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THE EFFECTS OF FUNGICIDES ON *FUSARIUM* SPP. AND THEIR ASSOCIATED MYCOTOXINS IN NATURALLY INFECTED WINTER WHEAT GRAIN

ABSTRACT

Field trials conducted at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (central part of Lithuania) in 2009 were aimed to evaluate the effect of fungicides on Fusarium Head Blight (FHB) in a naturally infected field. A single application of dimoxystrobin + epoxiconazole (Swing Gold), prothioconazole (Proline), metconazole (Juventus), tebuconazole (Folicur), prothioconazole + tebuconazole (Prosaro) was applied to winter wheat cv. 'Zentos' at the manufacturer's recommended doses at anthesis (BBCH 65). The FHB incidence and severity were assessed at milk and hard maturity stages. The percentage of *Fusarium* infected grain and deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin (T-2) concentrations in harvested grain were determined. In all fungicide treated plots a significant reduction of FHB incidence and severity was determined; however the fungicides did not exert any effect on the amount of *Fusarium*–infected grain as compared with the untreated control. A reduction of DON, ZEN and T-2 contents in grain was determined in tebuconazole treatments. *Fusarium avenaceum* (Fr.) Sacc, *F. culmorum* (W. G. Sm.) Sacc., *F. poae* (Peck) Wollenw, *F. sporotrichioides* Sherb. and *F. tricinctum* (Corda) Sacc were identified in wheat grain, *F. poae* was prevalent.

Keywords: Fusarium head blight, *Fusarium* spp., fungicides, mycotoxin, winter wheat

INTRODUCTION

Fungicidal effect on FHB or *Fusarium*-infected grain has been variable in different studies. Cultivar resistance, fungicide efficacy and timing, species of *Fusarium* and pathogen aggressiveness are probably some of the reasons for the variable effects of fungicides on FHB (Masterhazy et.al,

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2003). Menniti *et al.*, (2003) indicate that the epoxiconazole effectively reduces FHB incidence but is not very effective directly against *F. graminearum* and *F. culmorum* fungi. In *F. graminearum* artificially infected winter wheat an average dimoxystrobin+epoxiconazole efficacy for DON was 49.55 and 63.9% for a reduction in yield loss, however, it was found highly variable in different years and sites (Šip *et al.*, 2010). Metconazole and tebuconazole significantly reduced the amount of trichothecene-producing *Fusarium* in harvested grain (Edwards *et al.*, 2001). Tebuconazole has been proved to be highly effective in reducing *Fusarium* fungi infection by many researchers (Menniti *et al.*, 2003; Tvaruzek, 2004; Ioos *et al.*, 2005; Blandino *et al.*, 2006), and especially *F. culmorum* and *F. avenaceum* and DON contents (Simpson *et al.*, 2001), however under natural infection conditions this fungicide does not always exhibit sufficient efficacy against the *F. culmorum* or *F. graminearum* (Ioos *et al.*, 2005). The prothioconazole is mentioned as very effective against *F. graminearum* and *M. nivale* (Suty-Heinze, Dutzmann, 2004), and recently Lechoczki-Krasjak *et al.* (2008) reported that the prothioconazole +tebuconazole (Prosaro) was the most efficient and caused 80% reduction of the FHB symptoms.

The aim of this study was to investigate the effect of fungicides on the development of FHB in naturally infected wheat and on the *Fusarium* species composition and mycotoxin contamination.

MATERIALS AND METHODS

Field experiments

Field trials with a randomized block design and four replicates were done in winter wheat cv. 'Zentos' at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry in 2009. The treatments included untreated control and fungicide application with dimoxystrobin (133 g × l^{-1}) + epoxiconazole (50 g × l^{-1}) (Swing Gold 1.5 l × ha^{-1}), prothioconazole (480 g l^{-1}) (Proline 1.0 l ha^{-1}), metconazole (90 g × l^{-1}) (Juventus 1.0 l ha^{-1}), tebuconazole (250 g × l^{-1}) (Folicur 1.0 l ha^{-1}), prothioconazole (210 g × l^{-1}) + tebuconazole (210 g × l^{-1}) (Prosaro 1.0 l × ha^{-1}) at the manufacturer's recommended doses was done at anthesis (BBCH 65). The incidence and severity of FHB were recorded on 45 (3 × 15) ears per each plot, carrying out visual evaluations of the disease at milk (BBCH 75) and hard maturity (BBCH 89) stages. *Fusarium* spp. analysis. *Fusarium* species composition was evaluated at milk maturity stage by plating 100 *Fusarium* affected seeds from each plot. Percentage of *Fusarium* infected grain was evaluated by plating 100 randomly selected seeds harvested from affected ears at hard

maturity stage and plating 400 randomly selected seeds taken from each 1 kg sample of mechanically harvested grain with a small plot harvester at hard maturity stage. Seeds were sterilised for 3 min in 1.0 % NaOCl solution, rinsed three times in sterile water and dried before plating on Petri dishes with potato dextrose agar (PDA) and incubated for 7-8 days at 26±2 °C (Mathur, Kongsdal, 2003). The overgrown Fusarium colonies were isolated, purified and identified according to the manuals of Nelson *et al.* (1983) and Leslie *et al.* (2006) by visual and microscopic observation of single spore cultures. Colonies with confused indications were attributed to *Fusarium* spp. The Fusarium infection level of grain was evaluated in percent from analysed grain.

Analysis of mycotoxins

Grain samples were analysed for contamination by deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin. The analysis was done by the ELISA (enzyme-linked immunosorbent assay) method (Wilkinson *et al.*, 1992). The Veratox test kits (Neogen Corporation, Scotland), approved by the AOAC Research Institute (Certificate N 950702) were used for the analysis. The optical densities of samples and controls from standard curve were estimated by a photometer Multiskan Ascent, using a filter of 650 nm. Measured absorbances were automatically converted to the mycotoxin concentration units - $\mu g \times kg^{-1}$. 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 $\mu g \times kg^{-1}$ (ppb); ZEN – 10.0 $\mu g \times kg^{-1}$ (ppb); T-2 toxin – 7.5 $\mu g \times kg^{-1}$ (ppb).

Statistical analysis

ANOVA was applied for the statistical processing of data. For data significance the Fisher test was used. Averages for the other data were calculated (Tarakanovas, Raudonius, 2003).

Meteorological conditions

The end of May and June – July was rainy and moderately warm, consequently the conditions were conducive to the spread and development of FHB in cereals.

RESULTS

Visual FHB assessment at milk maturity stage (BBCH 75) showed significant differences in disease incidence and severity among the untreated control and fungicide applied plots (Table 1). *F. avenaceum, F. culmorum* and *F. poae* were the most frequent *Fusarium* species in FHB affected seeds at BBCH 75.

Table 1

FHB incidence, severity and *Fusarium* species composition in affected wheat grain at milk maturity stage (BBCH 75) as influenced by fungicide application at anthesis (BBCH 65)

Treatment	FHB		*Fusarium* species composition
	Incidence [%]	Severity [%]	
Untreated	86.1	6.7	*F. avenaceum, F. culmorum, F. poae, F. sporotrichioides, F. tricinctum, F. graminearum, Fusarium* spp.
Dimoxystrobin (133 g × l^{-1}) + epoxiconazole (50 g l^{-1})	73.9	2.7**	*F. poae, F. culmorum, F. sporotrichioides, F. tricinctum, Fusarium* spp.
Prothioconazole (480 g × l^{-1})	68.3*	3.1**	*F. tricinctum, F. poae, F. culmorum, F. sporotrichioides*
Metconazole (90 g l^{-1})	69.4*	3.0**	*F. avenaceum, F. poae, F. culmorum, F. sporotrichioides*
Tebuconazole (250 g ×l^{-1})	72.8*	3.0**	*F. culmorum, F. poae, F. sporotrichioides, F. avenaceum, F. graminearum*
Prothioconazole (210 g ×l^{-1}) + tebuconazole (210 g × l^{-1})	60.0**	2.5**	*F. poae, F. culmorum, F. avenaceum, F. sporotrichioides,*
Significance (P-value)	0.0167	0.0001	
$LSD_{5\%}$	12.92	1.48	
$LSD_{1\%}$	17.86	2.05	

*Significant difference from untreated at $P \leq 0.05$ probability level; ** Significant difference from untreated at $P \leq 0.01$ probability level

Table 2

FHB incidence, severity and percentage of *Fusarium* infected grain in affected wheat ears at hard maturity stage (BBCH 89) as influenced by fungicide application at anthesis (BBCH 65)

Treatment	FHB		*Fusarium* infected grain [%] ± Sd
	Incidence [%]	Severity [%]	
Untreated	89.2	4.2	64.0 ± 10.8
Dimoxystrobin (133 g l^{-1}) + epoxiconazole (50 g l^{-1})	74.2**	1.7**	12.0 ± 5.7
Prothioconazole (480 g l^{-1})	75.8*	1.7**	12.0 ± 6.1
Metconazole (90 g l^{-1})	72.5**	1.7**	21.0 ± 5.5
Tebuconazole (250 g l^{-1})	69.2**	1.5**	26.0 ± 8.9
Prothioconazole (210 g l^{-1}) + tebuconazole (210 g l^{-1})	71.7**	1.8**	8.0 ± 5.2
Significance (P-value)	0.0100	0.0000	
LSD5%	10.05	0.93	
LSD1%	13.89	1.28	

*Significant difference from untreated at $P \leq 0.05$ probability level; ** Significant difference from untreated at $P \leq 0.01$ probability level

At hard maturity stage (BBCH 89), significant differences in disease incidence and severity persisted among the untreated control and fungicide applied plots (Table 2). Percentage of *Fusarium* infected grains from affected ears markedly differed in the untreated control and fungicide applied plots as well. At hard maturity stage (BBCH 89), a significant reduction in *F. sporotrichioides* was observed for all fungicide treated plots (Table 3). Prothioconazole, metconazole and dimoxystrobin + epoxiconazole and prothioconazole + tebuconazole significantly reduced *F. culmorum*. Metconazole and prothioconazole + tebuconazole significantly reduced *F. tricinctum*. All fungicides had no impact or only inappreciably influenced *F. poae* and *F. avenaceum* infection in grain.

Table 3.

Effect of fungicide treatments on percentage infected grain by *F. avenaceum, F. culmorum, F. poae, F. sporotrichioides, F. tricinctum* and other *Fusarium spp.* in affected wheat ears at hard maturity stage (BBCH 89)

Treatment	*Fusarium* infected grain (%) ± Sd					
	F. avenaceum	*F. culmorum*	*F. poae*	*F. sporo-trichioides*	*F. tricinctum*	*Fusarium spp.*
Untreated	6.0 ± 5.4	10.0 ± 5.8	10.0 ± 4.7	22.0 ± 8.1	6.0 ± 3.5	10.0 ± 8.2
Dimoxystrobin (133 g l^{-1}) + epoxiconazole (50 g l^{-1})	2.0 ± 3.2	0.0	5.0 ± 2.6	1.0 ± 1.6	4.0 ± 3.5	0.0
Prothioconazole (480 g l^{-1})	1.0 ± 1.6	0.0	8.0 ± 4.6	1.0 ± 1.6	2.0 ± 3.2	0.0
Metconazole (90 g l^{-1})	2.0 ± 2.1	0.0	12.0 ± 5.2	4.0 ± 3.5	0.0	0.0
Tebuconazole (250 g l^{-1})	3.0 ± 2.4	4.0 ± 6.3	5.0 ± 3.5	8.0 ± 4.6	5.0 ± 3.5	1.0 ± 1.6
Prothioconazole (210 g l^{-1}) + tebuconazole (210 g l^{-1})	2.0 ± 2.1	0.0	6.0 ± 3.5	0.0	0.0	0.0

Table 4

Percentage of *Fusarium* infected grain and DON, T-2 and ZEN content in harvested grain at full maturity stage as influenced by fungicide application at anthesis (BBCH 65)

Treatment	*Fusarium* [%] ± Sd	Mycotoxin [µg × kg^{-1}] ± Sd]		
		DON	T-2	ZEN
Untreated	16.0 ± 7.0	106.0±0.7	9.9±0.3	11.6±1.3
Dimoxystrobin + epoxiconazole	21.8 ± 6.6	108.9±1.0	9.4±0.7	10.7±0.1
Prothioconazole	16.5 ± 5.6	109.7±2.5	9.6±0.1	10.8±0.1
Metconazole	21.0 ± 8.5	111.5±1.0	11.4±0.7	10.5±0.3
Tebuconazole	18.8 ± 7.6	0	0	0
Prothioconazole + tebuconazole	19.0 ± 7.1	107.4±4.0	11.1±0.6	0

Percentage of *Fusarium* infected grain varied from 16.0% to 21.8% in harvested grain at full maturity stage (Table 4). No significant difference in *Fusarium* infection level on harvested grain was observed among treatments. Mycotoxins DON, T-2 and ZEN were not detected in tebuconazole

treated plots. ZEN was not detected in plots treated with prothioconazole + tebuconazole as well. In other treatments DON content varied from 106.0 µg × kg^{-1} to 111.5 µg × kg^{-1}, T-2 – from 9.4 µg × kg^{-1} to 11.4 µg × kg^{-1} and ZEN – from 10.5 µg × kg^{-1} to 11.6 µg × kg^{-1}.

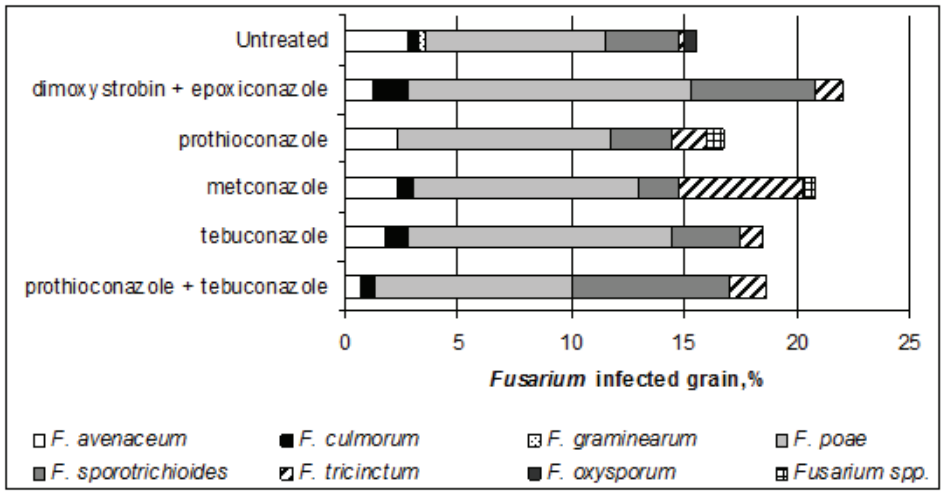

Fig. 1. Influence of fungicide application at anthesis (BBCH 65) on *Fusarium* infection in harvested wheat grain.

F. graminearum and *F. oxysporum* were detected only in harvested grain from untreated plots (Fig. 1). In prothioconazole treated plots grain was no infected with *F. culmorum*. The other *Fusarium* species varied irrespective of fungicide application, *F. poae* was prevalent.

DISCUSSION

Our experimental findings indicate that the treatments with fungicides, carried out at anthesis, led to the reduction in FHB incidence by on average 20.9% and severity by on average 59.5% from the untreated control in 2009. The results obtained are in agreement with those of Menniti *et al.* (2003), Tvaruzek (2004), Ioos *et al.* (2005), Blandino *et al.* (2006), Suty-Heinze, Dutzmann (2004) and other, who observed similar effect of triazole fungicides on FHB.

Compared with untreated control, all fungicides used reduced the amount of *Fusarium* infected grains in ears at hard maturity stage from 59.4 to 87.5; however significant effect of all fungicides was established only on *F. sporotrichioides* – one of the main T-2 toxin producers. Prothioconazole, metconazole and dimoxystrobin + epoxiconazole and prothioconazole + tebuconazole significantly reduced the amount of *F. culmorum* infected grain and only metconazole and prothioconazole + tebuconazole significantly reduced *F. tricinctum*. As was previously indicated Edwards *et al.* (2001) metconazole

and tebuconazole effectively reduced the amount of trichothecene-producing *Fusarium* species, Menniti *et al.* (2003) have indicated that epoxiconazole is effective against *F. culmorum*, which explains the results obtained by us. Prothioconazole has been reported as effective against *F. graminearum* (Suty-Heinze, Dutzmann, 2004), but our investigations showed its good effect against *F. culmorum* too.

Mycological analyses of harvested grain exhibited no significant effect on *Fusarium* infection level among the treatments. *F. poae* prevailed in grain and this could be explained by the insufficient influence of fungicides against *F. poae.*

The tebuconazole application at anthesis led to significantly lower DON, T-2 and ZEN contamination in harvested grain, whereas application of tebuconazole + prothioconazole significantly reduced only ZEN. Edwards *et al.* (2001) reported that metconazole and tebuconazole effectively reduced DON contamination in grain, but in our field trials only tebuconazole proved to be effective.

CONCLUSIONS

Winter wheat application with prothioconazole, metconazole, tebuconazole, dimoxystrobin + epoxiconazole and prothioconazole + tebuconazole at anthesis (BBCH 65) resulted in a significant reduction of FHB incidence and severity at milk (BBCH 75) and hard (BBCH 89) maturity stages. The application with tebuconazole gave a significant reduction of deoxynivalenol, T-2 toxin and zearalenone content in grain.

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