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The effect of fungicides on rye and triticale grain contamination with *Fusarium* fungi and mycotoxins

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Abstract

The study on the control of Fusarium head blight (FHB) in naturally infected winter rye and winter triticale crop was conducted at the Lithuanian Institute of Agriculture during the period 2004–2006. Our experimental findings indicate that the incidence of FHB, grain infestation with *Fusarium* spp. and mycotoxin deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin (T-2) contamination were varied under cultural (rye, triticale) and environmental (year) conditions and fungicide applications. Irrespective of the fungicide treatment, on rye and triticale the most common *Fusarium* species of the FHB complex were *F. avenaceum*, *F. poae* and *F. culmorum*. An increase in rye grain infestation with *Fusarium* fungi and DON and T-2 toxin contamination in azoxystrobin applied plots was observed.

Keywords: rye, triticale, grain, Fusarium head blight, *Fusarium* spp., mycotoxin.

Introduction

Rye and triticale are economically important crops in Europe, however, in Lithuania rye accounts for 4% and triticale 5% of the total arable crops. Rye is used for bread-making and animal feed and triticale is used primarily for animal feed. Fusarium head blight (FHB) of cereals caused by *Fusarium* spp. is known as a disease leading to considerable reduction of grain yield and grain contamination with secondary metabolites of the pathogens (Pirgozliev et al., 2003; Desjardins, 2006). *Fusarium* species can result in reduction of small-grain cereals grain quality, either by affecting grain processing qualities or by producing a range of toxic metabolites that have adverse effects on humans and livestock. About 20 species of *Fusarium* as well as *Monographella nivalis* damage wheat, triticale and rye wherever these crops are grown. The most common of these are *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. sambucinun* var. *coeruleum*, *F. srookwellense*, and *F. sporotrichioides*, *F. poae*, *F. tricinctum* (Arseniuk et al., 1993; Miedaner, 1997; Nicholson et al., 2003; Henriksen, Elen, 2005). As Kiecana and Mielniczuk (2010) reported, in Poland *F. avenaceum*, *F. culmorum* and *F. sporotrichioides* were the largest threat to rye head.

Fungicidal effect on FHB or *Fusarium*-infected grains has been variable in different studies. Cultivar resistance, fungicide efficacy, timing, species of *Fusarium* and pathogen aggressiveness are probably some of the reasons for the variable effects of fungicides on FHB (Mesterhazy et al., 2003; Henriksen, Elen, 2005). Fungicide treatment and agricultural management practices only reduce the damage, but they cannot prevent yield and quality losses (Miedaner, 1997). Fungicide use in wheat artificially inoculated with *F. culmorum* and *F. graminearum* under field conditions can reduce FHB severity at most 77% and mycotoxin content by 89% (Haidukowski et al., 2005). Lower levels of at most 70% effectiveness have been reported for fungicide control in field conditions for naturally infected wheat (Stack, 2000). In winter rye the correlation between FHB rating and deoxynivalenol (DON) content was moderate and varied with the environment (Miedaner et al., 2003). Effective chemical control of FHB under field conditions has generally been inconsistent. Glasshouse and field trials conducted to assess the efficacy of fungicides against FHB yielded conflicting results. One possible reason for the inconsistent control of FHB achieved by fungicides under field condition is the complex interaction, witch may occur between fungicide, *Fusarium* species and other ear colonising fungi, such as *Alternaria*, *Septoria*, *Cladosporium* and *Botrytis cinerea* (Pirgozliev et al., 2003). The effectiveness of fungicides against FHB (*F. graminearum*) is influenced by complex interactions between rainfall, temperature, fungicide concentration and the time of inoculation. However, some of the fungicides are ineffective against FHB and some have been shown to stipulate DON and nivalenol (NIV) production particularly at sub-optimal fungal growth conditions and low fungicide dosage (Jennings et al., 2000; Magan et al., 2002; Ramirez et al., 2004).

Most of FHB control researches were performed in a winter wheat crop, while little is known about the particularities of FHB control and mycotoxins (deoxynivalenol, zearalenone, T-2 toxin) accumulation in rye and triticale grain. The aim of this study was to investigate the influence of fungicides on the development of FHB on triticale and rye and on the *Fusarium* species composition and mycotoxin contamination of harvested grain.

Materials and methods

Field experiments. Field trials with winter rye cv. 'Duoniai', and winter triticale cv. 'Tornado' were done at the Lithuanian Institute of Agriculture during the period 2004–2006. The treatments were arranged in a randomized complete block design with four replications in plots of 25 m^2 (2.5 x 10). The treatments included untreated control and pro-piconazole 125 g ha^{-1} (Tilt); tebuconazole 250 g ha^{-1} (Folicur) and azoxystrobin 250 g ha^{-1} (Amistar) were applied at the anthesis (BBCH 65). Phenological growth stages were recorded according to Meier (1997). The fungicides were applied using a precision bicycle spryer. The incidence of naturally occurring FHB was recorded at soft dough stage BBCH 85 on 50 ears per plot. At hard maturity (BBCH 89), the plots were harvested with a plot harvester "Wintersteiger Delta" (Germany) and yields in t ha^{-1} were adjusted to 15% moisture content, thousand grain weight (TGW) was calculated with a seed counter "Contador" (Germany).

Fusarium spp. analysis. The internal grain infestation with *Fusarium* fungi was rated in rye and triticale on 96 samples per crop by agar plate method (Mathur, Kongsdal, 2003). The surface-sterilized seeds (200 per sample) were plated on a potato dextrose agar (PDA) and incubated at 26 ± 2ºC in the dark. The grain infection level was evaluated in percent (0% – all grains healthy, 100% – all grains infected). Microscopic studies of *Fusarium* fungi were carried out after 7–8 days. The purified single spore cultures of *Fusarium* species were identified on the basis of their cultural and morphological characteristics according to Gerlach, Nirenberg (1982), Nelson et al. (1983) and Leslie et al. (2006).

Analysis of mycotoxins. Grain samples (48 per crop) were collected at harvesting and analysed for contamination by deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2). The analysis was done by the CD-ELISA (competitive direct enzyme-linked immunosorbent assay) method (Wilkinson et al., 1992). The "Veratox®" quantitative test kits ("Neogen" corporation, Food Safety Diagnostics), 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", 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).

Statistical analyses. Anova was applied for the statistical data processing. The significance of data was determined by the Fisher's criterion with a significance level of $P \leq 0.01$ and 0.05. Significant differences from untreated in tables are marked as ** ($P \leq 0.01$) and * ($P \leq 0.05$). Averages for the other data were calculated (Tarakanovas, Raudonius, 2003).

Meteorological conditions. In 2004, the lack of precipitation was appreciable until the middle of June. In 2005, June was rainy and cool, while in July the weather became warmer and dry. Rainy weather at the end of July and at the beginning of August predominated both in 2004 and 2005. Conversely, in 2006 a shortage of rainfall was appreciable during the whole growing season, only before harvest rainy weather occurred (Figure).

Results and discussion

In rye and triticale crops the highest natural infection of FHB was recorded in 2004, in 2005 it was lower, while in 2006 no FHB symptoms were recorded in the trial field. The observed differences between rye and triticale infestation might have been influenced by their flowering dates, while in 2005 the flowering dates in both rye and triticale were closer (Figure). In 2004, the humid conditions during the flowering stage in rye resulted in more intense disease development, however triticale

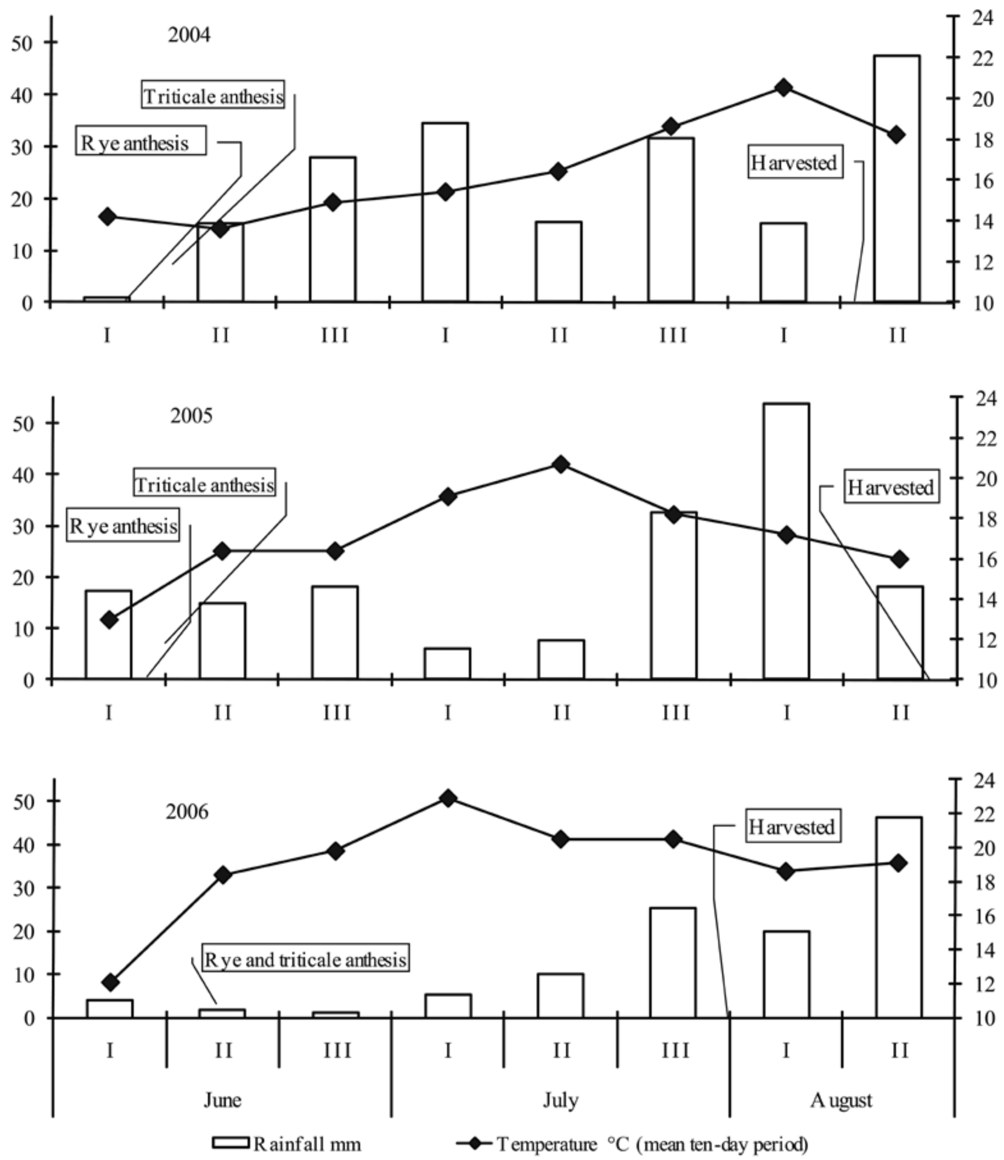

Figure. Meteorological conditions during 2004–2006, anthesis (BBCH 65) and harvest time of rye and triticale

flowered in dryer conditions, which might have determined that in 2004 the infection of FHB in rye was higher than that in triticale. The field-based studies conducted by Xu (2003) confirmed that warm and moist conditions during anthesis are the key facto for FHB development. Due to hot weather in 2005 and 2006, the flowering sage both in rye and triticale occurred at dry period and was short. The visual disease assessments after the application of different fungicides showed some variations in symptom development among the treatments (Table 1). In different years, the treatments used resulted in different levels of FHB. In 2004, in the untreated control in both rye and triticale and in 2005 in triticale the FHB incidence was higher than that in the fungicide applied plots. In 2004, the significant reduction in FHB incidence in fungicide treated plots was observed in all treatments except for tebuconazole treatment in triticale. In contrast, in 2005, the FHB incidence in rye was significantly lower in the untreated control in comparison with propiconazole and azoxystrobin applied plots. The disease incidence in tebuconazole treatment was comparable to the untreated control. The fungicide efficacy results obtained in the two years survey are contradictory. In many studies the effective chemical control of FHB under field conditions has generally been inconsistent. Glasshouse and field trials conducted to assess the efficacy of fungicides against FHB have yielded conflicting results (Jacobsen, 1977; Simpson et al., 2001; Magan et al., 2002; Mesterhazy et al., 2003; Pirgozliev et al., 2003). Control of FHB by fungicides has often proved to be erratic and it is possible that environmental factors and replacement of one pathogen by others may have contributed to this (Nicholson et al., 2003).

Table 1. The impact of the fungicide application at anthesis on FHB incidence on ears, grain infestation with *Fusarium* fungi, grain yield and TGW

Fungicides	FHB ears affected %	*Fusarium* spp. infected grain %	Yield t ha^{-1}	TGW g
		Rye		
		2004		
Untreated	40.0	18.3	6.06	38.25
Propiconazole	21.6**	19.0	6.47	39.22**
Tebuconazole	18.3**	14.8	7.06**	39.36*
Azoxystrobin	11.7**	42.5	6.90*	40.19**
		2005		
Untreated	5.0	1.5	5.77	40.03
Propiconazole	21.7	6.8	6.58**	40.97*
Tebuconazole	6.7	2.5	6.39*	41.30*
Azoxystrobin	20.0	10.0*	6.49**	41.10*
		2006		
Untreated	0	4.8	4.26	35.95
Propiconazole	0	8.5	4.25	35.94
Tebuconazole	0	7.3	4.35	35.90
Azoxystrobin	0	5.0	4.26	35.88
		Triticale		
		2004		
Untreated	25.0	5.8	8.48	50.58
Propiconazole	8.3**	4.0	8.73**	52.06*
Tebuconazole	15.0	1.5	9.14**	52.06*
Azoxystrobin	5.0**	5.0	9.40**	53.15**
		2005		
Untreated	11.7	11.0	7.17	42.94
Propiconazole	6.7	5.0	8.11**	43.57
Tebuconazole	2.5	8.2	7.79*	43.24
Azoxystrobin	3.3	9.0	8.16**	44.13**
		2006		
Untreated	0	4.3	4.73	45.52
Propiconazole	0	5.8	5.02	46.02
Tebuconazole	0	2.5	5.10*	45.39
Azoxystrobin	0	2.3	5.30**	46.67*

Note. * – significant difference from untreated at 95% probability level, ** – significant difference from untreated at 99% probability level.

The highest grain infestation with *Fusarium* fungi was in 2004, occurred in line with the highest FHB incidence in that year. In 2005 and 2006, the grain infestation was low. In spite of the absence of FHB symptoms on ears in 2006, the infected grain in different treatments in rye amounted to 5–8.5 % and in triticale 2.3–4.3%, respectively. Despite the fact that in 2004 the incidence of FHB on rye in untreated plots was significantly higher than in fungicide treated plots, the grain infestation with *Fusarium* fungi was comparable in propiconazole and tebuconazole and much higher in azoxystrobin treatment. Both in 2004 and 2005, the rye grain infestation was signally higher in azoxystrobin applied plots compared with propiconazole and tebuconazole treatments and untreated plots. In triticale crop the incidence of FHB and grain infestation with *Fusarium* fungi were lower compared with rye grain. The contradictory results on triticale and rye susceptibility to FHB have been reported. Arseniuk et al. (1999) indicated that winter triticale genotypes were the least susceptible to FHB averaging 9.4% with winter rye being intermediate (13.5%) between triticale and the most susceptible wheat (30.1%). Conversely, based on the symptoms on harvested grains, Martin et al. (1991) found triticale to be more susceptible than wheat. Cultural susceptibility might be influenced of the complex interactions between environmental factors as well as associated mycoflora which can occur on ripening ears of cereals (Magan et al., 2002; Pirgozliev et al., 2003).

Application of fungicides resulted in significant yield and TGW increase in 2004 and 2005 (with little exception) both in rye and triticale; however in 2006 the increase was significant only in triticale tebuconazole and azoxystrobin treatments (Table 1). It is hypothesized that FHB could influence the reduction of rye and triticale grain yield only in 2004, when the FHB infection was reduced by fungicide use, while in 2005 and in 2006 the yield and TGW increase was obtained due to foliar disease control by fungicides used. In central Europe, severe natural epidemics of FHB occur once or twice a decade only, but it can sharply reduce yield and quality traits of cereals (Miedaner et al., 2001). FHB can have an adverse effect on several yield components resulting in significant loss of total yield. The reduction in kernel number, kernel weight per head and 1000 kernel weight for winter wheat triticale and rye were 13.9%, 8.8% and 11.4%, respectively (Arseniuk et al., 1999). Surveys carried out between 1951 and 1985 recorded 19 FHB outbreaks with wheat grain yield reduction of 5–15% in years when moderate epidemics of FHB were recorded and up to 40% in years when disease epidemics were severe (Pirgozliev et al., 2003).

Fusarium culmorum in 2004 was the most frequent species isolated from all rye and triticale untreated and fungicide treated grain samples except for tebuconazole treated triticale. *F. sporotrichioides* was common in all rye grain samples, however, on triticale grain it was detected only on azoxystrobin treated grain (Tables 2 and 3). *F. graminearum* was common on rye grain too, except for azoxystrobin treated. Apart from *F. culmorum*, triticale grain appeared to be infested with *F. poae* and *F. heterosporum*.

Table 2. Mycotoxin contamination level and *Fusarium* fungi composition of winter rye grain

Fungicides	Mycotoxins μg kg^{-1}			*Fusarium* composition on grain
	DON	ZEN	T-2	
2004				
Untreated	<100	0	23.5	*F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. sambucinum*, *F. sporotrichioides*
Propiconazole	281.7*	0	53.8	*F. culmorum*, *F. graminearum*, *F. sambucinum*, *F. semitectum*, *F. sporotrichioides*
Tebuconazole	578.1**	0	42.1	*F. culmorum*, *F. graminearum*, *F. sporotrichioides*
Azoxystrobin	634.0**	0	134.1**	*F. culmorum*, *F. poae*, *F. semitectum*, *F. solani*, *F. sporotrichioides*
2005				
Untreated	<100	<10	<7.5	*F. avenaceum*, *F. semitectum*, *F. graminearum*
Propiconazole	128.2	<10	<7.5	*F. avenaceum*, *F. equiseti*, *F. heterosporus* *F. poae*, *F. sporotrichioides*
Tebuconazole	<100	<10	<7.5	*F. equiseti*, *F. semitectum*, *F. graminum* *F. sporotrichioides*
Azoxystrobin	165.6	10.3	<7.5	*F. avenaceum*, *F. graminearum*, *F. graminum*, *F. poae*
2006				
Untreated	0	0	<7.5	*F. avenaceum*, *F. culmorum*, *F. sporotrichioides*
Propiconazole	0	0	<7.5	*F. avenaceum*, *F. poae*, *F. sporotrichioides, F. equiseti*
Tebuconazole	102.6	0	<7.5	*F. avenaceum*, *F. poae*, *F. sporotrichioides*
Azoxystrobin	110.0	0	<7.5	*F. sporotrichioides*, *F. tricinctum*, *Fusarium* spp.

Note. Explanation under Table 1.

Conversely, in 2005, on both rye and triticale grain samples the most frequent were *F. avenaceum*, *F. poae*, *F. sporotrichioides* and *F. equiseti. F. graminearum* was found only on untreated rye and azoxystrobin treated rye and triticale grain. Despite the absence of FHB symptoms on ears in 2006, various compositions of *Fusarium* species were identified on rye grains. *F. sporotrichioides* was common in all treatments, *F. avenaceum* and *F. poae* prevailed in most treatments, and *F. culmorum* was found only in untreated grain, while in that year in triticale grain *F. poae* and *F. sporotrichioides* were identified in all treatments. Irrespective of the fungicide treatment, on rye and triticale the most common *Fusarium* species of the FHB complex in 2004 was *F. culmorum*. This pathogen is known to be more common in temperate climates. Studies performed in Scandinavia, Hungary, Poland and other mid-European countries identified *F. avenaceum* and *F. poae* as the most important species as well as the dominance of particular species in infecting rye and triticale heads was determined by the weather conditions (Lukanowski, Sadowski, 2002; Kiecana, Mielniczuk, 2010). The harvested grain analysis showed that these species were the most common on rye and triticale grain both in 2004 and 2005. *F. graminearum* was identified on rye grain and in 2005 on triticale too. This species is known as the most toxic fungi and is pointed to an increase in the importance of *F. graminearum* as a major pathogen of wheat in temperate climates. In the Netherlands *F. graminearum* was the primary species for two consecutive years (2000–2001) which suggests a dramatic shift in the composition of the FHB complex (Waalwijk et al., 2003).

Table 3. Mycotoxin contamination level and *Fusarium* fungi composition of winter triticale grain

Fungicides	Mycotoxins μg kg^{-1}			*Fusarium* composition on grain
	DON	ZEN	T-2	
2004				
Untreated	419.6	0	47.7	*F. culmorum*, *F. heterosporum*
Propiconazole	361.3	0	47.8	*F. culmorum*, *F. poae*
Tebuconazole	240.6*	0	51.0	*F. poae*
Azoxystrobin	170.5*	0	0	*F. culmorum*, *F. sporotrichioides*
2005				
Untreated	169.0	11.0	<7.5	*F. avenaceum*, *F. poae*, *F. sporotrichioides*
Propiconazole	<100	10.9	<7.5	*F. equiseti*, *F. poae*
Tebuconazole	<100	0	<7.5	*F. avenaceum*, *F. poae*
Azoxystrobin	<100	0	<7.5	*F. avenaceum*, *F. equiseti*, *F. graminearum* *F. poae*, *F. sporotrichioides*, *F. solani*
2006				
Untreated	160.5	10.2	<7.5	*F. poae*, *F. sporotrichioides*
Propiconazole	164.5	10.6	<7.5	*F. poae*, *F. sporotrichioides*
Tebuconazole	163.1	10.4	<7.5	*F. poae*, *F. sporotrichioides*
Azoxystrobin	123.1	11.7	8.2	*F. poae*, *F. sporotrichioides*

Note. Explanation under Table 1.

The assay of mycotoxin contaminations yielded contradictory results. The most contaminated with DON, ZEN and T-2 grain both rye and triticale was in 2004 when the FHB was intense, while in 2005 and 2006 only low levels of mycotoxins were found. DON content in rye grain samples from fungicide treated plots was significantly higher compared with untreated. The most severe grain contamination with DON was in azoxystrobin and tebuconazole treatments. The significant increase in T-2 was found in azoxystrobin treatment. In 2004, triticale grain contamination with DON was significantly lower in grain samples from tebuconazole and azoxystrobin treated plots. Summarised data on the presence of mycotoxins in rye and triticale grain showed DON to be the most common in both rye and triticale. The more intensive contamination with T-2 was found in rye, and a significant increase was determined in azoxystrobin treatment. In our trials only traces of ZEN were detected. In many studies the contradictory results of fungicide effect on FHB, grain infestation and mycotoxin content are yielded. Data from this study indicate an increase in rye grain infestation with *Fusarium* fungi contamination with DON and T-2 in azoxystrobin applied plots at anthesis. This agrees with the results from many studies (Simpson et al., 2001; Pirgozliev et al., 2002; Pirgozliev et al., 2003). The fungicides containing tebuconazole have been reported as the most effective to reduce FHB and *Fusarium* infection in the grain (Mesterhazy et al., 2003; Pirgozliev et al., 2003). Conversely, Pirgozliev et al. (2002) reported that fungicides affected the DON concentrations indirectly by influencing the amount of *Fusarium* species in grain. For mycotoxin risk assessment, the importance of *Fusarium* fungi in the deoxynivalenol, nivalenol, and zearalenone contamination of cereal grains warrants further study (Desjardins, 2006). This study indicated that FHB can pose a significant threat for small grained cereals. Due to the lack of consistently effective control measures, the results of the present study indicated a need for regular screening for *Fusarium* fungi composition and mycotoxin contamination.

Conclusion

Our experimental findings indicate that the incidence of FHB, grain infestation with *Fusarium* spp. and mycotoxin contamination were varied under cultural (rye, triticale) and environmental (year) conditions and fungicide applications. Irrespective of the fungicide treatment, the most common *Fusarium* species of the FHB complex on rye and triticale were *F. avenaceum*, *F. poae* and *F. culmorum*. Our experimental evidence suggests an increase in rye grain infestation with *Fusarium* fungi and contamination with DON and T-2 toxin in azoxystrobin applied plots. The control of *Fusarium* fungi and the prevention of increased mycotoxin contaminations are essential for consumers' protection from toxic contaminants. For mycotoxin risk assessment, the importance of *Fusarium* fungi and trichotecenes and zearalenone contamination of cereal grains warrants further study.

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Fungicidų poveikis rugių ir kvietrugių grūdų užterštumui *Fusarium* grybais bei mikotoksinais

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Santrauka

Straipsnyje apibendrinti rugių ir kvietrugių varpų fuzariozės kontrolės tyrimų, 2004–2006 m. atliktų Lietuvos žemdirbystės institute, duomenys. Nustatyta, kad varpų fuzariozės išplitimas, nukultų grūdų pažeidimas *Fusarium* genties grybais ir užterštumas mikotoksinais deoksinivalenoliu (DON), zearalenonu (ZEN) bei T-2 toksinu įvairavo priklausomai nuo javų rūšies, aplinkos sąlygų ir panaudotų fungicidų. Iš rugių ir kvietrugių varpų fuzariozę sukeliančių *Fusarium* spp. komplekso dažniausi aptikta *F. avenaceum, F. poae* bei *F. culmorum*. Fungicidų poveikio rugių ir kvietrugių grūdų užterštumui *Fusarium* grybais bei mikotoksinais rezultatai buvo prieštaringi. Didesnis rugių grūdų užterštumas *Fusarium* grybais ir DON bei T-2 toksinu nustatytas azoksistrobinu apdorotų laukelių javų grūduose visais tyrimų metais, o kvietrugių didžiausias užterštumas DON buvo fungicidais neapdorotų laukelių javų grūduose.

Reikšminiai žodžiai: rugiai, kvietrugiai, grūdai, varpų fuzariozė, *Fusarium* spp., mikotoksinai.