food and forage safety we referred to the EU document No. 1126/2007 (European Commission Regulation, 2007), in which it is specified that the maximum allowable concentration for DON in unprocessed grain (except for hard wheat, oats and maize) is 1250 μg kg^{-1}, in cereal intended for direct human consumption –750 μg kg^{-1}, for ZEN – in unprocessed grain (except for maize) – 100 μg kg^{-1}, intended for direct human consumption – 75 μg kg^{-1}. The T-2 assessment was based on research-recommended concentrations in grain and grain products 100 μg kg^{-1} (Eriksen, Alexander, 1998).

DNA extraction. Total DNA from wheat grain samples was quantified using "NucleoSpin® Plant II Kit" ("Macherey-Nagel", Germany) according to manufacturer's instructions.

Amplification of Tri5 gene. PCR with TOX5-1 and TOX5-2, specific to the *Tri5* gene locus, were performed for 30 cycles (1 min denaturation at 94ºC, 1 min annealing at 55ºC, 1 min extension at 72ºC), 10 min final elongation and storage at 4ºC. Amplicons were separated on 1.0% agarose for gel electrophoresis in a TAE buffer at 80 V, 120 mA. TOX5-1 and TOX5-2 allowed the amplification of a 658-bp region of *Tri5* gene (Niessen, Vogel, 1998).

Methods of statistical data analysis. The experimental data were processed using analysis of variance and correlation-regression methods recommended in agronomy science. Significance of the differences between the means was determined according to the least significant difference (LSD) at 0.05 probability level. The data were processed using the software *ANOVA*, *SPLIT-PLOT* and *STAT-ENG* from the package *SELEKCIJA* (Tarakanovas, Raudonius, 2003).

Meteorological conditions. In 2005, the amount of rainfall that fell during the cereal flowering period was low (Fig. 1). During winter cereal anthesis, there were a few days conducive to *Fusarium* fungi spread (with a relative air humidity of about 80%). In 2006, the spring was cold, dry and delayed for about one and a half weeks, therefore winter cereals flowered slightly later than in 2005; however, an especially hot June and July not only advanced anthesis of spring cereals but also accelerated grain ripening of all cereals by almost ten days compared with long-term data. In June and July, the weather conditions were adverse for *Fusarium* fungi occurrence in cereals. In 2007, the spring was very early and changeable. In the last five-day period of May, the second half of June and the first ten-day period of July the weather was rather wet, relative air humidity persisted around 80%, which resulted in more favourable conditions for *Fusarium* fun-



WW – winter wheat, SW – spring wheat

Figure 1. Meteorological conditions from wheat anthesis to full maturity stage (June to August) 2005–2008

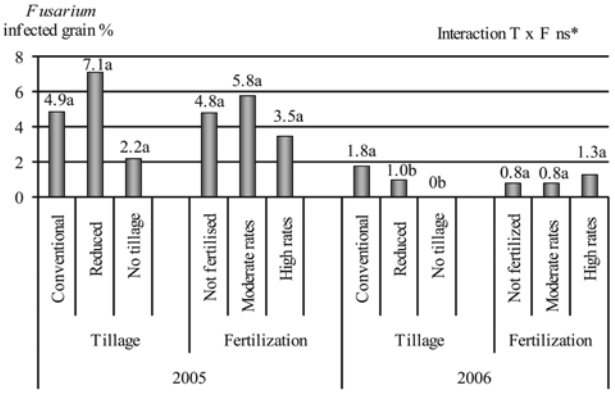
gi occurrence in cereals than in 2005–2006. In 2008, the spring was a month earlier compared with the long-term data. During cereal anthesis, the weather was warm and dry until mid June and later it was moderately wet.

Results and discussion

Winter wheat. Mean values for the winter wheat grain infection with *Fusarium* spp. are shown in Figure 2, deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin (T-2) concentrations in Table 2. *Fusarium* infection level in harvested grain ranged from 2.2% to 7.1% in 2005 and from 0% to 1.8% in 2006.

The highest infection level (7.1%) (Fig. 2) and DON content (166.3 μg kg^{-1}) (Table 2) were observed in the grain harvested in the reduced tillage plots in 2005. In 2006, *Fusarium* infection level correlated with DON content ($r = 0.789$, $P < 0.05$) and was significantly ($P = 0.01$) lower in the reduced and no-tillage treatments (1.0% and 0% respectively). *Fusarium* species composition in grain varied irrespective of the tested factors. *F. poae* was the most prevalent species in both years, followed by *F. avenaceum* and *F. sporotrichioides*.

Mean values for ZEN were close to limit of detection in 2005 and ranged from 21.6 to 32.3 μg kg^{-1} in 2006 (Table 2). T-2 content was significantly ($P = 0.03$) lower in no-tillage treatments (3.0 μg kg^{-1}) than in reduced and conventional treatments in 2005, which coincided with the lowest *Fusarium* infection level (2.2%). In 2006, T-2 content in no-tillage treatments was greater (14.1 μg kg^{-1}) than in conventional.



Note. Means followed by the same letters within each treatment are not significantly different at $P \leq 0.05$; ns* – not significant at $P \leq 0.05$.

Figure 2. *Fusarium* spp. infection level in winter wheat grain as influenced by different tillage (T) and fertilization (F) practices in 2005–2006

Table 2. Deoxynivalenol, zearalenone and T-2 toxin concentrations (μg kg^{-1}) in winter wheat grain samples as influenced by different tillage and fertilization practices in 2005–2006

Treatments	Deoxynivalenol		Zearalenone		T- 2 toxin	
	mean	range	mean	range	mean	range
2005						
Conventional tillage	<100	<100–163.0	7.1[a] a	0.0–10.8	11.6 a	10.9–12.2
Reduced tillage	166.3	136.0–182.0	7.3 a	0.0–11.1	13.2 a	12.5–14.0
No tillage	116.7	110.0–126.0	10.8 a	10.5–11.1	3.0 b	0.0–8.9
Not fertilized	136.3	110.0–163.0	10.7 a	10.5–10.8	11.2 a	8.9–12.5
Moderate rates	(113.7)[b]	<100–182.0	7.1 a	0.0–10.7	8.3 a	0.0–13.0
High rates	(120.7)	<100–181.0	7.4 a	0.0–11.1	8.3 a	0.0–14.0
2006						
Conventional tillage	107.6	106.9–108.3	32.3 a	10.4–43.6	<7.5	<7.5–10.7
Reduced tillage	<100	<100–109.6	21.6 a	18.3–25.1	13.1	10.2–15.2
No tillage	<100	0.0–<100	31.1 a	27.5–34.8	14.1	13.4–14.6
Not fertilized	<100	<100–109.6	29.6 a	18.3–42.9	(9.1)	<7.5–13.4
Moderate rates	<100	0.0–107.5	32.0 a	21.4–43.6	13.1	10.7–14.6
High rates	<100	0.0–108.3	23.4 a	10.4–34.8	(12.2)	<7.5–15.2

Notes. Means followed by the same letters within each column and within each treatment are not significantly different at $P \leq 0.05$. [a] – some means of mycotoxins are below limit of detection because of inclusion of samples with zero values; [b] – means in parentheses include samples with values below limit of detection.

Winter wheat stand density was similar in all treatments in 2005; while in 2006 it was significantly higher in conventionally (509 productive tillers m^{-2}) tilled plots than reduced (459 productive tillers m^{-2}) and no-tilled (465 productive tillers m^{-2}) plots (Table 3). The percentage of *Fusarium* infected grain ($r = 0.935$, $P < 0.01$) and DON content ($r = 0.836$, $P < 0.01$) positively correlated with the number of plants m^{-2}. T-2 toxin content negatively correlated with the productive tillers m^{-2} (-0.592, $P < 0.01$). In 2005, the grain yield was nearly twice as high as that in 2006. A significant yield increase was observed in the fertilized winter wheat in both years (8.3 and 8.8 t ha^{-1} in 2005, 4.2 and 4.5 t ha^{-1} in 2006) and in the conventional tillage treatments (4.6 t ha^{-1}) in 2006.

Meteorological conditions in 2005 and 2006 did not favour FHB development in winter wheat, because the flowering period was droughty and short (Fig. 1). The *Fusarium* infection level in grain samples was low and the concentrations of all mycotoxins tested in most cases were very close to the limit of detection. Those conditions were unfavourable for examination of tillage and fertilization treatments' effect on *Fusarium* infection and mycotoxin production. The studies done by other researchers confirmed that favourable weather conditions are more important for FHB development and DON production than tillage and fertilizer treatments (Schaafsma et al., 2001; Lori et al., 2009).

Some increase in *Fusarium* infection level and DON content in the reduced tillage treatments was seen in 2005, which supports the findings of Schaafsma et al. (2001), suggesting that the average DON levels in reduced tillage systems were always higher than in no-tillage or conventional tillage systems, but the evident effects were only in the year (1997) following epidemic.

In 2006, hot and dry weather conditions during June and July were inimical not only to the development of *Fusarium* fungi, but also influenced plant development and accelerated grain ripening. A significant correlation between *Fusarium* infection levels, DON content and number of plant m^{-2} was obtained, while T-2 toxin was negatively correlated with the number of productive tillers. The lower stand density and accelerated grain ri-

pening seem to have resulted in lower *Fusarium* infection, DON content and grain yield in reduced and no-tillage treatments. Temperature and water availability are two important environmental factors affecting fungal growth and development. Denser crop stands favour disease development by shading the soil surface and creating a moist micro-environment. Moreover, the proximity of plants to one another is increased and allows for easier disease spread (Pearse, 2002). Instead of microclimatic conditions the presence of other species may have a direct effect on the development and growth of individual species. The infection process of different *Fusarium* species in wheat (*F. graminearum* – main DON or *F. langsethiae* – main T-2 producers) is different; therefore more comprehensive research is needed to understand the interaction between the different species of *Fusarium* colonizing the same plant (Klemsdal et al., 2008).

Table 3. Winter wheat stand density, a thousand kernel weight and grain yield as influenced by different tillage (T) and fertilization (F) practices in 2005–2006

Treatments	Productive tillers m^{-2}		Thousand kernel weight g		Grain yield t ha^{-1}	
	2005	2006	2005	2006	2005	2006
Conventional tillage	554 a	509 a	48.1 a	27.5 a	8.1 a	4.6 a
Reduced tillage	495 a	459 b	48.2 a	25.5 a	7.3 a	4.0 b
No tillage	559 a	465 b	48.0 a	28.4 a	7.3 a	3.3 b
Not fertilized	456 a	452 a	47.8 a	29.1 a	5.6 b	3.3 b
Moderate rates	552 a	492 a	48.7 a	26.0 b	8.3 a	4.2 a
High rates	599 a	489 a	48.0 a	26.2 b	8.8 a	4.5 a
Interaction T × F	ns	ns	ns	ns	ns	**

Note. Means followed by the same letters within each column and within each treatment are not significantly different at $P \leq 0.05$; ns – not significant at 0.05 probability level, ** – $P < 0.01$.

Spring wheat. *Fusarium* infection level in spring wheat grain ranged from 27.3% to 41.3% and was very similar during the 2006–2008 period (Table 4). High rates of fertilizers significantly increased *Fusarium* infection level only in 2007, but the same trends were noted in the other years too. The main *Fusarium* species infecting spring wheat grain were: *F. avenaceum*, *F. culmorum*, *F. poae* and *F. sporotrichioides*. High rates of fertilizers significantly increased *F. avenaceum* infection level in grain in 2006–2007 and *F. poae* in 2008. The highest *F. culmorum* infection level (13.7%) was observed in the grain harvested from no-tilled plots in 2006. During all three years, less *F. poae* infection level was recorded in no-tilled plots compared with conventionally tilled plots, but only in 2008 the differences were significant.

Significantly lower ZEN concentrations were observed in spring wheat grain samples harvested from no-tilled plots in 2006 and 2008 (<10 μg kg^{-1}) than in conventional and reduced tillage plots. While in 2008, T-2 content was significantly lower in conventional tillage treatments (8.2 μg kg^{-1}), which corresponds to the lowest percentage of grain infected with *F. sporotrichioides,* one of the main T-2 toxin producers (Table 4).

Table 4. Spring wheat grain infection with *Fusarium* fungi as influenced by different tillage and fertilization practices in 2006–2008

Treatments	Percentage of infected grain				
	Fusarium spp.	*F. avenaceum*	*F. culmorum*	*F. poae*	*F. sporotrichioides*
			2006		
Conventional tillage	35.7 a	8.7 a	7.5 a	3.2 a	6.4 a
Reduced tillage	39.4 a	12.0 a	7.3 a	2.4 a	8.7 a
No tillage	36.2 a	6.8 a	13.7 ab	1.8 a	7.3 a
Not fertilized	33.7 a	6.2 b	9.8 a	1.8 a	7.4 a
Moderate rates	37.3 a	9.8 a	9.5 a	2.6 a	7.0 a
High rates	40.3 a	11.5 a	9.1 a	3.0 a	7.9 a
			2007		
Conventional tillage	35.5 a	24.1 a	3.3 a	0.8 a	2.2 a
Reduced tillage	31.9 a	22.5 a	1.0 a	0.5 a	4.0 a
No tillage	31.8 a	23.5 a	2.5 a	0.0 a	2.6 a
Not fertilized	27.3 c	17.3 b	2.7 a	0.5 a	2.4 a
Moderate rates	30.6 b	19.8 b	1.9 a	0.3 a	4.0 a
High rates	41.3 a	33.1 a	2.2 a	0.5 a	2.3 a
			2008		
Conventional tillage	32.8 a	10.3 b	5.8 a	6.3 a	1.6 a
Reduced tillage	36.8 a	10.2 b	6.0 a	2.7 a	2.6 a
No tillage	34.4 a	21.3 a	3.1 a	1.4 b	3.8 a
Not fertilized	33.3 a	16.8 a	5.3 a	1.6 b	1.9 a
Moderate rates	36.1 a	12.4 a	4.8 a	3.3 ab	2.2 a
High rates	34.7 a	12.5 a	4.9 a	5.5 a	3.9 a

Notes. Means followed by the same letters within each column and within each treatment are not significantly different at $P < 0.05$. Interaction between tillage and fertilization was not significant at 0.05 probability level.

Test of fungal genomic DNA, for the presence or absence of *Tri5* gene, encoding the first step in the trichothecene (TRI) synthesis pathway, showed that spring wheat grains were contaminated by the producers of TRI in 2007 and 2008. High fertilizer rates resulted in the highest presence of TRI producers in the grain from conventionally tilled plots in both experimental years (Table 5). All grain samples from reduced tillage treatments were contaminated with TRI producers in both year, but in 2007 contamination level was higher in fertilized wheat than in not fertilized. Similar grain contamination was found in no-tilled plots except for not fertilized plots in 2007.

Table 5. Presence of polymerase chain reaction (PCR) products related to *Tri5* gene as influenced by different tillage and fertilization practices in 2007–2008

Treatments	Not fertilized		Moderate rates		High rates	
	2007	2008	2007	2008	2007	2008
Conventional tillage	−	−	−	−	+++	+++
Reduced tillage	+	+	++	trace	++	+
No tillage	−	+	++	+	+	trace

Notes. +++ strong band, ++ moderate band, + weak band, − no band. The concentration of amplicons is equivalent to the trichothecene contamination levels in grain.

DON concentrations in spring wheat grain samples ranged from <100 to 173.0 μg kg^{-1} in 2006, from 144.3 to 160.1 μg kg^{-1} in 2007 and from 102.9 to 229.3 μg kg^{-1} in 2008 (Table 6). In 2006, values below the limit of detection were detected in conventional and reduced tillage treatments, while in no-tilled treatment DON concentrations ranged from 146.0 to 173.0 μg kg^{-1}, depending on fertilizer rates. The highest *F. culmorum* infection level (13.7%) was detected in no-tilled treatment as well (Table 4). In 2008, grain samples from no-tillage treatments contained a significantly lower DON concentration (107.2 μg kg^{-1}) than from conventional (192.5 μg kg^{-1}) and reduced tillage (180.5 μg kg^{-1}) treatments.

Table 6. Deoxynivalenol, zearalenone and T-2 toxin concentrations (μg kg^{-1}) in spring wheat grain samples as influenced by different tillage and fertilization practices in 2006–2008

Treatments	Deoxynivalenol		Zearalenone		T- 2 toxin	
	mean	range	mean	range	mean	range
2006						
Conventional tillage	<100	<100	41.2 a	38.6–43.8	17.7 a	17.2–18.1
Reduced tillage	<100	<100–110.0	32.8 a	13.5–45.8	16.9 a	16.2–18.2
No tillage	163.0	146.0–173.0	3.5[b] b	0.0–10.5	18.3 a	17.4–19.2
Not fertilized	(106.5)[a]	<100–146.0	28.1 a	0.0–45.8	17.2 a	17.4–18.1
Moderate rates	<100	<100–170.0	27.6 a	0.0–43.8	17.5 a	16.3–18.3
High rates	(111.3)	<100–173.0	21.8 a	10.5–41.3	18.2 a	17.2–19.2
2007						
Conventional tillage	149.5 a	144.3–155.3	22.5 a	22.2–22.7	20.6 a	19.2–21.7
Reduced tillage	151.4 a	150.3–153.0	23.2 a	23.1–23.3	21.9 a	20.6–23.0
No tillage	156.9 a	152.0–160.1	22.4 a	21.0–24.6	21.0 a	20.8–21.1
Not fertilized	151.3 a	148.9–153.0	23.3 a	22.2–24.6	20.3 a	19.2–23.0
Moderate rates	155.2 a	150.3–160.1	22.3 a	21.0–23.3	21.3 a	20.8–22.0
High rates	151.2 a	150.8–158.6	22.5 a	21.7–23.2	21.8 a	20.8–23.0
2008						
Conventional tillage	192.5 a	134.6–229.3	12.0 a	11.6–12.3	8.2 b	7.9–8.4
Reduced tillage	180.5 a	102.9–220.8	12.5 a	11.9–12.9	12.6 a	11.9–13.6
No tillage	107.2 b	105.8–109.2	3.7 b	0.0–11.0	12.9 a	12.5–13.3
Not fertilized	180.1 a	105.8–220.8	7.8 a	0.0–11.9	11.6 a	7.9–13.6
Moderate rates	184.5 a	106.5–229.3	8.4 a	0.0–12.8	11.2 a	8.4–12.9
High rates	115.6 a	102.9–134.6	12.0 a	11.1–12.9	10.9 a	8.4–12.5

Notes. Means followed by the same letters within each column and within each treatment are not significantly different at $P \leq 0.05$. [a] – means in parentheses include samples with values below limit of detection; [b] – some means of mycotoxins are below limit of detection because of inclusion of samples with zero values.

Spring wheat stand density differed between tillage treatments. No-tillage resulted in significantly lower number of productive tillers in 2007 and 2008, the same trends were seen in 2006 too (Table 7). In winter wheat positive correlations were established between plant number and DON content, while in spring wheat the number of productive tillers m^{-2} was correlated with DON content (0.575, $P < 0.01$). T-2 toxin content negatively correlated not only with the productive tillers m^{-2} (−0.546, $P < 0.01$) like in winter wheat, but also with the number of plants m^{-2} (−0.724, $P > 0.01$). ZEN content did not appear to correlate with stand density components when the data were combined over 2006–2008 years; however, in 2006 it correlated with the number of plants m^{-2} (0.876, $P > 0.01$) and in 2008 with both number of plants (0.737, $P > 0.05$) and number of productive tillers m^{-2} (0.77, $P > 0.05$).

A significant reduction in a thousand kernel weigh in 2007 and grain yield in 2006–2007 was influenced by the interaction between no-tillage and zero fertilization treatments. In 2008, there were no significant interactions, but the trends were the same: a significantly lower thousand kernel weigh (41.8 g) was obtained in no-tillage and grain yield (2.7 t ha^{-1}) in no fertilized treatments (Table 7).

Field experiments verified that non-inversion tillage is increasing the FHB incidence and severity and DON contamination in grain (Dill-Macky, Jones, 2000; Fernandez et al., 2005; Lori et al., 2009). However, the influence of conservation tillage systems is indirect and increasing of disease is mostly influenced by leaving all or part of the *Fusarium*-infected crop residue on the soil surface after harvest (Dill-Macky, 2008; Maiorano et al., 2008).

In our experiment, plant residues of the pre-crops (pea and rape) as well as pre-pre-crops (spring barley and winter wheat) were collected and removed from the experimental field each year after harvest. It was expected that with the use of conventional crop rotation, unfavourable for wheat diseases, and removal of the pre-crops' straw from the fields we would get results influenced only by tillage and fertilization.

Spring wheat grain had on average 10 times higher *Fusarium* infection level than winter wheat and the infection levels were similar during 2006–2008; however, the tillage and fertilization treatments' effects were not clearly evident either. A significant influence of high fertilizer rates on grain infection with *Fusarium* spp. was observed only in 2007; in 2006 and 2008 similar trends persisted. An increase in trichothecene producers in fertilized spring wheat was also observed. This agrees with the studies conducted by Martin et al. (1991) who indicated that supplementary nitrogen increased the incidence of *Fusarium* infection in harvested grain. Other investigations also support those findings (Lemmens et al., 2004; Krnjaja et al., 2009).

Table 7. Spring wheat stand density, a thousand kernel weight and grain yield as influenced by different tillage (T) and fertilization (F) practices in 2006–2008

Treatments	Productive tillers m^{-2}			Thousand kernel weight g			Grain yield t ha^{-1}		
	2006	2007	2008	2006	2007	2008	2006	2007	2008
Conventional tillage	476 a	693 a	844 a	23.8 a	44.9 b	43.6 a	2.6 a	5.2 a	3.3 a
Reduced tillage	462 a	677 a	836 a	23.5 a	45.2 a	42.6 b	2.5 a	5.1 a	3.2 a
No tillage	455 a	621 b	725 b	25.7 a	43.6 c	41.8 c	2.2 b	3.0 b	3.1 a
Not fertilized	493 a	660 a	786 a	23.4 a	40.7 b	42.4 a	2.4 a	3.1 b	2.7 c
Moderate rates	446 a	667 a	815 a	24.1 a	45.2 a	43.0 a	2.5 a	4.6 a	3.2 b
High rates	453 a	664 a	804 a	25.3 a	47.8 a	42.6 a	2.4 a	5.6 a	3.7 a
Interaction T × F	ns	ns	ns	ns	*	ns	*	**	ns

Note. Means followed by the same letters within each column and within each treatment are not significantly different at $P \leq 0.05$; ns – not significant at 0.05 probability levels, ** – $P < 0.01$, * – $P < 0.05$.

The tillage methods had no significant influence on *Fusarium* infection level; however, some significant differences in the mycotoxin contents were observed between the tillage treatments. The concentrations of DON (in 2008) and ZEN (in 2006 and 2008) in spring wheat and T-2 toxin (in 2006) in winter wheat significantly correlated with the number of productive tillers or number of plants m^{-2} and were significantly lower in the no-tillage system. That shows indirect tillage effect on mycotoxin content.

Conclusions

1. In two-factorial tillage and fertilization field experiments carried out during 2005–2008, *Fusarium* infection level in harvested winter wheat grain ranged from 0% to 7.1%; in spring wheat from 27.3% to 41.3%. Deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin concentrations in winter wheat grain samples were <100–182.0 μg kg^{-1}, 0–43.6 μg kg^{-1} and 0–15.2 μg kg^{-1} and in spring wheat <100–229.3 μg kg^{-1}, 0–45.8 μg kg^{-1} and 7.9–23.0 μg kg^{-1}, respectively.

2. High fertilizer rates significantly increased spring wheat grain infection with *Fusarium* spp. in 2007 and similar trends were found in 2006 and 2008.

3. Tillage systems had no significant influence on *Fusarium* infection level; however, they had indirect effect on mycotoxin content in separate years. DON (in 2008) and ZEN (in 2006 and 2008) concentrations in spring wheat and T-2 toxin (in 2006) in winter wheat significantly correlated with the number of productive tillers or number of plants m^{-2} and were significantly lower in the no-tillage system.

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Žemės dirbimo ir tręšimo įtaka *Fusarium* infekcijai bei mikotoksinų kaupimuisi kviečių grūduose

S. Supronienė[1], A. Mankevičienė[1], G. Kadžienė[1], A. Kačergius[2], D. Feizienė[1], V. Feiza[1], R. Semaškienė[1], Z. Dabkevičius[1], K. Tamošiūnas[1]

[1]Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas
[2]Gamtos tyrimų centro Botanikos institutas

Santrauka

Varpų fuzariozė yra pasaulyje plačiai išplitusi javų liga, mažinanti grūdų derlių ir bloginanti jo kokybę. Tyrimų tikslas – nustatyti įvairių žemės dirbimo ir tręšimo būdų įtaką žieminių bei vasarinių kviečių grūdų vidiniam pažeidimui *Fusarium* genties grybais ir užterštumui mikotoksinais – deoksinivalenoliu (DON), zearalenonu (ZEN) bei T-2 toksinu. Dviejų veiksnių lauko bandymai 2005–2008 m. vykdyti Lietuvos žemdirbystės institute. Vidinis grūdų pažeidimas *Fusarium* genties grybais vertintas taikant agarizuotų terpių metodą. Grynosios grybų kultūros identifikuotos naudojant įvairius raktus. Mikotoksinų kiekis grūduose nustatytas ELISA metodu.
Meteorologinės sąlygos 2005–2008 m. nebuvo palankios varpų fuzariozei plisti, todėl *Fusarium* infekcijos lygis nukultuose žieminių kviečių grūduose buvo labai mažas (0–7,1 %), o vasarinių kviečių grūduose – vidutinis (27,3–41,3 %). DON (<100–182,0 μg kg^{-1}), ZEN (0–43,6 μg kg^{-1}) ir T-2 toksino (0–15,2 μg kg^{-1}) koncentracijos žieminių kviečių grūdų mėginiuose dažniausiai buvo artimos aptikimo ribai, vasarinių kviečių grūdų mėginiuose – kiek didesnės. Žemės dirbimo būdai neturėjo akivaizdžios įtakos *Fusarium* infekcijos lygiui grūduose, tačiau mikotoksinų produkcijos skirtumų buvo nustatyta. DON (2008 m.) bei ZEN (2006 bei 2008 m.) koncentracija vasariniuose kviečiuose ir T-2 toksino (2006 m.) – žieminiuose kviečiuose esmingai koreliavo su produktyvių stiebų arba augalų skaičiumi m^2 ir buvo iš esmės mažesnės taikant tiesioginę sėją. Didesnių normų trąšų esminė įtaka *Fusarium* spp. infekcijos lygiui vasarinių kviečių grūduose nustatyta 2007 m., tačiau ir 2006 bei 2008 m. užfiksuotos panašios tendencijos. Didesnis kiekis trichotecenus produkuojančių grybų nustatytas tręštuose vasariniuose grūduose, ypač taikant tradicinį arimą ir tręšiant didesniu kiekiu trąšų.

Reikšminiai žodžiai: žemės dirbimas, tręšimas, *Fusarium* spp., mikotoksinai, *Triticum aestivum*, grūdai.